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Investigating the Effect of Different Culture Media on the Growth of *Paramecium spp*. as the First Food for *Betta splendens* Larvae

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ABSTRACT

Background: Live feed is essential for the successful rearing of ornamental fish larvae because of its suitable size, digestibility, and nutritional value. Although *Artemia* is widely used, its high cost and limited availability encourage the search for affordable alternatives. *Paramecium* spp. is a promising live feed for early larval stages, but efficient and low-cost culture methods using locally available organic substrates remain insufficiently explored. **Methods:** Five organic materials (dry banana leaf, straw, banana peel, cabbage, and areca nut peel) were evaluated for *Paramecium* culture. Cell density was determined using a haemocytometer over four weeks, while pH, dissolved oxygen (DO), temperature, and light intensity were monitored. **Results:** Banana peel medium produced the highest *Paramecium* density (53,708 ± 15,471 cells mL^{-1}), significantly exceeding the other media ($P < 0.05$). Temperature, DO, and light intensity did not differ significantly among treatments ($P > 0.05$). Larvae fed *Artemia* showed the highest survival (54.17 ± 3.63%), final length (6.29 ± 0.10 mm), and weight (0.03 ± 0.00 mg), outperforming egg yolk and *Paramecium*. **Conclusion:** Banana peel is an effective, inexpensive substrate for culturing *Paramecium* spp. and represents a practical option for producing live feed as a supplementary feeding strategy in low-cost *B. splendens* larviculture.

Keywords: banana peel, *betta splendens* larvae, organic materials, *paramecium spp,* water quality

1. Introduction

The availability of cost-effective and nutrient-dense feed is crucial to the success of aquaculture's larval rearing process. Due to their high nutritional value, live feeds like Artemia nauplii have long been the standard in traditional feeding methods. However, the cost and particular hatching conditions of Artemia eggs restrict their widespread use, particularly among aquaculture practitioners on a small scale [1]. Consequently, a lot of farmers use egg yolk instead of Artemia because it is easier to obtain, but because of its poor nutritional profile, it frequently does not promote the best growth and survival of fish larvae [2]. This challenge emphasises the need for a more economical, sustainable, and effective substitute for traditional live feeds. A unicellular protozoan frequently employed in larviculture, *Paramecium spp*., is one such promising candidate. Numerous freshwater fish larvae can use *Paramecium spp*. Because it is small, high in proteins and essential fatty acids, and fits well in their mouth gap [3]. It is frequently used as the initial food source for species like *Betta splendens* and Danio rerio [4].

Betta splendens is one of the most well-known and popular ornamental fish species in the world. The species belongs to the family of Osphronemidae. They are native to Southeast Asia, specifically in Thailand. Geographically, the *Betta splendens* are indigenous to shallow freshwater swamps, rice fields, creeks, and

rivers [2]. It is considered one of the most important ornamental fish due to body colouration, shape, and economic as well as notable commercial value. *Betta splendens* is a fascinating perspective model for evolutionary, neurobiological, and behavioural studies. *Betta splendens* are farmed commercially for ornamental purposes. They are also a well-studied species due to their availability and other unique behavioural traits [5, 6, 7]. Although formulated dry diets are economically beneficial, they are less nutritionally advanced than required by larvae at initial developmental stages. Live feeds remain significant in larviculture because they facilitate better growth, development, and immunity of fish larvae [1]. Live feeds are extensively used in aquaculture due to their high protein content and nutritional lipid profile, including EFAs. All these nutrients are essential to maintain fish larvae for the proper development of the digestive system [8]. Conventionally, fish larvae are fed exogenous live feeds such as *Paramecium spp.,* rotifers, and Artemia spp. Naupli [4]. Live prey is considered highly suitable for the early feeding stages of fish larvae because of its appropriate size, digestibility, and nutritional value. Among the microorganisms used as live prey, *Paramecium* spp. have been utilised as an early food source for developing fish larvae. For instance, *Paramecium multinucleatum* has been successfully used as a live feed for *Danio rerio* larvae from 5 days post-fertilisation, demonstrating its suitability as prey during the early larval feeding stage [9]. *Paramecium* spp. are unicellular, free-living ciliates belonging to the phylum Ciliophora and are widely studied because of their simple cellular organisation and ecological importance. As ciliates occupy an important trophic position within aquatic microbial food webs, they contribute to the transfer of energy and nutrients from microorganisms to higher trophic levels [33,34]. *Paramecium* spp. are unicellular heterotrophic ciliates that obtain nutrients primarily through phagotrophy, ingesting microorganisms and particulate organic matter. Their relatively simple cellular organisation, short generation time, and well-characterised cellular processes have made them valuable model organisms for investigating fundamental biological mechanisms. Recent research has continued to demonstrate the usefulness of *Paramecium caudatum* as an experimental model for studying cellular and molecular processes, including gene function and protein localisation [10]. In addition, *Paramecium* spp. have considerable potential in ecotoxicological research because environmental contaminants can induce measurable changes in physiological and behavioural characteristics. Recent studies have demonstrated that exposure of *P. caudatum* to contaminants can alter cell motility, morphology, osmoregulation, and other physiological functions, supporting its application as a sensitive bioindicator for environmental toxicity assessment [11,12]. *Paramecium spp*. are usually obtained from nature, commercial suppliers, and research institutes. The growth and proliferation of this *Paramecium spp*. Rely on environmental conditions and the availability and content of cultural media. High concentration of organic and inorganic compounds in *Paramecium spp.* Culture media has a great favourable influence on *Paramecium spp.* Growth patterns and density [14]. Traditional culture media for *Paramecium spp*. Includes hay infusion and wheat grass with their essential nutritional factors, providing optimal conditions for growth [15]. However, there is limited research on the effectiveness of alternative culture media such as banana peel, straw, cabbage, and areca nut peel for enhancing Paramecium growth.

The main objective of the present study is to investigate the effect of different culture media on the growth of Paramecium using dry banana leaves, straw, banana peel, cabbage, and areca nut peel and determine its suitability for *Betta splendens* larval development as an initial feed. Additionally, this study aims to investigate suitable culture media for the growth and development of *Paramecium spp*. and to investigate the effect of water quality parameters on the growth of *Paramecium spp.* This addresses the knowledge gap by determining the most effective culture media for Paramecium species and assessing how they affect the performance of larvae in Betta splendens, a species with significant ornamental and scientific value.

2. Methods

2.1. Preparation and Evaluation of Culture Media for Paramecium spp. Growth

Five organic materials were used to prepare culture media for *Paramecium spp.* Growth (Figure 1). They were dry Banana Leaf (T1), Straw (T2), banana peel (T3), cabbage (T4), and areca nut peel (T5). All treatments were triplicated. Dry Banana leaves and straws were torn up and soaked in boiling water for 15 minutes to sterilise the materials and remove impurities. The soaked materials were then sun-dried for one day to reduce their moisture content, making them suitable for culture media. Banana peel, areca nut peel, and cabbage were cut into small pieces and soaked in boiling water for 15 minutes. Subsequently, all the materials were weighed using a scale and separated into equal quantities. Dry banana leaf (TR1, T1R2, T1R3)16g for each replicate, Straw (T2R1, T2R2, T2R3) 22g for each replicate, banana peel (T3R1, T3R2, T3R3) 50g for each replicate, cabbage (T4R1, T4R2, T4R3)61g for each replicate, areca nut peel (T5R1, T5R2, T5R3) 33g for each replicate. The quantity of each organic substrate was determined based on its dry matter content, decomposition characteristics, and preliminary observations of its suitability for supporting *Paramecium* spp. proliferation.

As the substrates differed considerably in water content and physical structure, the amount of each material was adjusted to provide approximately equivalent dry matter across treatments. Accordingly, 16 g of dry banana leaves, 22 g of straw, 50 g of banana peel, 61 g of cabbage, and 33 g of areca nut peel were used per replicate. This approach was adopted to minimise variation arising from differences in substrate mass while providing comparable amounts of biodegradable organic material for microbial decomposition and subsequent nutrient release, thereby enabling a more meaningful comparison of substrate type on *Paramecium* production.

Then 4.5L glass bottles were sterilised using 75% alcohol. Sterilised containers were filled with 2250 ml of boiled water. After that, equal quantities of each material were added to ensure consistency across all replicates. The containers were allowed to cool down to room temperature. Subsequently, initial water quality parameters, pH, Dissolved Oxygen (DO), Temperature, and LUX, were measured for all the samples.

Figure 1. Organic Materials and culture media used to grow *Paramecium spp*. ;(a) Dry banana leaf (T1); (b) Straw (T2); (c) Banana peel (T3); (d) cabbage (T4); (e) Areca nut peel (T5); (f).

2.2. Feed for Paramecium spp. And Paramecium spp. cell count

1ml of milk was added to all culture media 2 days after their preparation of culture media as the feed (nutrition source) for *Paramecium spp.* And it was continued at two-day intervals for a month. No artificial inoculum of *Paramecium* spp. was introduced. Instead, the cultures were allowed to undergo natural colonisation under identical atmospheric conditions at the Aquaculture Research Centre, where protozoa were presumed to originate from naturally occurring microorganisms associated with the organic substrates and the surrounding environment. All culture containers were maintained under the same environmental conditions throughout the experiment.

Before enumeration, samples from each culture were examined under a compound light microscope. *Paramecium* spp. were identified based on their characteristic morphological features, including their slipper-shaped body, uniform ciliary movement, and rapid gliding locomotion, following standard protozoological

identification keys. Only organisms exhibiting these characteristics were counted using a haemocytometer.To count the Paramecium cells, 1 ml volume of culture media was taken from the surface layer of the culture media by using a pipette dropper and diluted with distilled water. A drop of formalin was added to the solution. After that, a drop of methylene blue was added to the solution for staining purposes. Subsequently, 1 ml of the solution was transferred to the hemocytometer for counting Paramecium cells. Paramecium cells were counted under the Euromex MicroBlue Binocular Microscope in a zigzag pattern, with 9 cells counted, and an average was taken (Figure 2). Cultures were observed daily to observe for the presence of Paramecium. *Paramecium* count was taken at two-day intervals to determine the presence of *Paramecium* in the cultures. *Paramecium* counts were taken for a month.

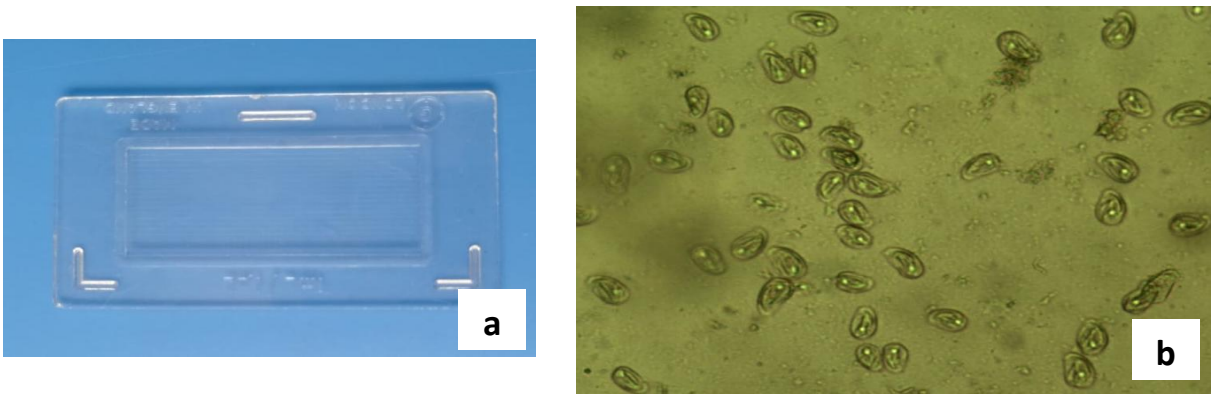

Figure 2. Hemocytometer (a) that use to count *Paramecium spp*. under microscope and Microscopic (microblue euromex) visibility of *Paramecium spp.* (b)

2.3. Determination of water quality measurement of the culture media

Water quality measurements, including pH, Temperature, DO, and LUX levels, were recorded daily till the *Paramecium spp.* Appeared in each culture medium [13,11]. After the *Paramecium spp*. had grown in every culture, water quality parameters and LUX were recorded at two-day intervals to see how water quality parameters affect the growth and development of the *Paramecium spp.*

2.4. Source of Betta splendens Brooders and larvae

Brooders were obtained from a fish farm in Piliyandala, Colombo, Sri Lanka. The fish were acclimatised for about 15 minutes in a water tank. Before introducing the water tanks, they were treated with salt water by dipping them in seconds for disinfection. Fish were reared separately in breeding tanks (60×20×20 cm^3) filled with conditioned water. They were fed on ground meat for a week. Fish were fed to satiation twice a day at 9:30 hr and 15:30 hr. After a week, 3 pairs of brooders were bred in tanks. The *Betta splendens* larvae used in the experiment were spawned from a cross between adult *Betta splendens* bred at the Aquaculture Research Centre. After 3 days of hatching, larvae were counted and separated into the experimental tanks.

Source of live feeds. *Paramecium spp.* and brine shrimp Artemia nauplii were the live feeds used for the experiment. Brine shrimp Artemia nauplii were used as one of the controls in this experiment. The *Paramecium spp*. was obtained from culture media that were prepared in the first experiment. The Artemia spp. were hatched from commercially available Aquaculture Artemia Cysts.

2.5. Feeding trials of the Betta splendens larvae

Three treatments with three replicates were used in this experiment (Table 1). Each was conducted using 3dpf *Betta splendens* larvae, and two treatments were considered as controls. After the eggs were hatching and the larvae started to swim in the water (3dpf), they were counted. Three treatments are as follows: brine shrimp Artemia spp.(C1), egg York (C2), *Paramecium spp*. (T3). An analytical scale was used to measure the total length (TL) of the *Betta splendens* larvae. The 3-pdf larvae (4 mm mean total length) were randomly distributed into the experimental tanks (45×30×24 cm^3). Three days before, water was filled into every tank for placing larval fish. In this experiment, 1 L was used for 15 *betta splendens* larvae were used in the tank. The differences in feeding volumes among treatments were due to the distinct physical characteristics, nutritional composition, feeding requirements, and potential impacts on water quality of each feed type. The feeding quantities were selected based on hatchery practices, preliminary observations of feed acceptance, and the need to maintain

suitable culture conditions. *Paramecium* spp. was supplied at approximately 10 cells larva^{-1} during the first 7 days after hatching and 20 cells larva^{-1} from day 8 onwards. In this study, 0.05 mL of *Paramecium* culture was provided per feeding, while *Artemia* nauplii and egg yolk were supplied at 5 mL and 0.5 mL per tank per feeding, respectively. Egg yolk was selected as a control because it is commonly used in larval fish culture and was prepared by dissolving it in distilled water before feeding. All diets were provided three times daily (09:00, 12:30, and 15:30 h) for 10 days, and 50% of the tank water was replaced every two days to maintain water quality. Based on estimated feed densities, the *Artemia* treatment supplied approximately 1,250 nauplii per feeding (≈2.5 mg dry biomass and 1.4 mg protein), whereas the egg yolk treatment supplied approximately 0.5 g fresh egg yolk (≈82 mg protein). The feeding levels were maintained within practical ranges to provide adequate food availability while minimizing organic loading and water quality deterioration, particularly in the egg yolk treatment due to its high protein and lipid content. Therefore, the diets were not standardized on an equal biomass, protein, or energy basis and represent comparisons among different practical feeding regimes rather than isoenergetic or isonitrogenous diets.

Table 1. Feeding for the rise of the *Betta splendens* larvae

Control 1 (Artemia nauplii)	Control 2 (egg York)	Treatment 3 (*Paramecium spp.*)
5ml (3x daily)	0.5ml (3x daily)	0.05ml (3x daily)

2.6. Water quality measurement

Initial water quality was taken, and then once every two days, water quality measurements consisting of pH, temperature, DO (Dissolved oxygen) and LUX (Light intensity) were recorded, and ammonia and nitrate were recorded weekly.

2.7. Determination of growth and survival

Before distributing larval fish into experimental tanks, the total length of at least 15% of larvae was measured using a ruler, and the total weight of 15% of larvae was measured using an analytical balance and recorded. Sampling was done after 10 days of stocking using the same percentage, and TL was recorded. From 20 hours before the last day of the experiment, larvae were not fed so that undigested feed in the gut would not influence the final physiology of the fish. Finally survival rate was also taken. The following relations were used to calculate the SGR, % survival, length-weight relationship, percentage length, and weight gain from the treatments.

(i) $$\text{Specific Growth Rate (SGR)} = (\log_e(final\ weight) - \log_e(initial\ weight)) \times 100\%$$

$$\text{Percentage Survival Rate (\%SR)} = \frac{\text{Final number of fish}}{The\ initial\ number\ of\ fish} \times 100\%$$

$$\text{Percentage Length Gained} = \frac{\text{Change in length of fish}}{The\ initial\ lengh\ of\ fish} \times 100\%$$

$$\text{Percentage Weight Gained} = \frac{\text{Change in weight of fish}}{Initial\ weight\ of\ fish} \times 100\%$$

(ii) Length–Weight Relationship (W) = aLb,

W= Weight of fish in grams
a = regression intercept
L= observed total length (mm)
b = regression slope

According to Le Cren's law (1951)

In estimating the value of the regression slope (b), the formula below was used:

$$b = \frac{n\sum(xy) - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2}$$

Where the parameters used are explained as:
x = Ln (L)
y = Ln (W)
∑xy = Ln (L) × Ln (W)
n = number of treatments $a =$
b = regression slope
In estimating the value of the regression intercept (a), the formula below was used:

$$a = \bar{Y} - b\bar{X}$$

Where,
a = regression intercept
b = regression slope
$\bar{x}$= mean In (L)
$\bar{y}$= mean In (W)

(iii) Condition Factor (C.F) = (W × 100)/L

W= Weight of fish in grams
L= observed total length (cm)

2.8. Data Analysis

All data were tested using Minitab 20 software; Analysis of variance one-way (ANOVA) to compare means of treatments. Significant differences were analysed with Tukey's HSD test to determine which groups differed. Significant statistical differences were considered for results with a p-value < 0.05.

3. Results

3.1. Selection of the Best Culture Medium for Paramecium spp. Growth

3.1.1. Variability of Paramecium spp. Count in different culture media

The number of *Paramecium spp*. in each treatment was monitored every two days for one month, with results presented as mean ± SE in Figure 3. Banana leaf (T1) 7,884 ± 2,337, straw (T2) 7,339 ± 1,881, banana peel (T3) 53,708 ± 15,471, cabbage (T4) 7,690 ± 2,355, and areca nut peel (T5) 3,544 ± 920. Statistical analysis revealed significant differences among treatments (P < 0.05). Banana peel (T3) exhibited the highest Paramecium count, significantly surpassing all other treatments.

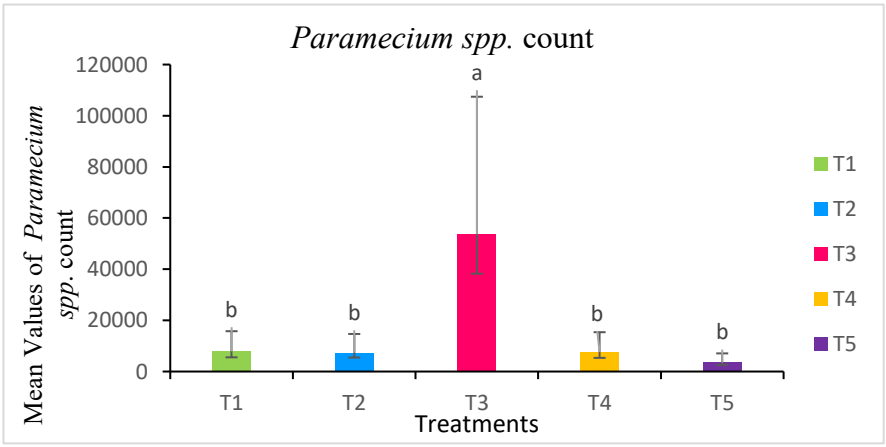

Figure 3. Variation of the *Paramecium spp.* Count in different selected media

3.1.2. Water quality parameters of culture media

Table 2. Temperature range (^{0}C), pH, Dissolved Oxygen, and LUX of culture media measured for 30 days in the same environmental conditions.

Variable	Banana leaf (T1)	Straw (T2)	Banana peel (T3)	Cabbage (T4)	Areca nut peel (T5)
Temperature (^{0}C)	31.80 ± 0.24^a	31.61 ± 0.20^a	31.63 ± 0.22^a	31.69 ± 0.20^a	31.62 ± 0.21^a
pH	7.47 ± 0.05^a	6.60 ± 0.10^{bc}	6.15 ± 0.16^c	6.18 ± 0.19^c	7.04 ± 0.04^{ab}
Dissolved Oxygen	2.37 ± 1.05^a	2.27 ±1.07^a	2.32 ±1.06^a	2.32 ± 1.06^a	2.36 ± 1.05^a
LUX	68.88 ± 2.25^a	68.88 ± 2.26^a	68.88 ± 2.27^a	68.88 ± 2.28^a	68.88 ± 2.29^a

The values are expressed as Mean ± Standard Error (SE). The values in the rows with different superscript letters are significantly different ($P < 0.05$).

The observed water quality parameters of the culture media are shown in Table 2. The statistical analysis showed that there was no significant difference in temperature between the treatments ($P > 0.05$). The banana leaf treatment had the highest pH value, 7.47 ± 0.05, and it was followed by areca nut peel with its pH value of 7.04 ± 0.04. DO levels did not differ significantly among the five treatments, indicating that oxygen availability in the water was almost similar in all treatments.

3.2. Growth Performance of Betta splendens *Larvae under Different Feeding Treatments*

The growth performance of larvae in terms of weight gain, final weight, and specific growth rate (SGR) is shown in Table 3. The growth rates in response to the treatments were determined based on the mean final length (mm) of fish at the end of the 10-day culture period. At the start of the experiment, 3dpf larvae measuring a mean TL of 4mm were randomly distributed into three treatments with three replicates. At the end of the experiment, the TL (final length) of the larvae expressed as mean ± SE are 6.29 + 0.10 for Artemia nauplii, 5.7 + 0.18 for egg yolk, and 5.89 + 0.20 for *Paramecium spp*. The mean length (mm) of larvae gained over the 10 days of period for each treatment. The result expressed as mean ± SE are 2.29 + 0.10 a for Artemia nauplii, 1.79 + 0.21 for egg yolk, and 0.89 + 0.20 a for *Paramecium spp.* The result of the study in terms of percentage of length gained expressed as mean ± SE are 57.31 + 2.60 for Artemia nauplii, 44.64 + 5.36 for egg yolk, and 47.22 + 5.01 for *Paramecium spp.*

When considering the final weight, at the beginning of the experiment, an initial mean wet weight (g) of the larvae, measuring 0.0033 g, was randomly distributed into three treatments with three replicates. At the end of the experiment, the final mean wet weight (g) of the larvae expressed as mean ± SE are 0.03 + 0.00 for Artemia nauplii, 0.01 + 0.00 for egg yolk, and 0.01 + 0.00 for *Paramecium spp.* The results show that there was a significant difference between treatments ($P<0.05$). The average wet weight (mg) gained by larvae for all treatments, expressed as mean ± SE, is 0.03 + 0.00 for Artemia nauplii, 0.01 + 0.00 b for egg yolk, and 0.01 + 0.00 for *Paramecium spp.* The results show that there was a significant difference between treatments ($P < 0.05$). Artemia nauplii resulted in a significantly higher final weight compared to *Paramecium spp*. and egg yolk, which did not differ significantly from each other. The results of the study show the percentage weight gain for all treatments, expressed as mean ± SE. The percentage weight gains were 777.95 ± 25.04 for *Artemia* nauplii, 158.87 ± 15.62 for egg yolk, and 342.42 ± 47.98 for *Paramecium spp.* Statistical analysis revealed a significant difference in weight gain for *Artemia nauplii* compared to egg yolk and *Paramecium spp.* ($p < 0.05$). *Artemia nauplii* provided the highest percentage weight gain, indicating it is the most effective feed in promoting larval growth. In comparison, *Paramecium spp.* Showed a significantly lower weight gain than *Artemia*, but it resulted in a higher weight gain than egg yolk.

The Specific Growth Rate (SGR) of *Betta splendens* larvae fed Artemia nauplii was significantly higher (2.14 ± 0.033) than those fed egg yolk (0.94 ± 0.06) and *Paramecium spp*. (1.44 ± 0.12), with no significant differences observed ($P < 0.05$).

Table 3. Growth Performance of *Betta splendens* Larvae under Different Feeding Treatments

Variable	C1 (Artemea)	C2 (Egg yolk)	T3 (Paramecium)	P value
Initial mean TL fish -1 (mm	4.00	4.00	4.00	-
Final mean TL fish - (mm)	6.29 + 0.10[a]	5.7 + 0.18[a]	5.89 + 0.20[a]	0.135
Mean length gain	2.29 + 0.10 [a]	1.79 + 0.21 [a]	1.89 + 0.20 [a]	0.135
% Length gain	57.31 + 2.60 [a]	44.64 + 5.36 [a]	47.22 + 5.01 [a]	0.135
Initial weight. fish	0.0033	0.0033	0.0033	-
Final weight. fish (g)	0.03 + 0.00 [a]	0.01 + 0.00 [b]	0.01 + 0.00 [b]	0.00
% Weight gain	777.95 + 25.04[a]	158.87 + 15.62[b]	342.42 + 47.98 [b]	0.00
Specific growth rate	2.14 + 0.03[a]	0.94 + 0.06[c]	1.44 + 0.12[b]	0.00
Survival rate (%)	54.17 + 3.63 [a]	5.83 + 0.83 [b]	7.50 + 1.44[b]	0.00
Length-weight (a-value)	-5.74 + 0.35[ab]	-5.23 + 1.67[a]	-9.84 + 0.60[b]	0.039
Length-weight (b-value)	1.19 + 0.19[ab]	0.25 + 0.99[b]	3.18 + 0.37[a]	0.04
Condition factor (C. F)	0.46 + 0.01[a]	0.15 + 0.01[b]	0.24 + 0.02[b]	0.00

The values are expressed as Mean ± Standard Error (SE). The values in the rows with different superscript letters are significantly different ($P < 0.05$).

3.2.1.Survival Rate and Length Weight Relationship of Betta splendens Larvae Fed with Different Diets

Survival rate and length weight relationship of *Betta splendens* larvae fed with selected Artemia, egg yolk, and Paramecium are presented in Table 3. The survival rate of *Betta splendens* larvae after 10 days was highest in the Artemia nauplii treatment (54.17 ± 3.63%), followed by *Paramecium* spp. (7.50 ± 1.44%) and egg yolk (5.83 ± 0.83%). Significant differences were observed among the dietary treatments ($P < 0.05$), indicating that Artemia nauplii resulted in significantly higher larval survival compared with *Paramecium* spp. and egg yolk.

The length–weight relationship of the treatments was determined. The result of the "a value" (regression intercept) of all treatments expressed as mean ± SE are -5.74 + 0.35 for *Artemia* nauplii, -5.23 + 1.67 for egg yolk, and -9.84 + 0.60 for *Paramecium spp.* The results show no significant difference among all the treatments ($P < 0.05$). Length–weight relationship (Regression coefficient) "(b) values" for the length-weight relationship were significantly different among the treatments ($P < 0.05$). *Paramecium spp*. (3.18 ± 0.37) exhibited the highest b value, indicating a more efficient weight-to-length relationship compared to egg yolk (0.25 ± 0.99), which had a lower b value. Artemia nauplii (1.19 ± 0.19) showed no significant difference when compared to either *Paramecium spp.* or egg yolk.

The condition factor (C.F.) of *Betta splendens* larvae showed significant differences among the treatments ($P < 0.05$). Artemia nauplii (0.46 ± 0.01) resulted in a significantly higher C.F. compared to both *Paramecium spp*. (0.24 ± 0.02) and egg yolk (0.15 ± 0.01), with no significant difference between *Paramecium spp.* and egg yolk.

3.2.2.Water Quality Assessment in different culture media

The water quality parameters measured throughout the experiment were optimal for the growth and survival of *Betta splendens* larvae, with temperature, pH, dissolved oxygen (DO), ammonia, and nitrate levels remaining within acceptable ranges for all treatments. The temperatures for all the treatments remained constant; there were no significant differences among them ($P > 0.05$). The temperature recorded for each group was as follows: 29.77 ± 0.08 for Artemia (C1), 29.66 ± 0.07 for Egg yolk (C2), and 29.68 ± 0.05 for *Paramecium spp.* (T3). The pH values were comparable across all treatments: C1 (Artemia) 7.50 ± 0.06, C2 (Egg yolk) 7.59 ± 0.05, and T3 (*Paramecium spp.*) 7.46 ± 0.08, with no significant differences ($P > 0.05$). The pH ranged between 7.0 and 8.0, which is considered optimal for *Betta splendens* larvae. The DO levels were consistent across treatments: C1 (Artemia) 6.69 ± 0.09 mg/L, C2 (Egg yolk) 6.56 ± 0.11 mg/L, and T3 (*Paramecium spp.*) 6.60 ± 0.12 mg/L, with no significant differences ($P > 0.05$). Ammonia concentrations were low and similar among the treatments: C1 (Artemia) 0.08 ± 0.03 mg/L, C2 (Egg yolk) 0.10 ± 0.03 mg/L, and T3 (*Paramecium spp.*) 0.07 ± 0.02 mg/L, with no significant differences ($P > 0.05$). Nitrate levels varied significantly between treatments ($P < 0.05$), with C1 (Artemia) having the highest nitrate concentration at 0.38 ± 0.11 mg/L, followed by T3 (*Paramecium spp.*) at 0.15 ± 0.04 mg/L, and C2 (Egg yolk) with the lowest at 0.07 ± 0.02 mg/L.

4. Discussion

4.1. Variability of Paramecium spp. Count in different culture media.

This study successfully investigated the effect of different culture media on the growth of *Paramecium spp.* and evaluated their suitability as the first feed for *Betta splendens* larvae. The first experiment focused on identifying an optimal culture medium to maximize Paramecium production, while the second experiment examined the impact of Paramecium as a live feed on larval growth and survival compared to alternative diets.

The results from the first experiment demonstrated that *Paramecium spp.* Exhibited the highest growth rate in the banana peel culture medium, outperforming other tested media, including dry banana leaves, straw, cabbage, and areca nut peel. Banana peel (T3) supported the highest *Paramecium spp.* Count, markedly exceeding all other treatments. In contrast, the counts recorded for T1, T2, and T4 were statistically similar to one another but considerably lower than T3. Areca nut peel (T5), though showing the lowest population, did not differ significantly from T1, T2, or T4. The exceptional performance of banana peel likely reflects its richer nutrient profile, which enhances *Paramecium* proliferation. Leaf substrates could support populations exceeding 40,000 cells/mL, aligning with the present findings for T3. Leaf litter substrates produced higher *Paramecium* counts compared to straw-based cultures, with values (~8,000 cells/mL) closely corresponding to those observed for T2 (7,339 ± 1,881). This finding indicates that banana peel provides a nutrient-rich environment, likely due to its organic content, which supports microbial activity and enhances Paramecium proliferation [17,18].

4.2. Water quality parameters of culture media

The water quality parameters, such as pH, temperature, and dissolved oxygen, played a crucial role in the culture's success, with optimal conditions correlating with increased Paramecium density. In the current investigation, all treatments maintained stable levels of temperature, pH, and dissolved oxygen (DO). Temperature readings across T1 to T5 ranged narrowly from 31.61°C to 31.80°C. The average water temperatures in the experiment for the five treatments from T1 to T5 ranged between 31.61°C and 31.80°C, with a general average of 31.67°C. Previous studies indicate that the optimal temperature range for the growth of Paramecium is between 24°C and 28°C. According to [19] when the growth is exponential, P. caudatum undergoes 1-3 divisions in 24 hours at optimal temperatures within these ranges. The pH levels varied from 6.15 to 7.47 across all treatments, indicating the organic materials in each treatment influenced the acidity or alkalinity of the water. The pH in T1 (banana leaf) was the highest, and in T3, it was the lowest. However, based on the data obtained, pH is not likely to be the factor influencing the growth of Paramecium, even though it is considered to affect culture conditions. In studies such as that by [20], which emphasised that physicochemical parameters, among them pH, can be highly influential on protist dynamics and performance under culture media conditions. In their experiment, Paramecium tetraurelia could be grown best. At about pH 6.5. Thus, optimal conditions may be close to the results one might have had, where high growth rates correlate with higher pH values. Similarly, DO levels were consistent across treatments, ranging between 2.27 and 2.37 mg/L. These short-term environmental conditions appeared adequate for *Paramecium spp.* Survival and proliferation, even though [11] noted that extended bacterial activity could eventually deplete oxygen availability.

DO levels did not differ significantly among the five treatments, indicating that oxygen availability in the water was almost similar in all treatments. The results indicate that water quality, in terms of DO, was not the major influence on the growth of *Paramecium spp.* During the study. According to research done by [22,11], the accumulation of metabolic waste products from bacteria can also decrease the availability of oxygen over time and thereby affect protozoan survival and reproduction. In our study, although immediate differences in DO were not significant, longer-term monitoring may reflect trends associated with the accumulation of wastes in the various substrates.

4.3. Growth Performance of Betta splendens *Larvae under Different Feeding Treatments*

Building on these findings, the second experiment assessed the impact of feeding *Betta splendens* larvae with Paramecium cultured in banana peel medium, comparing it with egg yolk and Artemia nauplii. Larvae fed with *Artemia* nauplii showed the highest weight gain, final weight, and specific growth rate (SGR) ($p < 0.05$), outperforming both egg yolk and *Paramecium spp.*-fed groups. *Artemia* nauplii, with their superior nutritional profile and active motility, are widely regarded as one of the most effective live feeds in larviculture [3,23] Larvae fed *Paramecium spp.* Demonstrated intermediate growth performance, with SGR and weight gain higher than the egg yolk group but lower than the *Artemia* group. Due to their small size, *Paramecium spp.* are particularly suitable for early-stage larvae [24]. In contrast, egg yolk, while readily available, lacks essential fatty acids and proteins critical for optimal larval growth, resulting in inferior growth performance [25].

4.4. Survival Rate and Length–Weight Relationship of Betta splendens Larvae Fed with Different Diets

Survival rates also differed significantly among treatments ($p < 0.05$), with *Artemia*-fed larvae achieving the highest survival (54.17 ± 3.63%), followed by *Paramecium spp.* (7.50 ± 1.44%) and egg yolk (5.83 ± 0.83%). Artemia nauplii provided the best survival rate, consistent with previous studies on various fish species [26]. Egg yolk, while easy to access, lacks the dynamic qualities of live prey, which affects its digestibility and feeding behaviour, leading to poorer growth and survival rates in fish larvae [27]. *Paramecium spp*., though less nutritious than Artemia, offers some advantages over egg yolk due to its mobility, stimulating the larvae's natural hunting instincts and supporting better growth and survival compared to egg yolk [28,4].

No significant differences ($p > 0.05$) were observed among treatments for final total length, mean length gain, or percentage length gain. However, the condition factor (C.F.) was significantly higher ($p < 0.05$) in the *Artemia* group compared to *Paramecium spp.* and egg yolk treatments, supporting earlier evidence that live feeds, especially *Artemia*, promote better health and growth in larvae [16].. Significant differences ($p < 0.05$) were also detected in the length-weight relationship across treatments. The highest b-value (3.18 ± 0.37), recorded in the *Paramecium spp.* group, indicates comparatively better weight gain relative to length than in the egg yolk group, aligning with previous reports that higher b-values reflect enhanced growth performance [30].

Artemia nauplii resulted in a significantly higher condition factor (C.F.) compared to both *Paramecium spp*. This result supports the findings of [31] where larvae fed Artemia exhibited better growth and condition than those receiving other feeds. Live feeds, especially Artemia, are known to improve the physiological condition of fish larvae by enhancing nutrient availability, contributing to better health and growth [32].

4.5. Water Quality Assessment in different culture media

Throughout the experiment, water quality parameters remained within optimal ranges for the growth and survival of *Betta splendens* larvae, with temperature, pH, dissolved oxygen (DO), ammonia, and nitrate at biologically acceptable levels. The temperature of all treatments was maintained within a stable range to ensure that external factors such as temperature did not affect the results of fish growth and survival rates. Even the uniformity of pH across treatments, variations in growth and survival are unlikely to be attributed to pH levels. The better growth observed in Artemia-fed larvae (C1) is likely due to the superior nutritional value of the feed rather than any pH variation in the water. All treatments maintained DO levels well above the minimum required for *Betta splendens* larvae, ensuring adequate oxygen for metabolic functions. Consequently, DO levels were not a limiting factor in growth or survival, with the observed differences likely related to feed quality. The ammonia levels were well below the toxic threshold for *Betta splendens* larvae, indicating that ammonia toxicity did not negatively impact growth or survival. The low ammonia concentrations across treatments suggest effective water quality management during the experiment. Despite the higher nitrate concentration in Artemia-fed larvae, this did not result in negative effects on growth or survival, suggesting that the increased metabolic activity from better growth may have contributed to the higher nitrate levels. Nitrate concentrations were still well below levels considered harmful to *Betta splendens* larvae, reinforcing that this parameter did not affect the observed differences in growth and survival. All values remained well below harmful thresholds for larvae. Overall, the water quality across treatments was sufficient to support healthy larval development, indicating that differences in survival and growth were primarily influenced by the nutritional quality of the feeds rather than environmental conditions.

The results showed that larvae fed with Paramecium exhibited superior growth rates compared to those fed with egg yolk, although Artemia remained the most effective diet overall. The enhanced performance of Paramecium-fed larvae suggests that this live feed provides adequate nutritional value and digestibility during early larval stages. The relatively lower growth observed in the egg yolk group may be attributed to its non-motile nature, which reduces feeding efficiency compared to actively swimming Paramecium and Artemia. Furthermore, water quality parameters were maintained more effectively in the Paramecium and Artemia treatments compared to the egg yolk treatment, where excess nutrients likely contributed to water deterioration.

5. Conclusion

This study demonstrated that banana peel culture medium supported the highest growth of *Paramecium* spp., which was significantly higher than that observed in the other culture media ($P < 0.05$). As an initial feed for *Betta splendens* larvae, *Paramecium* spp. showed potential to support larval growth and survival; however, its performance was considerably lower than that of Artemia nauplii, which resulted in the highest overall larval performance. Although water quality parameters remained within the optimal range across all treatments, differences in growth and survival were primarily associated with feed type. The findings suggest that banana

peel is a cost-effective and sustainable medium for culturing *Paramecium* spp. and that *Paramecium* spp. may serve as a supplementary feed option in larviculture systems, particularly during early larval stages. However, further optimisation of culture conditions, feeding strategies, and nutritional supplementation is required to improve its effectiveness before it can be recommended as a primary live feed, especially in resource-limited environments.

6. Data Availability Statement

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

7. Ethical Statement

I conducted my research at National Aquatic Resources Research and Development Agenc, Colombo 15, Sri Lanka, and it has the authority to do the aquaculture researchers in Sri Lanka. Under project number 1.1 this project was approved.

8. Author Contributions

W.A.N. Tahana: Conceptualization, methodology, experimental work, investigation, data collection, data analysis, interpretation of results, and writing–original draft preparation. K.M.S.A.K. Dehideniya: supervision,, guidance on data validation, and manuscript review and editing. A.M.A.N. Adikari: supervision, technical guidance, experimental design. K.L.W.T. Maduka: supervision, scientific guidance, validation.

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11. Conflict of Interest

The authors declare no conflict of interest.

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