BREEDING SUCCESS AND EFFECTS OF DIFFERENT DIETS ON THE GROWTH OF SIAMESE FIGHTING FISH, *BETTA SPLENDENS* REGAN, 1910

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Abstract

This study investigated the reproductive performance and subsequent growth rates of the Siamese fighting fish (*Betta splendens*). The breeding experiment compared the effects of two water types (tap water and 40% almond leaf extract water) and three nesting materials (Regiform , Plastic, and Banana leaf) on hatching and survival rates. Statistical analysis showed that the highest hatching rate (70.00%) occurred in tap water with a Plastic nest, while the highest survival rate (53.85%) was achieved in almond extract water with a Plastic nest, indicating the benefit of almond extract for fry survival. Additionally, a four-month growth trial was conducted on B. splendens fingerlings using five different diets: Daphnia (Diet I), Mosquito larvae (Diet II), Pellet feed (Diet III), Mixed feed (Diet IV), and Artemia cysts (Diet V). ANOVA results revealed that live and mixed feed sources—Mosquito larvae (83.72% weight gain), Mixed feed (80.49%), and Daphnia (74.42%)—resulted in significantly higher mean body weights compared to Artemia (P-values < 0.05). Conversely, Artemia showed the highest survival rate (100%), despite the lowest weight gain (41.46%). The findings offer valuable insights for optimizing breeding and feeding protocols in ornamental fish aquaculture.

Keywords: *Betta splendens*, breeding performance, growth rates, survival rates

Introduction

The genus *Betta* is a large group of small, colorful freshwater ray-finned fishes belonging to the gourami family, Osphronemidae. The best known *Betta* species is *Betta splendens*, the Siamese fighting fish, appeared to be small in size but considerable variation in size was noted. *Betta splendens*, Siamese fighting fish is native to tropical Southeast Asia including the northern Malay Peninsula, central and eastern Thailand, Cambodia, southern Vietnam and Myanmar (U.S. Fish and Wildlife Service, 2019).

They are cultured varieties produced through generations of selective breeding of the *B. splendens*. Therefore, selected breeding methods and captive breeding of Bettas are popular practices in the ornamental fish production market. During the breeding, Betta shows high parental care animal species. Bettas are bubble nest builders and nest guarders. The males build bubble nests at the surface of the water; these can be very large in mass, incorporation pieces of vegetation (Sandar Aung, 2017). Males behaviorally build bubble nests and care for developing eggs until they reach the larval stage.

Male *Bettas* may fight to claim territory, or to protect their eggs or offspring from rival males. The fish will spread his fins, shudder his body, extend his gill opercula and membranes, and generally appear much larger than his resting size (Krempels, 2015).

Nutrition plays an important role in maintaining good health and normal behavior, enhancing the external appearance and reproduction performance of ornamental fish (Keshavanath and Patil, 2006). For Siamese fighting fish, live diets include zooplankton, tubifex

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worms, *Daphnia*, mosquito larvae, and small aquatic insects, which are commonly used in rearing throughout its life span (Mejia *et al*., 2021). Since Siamese fighting fish is naturally carnivorous, they prefer a live diet over an artificial pellet diet (Sermwatanakul, 2019).

The knowledge of quantitative aspects such as length-weight relationship, condition factor, growth and mortality of fishes are important tools for studying fishing biology (Lizama, *et al*., 2002). Length-weight relationship study of a fish is a pre-requisite for assessing the population health status of the species (Le Cren, 1951). Fish can attain either isometric growth, negative allometric growth and positive allometric growth (Nehemia and Justin 2012). Condition factor (K-value) is also useful index for monitoring of feeding intensity, age and growth rates of fish (Anene, 2005). Fishes sufficiently fed would have K greater than equal to 1 (One) but when undernourished K is less than 1 (Aye Aye Aung *et al*., 2022).

Good nutrition is essential for promoting growth, health, and breeding performance in fish. To assess these phenomena, the readily available aquarium species Siamese fighting fish, Betta splendens, was chosen for the present study. Therefore this research aimed to: (1) investigate and compare the hatchability and fry survivability of *B. splendens* across different water types and nesting materials , (2) evaluate the overall breeding success under controlled conditions and (3) examine the effect of different diets on the subsequent growth performance of B. splendens fingerlings.

Materials and Methods

Experimental Site and Study Period

This study was conducted in the museum of Department of Zoology, Pyay University. This study was conducted during December, 2023 to August, 2024. Before the experiment, identification of fish was made according to Tawlar and Jhingarn (1991), and Jayaram (2013).

Breeding Performance of Siamese Fighting Fish

Maintenance of broodstock

Six pairs of healthy, seven-month-old Siamese fighting fish were sourced from local aquarium shops in Pyay Township, averaging 3.94±0.28 cm in total length and 0.95±0.12 g in body weight. Broodstock were individually maintained in 12×12×20 cm3 glass tanks filled with 0.8 L of water and fed dry pellets and Daphnia at 6% of their body weight, twice daily.

Experimental Design for Assessing Breeding Performance in Two Water Types and Three Nesting Materials

The breeding pairs were stocked at a ratio of one male to one female in aquarium tanks (30 x 30 x 45 cm^3). To compare the effects of water types, two conditions were used: tap water and 40% almond water. In each type of water, equal pieces of regiform, plastic, and banana leaf (8 x 8 cm^2) were placed on the water surface in each tank to facilitate bubble nest formation by the male.

Nursery of fry

The fry was fed with boiled egg yolk ten days after hatching and later, commercial pellet and artemia cysts. Care was taken not to over feed fry to avoid the pollution of water and water surface must be kept stable.

Breeding Performance in Different Batches

The breeders were given a rest period of 15 days after mating and parental care. Following this, they were prepared for the next breeding batch. The above-mentioned procedure was applied for every batch, with each pair being used as breeders for four consecutive batches.

Effect of Different Diets on the Growth Rate of Siamese Fighting Fish

Experimental Fish and Experimental Design

The offspring of Siamese fighting fish (50 individuals) (2.91±0.33cm) were obtained from the previous breeding performance experiment. The fishes were placed into glass-tank (24 x 12 x 18 cm3) filled with tap water. Five groups of glass-tank were prepared stocking each tank with 10 fish fingerlings of Siamese fighting fish. The fish in each tank were fed with each kind of diets. To avoid water pollution, food remained at the bottom of each aquarium was siphoned off after feeding. Total length and body weight of sample fish were measure the twice a month. The survival rate of fish was checked every day during the experiment. Total length and body weight of fish were measured by using Digital Calipers and Digital Balance respectively. The growth of body length and weight was estimated based on the sample fish randomly taken from the rearing aquarium to include all size groups of fish (small, medium and large fish).

Larvae Feed Preparation

During the experimental periods, the fishes were fed with different diets amounting 6% of body weight of fishes in each experimental tank and they were feed twice a day at 3% each. The fishes were fed with Daphnia (Diet I), Mosquito larvae (Diet II), Pellet feed (Diet III), Mixed feed (50% of Daphnia and 50% of pellet) (Diet IV) and Artemia (Diet V) for this experiment.

Assessment of water quality

Water temperature and pH were measured weekly using thermometer and pH meter respectively.

Statistical Analysis

Hatching rate (%) = $100 \text{ x } \frac{\text{Number of fry hatched}}{\text{Number of egg laid}}$ (Ricker, 1975)

Survival rate (%) = $100 \text{ x } \frac{\text{Final number of fish}}{\text{Initial number of fish}}$ (Ricker, 1975)

Length Gain (LG) = {(FL-IL) / IL x 100} (Bagenal, 1978)

Weight Gain (WG) = {(FW-IW) / IW x 100} (Bagenal, 1978)

Condition factor (K) = $\frac{W}{L^3} \times 100$ (Fulton, 1904)

The water types and multiple comparison of nest places (regiform, plastic and banana leaf) and the significant differences between the treatments of different diets on weight the data were subjected to ANOVA test (R software version 3.4).

Results

Nest Building of *Betta splendens*

Once the male Betta fish becomes interested in the female, it will begin constructing a bubble nest. The nest is made of air bubbles coated in saliva and is typically located under regiform, plastic, banana leaf. The nest is considered complete when the male stops working on it. As the female lays her eggs, the male collects them in his mouth and spits them into the nest. The bubble nest serves to protect the eggs until they hatch.

Hatching rate and survival rate of *Betta splendens* in different nesting sites in Almond water and Tap water

In the present study, the first and second batches of *Betta splendens* were observed in December and temperature parameter was 25-27˚C, while the third and fourth batches were observed in January and the temperature parameter was 24-27˚C.This study on *Betta splendens* demonstrated significant differences in hatching and survival rates based on nesting type and water conditions. In almond water, regiform nests yielded hatching rates ranging from 45.45% to 60.00%, with survival rates peaking at 46.67%. In contrast, plastic nests showed more consistent success, achieving hatching rates between 50.00% and 64.29%, along with survival rates reaching up to 53.85%. Banana leaf nests, however, generally exhibited lower performance, with hatching rates between 40.91% and 51.85% and survival rates peaking at 42.86%. When considering tap water, hatching rates increased across all nest types, with plastic nests achieving the highest rate of 70.00% in Batch 4, while survival rates remained lower, particularly in regiform nests at 31.82 %(Table1, Fig.1,2 and Plate1).

Table 1 Hatching and Survival rates of different nesting places in Almond water and Tap water conditions

Nesting sites		Almond water		Tap water	
	Batch	Hatching Rate	Survival Rate	Hatching Rate	Survival Rate
Regiform nest	Batch 1	50.00	46.67	55.00	31.82
	Batch 2	45.45	46.00	60.00	38.89
	Batch 3	50.00	45.45	54.55	33.33
	Batch 4	60.00	46.67	66.67	41.67
Plastic nest	Batch 1	50.00	53.85	59.09	38.46
	Batch 2	45.83	45.45	64.29	42.22
	Batch 3	54.17	46.15	62.50	37.33
	Batch 4	64.29	50.00	70.00	42.86
Banana leaf nest	Batch 1	46.15	41.67	52.94	31.11
	Batch 2	40.91	40.00	50.00	36.36
	Batch 3	45.00	40.00	50.00	32.73
	Batch 4	51.85	42.86	58.82	35.00

In almond water, no significant differences were found among the groups for hatching rates, while a significant difference emerged among tap water groups. In tap water, a significant difference was observed, particularly between the "Plastic and Banana" groups (Fig.3). For survival rates, almond water demonstrated significant differences among groups, particularly between "Plastic and Banana" and "Regiform and Banana" groups (Fig.4). While tap water showed a potential difference among groups.

Body length and weight of different diets on the experimental period (four to seven month old fish)

During this study period, April-July, 2024, water temperature ranges from 25-34˚C and pH value range from 6.5-7.5.The study reveals that fish length and weight varied across different diets over experimental period. For Diet I (Daphnia), fish length ranged from 3.08 ± 0.37cm to 3.83 ± 0.23cm, with weight increasing from 0.43 ± 0.12g to 0.75 ± 0.13g. Similarly, Diet II (Mosquito larvae) saw length ranging from 3.15 ± 0.37cm to 3.94 ± 0.28cm and weight from 0.43 ± 0.13g to 0.79 ± 0.16g. Diet III (Pellet feed) exhibited length changes from 3.20 ± 0.32cm to 3.74 ± 0.21cm and weight from 0.44 ± 0.09g to 0.71 ± 0.11g. In Diet IV (Mixed feed), length increased from 3.09 ± 0.36cm to 3.82 ± 0.38cm, and weight from 0.41 ± 0.14g to 0.74 ± 0.16g. Finally, Diet V (Artemia cysts) showed length variations from 3.24 ± 0.32cm to 3.80 ± 0.24cm and weight from 0.41 ± 0.09g to 0.58 ± 0.10g (Plate 2).

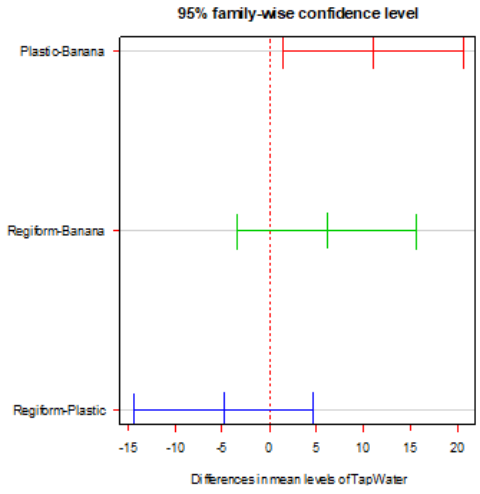


Figure.3 Multiple comparisons of mean differences between plastic, regiform, and banana nesting sites of hatching rate of tap water

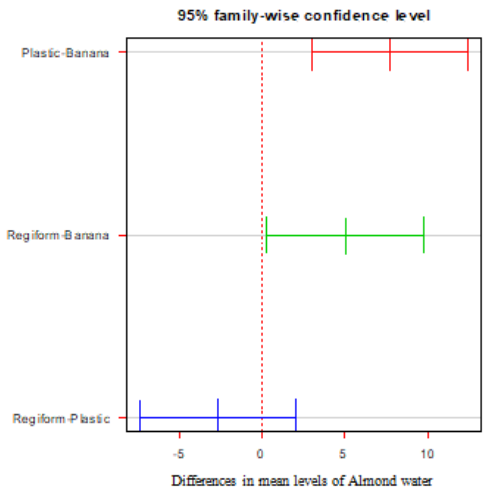
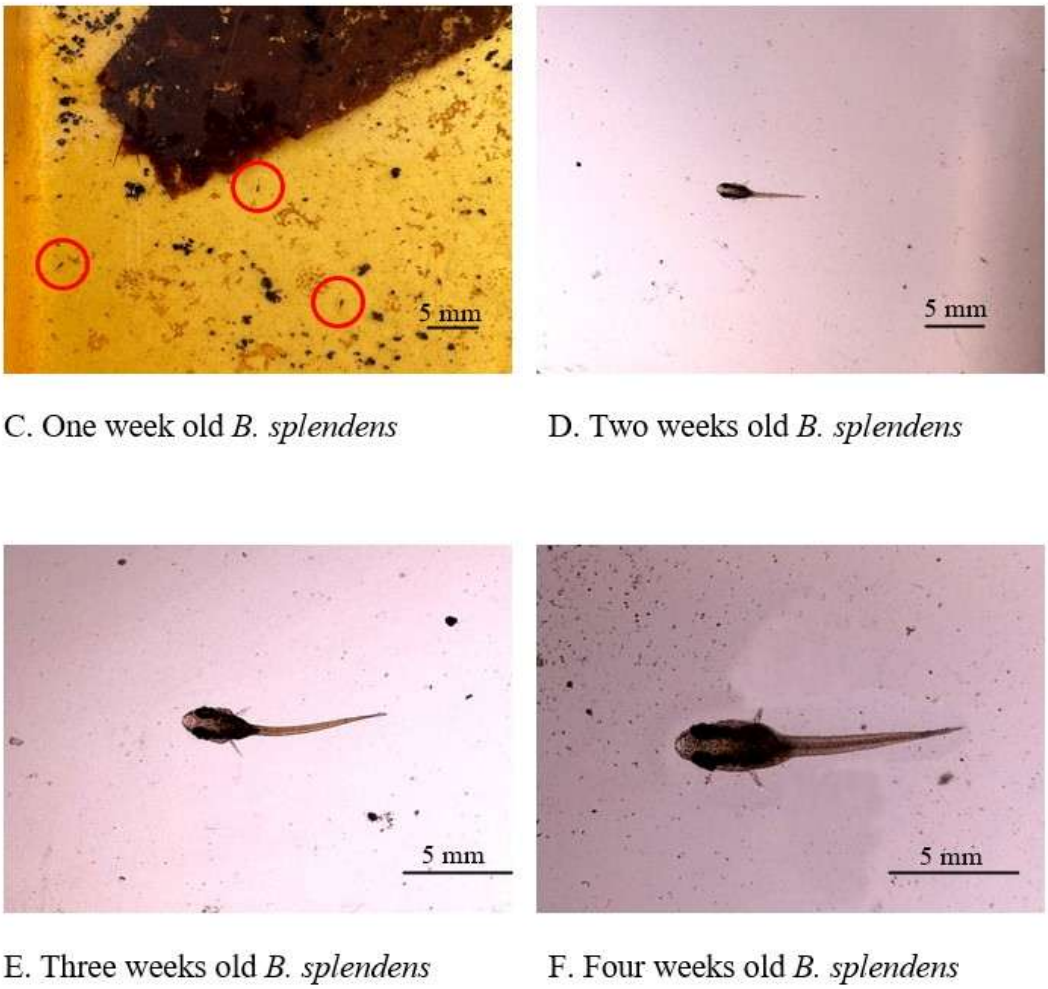


Figure.4 Multiple comparisons of mean differences between plastic, regiform, and banana nesting sites of survival rate of almond water



C. One week old *B. splendens* D. Two weeks old *B. splendens*

E. Three weeks old *B. splendens* F. Four weeks old *B. splendens*

Plate 1 Different age of *Betta splendens* in the tank

Table 2 Comparison of Weight Gain, Length Gain, Survival rate, K, r and b values of across different diets

Diet	Mean Weight Gain	Mean Length Gain	Survival rate	K-value	r-value	b-value
Diet I	74.42	24.35	80%	1.38	0.91	2.62
Diet II	83.72	25.08	80%	1.32	0.94	2.68
Diet III	61.36	16.88	90%	1.33	0.91	2.49
Diet IV	80.49	23.62	80%	1.38	0.91	2.56
Diet V	41.46	17.28	100%	1.11	0.86	2.07

Different diets based on weight gain, length gain, survival rates, K, r and b. Diet II has the highest weight gain at 83.72 and a mean length gain of 25.08. Diet III has the lowest length gain at 16.88. Diet V has the lowest weight gain of 41.46. Diet I and Diet II both had an 80% survival rate, with K values of 1.38 and 1.32. Diet III had a slightly better survival rate of 90% with a K value of 1.33. Diet IV also had an 80% survival rate, with K values of 1.38. However, Diet V

was the best, achieving a 100% survival rate, even though its growth potential (K value of 1.11) was lower. (Table 2, Fig.5).

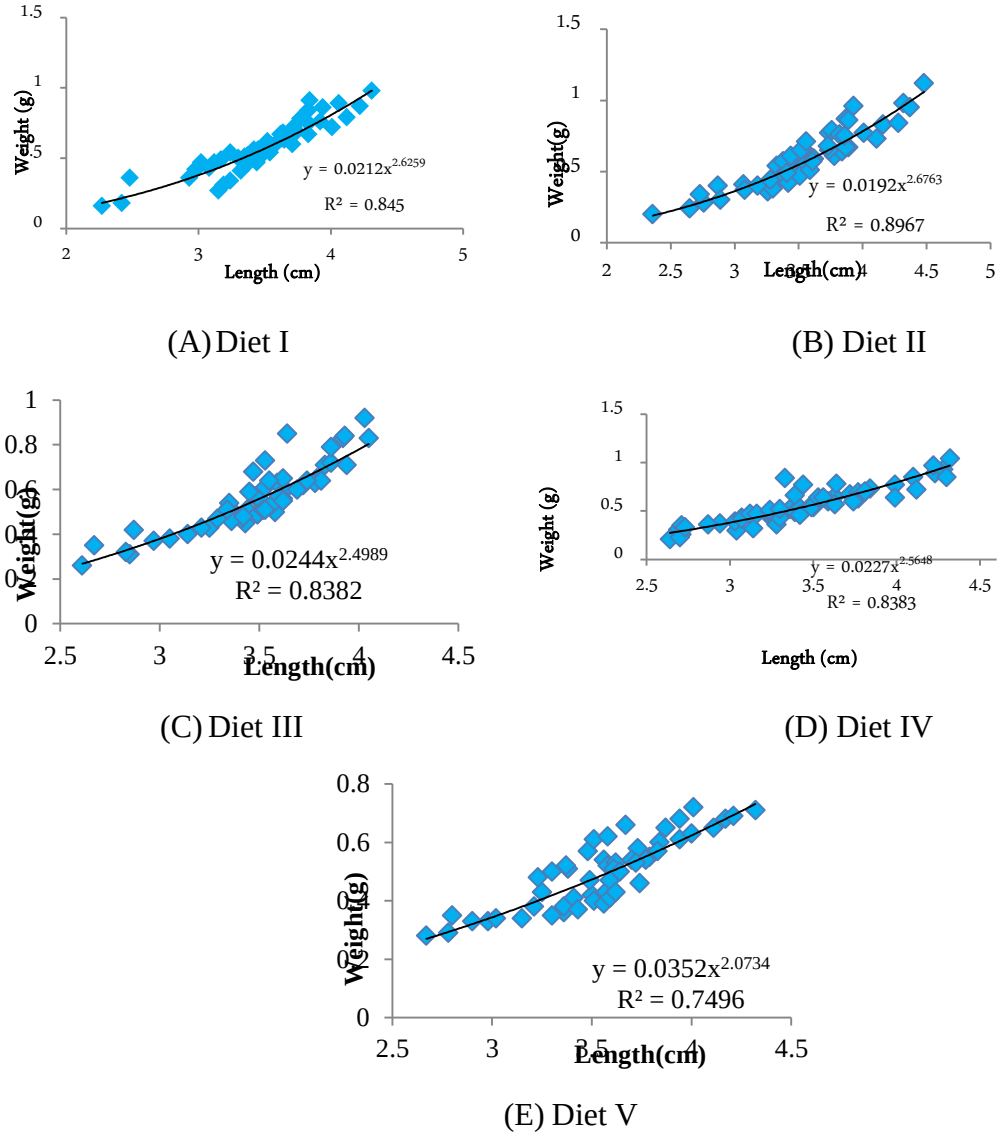
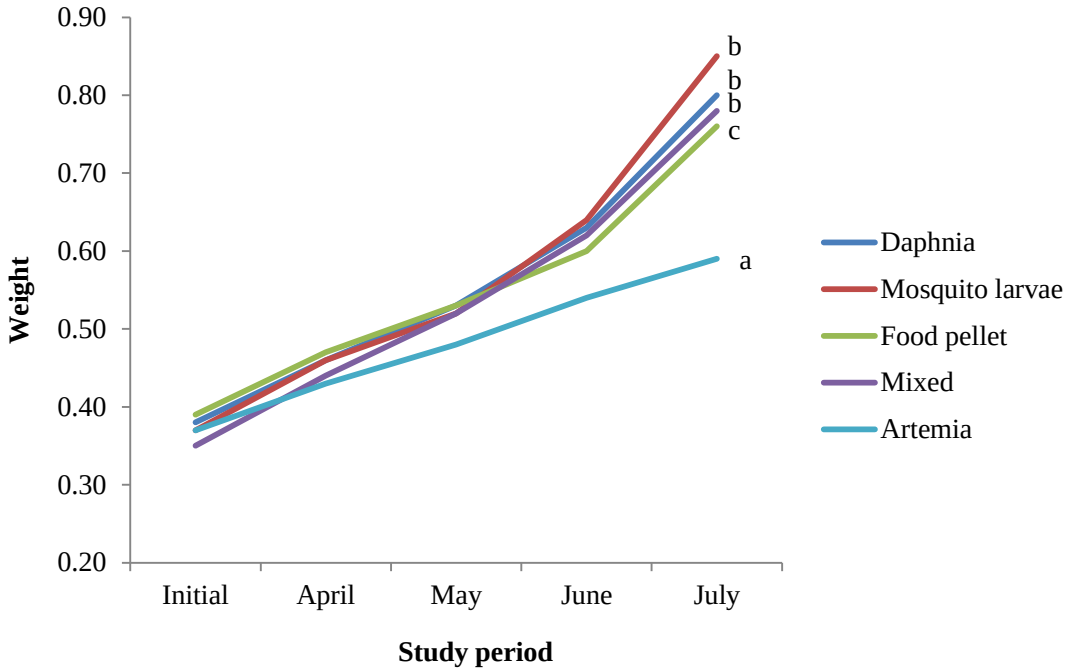


Figure.5 Relationship between body length and weight of *Betta splendens* fed with different diet

The ANOVA analysis assessed the variation in body weights of Betta splendens across different diet groups, revealing significant differences. Specifically, Daphnia, mosquito larvae, and a mixed diet showed significantly higher mean weights compared to Artemia, with p-values of 0.027, 0.007, and 0.045, respectively. Other diet comparisons did not yield statistically significant differences (Fig.6).



(a significant difference between b, a no significance different between c)

Figure.6 Comparison of body weight of *Betta splendens* fed with different diets

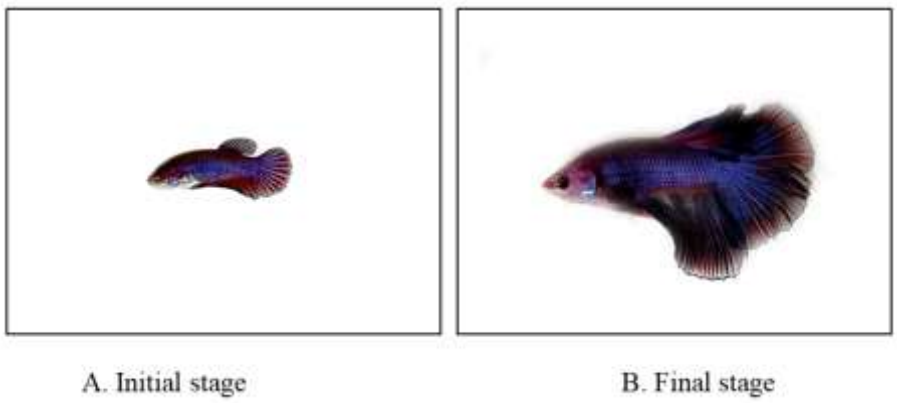


Plate 2 Initial stage and final stage of *Betta splendens* fish

Discussion

The Siamese fighting fish or Betta (*Betta splendens* Regan, 1910) is a popular aquarium fish that is commercially produced and exported throughout the world. Selective breeding of this species provides various brilliant colors, tail types and fin shapes (Thongprajukaew *et al*., 2018).

Patidar, (2022) reported, it's often best to purchase Betta fish from a reputable breeder depend on the age of fish. Breeding Betta fish works best when both mates are between four months and one year old. In the present study, pair of *Betta splendens* at the age of seven months old and in optimal health was chosen for breeding. So that, this results was similar to the above author.

Patidar, (2022) reported that Betta fish prefer water that's between 24˚C-28˚C, so unless they live in an area that has a warm climate all year round, they will need to install a heater. Warm water also aids in breeding Betta fish as it stimulates their urge to reproduce. *Betta splendens* were observed in four batches from December to January. It also specifies that the temperature during December and February ranged from 24 to 27 °C. So that, this results was similar to Patidar, 2022.

Srikrishnan *et al*., (2017) also reported that, females spawn under the nest, where the male waits to fertilize the eggs and typically 400-500 eggs are spawned by female fish. In Plastic nest, tap water reached a peak hatching rate of 70.00%, while almond water was lower at 64.29%. However, almond water sometimes had higher survival rates, particularly in the Plastic nest, where it reached 53.85%. In Banana leaf nest, hatching rates were lower for almond water compared to tap water. Tap water seems more effective for hatching, but almond water may support survival in certain conditions. Because of the chemical composition of almond water effect on embryonic growth and metabolic processes. The almond water did not significantly impact hatching rates among groups. In contrast, tap water showed significant differences, particularly between the "Plastic-Banana" nesting sites, indicating the influence of nesting sites on hatching success. For survival rates, almond water displayed significant differences, especially between the "Plastic-Banana" and "Regiform-Banana" nesting sites. In tap water, a potential different is noted between the "Plastic-Banana" nesting sites.

The effect of different diets on the growth performance of Siamese fighting fish was evaluated on the basis of weight gain (WG), length gain (LG), survival rate, condition factor (K) and length-weight relationship (LWR) using different diets. Sandar Aung, 2017 reported that the optimal water temperature of Siamese fighting fish keeping in 23-29°C and the pH value was 8. In the present study, water temperature recorded ranged from 25-34°C and pH value was 6.5-7.5 during the experimental period.

Zakes *et al*., (2006) reported that generally growth and survival rate increased with feeding frequency. In this experiment, the feeding frequency of two times per day was adopted during the experimental period. Mosquito larvae are the most common live diet for rearing Siamese fighting fish. They contain high amount of proteins and lipids that are notably suitable for feeding carnivorous fish, like Siamese fighting fish (Thongprajukaew *et al*., 2018). In this study, the highest weight gain of *Betta splendens* fed with mosquito larvae and lowest weight gain fed with Artemia. Length gain in *Betta splendens* fed mosquito larvae statistically was obtained better results than other diets. Lowest length gain was found in pellet feed. Therefore, this result was similar to above authors.

The condition factor (K) of a fish reflects physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren, 1951). Diet I (Daphnia) and Diet II (Mosquito larvae) both provided an 80% survival rate, with condition factors (K) of 1.38 and 1.32, respectively. Diet III (Pellet feed) achieved a 90% survival rate and a K value of 1.33. Diet IV (Mixed feed) also achieved an 80% survival rate and a K value of 1.38. Notably, Diet V (Artemia) excelled with a 100% survival

rate, although its K value of 1.11 indicates lower growth potential. Artemia may be best for survival, but other diets could support better growth.

Length-weight relationship is of great importance in fishery management. The correlation coefficient (r) was also estimated to determine the degree of linear relationship between the length and weight of samples (Sandar Aung, 2017). Costa and Araujo (2003) reported that r value of 0.99 was the highest value associated to the individuals that present highest weight for a given length. In this experiment, the highest values of correlation coefficient r = 0.94 was observed in Diet II (Mosquito larvae) and the lowest values of correlation coefficient r = 0.86 in Diet V (Artemia).

The significantly higher mean weights observed with Daphnia, mosquito larvae and the mixed diet suggest that these food sources provide essential nutrients, leading to better growth outcomes compared to Artemia. Because of the higher protein content and nutritional quality of these diets. Pellet feed did not show significant difference compare to Artemia.

The study investigated how different factors affect the breeding success and the growth of *Betta splendens*, a popular aquarium fish. This study show that the choice of nest material and water type significantly influenced reproductive outcomes, highlighting the importance of environmental conditions. . It found that diet plays a crucial role, with live food sources like Daphnia and mosquito larvae leading to better growth compared to Artemia. These results provide valuable insights for fish keepers and aquaculture enthusiasts, helping them understand the factors that contribute to the well-being and reproductive success of *Betta splendens* in captivity.

Conclusion

In conclusion, this study on the Siamese fighting fish (*Betta splendens*) highlighted important factors that affect their breeding success and growth. This study confirmed that fish aged between four months to one year are ideal for breeding and highlighted the importance of maintaining water temperatures between 24˚C and 28˚C. Additionally, the impact of different nesting sites and water types on reproductive success was explored, revealing distinct variations across batches and environments. For tap water, in plastic nesting sites produced the highest hatching rates, while almond water led to better survival rates. Live food sources such as Daphnia, mosquito larvae and mixed feed were found significant different compared to Artemia and pellet feed, although Artemia resulted in the highest survival rates. These results provide valuable insights for aquaculture practices and conservation efforts, emphasizing the multifaceted nature of factors influencing the health and reproductive success of *Betta splendens*.

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