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Original Article

# Nutritional Benefits of Innovative Papaya-based Zooplankton for Ornamental Fish Growth, Chiefly Guppy Fishes (*Poecilia reticulata*)

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## Abstract

Zooplankton plays a key role in linking primary producers to higher-level consumers within the aquatic food web. More than 75% of fish species consume zooplankton. The availability of live feed cultures is vital for the growth of aquaculture ventures. *Daphnia* and *Moina* are the two typical zooplankton species frequently used in fish care. A laboratory study was conducted to assess the growth and production of two specific zooplankton species, highlighting their potential as fish food. *Daphnia* and *Moina* are generally cultured with cow dung cake, yeast, and green pond water as growth resources. Our innovation introduces papaya leaves and stems into the zooplankton culture, as papaya leaves are a rich source of nutrients and vitamins. Observations over 10 days indicated significant growth of zooplankton when papaya leaves and stems were introduced. We studied guppy fish (*Poecilia reticulata*), one of the most popular ornamental fish, hypothesizing that live feed would impact the growth and colouration of guppies. Supporting our hypothesis, both male and female guppies exhibited more vivid colours and faster growth rates when fed a mixture of *Moina* and *Daphnia* cultured with papaya leaves and stems, compared to others. Zooplankton growth and water quality analysis of different fish aquaria also showed significant differences. We can conclude that a live mixture of *Daphnia* and *Moina*, innovatively cultured with papaya leaves and stems, provides nutritional benefits to the zooplankton, significantly enhancing the growth and colouration of guppy fish with fewer toxic effects. This innovation will make a revolution in the ornamental fish industry in the near future.

***Keywords:*** *Guppy Fishes; Innovative Technique; Papaya; Zooplankton*

## Introduction

Plankton derives from the Greek term "planktos," meaning "drifter" or "wanderer." These are aquatic organisms, including animals, plants, and bacteria, that live in the pelagic zones of oceans, seas, or freshwater environments and cannot swim against water currents (Ekelemu, 2010). Zooplankton refers to the animal component of plankton, many of which are too small to be observed individually without aid (Kirn *et al*., 2005). They serve as the primary food source for all fish, including larvae. Zooplankton includes a wide variety of animals, ranging from microscopic single-celled protozoa to jellyfish, and they inhabit nearly all surface water bodies globally, including subterranean aquifers (Sharma, 2022; Ashour *et al*., 2025).

The survival of a wide array of freshwater organisms, including fish, relies on zooplankton. In commercial fish farming, various fish species' fingerlings are fed on live or processed zooplankton to achieve optimal growth. In natural ecosystems, gut content analyses show that zooplankton make up a significant portion of many fish species' diets. As a live feed, zooplankton are favoured due to their high-quality protein and lipid content, critical for the growth and survival of fish fries and fingerlings (Velasco-

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Santamaría & Corredor-Santamaría, 2011; Shil *et al*., 2013). Often, the intensive rearing of fish larvae is facilitated by the provision of cultured zooplankton. Zooplankton culture techniques and sufficient production should be economically viable, as zooplankton are recognized as valuable resources for the growth and survival of commercially farmed fish species, including ornamental fishes. Recent years have seen an exploration of culture methods for various zooplankton, offering ample options for both small-scale and large-scale production of zooplankton. The artificial cultivation of cladocerans like *Diaphanosoma celebensis* (Khatoon *et al*., 2013), *Ceriodaphnia*, and *Moina* (Peña-Aguado *et al*., 2005), as well as copepods such as *Acrodiaptomus* (Temerova *et al*., 2002), *Acartiatonsa* (Øie *et al*., 2017), and *Centropageshamatus* (Jakobsen *et al*., 2016), along with rotifers (Folkvord *et al*., 2018) like *Brachionus* (Peña-Aguado *et al*., 2005; Maehre *et al.*, 2013), has been established to satisfy the demand for live food for fish fries and finger links in commercial aquaculture. The cultivation of zooplankton can also be carried out in wastewater with minimal or no pollution build-up from the surrounding environment (Nandini & Sarma, 2003; Nandini *et al*., 2004). Considering the benefits of zooplankton as a food source and the simplicity of their culture, this study was conducted to examine the potential growth and survival of two specific zooplankton species, *Moina micrura* (*Moina*) and *Daphnia pulex* (small) and *Daphnia magna* (large) (*Daphnia*), under laboratory conditions (Kar *et al*., 2017). The quality of water and availability of food within the system play a crucial role in the dynamics of zooplankton populations, particularly in a cultured setting (Yuslan *et al*., 2021).

The widely known ornamental fish, guppy fish (*Poecilia reticulata*), has been introduced in several countries for controlling mosquito populations and is often informally referred to as ‘mosquito fish’. It has been observed to successfully thrive in both freshwater and polluted environments (Ahmed & Bhuiyan, 1985). This species was brought to India as early as 1910 for mosquito control (Kalra *et al*., 1967). Guppy varieties are favoured among fish enthusiasts and hobbyists due to their vibrant colours and resilience. In aquaculture, proper nutrition is crucial to produce a healthy and high-quality product (Fah & Leng, 1986; Khan & Rahman, 2025), as feed constitutes 50%-80% of production expenses (FAO, 2017). Recent advancements in fish nutrition research have led to the creation of commercially balanced diets that support optimal growth and health in fish with less toxic effects (Giri *et al*; 2017). While there is a range of commercial feeds available on the market, their cost-effectiveness and suitability for various ornamental fish types remain unclear (Kumaratunga & Radampola, 2019).

Now a days, guppy fish is the most popular fish in the ornamental aquaculture trade, featuring a variety of colours and fin patterns. Similar to other species, the colouration in guppy fishes may reflect immune health and play a crucial role in mate selection. We investigated how live feed affects the colouration and growth of *P. reticulata*, proposing that if live feed influences these factors, there will be noticeable differences in colour intensity and growth rates among fish that are fed different kinds of live prey (Uribe *et al*., 2018). It has been proposed that these innovations will enhance the quality of live fish feed in the aquaculture industry as a greater variety of species with distinct feeding needs have been introduced recently into aquaculture practices (Komilus & Mufit, 2021).

Many farmers tend to struggle because they do not take sufficient care in preparing guppy fish. Key water quality parameters on fish farms include ammonia levels, oxygen content, water temperature, and water pH levels, which are the crucial water parameters for assessing water quality. Extreme pH levels threaten the survival of fish and reduce the fecundity rate. According to a study, specific water compositions can influence guppy fish production linked to particular genes. Additionally, temperature can impact the quantity of guppy fish produced and may also affect the sex ratio (Shah *et al*., 2017).

A laboratory study was conducted to assess the growth and production of two specific zooplankton species to showcase their potential as fish food. *Moina* and *Daphnia* are generally cultured with cow dung cake, yeast, and green pond water as growth resources (Kar *et al*., 2017; Sharma, 2022). However, water analysis revealed high toxicity in those cultured waters. Our innovation included the introduction of papaya leaves and stems into the zooplankton culture, as the papaya plant itself is a huge source of nutrients and vitamins like vitamins E, A, and C. Phytochemical analysis of papaya leaf extracts reveals the presence of alkaloids, flavonoids, phenols, saponins, steroids, and tannins. Additionally, papaya leaves contain compounds such as papain, chymopapain, cystatin, tocopherol, flavonoids, ascorbic acid, cyanogenic glucosides, and glucosinolates (De Oliveira & Vitória, 2011; Hamid *et al*., 2022; Ashaari *et al*., 2024). There have been several studies on *Daphnia* and *Moina* culture mixture that have been used as live fish food (Khan & Rahman, 2025). Many reports indicate that the growth of zooplankton relies heavily on yeast, starch, etc., which can inadvertently lead to fungal diseases affecting both the brooders and fries of fishes, chiefly guppies. But there is no report on any research that has been conducted to promote the growth of zooplankton without the use of yeast or other components as a medium.

Our objective is to promote the growth and nutritional value of zooplankton, especially *Moina* and *Daphnia* (zooplankton mixture), using solely papaya leaves and stems and without any starch or Baker's yeast, and fed to guppies from day 1 fries to adults. There is no such documentation of these being combined to boost the development of livebearers from day one fish fries. Therefore, this innovation would be beneficial if our research yields positive results compared to some conventional culture-produced zooplankton feeding to the guppy fishes. This live fish food is free from chemicals and synthetic additives, which will also help to reduce ammonia, nitrite, etc. in water and maintain a steady level of dissolved oxygen in water. This concept can provide a valuable resource for ornamental fish farmers, especially guppy breeders worldwide, as this live fish food is very affordable with minimal or no toxic side effects.

## Materials and Methods

### *Collection of Zooplankton*

During summer, samples of zooplankton from the freshwater oxbow lakes were taken to the laboratory and initially categorized by genus. Proper gear- plankton net was used for the collection of zooplanktons and the mesh size was 63-500µm (micrometer). (Figure 1). The segregated specimens were placed in separate plastic containers (25 litre [L] in volume) and plastic tubs (40L in volume) and were provided with various food sources. After establishing the culture medium and segregating the plankton into smaller groups, the study commenced with observations of the density of individuals in each container. The data regarding the density of individuals per unit space served as response variable for assessing growth in relation to the different food types as energy sources for the species.

### *Identification of Zooplankton*

In this study, two zooplankton species, *Daphnia* sp. and *Moina* sp., were selected after separating them from a mixed collection of zooplankton collected from the oxbow lakes adjacent to our college (Figure 1). In order to differentiate species within intricate aquatic ecosystems, zooplankton species identification is a crucial, multidisciplinary process that uses specialized morphological, molecular, and microscopic approaches. We used widely used references include works by Battish (1992); Murugan *et al*. (1998); Altaff (2004) and Manickam *et al*., 2018) for freshwater zooplankton species identification taxonomically. Then, with the help of a compound microscope (Olympus), we separate *Daphnia* and *Moina* to fulfil our requirement. Zooplankton abundances were expressed as individuals per cubic meter (m3) (Guermazi *et al*., 2023).

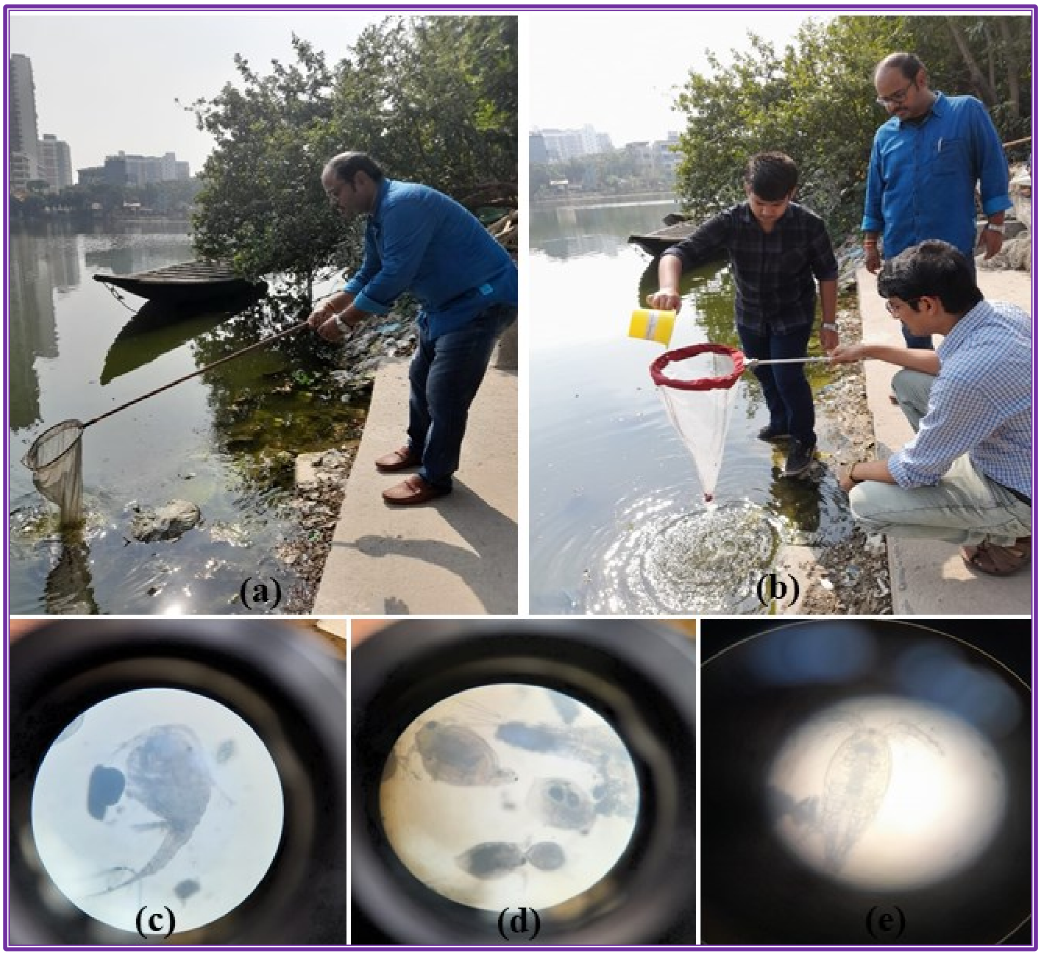


***Figure 1:*** *Collection of Zooplanktons*

Figure 1 Showed (a)- (b) Collection of Zooplanktons from the ox-bow lakes adjacent to our college. (c)-(e) Then samples of zooplankton were taken to the laboratory and initially categorized by genus depending on microscopic observation. *Daphnia* sp. and *Moina* sp., were selected after separating them from a mixed collection of zooplankton collected.

*Culture Set Up Preparation for Zooplanktons*

This study compares the zooplankton natality and abundance produced using cow dung cake and dry banana leaf mixture, baker's yeast and starch mixture, green water from the pond, and a very innovative method using papaya leaves and stems. This experiment was conducted at the Brahmananda Keshab Chandra College research laboratory (Figure 2). A total of nine (9) plastic tubs (40L in volume) with aeration (SoBo, China) were maintained for the culture medium. Before experimentation, tanks were washed thoroughly, dried and then filled with 10L of filtered water. The water was kept for 3 days. On the 4$^{th}$ day, three different kinds of food, viz., cow-dung cake (dry) and dry banana leaf mixture (20 gram [g] + 2L of water), baker's yeast and starch mixture (20g + 2L of water), and pure green water from the pond (2L), were introduced to the water tanks. For each culture type, 3 (three) plastic tubs were used as replicates. The culture was maintained for every 7 days under standard laboratory conditions. Before culture, the pH of the tank was calculated to be 6.5 (Kar *et al*., 2017). *Daphnia* and *Moina* (zooplankton mixture) were introduced in each water tank in nearly 500/m3 in amount (counted by Sedgewick–Rafter counting cell – 50 mm x 20 mm x 1 mm). Each replicate was introduced with 10 neonates of each of *Daphnia* and *Moina*. Following the initiation of growth of both *Daphnia* and *Moina*, the number of individuals was counted daily using Sedgewick-Rafter counting cells (50 mm x 20 mm x 1 mm). The water of each zooplankton culture aquarium was changed (30%) every week (after every seven days) and supplied with fresh food in each aquarium. The water temperature (°C) of the culture media was recorded by using a mercury thermometer (Kar *et al*., 2017; Ekelemu & Nwabueze, 2010).

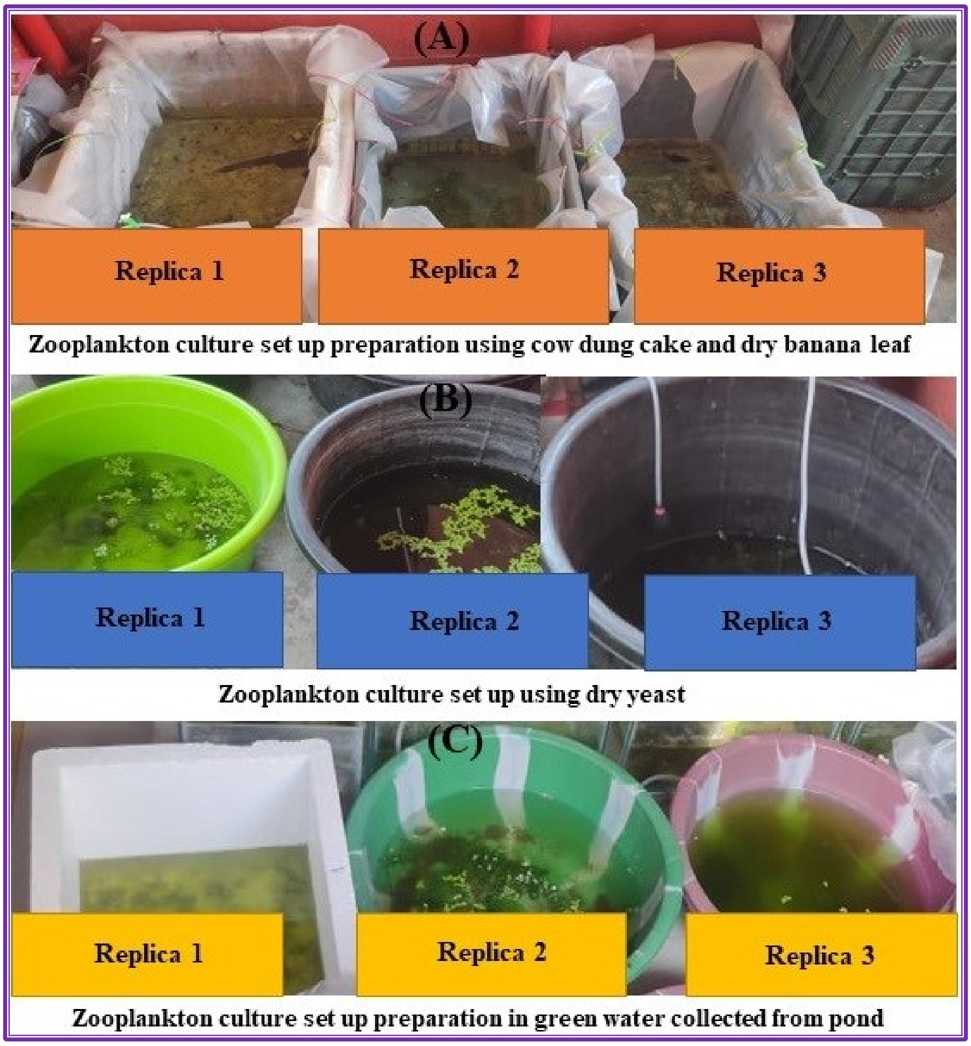


***Figure 2:*** *Culture Set Up Preparation for Zooplanktons*

Figure 2 showed (a) Zooplankton culture set up preparation using cow dung cake and dry banana leaf, (b) Zooplankton culture set up using dry yeast, (c) Zooplankton culture set up preparation in green water collected from the pond. All three different zooplankton culture setups are distributed in three replicates for each type.

*Use of an Innovative Technique of Zooplankton Culture*

We have used a very innovative technique to culture the zooplankton mixture (*Daphnia* and *Moina*). We all know that papaya leaves and stems contain alkaloids, flavonoids, phenols, saponins, steroids, and tannins. Papaya leaves also have compounds of papain, chymopapain, cystatin, tocopherol, flavonoids, ascorbic acid, cyanogenic glucosides, and glucosinolates (De Oliveira& Vitória, 2011; Hamid *et al.*, 2022). These components are directly or indirectly required for the growth of fish. It is also established that these components have a significant role in the increment of the fecundity rate of fishes. We especially targeted the fresh stems and leaves of the papaya tree and cleaned them properly with water. Then we add the leaves and stems together in each of the zooplankton-containing tanks after randomly tearing them. A total of three (3) plastic tubs (replicates) (40L in volume) with aeration (SoBo, China) were maintained for this innovative culture medium preparation. Each tank contains 25L of water and 2kg (kg- kilograms) of papaya leaves and stems (Figure 3). Zooplanktons (*Daphnia* and *Moina* mixture) were introduced in each water tank in nearly 500/m3 in number (counted using a Sedgewick–Rafter counting cell – 50 mm x 20 mm x 1 mm) (Ekelemu& Nwabueze, 2010). The culture was maintained for every 7 days under standard laboratory conditions. The water of each zooplankton culture aquarium was changed (30%) every week (after every seven days) and supplied with fresh food in each aquarium.

The water temperature (°C) of the culture media was recorded by using a mercury thermometer (Kar *et al*., 2017; Ekelemu & Nwabueze, 2010).


***Figure 3:*** *Use of an Innovative Method of Zooplankton Culture*

Figure 3 Showed (a)**-** (b)Culture set-ups were filled with 25 litres of water, and zooplanktons (*Daphnia* and *Moina* mixture) were introduced in water in nearly 500/m$^3$ in amount (counted using Sedgewick–Rafter counting cell- 50 mm x 20 mm x 1 mm). Then, 2 kgs of freshly collected and washed papaya leaves and stems were added to each zooplankton culture tank. (c)- (e) A total of three (3) plastic tubs (replicates) were arranged for this study. (c)- (g) A high increment of *Daphnia* and *Moina* mixture population was observed (marked by circles) and counted daily using Sedgewick–Rafter counting cell- 50 mm x 20 mm x 1 mm.

*Guppy Fish Set Up Preparation*

Two different brooder strains of guppies, viz. chilli mosaic dumbo ear and full black guppies with uniform size (2.955cm in length and nearly 310mg in weight; cm- centimeter, mg- milligram) of 3 months old were obtained from a reputed guppy fish farm (Guppywala, Kerala). Guppy brooders were maintained in two separate breeding setups with 5 pairs of fish in each (Figure 4). Our first target was to make a suitable breeding set-up where they could breed well so that we would get a huge number of fries with proper health conditions. Brooder fishes were fed with live zooplankton *ad-libitum*, 2 times daily at 9.00 a.m. and 4.30 p.m. for nearly 6 weeks and daily food consumption was recorded. After the completion of 6 weeks, we got a good number of fries from the two setups of different strains. We were using sufficient aquatic plants to collect the proper number of fries as well (Kumaratunga &Radampola, 2019; Ashaari *et al*., 2024).


***Figure 4:*** *Guppy Fish Set Up*

Figure 4: (a) Two different brooder guppy strains viz. chilli mosaic dumbo ear (b) and full black guppies (c), with more or less uniform size (2.955 cm in length and nearly 310 mg in weight) of 3 months old were obtained from an ornamental fish farmer. They were maintained in two separate breeding setups with 5 pairs of fish in each

*Maintenance of Guppy Fishes*

Guppy fish were descendants by reproduction in the laboratory. Fish were obtained randomly at 30 days old from the same population of offspring of a pair of guppies, *P. reticulata*. Physical and chemical conditions of the water were 25˚C ± 0.5˚C, pH 7.2 ± 0.1, 154.5 mg/L$^{-1}$ $CaCO_3$ and 5.5 ± 0.5 mg/L$^{-1}$ $O_2$. Food waste and faeces were removed by syphon daily (Arce *et al*., 2018). At the start of the experiment, fish were placed in 20L tanks and fed daily *ad libitum* twice a day with each of the experimental diets (Figure 4). The fishes were removed from the experiment at 90 days, and the Absolute Growth Rate (AGR) was estimated as the difference between the final and the initial size, divided by the duration of the experiment. Fish were weighed with a plate balance (OHAUS; 0.01gm), measured with callipers (0.01mm; mm- millimetre), and sex was determined by gonophore identification.

*Experimental Diets for Guppy Fishes*

The *Daphnia* and *Moina* mixture was maintained properly in four different culture setups and used for feeding in live conditions. There was no requirement of preservation of those zooplankton. Feeding was started from day 1 guppy fry, and it was maintained for 1-month, 2-months, and 3-months-old guppy fish with four different cultured zooplanktons, twice a day. All the feeding procedures were done by using a 10ml dropper. Day 1 fries (n= 10) were fed with 200 m$^3$, measured by Sedgewick – Rafter counting cell), twice a day. It was followed by an increment of one drop per month. The growth rate and colour enhancement were calculated (Mic *et al*., 2023).

*Biochemical Quantification of Zooplankton*

This study to compare the quantity of *Daphnia* and *Moina* mixture, produced using cow dung, baker's yeast, and starch mixture, pure green pond water, and papaya leaves and stems as sources of organic materials for the growth of zooplankton (*Daphnia* and *Moina* mixture), was conducted in our laboratory setups. The study was maintained for every seven (7) days. The number of individuals was counted daily and recorded statistically (Ekelemu& Nwabueze, 2010). Zooplankton abundances were expressed as individuals per cubic meter (m3).

*Water Parameters Analysis*

pH, water temperature, dissolved oxygen (DO), ammonia, and nitrite amounts of two different guppy set-ups fed with *Daphnia* and *Moina* mixture, cultured with cow dung, green pond water, dry Baker's yeast medium, and papaya leaf and stems, were analysed to measure any toxicity. All the data were measured monthly and statistically analysed. A digital pH meter (pocket type, brand name – HANNA) was used to estimate the pH of water. A regular mercury thermometer was used for water temperature measurement. A portable dissolved oxygen meter, polarographic type probe water quality tester for aquarium tank, Lutron Model: DO-5509 (Range: 0 to 20 mg/L) was used to analyse dissolved oxygen in all fish tanks in different month intervals. A water testing kit (NICE – 3X 200 tests) was used for the estimation of nitrite and ammonia in water.

*Colour Measurements and Statistical Analyses*

At every time interval, photographs were captured, keeping them in separate small photogenic tanks. Fish body colour was analyzed. All images were transformed into 8-bit greyscale (Schweitzer *et al*., 2015) and analyzed for colour using ImageJ software (Schneider *et al*., 2012). This software measures the colour intensity of an image in greyscale; specifically, each pixel (PX) is assigned a value corresponding to a greyscale level. Each pixel is given a number ranging from 0 to 255, where 0 signifies black and 255 signifies white. Values closer to zero indicate a higher colour intensity, while higher values imply lower intensity.

## Result

*Identification of Zooplankton*

We identified *Daphnia* and *Moina* at the genus level using a compound microscope. The abundance and the species richness of the zooplankton at each medium were assessed in triplicate. As zooplankton mixture, especially *Daphnia* and *Moina* mixture are used as feed for guppies after treating them with four different feeding media, we did not go for in depth study of zooplankton at the species level.

*Estimation of the Growth of Zooplankton*

All four different culture media containing zooplanktons, including cow dung media, Baker's yeast media, green pond water media and innovative papaya leaf and stem treated media, remained live in the culture vessels for the whole study period. The increase in the number of zooplanktons (*Daphnia* and *Moina* mixture), varied considerably regarding their growth depending on the different culture media. The contingency table showing the zooplankton quantity per different culture medium (Table 1) signifies the growth of zooplankton in different media. Papaya leaf and stem treated medium as a food resource appeared to be significantly different from the other three, as the differences among the conventional three media were not markedly observed (Ekelemu & Nwabueze, 2010).

***Table 1:*** *Contingency Table Showing the Zooplankton Quality and Growth Per Different Culture Medium*

| | **Baker's Yeast Media** | **Organic Manure Media** | **Green Water Media** | **Innovative Papaya Leaf and Stem-Treated Media** |
|---|---|---|---|---|
| Total Number of Taxa *(S)* | 2 (Daphnia & Moina) | 2 (Daphnia & Moina) | 2 (Daphnia & Moina) | 2 (Daphnia & Moina) |
| Number of Individuals *(N)* | 250 | 251 | 278 | 290 |
| Taxa Richness *(d)* | 2.1735 | 2.1738 | 2.1322 | 1.9400 |
| Shannon Weiner Growth Index *(H)* | 1.0344 | 1.0342 | 1.0318 | 1.0671 |
| Evenness Index *(E)* | 0.9286 | 0.9283 | 0.9263 | 0.9888 |

*Analysis of the Quantity of Zooplankton Per Culture Media*

Quantity of zooplanktons, specially *Daphnia* and *Moina,* mixture of four different culture media, including cow dung media, Baker's yeast media, green pond water media, and innovative papaya leaf and stem treated media, remained live in all the culture vessels and analysed every seven days interval. Data clearly validate a significantly higher number of individuals found in papaya leaf and stem-treated media than in the other three media. This result clearly proves the nutritive value of papaya leaf and stems over the other three media (Figure 5). Zooplankton abundances were expressed as individuals per cubic meter ($m^3$). According to Sherman *et al*. (2002), zooplankton serve as environmental indicators that provide information on the quantity of fish and other predators as well as the state of the ecosystem. Because of their important role in the food chain, zooplankton also affect fish recruitment and stocks (Ashaari *et al*., 2024). Changes in zooplankton abundance may have a domino effect that affects fish stocks as well (Lomartire *et al*., 2021).

**Analysis of quantity of Zooplankton per culture media (Initial volume was 500/$m^3$ /culture medium)**

| Day | Zooplankton cultured with Cow dung (/$m^3$) | Zooplankton cultured with dry Yeast (/$m^3$) | Zooplankton cultured with green pond water (/$m^3$) | Zooplankton cultured with papaya leaf and stem (/$m^3$) |
|---|---|---|---|---|
| 1 | 500 | 500 | 500 | 500 |
| 2 | 485 | 480 | 479 | 491 |
| 3 | 431 | 410 | 438 | 470 |
| 4 | 370 | 355 | 365 | 440 |
| 5 | 325 | 301 | 338 | 405 |
| 6 | 289 | 260 | 280 | 390 |
| 7 | 271 | 205 | 237 | 371 |

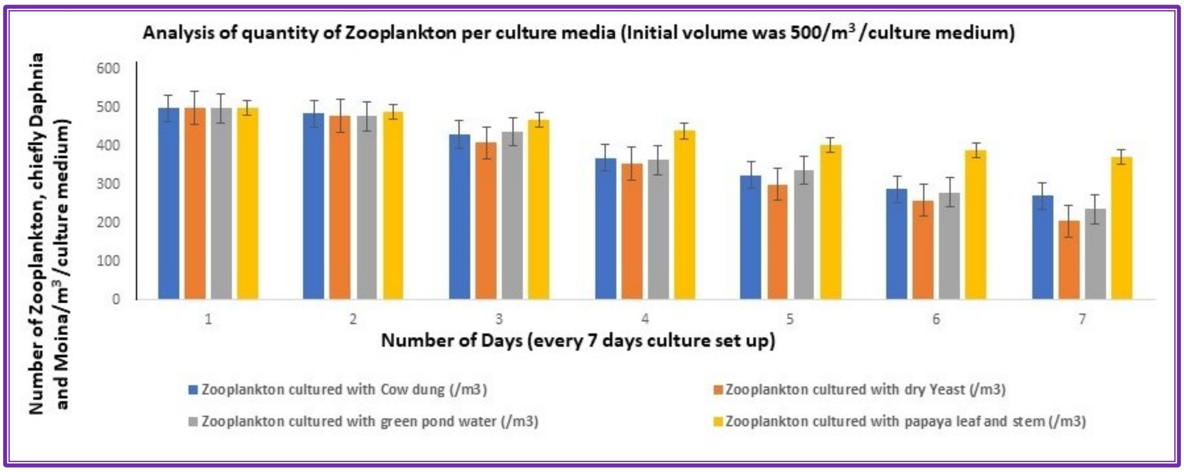


***Figure 5:*** *Analysis Of Quantity of Zooplankton Per Culture Media*

Both the table and graph showed that a significant number of individuals were found in papaya leaf and stem cultured media than the other three media. This result clearly proves nutritive value of papaya leaf and stems over other three media. Significance, *$p<0.05$ in papaya leaf and stems (set 4) vs. dry yeast fed zooplanktons (set 2)

*Water Analysis After Using Different Cultured Zooplanktons*

The pH, water temperature, ammonia, DO, and nitrite toxicity of the two different guppy fish tanks at different month intervals were tested, and data were analysed. The results showed that there was no significant variation in the pH and water temperature in different fish tanks fed with four different cultured zooplankton mixtures. However, significant variation was observed in papaya leaf and stem-fed zooplankton that were used to feed the two different guppy strains at different month intervals in relation to the yeast-fed zooplankton (Figure 6).

Result of DO showed a significant decrease in the DO level at the Yeast cultured zooplankton fed fish aquarium compared to the other three different experiment tanks of two different guppies. Maintenance of a steady state level of DO was also observed in the use of papaya leaf and stem cultured zooplankton-fed fishes (Figure 7). It was observed that the papaya leaf and stem-fed zooplankton have less or no toxicity increment capacity over the yeast-fed zooplankton (using papaya leaf and stem cultured zooplankton-fed fishes). High concentrations of ammonia in the water make it difficult for fish to eliminate ammonia from their bodies. High amounts of ammonia can cause stress, gill and internal organ damage, and eventually death. Graphical presentation (Figures 8A & B) gave a positive signal to a significant level of ammonia decrement after three regular monthly intervals. Nitrite is toxic to fish because it binds with the haemoglobin in fish's blood to form methaemoglobin. Haemoglobin carries oxygen through the body while methaemoglobin does not, so fish in high nitrite waters may suffocate even if sufficient oxygen is present. Graphs (Figures 8C & D) gave a positive signal to a significant level of nitrite decrement or no production of nitrite after using papaya leaf and stem-fed zooplankton culture-treated fishes monthly.

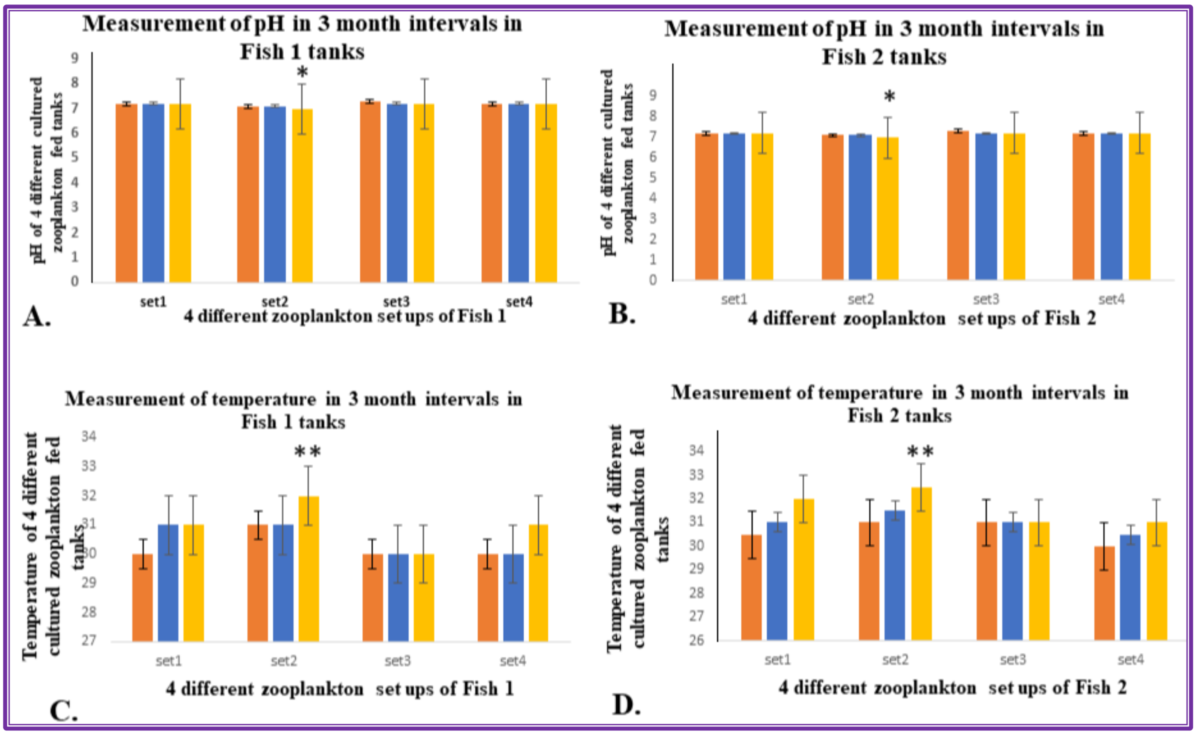


***Figure 6:*** *Measurement of pH and Water Temperature in Different Fish Tanks Fed with Four Different Cultured Zooplanktons*

A & B show the variation in the pH of water in two different fish tanks fed with four different cultured zooplanktons at three-month intervals. C & D shows the variation in the water temperature in two different fish tanks fed with four different cultured zooplanktons at three-month intervals. Significant level of variation was observed in papaya leaf and stem fed zooplanktons that were used to fed the two different guppy strains at different month intervals in relation to the yeast fed zooplanktons. Significance, *$p<0.05$ in yeast-fed zooplanktons (set 2) vs. papaya leaf and stem-fed zooplanktons (set 4) in water pH analyses and **$p<0.001$ in yeast-fed zooplanktons (set 2) vs. papaya leaf and stem-fed zooplanktons (set 4) in water temperature analyses. Set 1: organic manure (cow dung) media fed zooplanktons, set 2: Baker's yeast' media fed zooplanktons, set 3: green water media fed zooplanktons, and set 4: papaya leaf and stem fed zooplanktons.

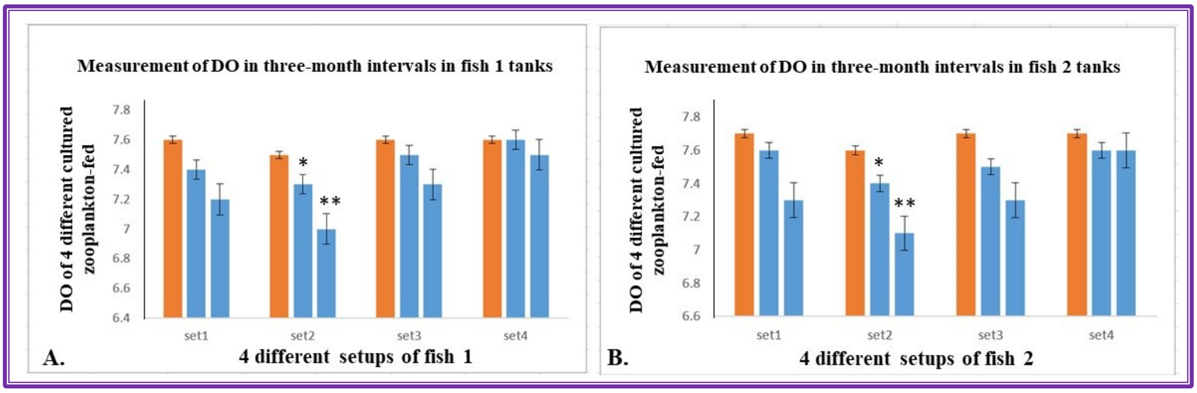


**Figure 7:** *Measurement of Dissolved Oxygen (DO) In Two Different Fish Tanks Fed with Four Different Cultured Zooplanktons*

The result of DO showed a significant decrease in DO level at the yeast-cultured zooplankton-fed fish aquarium compared to the other three different experiment tanks of two different guppies. Maintenance of a steady state level of DO was also observed in the use of papaya leaf and stem cultured zooplankton-fed fishes. Significance, **p*<0.05 in yeast-fed zooplanktons (set 2) vs. papaya leaf and stem fed zooplanktons (set 4) in water pH analyses and ***p*<0.001 in yeast-fed zooplanktons (set 2) vs. papaya leaf and stem fed zooplanktons (set 4) in water temperature analyses. Set 1: organic manure (cow dung) media fed zooplanktons, set 2: Baker's yeast media fed zooplanktons, set 3: green water media fed zooplanktons, and set 4: papaya leaf and stem fed zooplanktons.

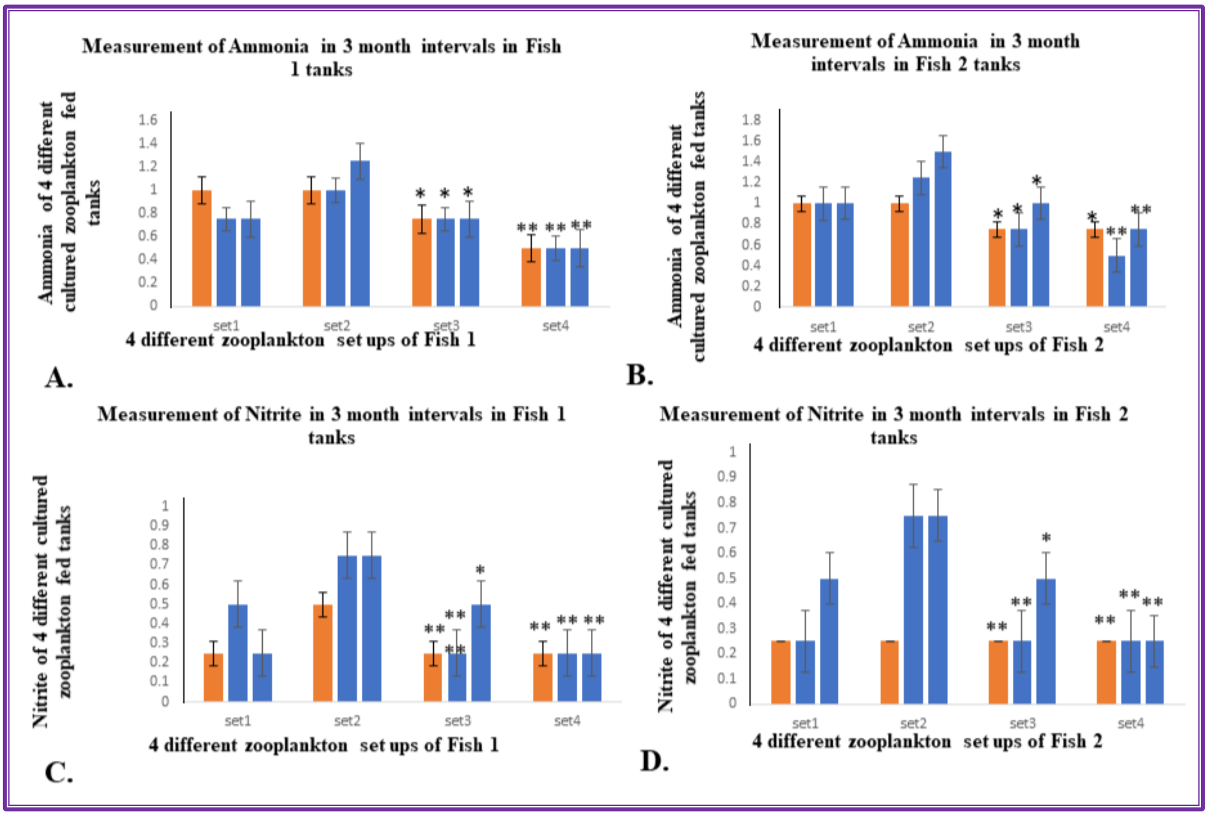


***Figure 8:*** *Measurement of Ammonia and Nitrite Amount (As Water Toxicity Level) in Different Fish Tanks Fed with Four Different Cultured Zooplanktons*

A & B shows the variation in the ammonia toxicity of water in two different fish tanks fed with four different cultured zooplanktons at three months intervals. C & D shows the variation in the nitrite level in two different fish tanks fed with four different cultured zooplanktons at three months intervals. All the graphs show the positive indication that the papaya leaf and stem-fed zooplanktons has less or no toxicity increment capacity over the yeast-fed zooplanktons. A & B gave a positive signal to a significant level of ammonia decrement after using papaya leaf and stem-fed zooplankton culture fed fishes in month basis. C & D gave a significant indication of the level of nitrite decrement or no production of nitrite after using papaya leaf and stem-fed zooplankton culture-treated fishes in month basis against yeast-fed zooplanktons. Significance, *$p<0.05$ in green pond water (set 3) vs. yeast-fed zooplanktons (set 2) in both ammonia and nitrite level measurement and **$p<0.001$ in papaya leaf and stem-fed zooplankton culture-treated fish tanks (set 4) vs. yeast-fed zooplanktons (set 2) in in both ammonia and nitrite level analyses. Set 1: organic manure (cow dung) media fed zooplanktons, set 2: Baker's yeast media fed zooplanktons, set 3: green water media fed zooplanktons and set 4 papaya leaf and stem fed zooplanktons.

*Analysis of the growth of guppy fishes*

Growth is a good indicator of the health of an organism. The growth rate of fish is affected by food quality, which is essentially determined by protein quality. During the time of the experiment, better foraging ability was observed in the order of the age of two different types of guppy fish. At the end of the experiment, better growth in terms of weight was found at one-month, two-month, and three-month intervals (Table 2). It was found that the growth rate of the two strains of guppy fish was higher in live *Daphnia Moina* mixture-fed guppy fish cultured in innovative papaya leaf and stem culture media than in the other three different cultured zooplankton-fed fishes. The length differentiation of day 1 fish fries, 1-month-old, 2-month-old, and three-month-old stage fishes was also recorded and given in Table 3 and in Figure 9. Body weight and body length were observed in two types of fish at three different month intervals and gave a significant level of changes in guppy fish, especially in live *Daphnia Moina* mixture-fed guppy fishes cultured in innovative papaya leaf and stem culture media compared to the other three sample foods. Results confirm that *Daphnia* and *Moina* mixture cultured with papaya leaf and stem directly enhances the growth of fingerlings, juveniles, and adults in respective month intervals.

***Table 2:*** *Measurement of body weight of chilli mosaic dumbo ear guppy (n=10) and full black guppy fishes (n=10)*

| Body Weight of chilli mosaic dumbo ear guppy (n=10) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| After 1 month | | | | After 2 months | | | | After 3 months | | | |
| Fed with Baker's yeast' media treated zooplanktons | Fed with organic manure media treated zooplanktons | Fed with green water media fed zooplanktons | Fed with Daphnia papaya leaf and stem fed zooplanktons | Fed with Baker's yeast' media treated zooplanktons | Fed with organic manure media treated zooplanktons | Fed with green water media fed zooplanktons | Fed with Baker's yeast' media treated zooplanktons | Fed with Baker's yeast' media treated zooplanktons | Fed with organic manure media treated zooplanktons | Fed with green water media fed zooplanktons | Fed with Daphnia papaya leaf and stem fed zooplanktons |
| 0.34 gm | 0.3 gm | 0.31 gm | 0.46 gm | Male- 0.74 gm Female- 0.95 gm | Male- 0.8 gm Female- 1 gm | Male- 0.89 gm Female- 1.1 gm | Male- 1 gm Female- 1.2 gm | Male- 0.9 gm Female- 1.1 gm | Male- 1.1 gm Female- 1.65 gm | Male- 1.6 gm Female- 1.75 gm | Male- 1.9 gm Female- 2.1 gm |

n=10 (number of individuals to take the average values)

| Body Weight of full black guppy (n=10) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| After 1 month | | | | After 2 months | | | | After 3 months | | | |
| Fed with Baker's yeast' media treated zooplanktons | Fed with organic manure media treated zooplanktons | Fed with green water media fed zooplanktons | Fed with Daphnia papaya leaf and stem fed zooplanktons | Fed with Baker's yeast' media treated zooplanktons | Fed with organic manure media treated zooplanktons | Fed with green water media fed zooplanktons | Fed with Baker's yeast' media treated zooplanktons | Fed with Baker's yeast' media treated zooplanktons | Fed with organic manure media treated zooplanktons | Fed with green water media fed zooplanktons | Fed with Daphnia papaya leaf and stem fed zooplanktons |
| 0.35 gm | 0.37 gm | 0.43 gm | 0.52 gm | Male- 0.78 gm Female- 1.1 gm | Male- 0.81 gm Female- 1.25 gm | Male- 0.8 gm Female- 1.2 gm | Male- 1.1 gm Female- 1.45 gm | Male- 0.92 gm Female- 1.2 gm | Male- 1.2 gm Female- 1.7 gm | Male- 1.45 gm Female- 1.9 gm | Male- 1.87 gm Female- 2.2 gm |

n=10 (number of individuals to take the average values)

1. Baker's yeast' media fed zooplanktons,
2. organic manure media fed zooplanktons,
3. green water media fed zooplanktons
4. papaya leaf and stem fed zooplanktons

***Table 3:*** *Measurement of body length or size of chilli mosaic dumbo ear guppy (n=10) and full black guppy fishes (n=10)*

| Body Length or Size of chilli mosaic dumbo ear guppy (n=10) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 1 | | | | After 1 month | | | | After 2 months | | | | After 3 months | | | |
| I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV |
| 0.3 cm | 0.3 cm | 0.3 cm | 0.3 cm | 0.5 cm | 0.6 cm | 0.6 cm | 0.7 cm | M- 0.7 cm F- 0.8 cm | M- 0.8 cm F- 0.9 cm | M- 0.8 cm F- 0.9 cm | M- 0.8 cm F- 1.0 cm | M- 1.1 cm F- 1.3cm | M- 1.2 cm F- 1.3 cm | M- 1.3 cm F- 1.5 cm | M- 2 cm F- 2.2 cm |

| Body Length or Size of full black guppy (n=10) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 1 | | | | After 1 month | | | | After 2 months | | | | After 3 months | | | |
| I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV |
| 0.4 cm | 0.4 cm | 0.4 cm | 0.4 cm | 0.6 cm | 0.7 cm | 0.7 cm | 0.8 cm | M- 0.9 cm F- 1.1 cm | M- 1 cm F- 1.3 cm | M- 1.1 cm F- 1.4 cm | M- 1.2 cm F- 1.5 cm | M- 1.2 cm F- 1.5cm | M- 1.5 cm F- 1.9 cm | M- 1.7 cm F- 2 cm | M- 2.2 cm F- 2.5 cm |

1. Baker's yeast' media fed zooplanktons, (I)
2. organic manure media fed zooplanktons, (II)
3. green water media fed zooplanktons (III)
4. papaya leaf and stem fed zooplanktons (IV)

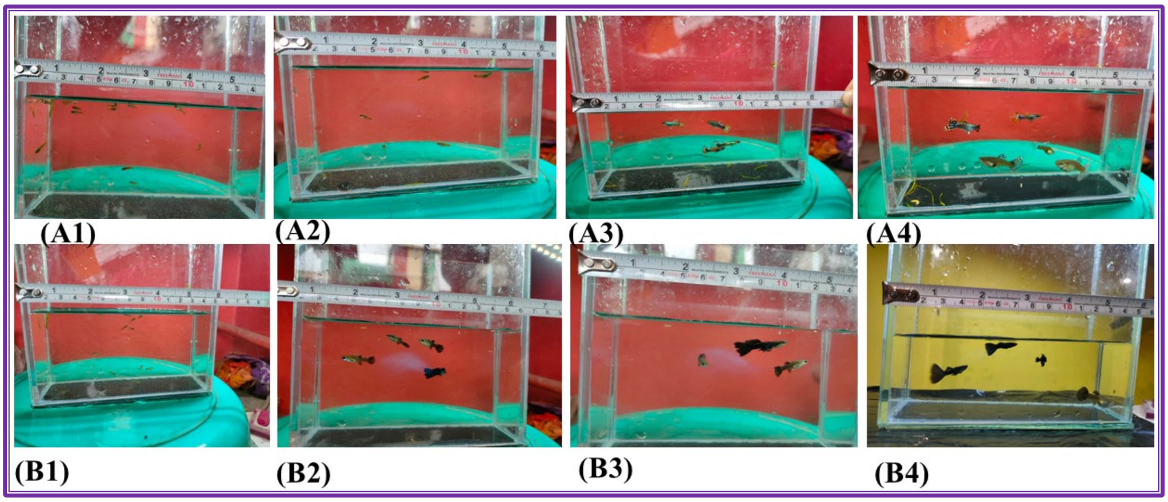


***Figure 9:*** *Size Measurement of 2 Types of Guppy Fish at Three Different Month Intervals, Fed with Papaya Leaves and Stem-Fed Zooplankton, as it Gives Significant Differences*
*A1- 1 day old chilli mosaic guppy, A2- 1 month old chilli mosaic guppy, A3- 2 months old chilli mosaic guppy, A4- 3 months old chilli mosaic guppy; B1- 1 day old full black guppy, B2- 1 month old full black guppy, B3- 2 months old full black guppy, B4- 3 months old full black guppy.*

*Colour Measurements and Statistical Analyses*

At the end of the experiment, we estimated the colour variations in both guppy strains fed with four different cultured zooplankton mixtures. The result showed the most approximate increment of colour with proper fin patterns in the innovative papaya leaf and stem cultured zooplankton-fed fishes than the other three conventionally cultured zooplankton-fed fishes, especially in the fishes fed zooplankton cultured with baker's yeast (Figure 10).

It was observed that the survival rate of the innovative papaya leaf and stem cultured zooplankton-fed fishes was almost 100%, which truly signifies our innovation. As we know, *Daphnia* and *Moina*, like zooplankton, provide two primary vitamins, A and D, so *Daphnia* and *Moina* have immense efficacy in increasing fish growth, colouration, and survival rate. Vitamin A is essential for the growth and development of fish, and it also serves as an excellent anti-infective agent.

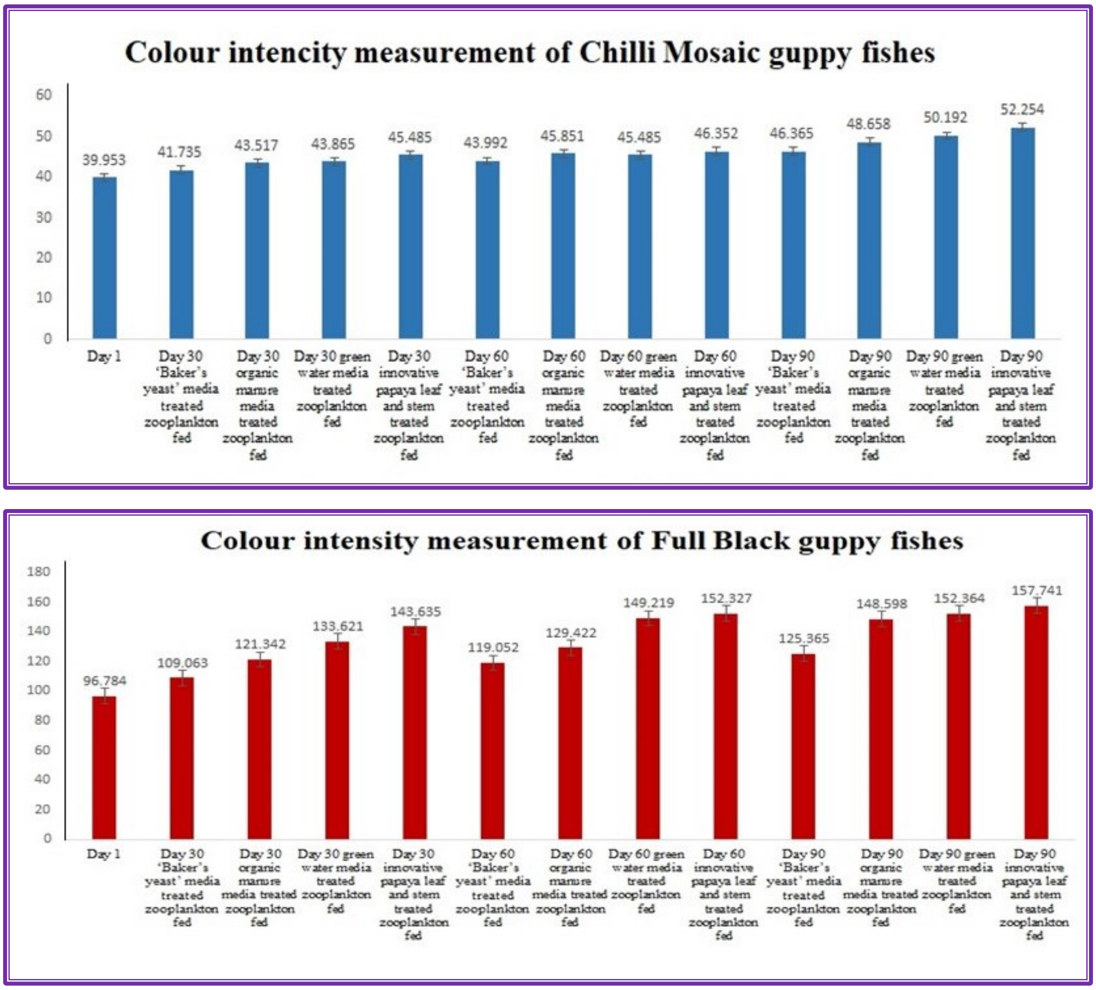


***Figure 10:*** *Body Colour Intensity Measurement of Two Different Guppy Strains at Three Different Month Intervals, Fed with Four Differentially Cultured Zooplanktons*

Both A (chilli mosaic guppy) & B (full black guppy) showed that a sufficient increment of colour in the innovative papaya leaf and stem cultured zooplankton fed fishes than the other three conventionally cultured zooplankton fed fishes, especially in the fishes fed zooplankton cultured with yeast at three gradual month intervals.

## Discussion

In the fish farming sector, nutrition plays a vital role from an economic perspective, as feed accounts for 40%-50% of production costs (De Silva & Anderson, 1995). Consequently, feed expenses should remain manageable for a sustainable industry. The costs associated with feed designed for specific ornamental species differ significantly from those for food fish (Tamaru & Ako, 1999). However, many small-scale aquarium hobbyists in India tend to choose fish feed based solely on its price (personal observations). Therefore, this study demonstrates that live feed like zooplanktons, chiefly *Daphnia* and *Moina*, are more beneficial for guppies in indoor aquariums when compared to alternative diets like optimum, etc. Further research is needed to evaluate the effectiveness of these diets for guppies in outdoor ponds, as the results would be invaluable for guiding management practices for small-scale ornamental fish farmers (Kumaratunga & Radampola, 2019).

Guppy fish are typically recognized as resilient fish due to their capacity to adapt to a variety of biotic and abiotic environments (Fernando & Phang, 1994). Throughout the experimental duration, no instances of mortality were observed in this study. The average food intake was approximately 10-12% of body weight for fish receiving different diets, and there were no significant differences between the treatments. At the start of the experiment, daily food intake (body weight per day) for the brooder guppies was elevated and decreased over time for all feeding regimens (evaluated from their release of faecal material). In the initial days, determining the precise quantity of food needed to adequately satisfy the fish proved challenging, which may have led to overfeeding during that period. Typically, food intake is higher at the beginning of the growth phase (from day 1) and tends to decrease as the fish increase in size. This pattern is commonly noted and has been recorded in two different guppy species (De Silva & Perera, 1985).

*Daphnia*, *Moina*, and other zooplankton found in freshwater environments are highly valued and ideal as fish feed (Karaçuha& Aral, 2011; Umi *et al*., 2024). These organisms are suspension feeders, utilizing a filtering system that includes phyllopod-broad, leaf-like appendages that generate water currents. *Daphnia* and *Moina* are crustaceans known for their high nutritional quality, rapid growth, and reproductive rates. In this study, we demonstrated that live food (chiefly *Daphnia* and *Moina* mixture) serves as a highly effective alternative for aquaculture, cultured in a very innovative way compared to other conventional techniques. All live food types are potentially viable for ornamental fish production, and we showcased the beneficial effect of live food on the colouration and growth of *P. reticulata* (Kumaratunga &Radampola, 2019).

An analysis of the phytochemicals in papaya leaf extracts reveals the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, papain, chymopapain, cystatin, tocopherol, flavonoids, ascorbic acid, cyanogenic glucosides, and glucosinolates (De Oliveira& Vitória, 2011; Hamid *et al*., 2022). Although some research has been conducted on *Daphnia* and *Moina* as fish feed, there have been limited investigation methods for enhancing the growth of zooplankton without relying on yeast or other factors as a medium. Numerous studies have indicated that the growth of zooplankton is largely dependent on Baker's yeast; however, yeast can inadvertently lead to fungal infections in both guppy brooders and fry.

Our target was to promote the growth of zooplankton, particularly *Daphnia* and *Moina*, using only papaya leaves and stems without any starch, in a very innovative way. Therefore, if our study yields positive results compared to some controls, it could be a promising strategy. Since *Daphnia* and *Moina* will be entirely nourished by papaya leaves and stems, we will not use any yeast extract as supplemental food for their growth. This method is advantageous as it makes the zooplankton, along with the organic mixture, a rich source of growth molecules without any adverse effects. Since the zooplankton will be fed exclusively on papaya leaves and no yeast was introduced, there is no risk of fungal infections that could harm the fish.

High survival rates are crucial for the successful production of economically valuable fish species. All test subjects exhibited a survival rate of 100% when fed the diets tested. Colouration serves as a signal for communication among species. Certain animals, such as fish, display ornaments based on carotenoids, which are perceived as reliable indicators of health and resistance to parasites. Fish require dietary sources of carotenoid pigments. Both male and female *P. reticulata* exhibited more vibrant colours when fed with a *Daphnia* and *Moina* mixture cultured with papaya stems and leaves compared to those given a *Daphnia* and *Moina* mixture cultured in cow dung, Baker's yeast medium, and green pond water. This variation may stem from differences in diet digestibility and the stimulation of fish feeding behaviours. Furthermore, live food typically avoids processes like drying, freezing, or packaging that diminish its nutritional quality. Conversely, the increased colour vibrancy associated with live food, as opposed to commercial flakes, might be linked to the crustaceans used (*Daphnia* and *Moina*), which are filter feeders obtaining carotenoids from the microalgae in their environment. Growth, as indicated by the fish size in every month interval and fin sizes too, serves as an effective measure of an organism's health. The growth rate of fish is influenced by the quality of their food, which fundamentally relies on protein quality. In this investigation, both male and female guppy fish (*P. reticulata*) fed with *Daphnia* and *Moina* mixture, cultured using fresh papaya stem and leaves, experienced quicker growth compared to those conventionally cultured zooplankton mixtures. Live food acts as a capsule that contains essential components of a balanced diet while maintaining its integrity when consumed by fish (i.e., it does not dissolve in water).

Modern studies revealed that zooplankton, particularly cladoceran crustaceans like *Daphnia*, *Moina*, etc., are a natural, high-quality source of DHA (Docosahexaenoic acid) and EPA (Eicosapentaenoic acid) (Sultana *et al*., 2023). They acquire these fats by consuming phytoplankton. Zooplankton are exceptionally rich in proteins (often 40%–50%+ of dry weight) and contain a well-balanced profile of essential amino acids. On the contrary, papaya leaves are rich in bioactive compounds and contain significant amounts of crude protein (approximately 16%–25% dry matter). They also contain various amino acids that help to improve immunity. This is thus an innovative combination for the modern fish feed industry.

*Carica papaya* (the papaya plant) generates a proteolytic enzyme named papain, recognized for its numerous health advantages. The heightened fish appetite, better feed consumption, and greater nutrient digestibility noted in this study may be linked to the presence of papain in papaya leaf meal, which consequently promoted the growth of the experimental fish. Nonetheless, additional studies are required to thoroughly investigate the possible advantages of papaya leaf and stem supplementation in fish feed, chiefly in zooplankton culture. Findings from this study confirm that *Daphnia* and *Moina* mixture cultured with papaya leaf and stem directly enhance the growth of fingerlings, juveniles, and adults in respective month intervals. Grishma Tewari and colleagues (2018) showed that the extract from papaya leaves enhanced the growth of *Cyprinus carpio* (Tewari *et al*., 2018). Additionally, Hamid *et al* (2022) discovered notable enhancements in weight gain, specific growth rate (SGR), and feed efficiency of red hybrid tilapia with this inclusion level and advised that the recommended inclusion level of papaya leaf extract as a feed additive to encourage the growth of red hybrid tilapia fry is between 1% and 2% (Mandal *et al*., 2025). This indicates that papaya leaf meal-fed zooplankton assists the fish in increasing body weight to a certain degree, as the quality of the feed greatly influences conversion efficiency. This result significantly contributes to the aquaculture science and the aquaculture industry.

Water parameters like ammonia and nitrite levels gave a positive indication of a significant level of decrement after using papaya leaf and stem cultured zooplankton for the guppy fish feeding over the three conventionally cultured zooplankton-fed guppies in three regular month intervals. This indicates a positive approach towards less toxic effects in innovative zooplankton-fed guppy aquariums over others. The result of body growth of both chilli mosaic dumbo ear and full black guppies was higher in live *Daphnia Moina*-fed fishes cultured in innovative papaya leaf and stem culture media than the other three different cultured zooplankton-fed fishes of the aquaria. Body weight and body length as parameters of growth of the fishes were observed in two types of fishes at three different month intervals and showed significant levels of changes in guppy fishes, especially in live *Daphnia*-*Moina*-fed fishes cultured in innovative papaya leaf and stem culture media compared to the other three conventionally cultured zooplanktons. The approximate colour variations and fin sizes and shapes in both guppy strains fed with four different cultured zooplanktons were also experimented with. The result showed the most approximate increment of colour with proper fin patterns in the innovative papaya leaf and stem culture media-fed fishes than the other three conventionally cultured zooplankton-fed fishes, significantly in the fishes fed with zooplanktons cultured with Baker's yeast.

*Limitations*

The only limitation of this research work is the collection of fresh, high-quality zooplankton, especially Moina and Daphnia.

*Future Scope*

Zooplankton fed on papaya leaves and stems would be a better option for the modern ornamental fish industry, as they can be used as live feed and may be more nutritious than the other available feeds in the market.

## Conclusion

All the experimental data support our revolutionary idea that papaya leaves and stems increase the quality and growth of live zooplankton, chiefly *Daphnia* and *Moina* mixtures, over other conventional culture protocol-made zooplankton. This cultured zooplankton (*Daphnia* and *Moina* mixture) is also able to increase the growth, colour, fin size, etc., of guppy fish, which could be helpful for guppy fish farmers to make their fish farm cost-effectively with fewer toxic side effects. This data significantly contributes to the aquaculture science and the aquaculture industry. This study could recommend the use of papaya leaves and stems for the culture of zooplanktons in this innovative way for the modern ornamental fish industry in the future.

## Conflict of Interest

The authors declare that they have no competing interests.

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