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Exploring the Freshwater Cladocera (*Pleuroxus* spp.) as New Potential Live Feed in Aquaculture

(Meneroka *Cladocera* Air Tawar (*Pleuroxus* spp.) sebagai Suapan Hidup Berpotensi Baharu dalam Akuakultur)

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ABSTRACT

In freshwater lakes, cladocera play a crucial role in the food chain, as they are the primary source of nutrition for nearly all aquatic species. *Pleuroxus* spp. are very suitable for aquaculture due to their ease of handling, rapid growth, non-selective filter feeder, high nutritional value, and remarkable ability to adapt to many environmental conditions. Silver barbs are in high demand in the aquaculture business due to their excellent flavours and rapid development rate. In this study, *Pleuroxus* spp. was cultured in a control condition with water quality maintained at the room temperature of 26 ± 1 °C and pH of 7.0 ± 0.5. Five treatments of *Pleuroxus* spp. were prepared, which comprised of T1 (control) = unfed, T2 = coffee grounds, T3 = corn flour, T4 = wheat flour, and T5 = sugarcane waste. *Pleuroxus* spp. were fed with a selected diet solutions at a concentration of 0.5 g/L daily. The result shows that *Pleuroxus* spp. given T3 (24.88 ± 5.48 ind/mL) had a greater population density than T4 (22.13 ± 4.83 ind/mL), T2 (21.06 ± 5.27 ind/mL), and T5 (20.56 ± 4.92 ind/mL) dietary treatments. *Pleuroxus* spp. given T3 had the greatest survival rate (97.9 ± 9.8%) when compared to other diets, T4 (86.81 ± 19.07%), T2 (82.71 ± 16.56%), and T5 (75.00 ± 21.08%). Silver barb showed the highest amount of daily weight gain in treatment T3 (0.11 ± 0.001 mg/days; $p < 0.05$) compared with other dietary treatments. The maximum specific growth rate (SGR) (%) was achieved when fed with T3 (13.37 ± 0.02%; $p < 0.05$). The feed conversion ratio (FCR) was shown to be significantly different ($p < 0.05$). The FCR of larvae fed with different nutritional treatments ranged from 1.22 to 4.12, depending on the treatment. In comparison to other diets, the T3 treatment (1.22 ± 0.03) achieved the highest FCR ratio. In short, pre-feeding of zooplankton with prospective diets has built a baseline of improved aquaculture feeding procedures that may be employed in the future aquaculture businesses.

Keywords: Aquaculture; *Cladocera*; enrichment; life history parameter; survival rate

ABSTRAK

Di tasik air tawar, *cladocera* memainkan peranan penting dalam rantaian makanan, kerana ia merupakan sumber pemakanan utama untuk hampir semua spesies akuatik. *Pleuroxus* spp. sangat sesuai untuk akuakultur kerana ia mudah dikendalikan, tumbesaran pesat, pemakan penapis tidak selektif, nilai nutrisi yang tinggi serta keupayaan luar biasa untuk menyesuaikan diri dalam banyak keadaan persekitaran. Lampan jawa mendapat permintaan tinggi dalam perniagaan akuakultur kerana rasa yang sangat baik dan kadar tumbesaran yang pesat. Dalam kajian ini, *Pleuroxus* spp. dibiakkan dalam keadaan terkawal dengan kualiti air dikekalkan pada suhu bilik 26 ± 1 °C dan pH 7.0 ± 0.5. Lima rawatan *Pleuroxus* spp. telah disediakan, yang terdiri daripada T1 (kawalan) = tidak diberi makan, T2 = serbuk kopi, T3 = tepung jagung, T4 = tepung gandum dan T5 = sisa tebu. *Pleuroxus* spp. diberi makan dengan larutan diet terpilih pada kepekatan 0.5 g/L setiap hari. Keputusan menunjukkan bahawa *Pleuroxus* spp. diberi T3 (24.88 ± 5.48 ind/mL) mempunyai kepadatan populasi yang lebih besar daripada rawatan pemakanan T4 (22.13 ± 4.83 ind/mL), T2 (21.06 ± 5.27 ind/mL) dan T5 (20.56 ± 4.92 ind/mL). *Pleuroxus* spp. diberikan T3 mempunyai kadar kemandirian hidup yang paling tinggi (97.9 ± 9.8%) jika dibandingkan dengan diet lain, T4 (86.81 ± 19.07%), T2 (82.71 ± 16.56%) dan T5 (75.00 ± 21.08%). Lampan jawa menunjukkan jumlah pertambahan berat harian tertinggi dengan rawatan T3 (0.11 ± 0.001 mg/hari; $p < 0.05$) berbanding rawatan diet lain. Kadar tumbesaran khusus maksimum (SGR) (%) dicapai apabila diberi T3 (13.37 ± 0.02%; $p < 0.05$). Nisbah penukaran suapan (FCR) ditunjukkan berbeza dengan ketara ($p < 0.05$).

FCR larva yang diberi makan dengan rawatan pemakanan yang berbeza adalah antara 1.22 hingga 4.12, bergantung kepada rawatan. Berbanding dengan diet lain, rawatan T3 (1.22 ± 0.03) mencapai nisbah FCR tertinggi. Secara ringkasnya, pra-pemakanan zooplankton dengan diet prospektif telah membina garis asas prosedur pemakanan akuakultur yang lebih baik yang mungkin digunakan dalam perniagaan akuakultur pada masa hadapan.

Kata kunci: Akuakultur; *Cladocera*; kadar kemandirian; pengayaan; parameter sejarah hidup

INTRODUCTION

The variety of zooplankton in the aquatic environment is one of the most important biological markers of the aquatic environment. In order to maintain a healthy ecosystem, zooplankton species need to be diverse (Manickam et al. 2018). In freshwater lakes, zooplankton play a crucial role in the food chain, as they are the primary source of nutrition for nearly all aquatic species (Karpowicz et al. 2020). The nutrition provided by zooplankton species includes proteins, lipids, and critical fatty acids required for the growth performance of larvae fish (Manickam et al. 2018). There has been research in the past applying mass culture production for marine and freshwater hatcheries to culture zooplankton (Radhakrishnan et al. 2020). Zooplankton may have substantial influence on their long-term viability of any larvae rearing operation since they contain rich biochemical ingredients and are readily digested and consumed by aquatic species (Radhakrishnan et al. 2017; Rasdi et al. 2021).

Cladocera, generally known as water fleas, represent the freshwater microfauna that provides critical biological support for the larval environment through supplying appropriate biochemical content for the growth of the larvae of the cultivated aquatic larvae species (Rizo et al. 2017). Cladocera species are well-suited for aquaculture because they are easy to handle, grow quickly, non-selective filter feeder for bacteria, high in nutrition, and capacity to adapt to a broad variety of environmental circumstances. As a result, it is critical to establish culture conditions suitable for bulk production of cladocerans. The availability and cost of food influence the growth and reproduction of cladocerans (Nandini, Sarma & Dumont 2021). Increased feeding rates resulted in an increase in zooplankton absorption efficiency, implying that increasing population growth rates in cladocera culture may be favorable for larvae rearing.

Exogenous feeding in fish larvae starts once the yolk sac has been absorbed, and the larvae are capable of consuming, digesting, and assimilating food particles. This occurs when the mouth and anus are fully or nearly depleted of yolk (Yúfera & Darias 2007). According to a previous study, food particle size is one of the most important aspects that contributes to effective feeding efficiency (El Hag et al. 2012). The size of food provided must be considerable, but should be somewhat slightly smaller than the mouth gape in order for the larvae to be able to ingest the food. *Barbonymous gonionotus* (Bleeker 1849) is a native freshwater carp found across South-east Asia, ranging from the Chao Phraya basins to the Peninsular of Malaysia, Sumatra, and Java (FAO 1996; Kottelat & Whitten 1996). It may be found in the benthopelagic zone, mainly from the midwater depth to the bottom of reservoirs, where it lives in the benthopelagic zone. The fish has been cultivated in a variety of locations throughout the world, most notably Southeast Asia (Ismail et al. 2019).

Silver barbs are in high demand in the aquaculture business due to their excellent flavor and rapid development rate. Hatchery systems that can produce high and consistent yields while also providing the aquaculture sector with reliable and effective feeding strategies are becoming increasingly important as interest in this species' culture grows. Research efforts should be focused on how to feed larvae the right way, how to improve and develop their larval diets, and how to use enriched live food as a supplement. It is thought that the development of a new live food species for silver barb larvae could help to increase the production of this fish by lowering the mortality rate during the larval stages and cutting down on the grow-out cost of this fish.

Nevertheless, during larviculture, it is vital to select a suitable diet with sufficient nutrients in order to provide nutritionally balanced meals for the fish larvae (Ismail et al. 2019). It is necessary to supplement zooplankton with nutrient-rich feed containing protein, lipids, and fatty acids. Hence, it is crucial to comprehend the alterations in fatty acid composition and the impact of enrichment on vital fatty acids (ARA, EPA and DHA) for fish larvae, as various species of zooplankton exhibit diverse nutritional profiles (Ramlee et al. 2021). The nutritional condition of zooplankton can be manipulated by pre-feeding them using 'bioencapsulation' or 'enrichment' techniques, which take advantage of their basic eating tendencies (Yuslan et al. 2022a). By employing enrichment techniques, it is possible to provide fish larvae with vital nutrients that are deficient in zooplankton, as well as prophylactics and treatments, through the use of zooplankton live food (Samat et al. 2020).

The purpose of the research was to identify potential zooplankton species of the lake located at Lake K11, Universiti Tunku Abdul Rahman campus (UTAR), Kampar, Perak. Thus, this study was established to discover new potential zooplankton to be cultured as live feed in aquaculture industries. Additionally, in this study, we also feed and enriched *Pleuroxus* spp. with different diet treatments in order to determine suitable diets on growth and production of *Pleuroxus* spp. Therefore, in view of the growing demand of cladocerans as live farm feed in aquaculture as well as alternative food supplementations for fish fry rearing and rising need for utilizing potentials of available agriculture by product, we enhanced *Pleuroxus* spp. nutritional quality by enriching them with different diets that comprised T1 (control) = unfed, T2 = coffee grounds, T3 = corn flour, T4 = wheat flour and T5 = sugarcane wastes, to determine its efficiency when being consumed by a freshwater fish larvae, silver barb, *Barbonymous gonionotus* (Bleeker 1849).

MATERIALS AND METHODS

SAMPLE COLLECTION

The sampling location for determining primary output was an abandoned tin mine located near Lake K11 on the campus of Universiti Tunku Abdul Rahman (UTAR) in Kampar, Perak. Kampar is situated on Peninsular Malaysia's north coast, with coordinates of 4.3° North latitude, 101.14° East longitude, and a height of 63 meters above sea level (Figure 1). Kampar is surrounded by disused mine lakes and mountains in a rainforest setting. Plankton net with mesh 30 µm size was used to collect zooplankton since, according to Makabe, Tanimura and Fukuchi (2012), more zooplankton can be collected when using a plankton net with mesh sizes of 100 µm and below. The collection of samples was conducted horizontally by two independent towing methods: boat towing and hand towing (Schwoerbel 2016). Three sanitized 50 mL bottles were used to collect samples from 200 mL of the collective plankton container (bottle collector at the end of plankton net) at each sampling location. To inhibit bacterial breakdown during the preservation process, a 40% formalin solution was used (Ramlee, Suhaimi & Rasdi 2022).

SAMPLE ISOLATION AND IDENTIFICATION

The samples were taken to the Plankton laboratory, Universiti Malaysia Terengganu for examination and species identification after they were collected. All of the samples were examined with an OLYMPUS compound light microscope (Model: CX23LEDRFS1, Olympus Corporation, Tokyo, Japan). Photos of plankton samples were taken by using a Dino-lite digital microscope, with a Dino-capture 2.0 software. Although the number of zooplankton collected was not recorded, cladocerans predominated in the samples. Cladocera specimens were grouped by species and acclimated to laboratory conditions. In the following two days, cladocerans were cultivated to serve as stocking animals for the various tests conducted in this work.

SAMPLE CULTIVATION AND DIET PREPARATION

Pleuroxus spp. were initially cultivated in 0.1 L flask and then transferred to a bigger tank for mass culture. All feeding trials were carried out in the hatchery of the Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu (UMT), Malaysia. Individual *Pleuroxus* spp. were cultured for several generations (one *Pleuroxus*

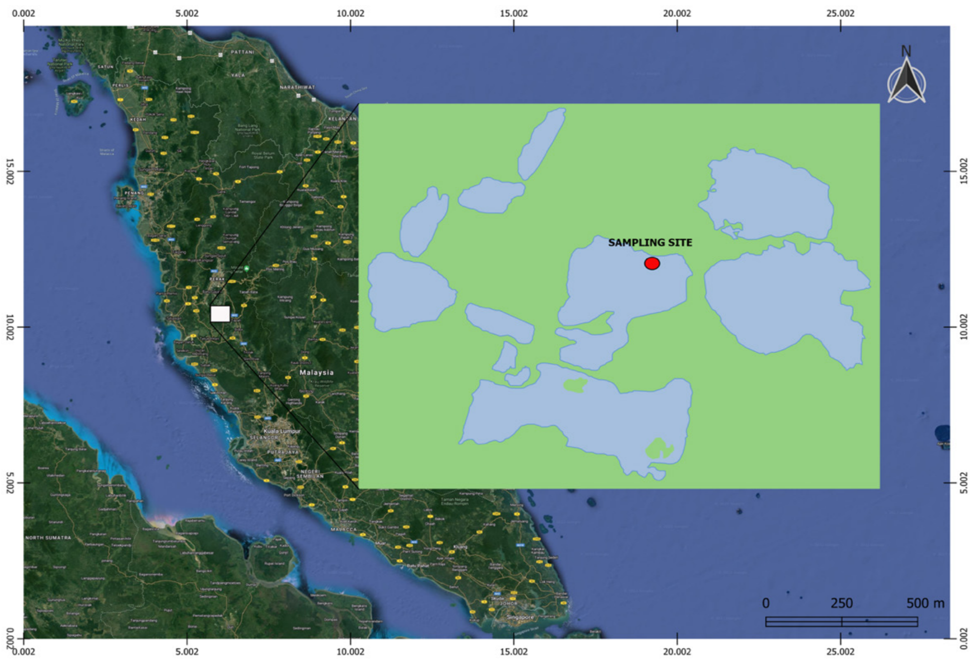


FIGURE 1. Sampling location at UTAR campus lake K11

spp./10 mL) to find species with optimum development and productivity (Figure 2). This stock culture of *Pleuroxus* spp. was started in a 0.1 L flask. After the density reached 2 individuals per millilitre of culture, the cultures were transferred into two 2-tonne tanks for mass culture, and the process was repeated.

Before the experiment, each diet was mixed and ground into a powder form (< 0.1 µm) for about 3 min at full speed in a blender. The powdered feeds were weighed and then diluted with freshwater. Before being placed into the *Pleuroxus* spp. culture tank, the feed mixture was sieved with a 50 µm net (Loh et al. 2013). *Pleuroxus* spp. were given the diet solution at a concentration of 0.5 g/L (Rasdi et al. 2018).

In order to make sure that the culture water was free of any chlorine that could harm the culture of zooplankton, anti-chlorine stones were added to the tanks which were heavily aerated. Before the experiment, 4 adults and 4 neonates of *Pleuroxus* spp. were harvested and inoculated into each tank where the experiment was taking place (Rasdi et al. 2018). The experiments were conducted in triplicate, in which the culture tanks were divided into five tanks, one for each of the five treatments of diets including the control (unfed). Every two days, 50% of the water was exchanged, and the water quality was maintained at the room temperature of 26 ± 1 °C and pH of 7.0 ± 0.5 (Islam et al. 2017; Yuslan et al. 2021). *Pleuroxus* spp. were fed with 5 diets which comprised T1 (control) = unfed, T2 = coffee grounds, T3 = corn flour, T4 = wheat flour and T5 = sugarcane wastes. For T1, *Pleuroxus* spp. was cultivated in dechlorinated freshwater culture medium without the addition of any food sources.

POPULATION GROWTH AND SURVIVAL RATES OF *Pleuroxus* spp.

The population density of *Pleuroxus* spp. in 0.1 tonne tanks was 8 individuals/mL with a diameter range of 100-200 µm (Suhaimi et al. 2022b) was evaluated daily for 15 days until all animals in the generation perished. To maintain water quality, the YSI meter (Model Professional Plus: 13M100666, YSI Inc., USA) was used daily to check the water parameters. A one-milliliter sample of *Pleuroxus* spp. was collected from the culture tank, and the population of *Pleuroxus* spp. was estimated using a dissecting microscope (Nikon model SMZ 1500, Tokyo, Japan), and the results were documented. The procedure was repeated three times to ascertain the total number of *Pleuroxus* spp. Eight individual neonates were picked from the culture tank and placed in a 30 mL Petri dish with fresh water (dechlorinated) in triplicates before the survival rate of *Pleuroxus* spp. was calculated. To minimize overfeeding,

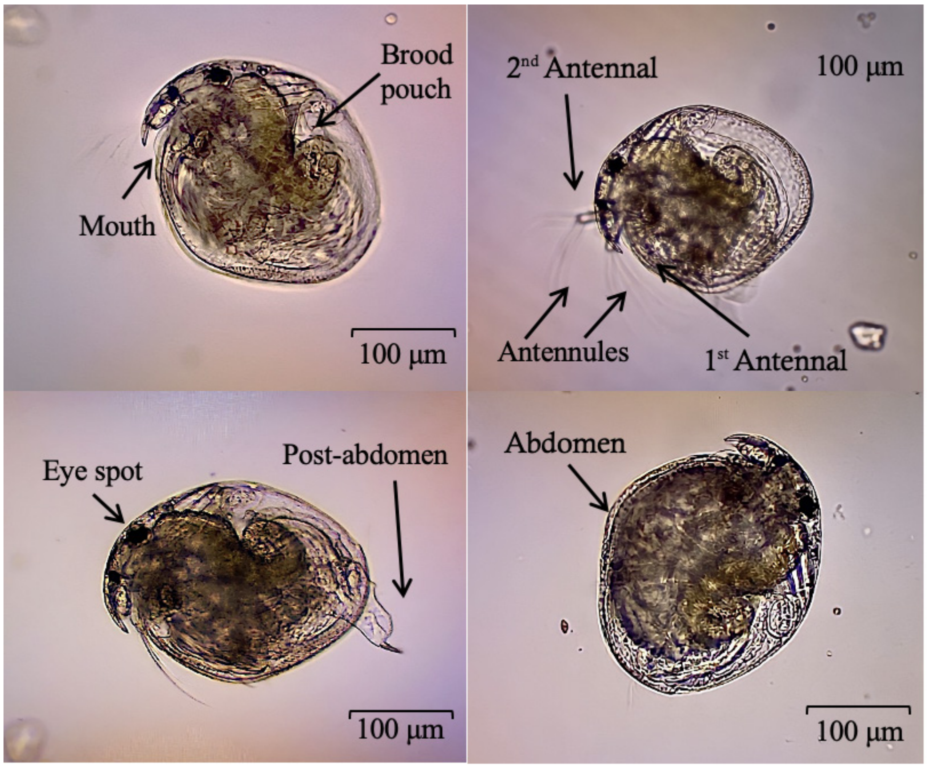


FIGURE 2. Lateral view of *Pleuroxus* spp. under a compound microscope

the neonates in Petri dish were given feeds daily (one drop of feed from the concentration of 0.5g/L) (T1 = unfed, T2 = coffee grounds, T3 = maize flour, T4 = wheat flour, and T5 = sugarcane wastes) that had been produced in the form of liquid medium. *Pleuroxus* spp. were given the diet solution at a concentration of 0.5 g/L daily (Rasdi et al. 2018; Yuslan et al. 2022b). Every two days, the survival rate of *Pleuroxus* spp. from neonates to adults was determined. The following equation was used to compute these survival rates (Suhaimi et al. 2022b):

$$Survival\ rate\ (\%) = \left(\frac{Total\ number\ in\ sample\ taken\ every\ two\ days}{Total\ number\ initial\ density\ in\ tank}\right) \times 100$$

DETERMINATION OF LIFETABLE PARAMETER OF *Pleuroxus* spp.

Eggs of the ephippium genus were found in all diets, and the time of hatching and hatching rate of these eggs were recorded. For each treatment, 20 eggs were removed from each tank and deposited in a 30 mL Petri dish with three replicates. The water quality in the Petri dish also was maintained at the room temperature of 26 ± 1 °C and pH of 7.0 ± 0.5 (Islam et al. 2017; Yuslan et al. 2021). Every 2 h, eggs were inspected for the presence of neonates. The inspections lasted more than 24 h to check that all of the eggs had hatched. *Pleuroxus* spp. fed with various diets had their hatching rates measured by collecting and counting their unhatched eggs after 48 h according to Pan et al. (2014) study.

$$Hatching\ rate\ (\%) = \left(\frac{Number\ of\ eggs\ hatched}{Initial\ number\ of\ eggs\ from\ each\ gravid\ female}\right) \times 100$$

Every day after hatching, *Pleuroxus* spp. neonates were observed in a Petri dish to assess their longevity. To minimize overfeeding, freshly prepared food was delivered everyday using a plastic dropper. Daily monitoring of the developmental phases had also been carried out in order to establish the time span of development from neonates to adults. The developmental phases from neonates to adults of enriched *Pleuroxus* spp. were monitored daily under a dissecting microscope by observing the appearance of antennae, a pair of mandibles, a pair of first maxillae (maxillulae) and changes in body structure and sizes from day one of hatching (neonates) until they grow (juvenile; adult) to measure the development time.

Therefore, to determine the number of offspring per brood pouch, total of spawning during the whole lifespan, and the average of lifespan, five adult *Pleuroxus* spp. (parthenogenesis) from the stock cultures were selected and placed in triplicates in 0.05 L beakers with aluminium foil covers with a ventilation hole, for viewing the fresh eggs in a female's brood pouch. To minimize overfeeding, the berried females of *Pleuroxus* spp. were given feeds daily (one drop of feed from the concentration of 0.5 g/L) (T1 = unfed, T2 = coffee grounds, T3 = maize flour, T4 = wheat flour, and T5 = sugarcane wastes) that had been produced in the form of liquid medium. *Pleuroxus* spp. were given the diet solution at a concentration of 0.5 g/L daily (Rasdi et al. 2018). Each day, the females were inspected and the neonates that emerged from each container were gathered using a 40 µm mesh net. The neonates were then counted using a dissecting stereo microscope (Nikon model SMZ 1500) in a Sedgewick-Rafter chamber (Pyser-SGI, Edenbridge, UK). The daily total averages of offspring development and the number of offspring per brood pouch were determined in triplicates. The total spawning during the whole lifespan was recorded and the average lifespan of the females was calculated by taking the mean of all individual lifespans within a 16-day period, specifically before the entire cohort had died.

FISH STOCKING AND EXPERIMENTAL DESIGN

This study was conducted at the hatchery of Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu. The stock of silver barb fish larvae was supplied by the hatchery of the Faculty of Fisheries and Food Science. A hundred silver barb larvae, ten days after hatching, with an estimated mouth gape of widening from 300 to 400 microns (Ismail et al. 2019), were divided into 20 fish per tank and prepared in triplicate in aquariums containing a ten-liter water volume (Islam et al. 2017; Suhaimi et al. 2022a). Before experiments were started, silver barb larvae were initially acclimated to lab-conditions at a temperature of 25 ± 1 °C and a pH of 7 ± 0.5, while receiving continuous aeration for 24 h without feeding. The experiment was carried out for 30 days in order to determine the effect of diet that was provided to the larvae (Islam et al. 2017). It was necessary to monitor the water quality on a regular basis, and a 30% water exchange was carried out twice a week to keep the conditions of the water stable. The YSI meter was used to monitor the water quality twice a week.

DIET PREPARATION AND FEEDING MANAGEMENT

The larvae in the treatment tanks were fed according to the following regime: 70 mg at 8 am, 50 mg at 11 am, and 50 mg at 4 pm (with a total of 170 mg/day) to keep them from getting over feeding. Plankton nets were used to sieve *Pleuroxus* spp. before being fed to silver barb larvae. Sieved *Pleuroxus* spp. were found at different stages of development, from neonates to adults, and were of different sizes, from 100 m to 200 m. They were rinsed and sieved to eliminate excess water, and then weighed using an electronic scale before feeding to silver barb larvae. To ensure that the *Pleuroxus* spp. was sufficiently enriched, the zooplankton was supplemented in the enrichment tanks for 2 h before the feeding trial (Estévez & Giménez 2017).

GROWTH PERFORMANCE OF SILVER BARB FED WITH ENRICHED *Pleuroxus* spp.

Ten fish larvae were collected randomly once every two days from the culture tank to calculate the growth performance of silver barb larvae. The fish larvae were weighed with the use of an electronic balance. The survival rate of the fish larvae was estimated daily to determine their survivability when fed with *Pleuroxus* spp. To determine the survival rate, total weight gain and the average daily gain as well as the feed conversion ratio (Islam et al. 2017; Limbu et al. 2016; Suhaimi et al. 2022a). The following formulae were used:DATA ANALYSIS

All data were recorded and presented as mean ± standard deviation. The collected data were tested for normality, homogeneity, and independence to meet the ANOVA hypotheses (IBM SPSS Version 25.0, 2017). All the data were analysed by one-way analysis of variance (ANOVA) to evaluate the effect of the different diets on *Pleuroxus* spp. population density, survival rate, and reproductive efficiency, and the effects of the enriched *Pleuroxus* spp. on the growth performance and survival rate of silver barb larvae. The variances were significant at $p < 0.05$ level. The post-hoc Tukey test was conducted when the main treatment impact was significant.

i) $$Survival\ (\%) = \left[\frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish}\right] \times 100$$

ii) $$Weight\ gain\ (mg) = (Final\ weight - Initial\ weight)$$

iii) $$Average\ daily\ gain\ \left(\frac{mg}{day}\right) = \left[\frac{Mean\ final\ weight - Mean\ initial\ weight}{Time\ interval\ (days)}\right]$$

iv) $$Specific\ growth\ rate\ (\%) = \left[\frac{lnln\ final\ weight - lnln\ initial\ weight}{Days\ of\ culture}\right] \times 100$$

v) $$Feed\ conversion\ ratio\ (FCR) = \left[\frac{Total\ feed\ consumed\ (mg)}{Fish\ weight\ (mg)}\right]$$

RESULTS AND DISCUSSION

POPULATION DENSITY, SURVIVAL RATE AND LIFE TABLE OF *Pleuroxus* spp.

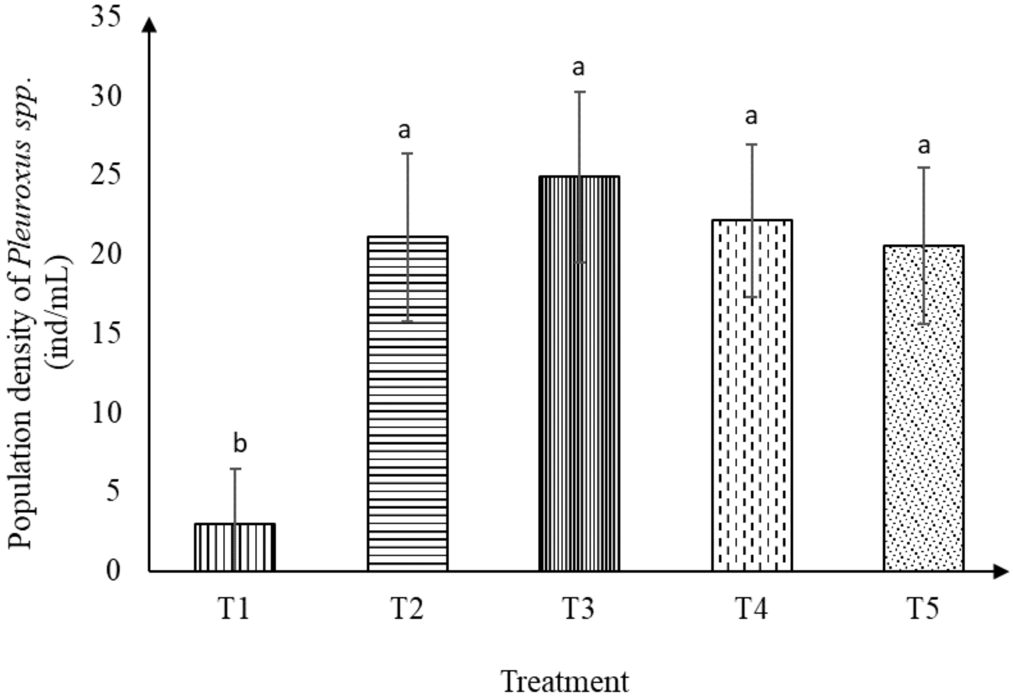
Figure 3 depicts the total population density increase of *Pleuroxus* spp. under each feeding treatment after 16 days of cultivation till the entire cohort dies. *Pleuroxus* spp. population density was considerably affected by feeding them with different types of pre-enriched diets ($p < 0.05$). *Pleuroxus* spp. given T3 (24.88 ± 5.48 ind/mL) had a greater population density than *Pleuroxus* spp. fed T4 (22.13 ± 4.83 ind/mL), T2 (21.06 ± 5.27 ind/mL), and T5 (20.56 ± 4.92 ind/mL) dietary treatments. Unfed *Pleuroxus* spp. had the lowest population in T1 (2.93 ± 3.4 ind/mL).

The survival percentage of *Pleuroxus* spp. was significantly affected with a different diet provided (Figure 4). *Pleuroxus* spp. given T3 had the greatest survival rate (97.9 9.8%) when compared to *Pleuroxus* spp. fed other diets, T4 (86.81 ± 19.07%), T2 (82.71 ± 16.56%), and T5 (75.00 ± 21.08%), while unfed (T1) *Pleuroxus* spp. had the lowest survival rate (39.58 ± 38.34%).

Pleuroxus spp. fed with different diets had substantially varying hatching times ($p > 0.05$) (Table 1). When compared to other diets, *Pleuroxus* spp. fed with T3 take the lowest time to hatch their eggs (1.76 ± 0.18 days). *Pleuroxus* spp. treated with T5 had the longest duration (2.10 ± 0.09 days), followed by those left unfed (2.79 ± 0.20 days). There was a statistically significant hatching rate with the varied diets ($p < 0.05$) (Table 1). *Pleuroxus* spp. treated with T3 produced a greater hatching rate (86.67 ± 2.88%). Meanwhile, the lowest hatching rate was seen when *Pleuroxus* spp. were fed T5 (63.33 ± 7.63%), or when they were left unfed (30.00 ± 5.00%).

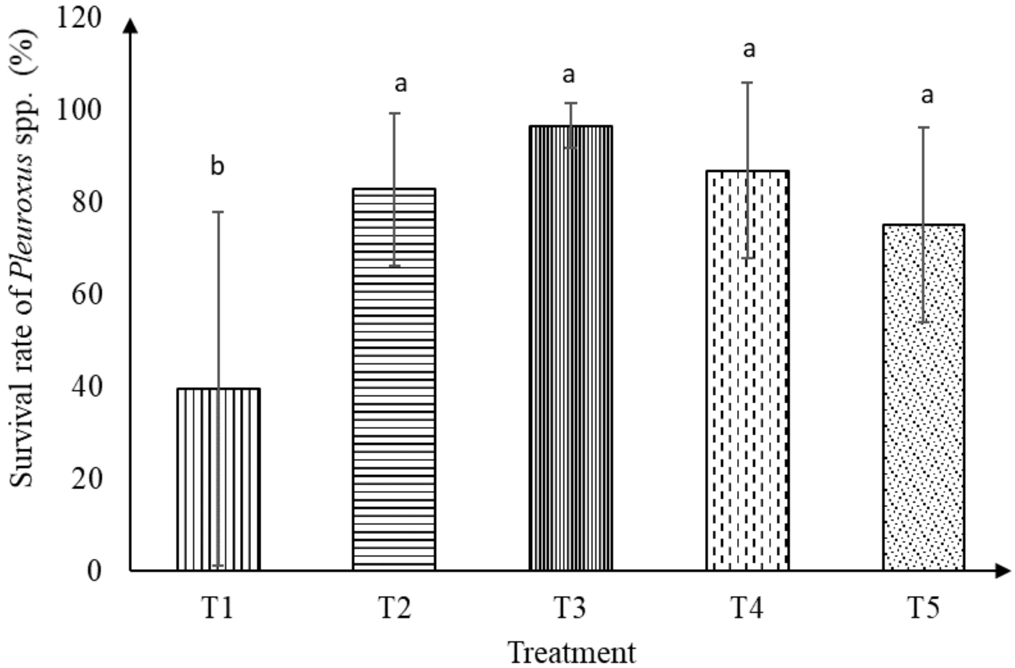
Pleuroxus spp. generation period from neonates to juveniles was influenced by the varied diets employed (Table 1). *Pleuroxus* spp. given T3 exhibited the quickest development (3.25 ± 0.95 days) whereas *Pleuroxus* spp. fed T5 showed the slowest development (4.15 ± 0.05 days). However, as compared to *Pleuroxus* spp. fed with any of the three diets, the longest development period of neonates till juveniles were found in unfed, T1 *Pleuroxus* spp. (6.36 ± 0.13 days). On the other hand, the different types of feed employed altered the development time of *Pleuroxus* spp. from juvenile to adult ($p > 0.05$). *Pleuroxus* spp. given T3 (3.09 ± 0.09 days) developed more rapidly from juvenile to adult than those fed other diets. T5 was utilized as a feed for the longest duration (3.57 ± 0.17 days).

Hence, *Pleuroxus* spp. generation time from neonates to adults differed considerably across the meals tested ($p < 0.05$). *Pleuroxus* spp. took the shortest amount of time for neonates to develop into adults when fed T3 (9.17 ± 0.07 days), and the longest time from neonates to adults when fed T5 (12.35 ± 0.16 days). The diets employed had a substantial impact on the number of offspring generated per parthenogenic mother ($p < 0.05$). When fed with T3, the most offspring were produced and the most spawns were generated (8.67 ± 1.52), (9.33 ± 0.57). The T3-fed *Pleuroxus* spp. had the largest mean lifetime (14.33 ± 1.52 days), whereas the unfed *Pleuroxus* spp. had the shortest (8.00 ± 2.00 days).



Different small letters indicate significant difference among different treatments ($p < 0.05$)

FIGURE 3. Population density of *Pleuroxus* spp. fed with different diets



Different small letters indicate significant difference among different treatments ($p < 0.05$)

FIGURE 4. Survival rate of *Pleuroxus* spp. fed with different diets

TABLE 1. Life table of *Pleuroxus* spp. fed with organic-based diet

Life table	Diets				
	T1	T2	T3	T4	T5
Hatching rate	30.00±5.00[c]	73.33±2.88[a,b]	86.67±2.88[a]	80.00±8.66[a]	63.33±7.63[b]
Hatching time	2.79±0.20[b]	2.02±0.09[a]	1.76±0.18[a]	1.93±0.04[a]	2.10±0.09[a]
Generation time neonate to juvenile	6.36±0.13[b]	3.80±0.08[a]	3.25±0.95[a]	3.48±0.58[a]	4.15±0.05[a]
Generation time juvenile to adult	-	3.21±0.04[a,b]	3.09±0.09[b]	3.45±0.23[a,b]	3.57±0.17[a]
Generation time neonate to adult	-	10.80±0.05[b]	9.17±0.07[d]	9.57±0.06[c]	12.35±0.16[a]
Offspring production	3.67±0.57[b]	7.33±1.15[a,b]	8.67±1.52[b]	8.00±1.73[b]	6.33±1.53[a,b]
Spawning	2.00±1.00[b]	7.33±2.08[a]	9.33±0.57[a]	7.00±1.73[a]	6.33±1.52[a]
Lifespan	8.00±2.00[b]	13.00±1.00[a]	14.33±1.52[a]	13.33±1.15[a]	13.33±2.08[a]

All values show mean ± standard deviation ($n = 3$). The different small letters indicate significant differences between different diets ($p < 0.05$)

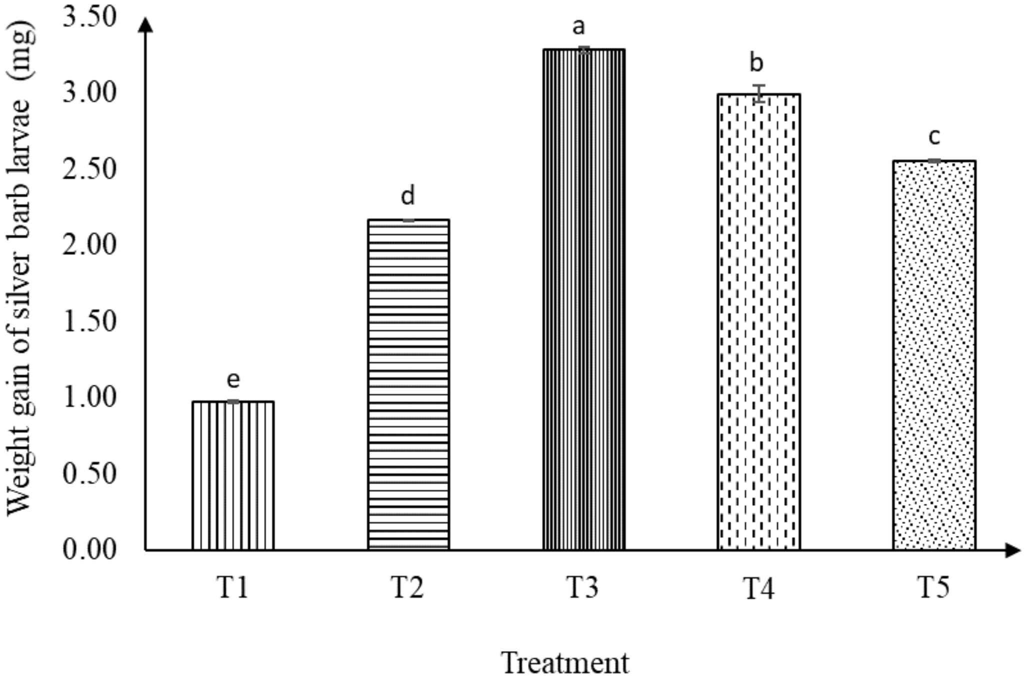
GROWTH PERFORMANCE OF SILVER BARB FED WITH *Pleuroxus spp.*

Total weight gain of silver barb larvae after being cultured for 30 days was significantly different when fed with enriched *Pleuroxus* spp. ($p < 0.05$) (Figure 5). The highest weight gain was measured in treatment T3 (3.28 ± 0.02 mg), compared with other treatments, respectively. Hence, the lowest weight gain occurred in T1 (0.97 ± 0.01 mg), which is when being fed with unenriched *Pleuroxus* spp.

Average daily gain or average daily weight gain of silver barb larvae when fed with enriched *Pleuroxus* spp. was varied among all the treatment diets ($p < 0.05$) (Figure 6). The highest amount of daily gain was obtained in treatment T3 (0.11 ± 0.001 mg/days) compared with other dietary treatments. Meanwhile, the lowest average daily gain data was observed in T1, when the results only showed an average of 0.03 ± 0.001 mg/days of daily weight gain compared to other treatments.

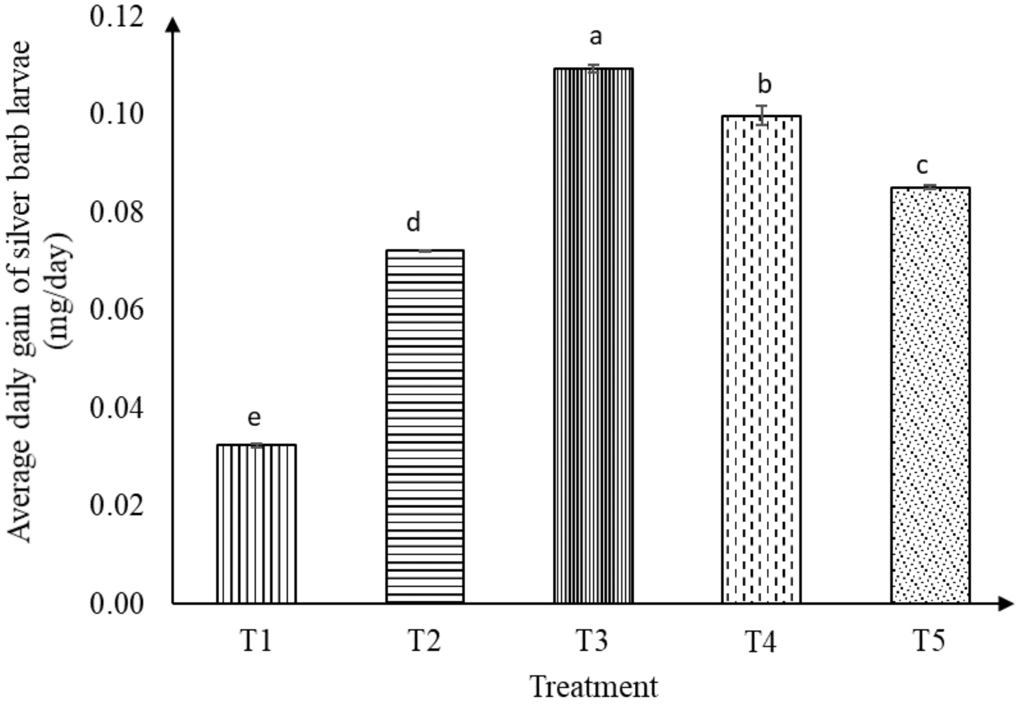
Changes in *Pleuroxus* spp. enrichment affected the specific growth rate of silver barb larvae ($p > 0.05$) (Table 2). According to the results, the maximum SGR (%) was achieved when fed with T3 (13.37 ± 0.02%). When larvae were given unenriched live food, the lowest SGR (%) was recorded in T1 (9.46 ± 0.04%). When comparing all the dietary treatments employed in this experiment, the feed conversion ratio was shown to be significantly different ($p < 0.05$). The FCR of larvae fed with different nutritional treatments ranged from 1.22 to 4.12, depending on the treatment. In comparison to other diets, the T3 treatment (1.22 ± 0.03) achieved the highest FCR ratio. Table 2 shows the survival rate of silver barb larvae at the conclusion of the trial period. It was discovered that the varied enrichment methods utilized in *Pleuroxus* spp. had a substantial influence on the survival rate of silver barb larvae ($p < 0.05$). Comparing different diets, the larvae fed with treatment T3 had the greatest survival percentage (91.67 ± 2.88%), followed by the other diets, respectively ($p < 0.05$). In contrast, T1 had the lowest survival rate (75.00 ± 5.00%) when fed with *Pleuroxus* spp. only without any enrichment as a growth booster for the larvae.

The outcomes of research on *Pleuroxus* spp. fed with different diets indicated that feeding types were crucial for optimal production. According to the findings of the current study, *Pleuroxus* spp. fed with maize flour (T3) had a greater population density and survival rate than *Pleuroxus* spp. fed with other feeding treatments. Maize flour can be used as a dietary source that is supplemented with nutrients to combat protein energy malnutrition and deficits in macronutrients (Bamidele & Fasogbon 2020). The composition of maize flour was determined to be 3.3% ash, 12.45% protein, 2.97% crude fiber, and 60.23% carbohydrate content (Fouzi, Surakshima & Withanage 2021). Maize is an indigenous crop that is readily accessible and may be easily obtained. The fish larvae that were fed with *Pleuroxus* spp., which have greater protein, fat, and carbohydrate contents, exhibited quick growth, high fertility, and improved nutritional value. Manipulation of the culture condition and feed such as optimum water parameters, utilizing varied grades and varieties of food, might improve the population density of cladocerans (Hakima, Khémissa & Boudjéma 2013; Yuslan et al. 2021). Furthermore, Gómez et al. (2012) found that the quality of the food fed to *Daphnia magna* affects growth performance. This study showed that feed quality had a



Different small letters indicate significant difference among different treatments ($p < 0.05$)

FIGURE 5. Weight gain of silver barb larvae after being fed with enriched *Pleuroxus* spp.



Different small letters indicate significant difference among different treatments ($p < 0.05$)

FIGURE 6. Average daily gain of silver barb larvae after being fed with enriched *Pleuroxus* spp. for 30 days

TABLE 2. Growth analysis of silver barb larvae, fed with enriched *Pleuroxus* spp.

Growth performance	Treatments				
	T1	T2	T3	T4	T5
Specific Growth Rate (SGR)	9.46±0.04[e]	12.02±0.01[d]	13.37±0.02[a]	13.07±0.06[b]	12.55±0.01[c]
Feed Conversion Ratio (FCR)	4.12±0.05[a]	1.85±0.01[b]	1.22±0.01[e]	1.34±0.03[d]	1.57±0.01[c]
Survival rate	75.00±5.00[c]	76.67±2.89[b,c]	91.67±2.88[a]	88.33±2.89[a,b]	86.67±7.63[a,b,c]

Different letters on the same row indicates significant difference ($p < 0.05$). All values show mean ± standard deviation ($n = 3$). The different small letters indicate significant differences between different diets ($p < 0.05$)

direct impact on the growth performance of *Pleuroxus* spp., because growth performance of *Pleuroxus* spp. was the highest with maize flour (T3) compared to other diets.

Utilizing the biofloc technology (BFT) method, giant river prawns may be effectively developed using corn flour starch as a source of carbon and energy during the nursery stage. Corn starch improved the survival, net income, and benefit-cost ratio of giant river prawns, as well as their growth and FCR (Hosain et al. 2021). A previous study by Wang et al. (2017) concluded that supplementing feeds for the copepod, *Tisbe furcata* with corn flour is recommended because the highest population densities of *T. furcata* were achieved by supplementing feeds with corn flour without negatively affecting the environment in the experimental tanks.

According to Ramadan et al. (2020), wheat flour may induce cyclic parthenogenesis, and each of them can be utilized to grow *Daphnia carinata* in aquaculture hatcheries as a live, affordable food source. As with any live feed, commercialization and usage of cladocerans in intensive aquaculture is dependent on standardized culture techniques in order to reliably and cost-effectively produce high quality feed organisms in large enough quantities to meet the needs of the industry. The white worm, *Enchytraeus albidus* (Henle, 1837), a marine oligochaete, was investigated as a potential live feed by examining its production rate and nutritive values when fed coffee grounds. The results showed that cultures fed with coffee grounds produced higher yields than cultures fed alternative diets (Fairchild, Bergman & Trushenski 2017).

The study results showed that all meals examined were tolerated mostly by the silver barb larvae. In all treatments, the overall survival rate was better than 70%. The larvae in the treatment T3 showed better growth performance results and had the highest weight gain compared to other treatments. Live food was more preferred than micro pellet by fish larvae because of the digestion and attraction of live food action with jerky movements (Gogoi, Safi & Das 2016; Suhaimi et al. 2022a). Results of this study were also supported by Anizah et al. (2017), where manufactured diets may not be able to completely substitute live feed. *Barbonymus gonionotus* larvae fed with plankton developed the greatest length (28.06 ± 0.38 mm), ultimate weight (135.00 ± 3.05 mg), and survival rate (92%) when grown for 28 days, as reported by Ahammad et al. (2009).

The use of feed derived from plants such as corn flour, wheat flour, coffee grounds, and sugarcane waste has contributed significant results to the development of diets for fish larvae. Therefore, the use of plant-based diets in previous studies as a supplement for live feed also exhibited a significant contribution to aquaculture fish production (Jusoh et al. 2020; Suhaimi et al. 2022a). Yousefian et al. (2013) previously discovered that common carp (*Cyprinus carpio*) larvae fed artificial meals consisting of diets derived from plants had a greater weight and survival rate of more than 70%. In the current study, larvae fed enriched *Pleuroxus* spp. survived at a rate of greater than 70% through the 20-day rearing period.

CONCLUSION

It was found that using pre-enriched live food as a viable new food source for culturing silver barb, *Barbonymus gonionotus* larvae, boosted the growth performance and survival of the fish. As a result, all diets derived from plants were approved for usage since they are simple to handle and can be stored for an extended period of time without the need for any extra medium or chemical for preservation. The outcomes of the present experiment demonstrated that the maize flour (T3) could be used as the best option as an additive for *Pleuroxus* spp. feed for hatchery owners. Therefore, other diets such as coffee grounds (T2), wheat flour (T4), and sugarcane waste (T5) are also adaptable feed to culture *Pleuroxus* spp. to enhance their reproduction and nutritive value. An important aspect of this study was to demonstrate the relevance of pre-feeding zooplankton with prospective diets to build a baseline of improved aquaculture feeding procedures that may be employed in the future aquaculture business.

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