Int. J. Aquat. Biol. (2025) 13(4): 61-70
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com
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Original Article

Production performance of cladoceran *Moina micrura* fed *Pleurotus florida* mushroom byproducts

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Abstract: Cladoceran *Moina micrura* is an important live food organism in aquaculture. It provides essential nutrients such as proteins, lipids, and amino acids for fish and crustacean larvae. The composition of their diet primarily influences their growth and reproduction. The spent mushroom substrate (SMS), mushroom stalk waste (MSW), and mushroom fruiting body (MFB) have the potential to serve as an alternative food source due to their nutrient content. However, their efficiency in supporting *M. micrura* production remains unclear. Therefore, this study aimed to evaluate the effects of *Pleurotus florida* mushroom byproducts on the life history and population dynamics of *M. micrura*. A controlled experiment was conducted using different diets provided at 1000, 500, and 250 mg/L concentrations. The positive control was provided with baker's yeast, while the negative control was provided with tap water without feed. Selected life history variables were measured to assess the production performance. The results showed that *M. micrura* fed 1000, 500, and 250 mg/L of SMS, as well as 500 mg/L of MSW and MFB, exhibited better production and longevity. The findings suggest that SMS and MSW have the potential to be alternative food sources in *M. micrura* production. Moreover, the high cost of MFB makes it less practical for large-scale use.

Article history:
Received 21 April 2025
Accepted 11 June 2025
Available online 25 August 2025

Keywords:
Mushroom byproducts
Mushroom stalk waste
Reproduction
Spent mushroom substrate

Introduction

Zooplankton play a significant role in aquaculture as a primary food source for fish and crustacean larvae. It provides essential nutrients, including proteins, lipids, and amino acids (Hasan et al., 2023). Commonly used live food organisms in aquaculture are rotifers and *Artemia* because of their high digestibility and nutritional value. However, *Artemia* production is often challenging in developing countries due to high costs, low availability, and unfavorable hatching conditions (Kamrunnahar et al., 2019). As an alternative, *Moina* has gained attention as a natural live food with a comparable nutritional profile to *Artemia*, which contains protein that usually averages 50% and 20-27% fat in dry weight (Rottmann et al., 2003).

Moina is not widely available in large quantities from natural habitats. However, it can be effectively cultured using byproducts from agricultural, animal, and food industries (Loh et al., 2013; Mubarak et al., 2017). Different factors influence their population dynamics in the natural environment. These include water quality, food availability, and population density. However, food sources are the primary determinant of their growth and reproduction (Kabery et al., 2019). Previous studies investigated different food sources to optimize the production of *Moina*, such as phytoplankton, yeast, agro-industrial residues, and fish waste (Alva-Martinez et al., 2007; Morales-Ventura et al., 2012; Kushniryk et al., 2015; Manklinniam et al., 2018; Latib et al., 2020; Suhaimi et al., 2022). Some of these agro-industrial byproducts successfully applied to *Moina* culture were rice bran and soybean meal, which have shown potential to enhance population density and reproductive performance (Suhaimi et al., 2022). Further exploration of other agricultural byproducts, such as those derived from mushrooms, may open up new

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DOI: https://doi.org/10.22034/ijab.v13i4.2502

opportunities for sustainable *Moina* production.

Mushroom production has increased globally since 2000, currently standing at 44 million tons, with the Asian continent accounting for 95% of the total output (Bijla and Sharma, 2023). One widely cultivated mushroom is the oyster mushroom (*Pleurotus* sp.), due to its high nutritional value, which includes proteins, carbohydrates, fiber, vitamins, and minerals (Raman et al., 2020). As this industry advances, it generates large amounts of waste known as spent mushroom substrate (SMS) and mushroom stalk waste (MSW). Approximately 5 kg of wet byproducts of SMS are produced for every 1 kg of fresh mushrooms harvested (Gao et al., 2021). Meanwhile, MSW accounts for 25-33% of total mushroom production (Chou et al., 2013). This poses a significant challenge, as improper disposal of these byproducts leads to environmental issues, including soil contamination and air and water pollution (Jiang et al., 2017; Lam et al., 2019).

SMS is a byproduct of mushroom cultivation that consists of composted organic materials and fungal mycelium left after harvesting (Phan and Sabaratnam, 2012). It is generally treated as agricultural waste and disposed of without proper utilization. Several studies have demonstrated the potential of SMS in various applications, including biofertilizers, animal feed, renewable energy sources, and pollution bioremediation (Leong et al., 2022). In aquaculture, SMS has been successfully incorporated into fish diets, yielding improvements in growth performance, immune response, and disease resistance (Van Doan et al., 2017; Chirapongsatonkul et al., 2019; Lee et al., 2022). Similarly, MSW is usually discarded after mushroom harvest (Adejonwo et al., 2021). However, it has been successfully applied as a feed ingredient in fish diets, replacing conventional feed components such as rice bran and fish meal (Muin et al., 2013, 2014, 2015) and as a prebiotic and an immunostimulant for fish (Abd Rahman et al., 2012; Ching et al., 2021). Given the rich nutritional profile of mushrooms, MSW could also be a valuable food source for *Moina* culture.

Even with the promising applications of SMS and MSW in aquaculture, research has primarily focused on their inclusion in fish diets for grow-out culture. There is a lack of studies investigating their potential as a diet for live food organisms in hatchery production, such as *M. micrura*. Therefore, this study evaluated the effects of *Pleurotus florida* mushroom byproducts on the production performance of *M. micrura* and aimed to assess their potential for promoting sustainable practices in the aquaculture and agriculture industries by turning waste into a valuable resource.

Materials and Methods

Food preparation: The mushroom byproducts (SMS and MSW) and mushroom fruiting body (MFB) were sourced from Quisha's Mushroom Farm, Impao, Isulan, Sultan Kudarat, Philippines. The SMS comprised a mixture of 20% rice hull and 80% sawdust, which was left over from several cultivation cycles. On the other hand, MSW was the discarded lower part of the mushroom products after processing. Samples were sun-dried for 4 to 5 days, then blended into powdered form using an electric grinder (Western Kitchen 800Y, China) for approximately 3 minutes at maximum speed. The powdered diet was weighed and diluted in tap water. The diet solutions were then sieved using a filter net with a 50 μm mesh size (Loh et al., 2013) before being transferred to the cultured container.

Culture of *M. micrura*: The *Moina micrura* starter was collected from a pure culture of the Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (BFAR-NFFTC), located at the CLSU Compound, Science City of Muñoz, Nueva Ecija, Philippines. A method of Loh et al. (2013) was used in the culture with slight modifications. Breeding females were individually placed in a circular plastic container (100 ml capacity) filled with tap water (50 ml/container) using a blunt pipette for isolation. Baker's yeast at 20 mg/L was supplied to the container as a food source throughout the breeding period. Matured *M. micrura* began breeding overnight after being introduced into the container. Neonates under 24 hours old were collected

and distributed to experimental containers, with one neonate per container. Each container was filled with 50 mL of experimental diets (SMS, MSW, and MFB, each with three concentrations: 1000, 500, and 250 mg/L), baker's yeast for the positive control, and tap water for the negative control. The diets were replaced with fresh media every two days using a pipette. The containers were kept in a well-ventilated area at 28-30°C under natural light conditions. Neonates of *M. micrura* were counted and removed daily throughout the experiment. Mortality and fecundity were monitored to evaluate life history and population dynamics. The experiments were terminated upon the complete mortality of all *Moina* individuals.

Life table demography: Life table demographics serve as an essential tool for describing the life cycle of zooplankton in response to continuously changing environmental conditions (Stearns, 1976). Different life history variables were selected for this study, including survival period, initial age of reproduction, average longevity, gross reproduction rate, net reproduction rate, generation time, and intrinsic rate of population increase (Chuah et al., 2007). The data collected was recorded in Excel (Microsoft Office 365) for easy computation of the result. The following definitions apply: initial age of reproduction (days) = the time when a female begins producing her first batch of neonates; longevity (days) = the average number of days the female survives. Survivorship and reproduction were calculated based on Krebs's (1985) standard procedures as follows:

Average Longevity (AL) = $\sum n_x/n$

Gross Reproduction Rate (GRR) = $\sum m_x$

Net Reproduction Rate (Ro) = $\sum l_x m_x$

Generation Time (T) = $\sum l_x m_{x\,x}/Ro$

Survivorship = $\sum l_x$

Life expectancy (ex) = T_x/n_x

Where, n_x = number of individuals alive for each age class, m_x = the age-specific fecundity, number of neonates produced per surviving female at age x), l_x = the proportion of individuals surviving to age x, T_x = generation time at age x, and n = the number of replicates. The intrinsic rate of population growth (r) was first estimated using the formula (Xi et al., 2005) of r – rough = $\ln R_0/T$, and for the final calculation, the equation was: $\sum e^{-rx} l_x m_x = 1$.

Statistical analysis: JASP (Version 0.19.3) computer software was used for data analysis. Before the analysis, Levene's test was performed to assess the homogeneity of variances. Following the confirmation of equal variances, Analysis of Variance (ANOVA) was employed to determine if there were significant differences between the treatments, and post hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test to determine specific group differences. When the data did not satisfy the ANOVA assumptions, the Kruskal-Wallis test was employed, followed by Dunn's test if significant differences were detected. A p-value of less than 0.05 (P<0.05) was considered statistically significant.

Results

This study evaluated the effects of *P. florida* mushroom and its byproducts on the production performance of *M. micrura*, measuring different parameters of life history and population dynamics. The results showed significant differences in different diet types at different concentrations (Table 1). Moreover, a negative control (no diet) and 1000 mg/L of MSW and MFB resulted in no offspring production.

Effect on reproductive parameters: There were no significant differences (P>0.05) in the average initial age of reproduction across all diets except for 250 mg/L of MSW and MFB (Table 1). SMS (1000, 500, and 250 mg/L), MSW (500 mg/L), MFB (500 mg/L), and (+) control had an earlier reproductive onset (2.00 days) compared to 250 mg/L of MFB and MSW, in which reproduction occurred at 3.00 and 3.33 days, respectively. Moreover, 1000 mg/L of MSW and MFB inhibited reproduction.

The highest fecundity was observed in *Moina* fed 1000 mg/L of SMS diet followed by 500 mg/L of SMS, MFB, and MSW (Fig. 1). The cumulative birth rate of *Moina* fed experimental treatments was higher than that of the control except for 250 mg/L of MSW and MFB (Fig. 2). In reproductive success (net reproduction rate and gross reproduction rate), a

Table 1. Life table of Moina micrura fed with different diets at different concentrations.

Different diet types	Concentration mg/L	Life History Variables					
		Average initial age of reproduction (days)	Average longevity (days)	Net reproduction rate	Gross reproduction rate	Generation time (days)	Intrinsic rate of population increase (d^{-1})
(-) Control (No Diet)	0	-	-	-	-	-	-
(+) Control (Baker's Yeast)	20	2.00±0.00^{a}	5.00±0.00^{a}	31.67±6.96^{bc}	29.67±5.46^{bcd}	3.24±0.12^{a}	1.23±0.06^{a}
Spent mushroom substrate (SMS)	1000	2.00±0.00^{a}	6.00±0.00^{a}	50.00±2.52^{a}	50.00±2.52^{a}	3.68±0.09^{ab}	1.30±0.05^{a}
	500	2.00±0.00^{a}	6.00±0.00^{a}	44.33±3.18^{ab}	44.33±3.18^{ab}	3.71±0.14^{ab}	1.24±0.06^{a}
	250	2.00±0.00^{a}	6.00±0.00^{a}	43.00±4.62^{abc}	43.00±4.62^{abc}	3.69±0.13^{ab}	1.25±0.08^{a}
Mushroom stalk waste (MSW)	1000	-	4.33±1.00^{a}	-	-	-	-
	500	2.00±0.00^{a}	7.00±0.00^{a}	43.33±5.36^{abc}	43.33±5.36^{abc}	4.19±0.06^{bc}	1.14±0.06^{ab}
	250	3.33±0.33^{b}	6.33±0.33^{a}	23.33±3.84^{c}	23.33±3.84^{d}	4.52±0.07^{c}	0.73±0.03^{c}
Mushroom fruiting body (MFB)	1000	-	5.00±1.73^{a}	-	-	-	-
	500	2.00±0.00^{a}	7.00±0.00^{a}	42.33±2.03^{abc}	42.33±2.03^{abc}	3.88±0.15^{b}	1.23±0.03^{a}
	250	3.00±0.00^{b}	6.33±0.33^{a}	24.67±0.88^{bc}	24.67±0.88^{cd}	3.82±0.13^{b}	0.89±0.02^{bc}

Data expressed as means with the standard error of three replicates. Values in the same column showing different superscripts are significantly different (P<0.05). Dash (-): No offspring were produced.

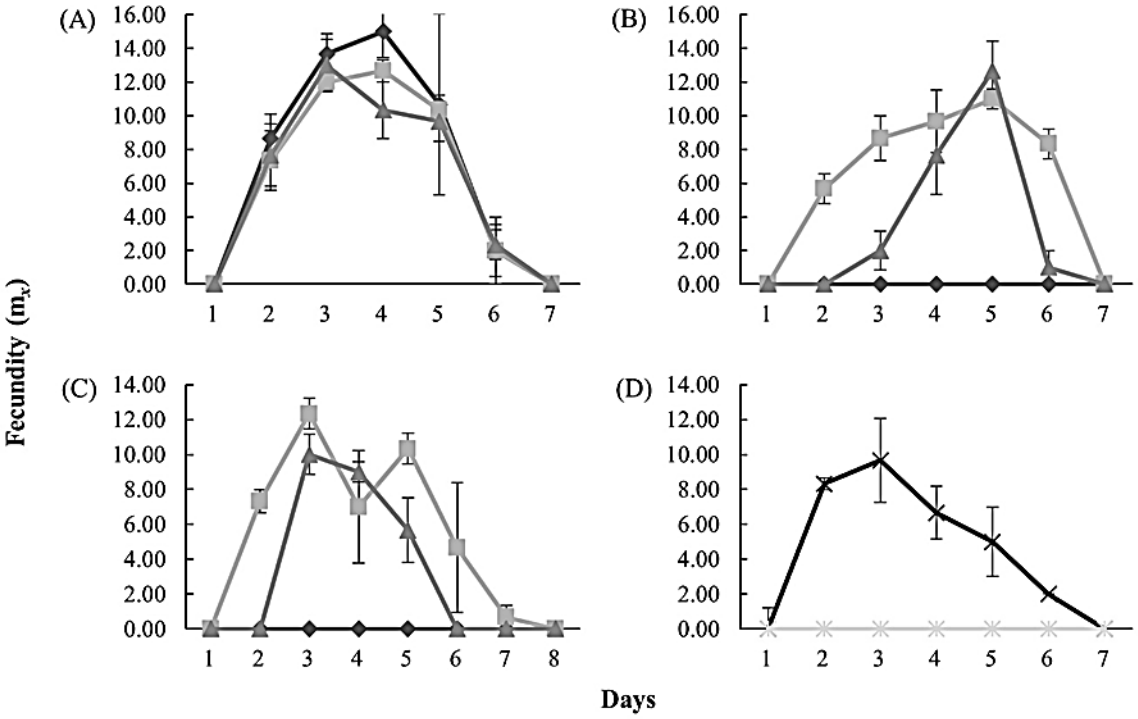

Figure 1. Fecundity of *Moina micrura* fed with different diets (A) spent mushroom substrate, (B) mushroom stalk waste, (C) mushroom fruiting body, (D) positive control (baker's yeast) and negative control (no diet) at different concentrations (—◆—) 1000 mg/L, (—■—) 500 mg/L, (—▲—) 250 mg/L, (—✕—) 20 mg/L, (—✳—) 0 mg/L. Error bars indicate means ± standard error.

significant variation was observed between diets at different concentrations (Table 1). Reproduction rates were higher for SMS at 1000 mg/L, but not significantly different from other treatments, except for the (+) control and 250 mg/L of MSW and MFB, whereas 1000 mg/L of MSW and MFB completely inhibited reproduction. It was observed that the reproduction rates decreased as the diet concentration decreased. All treatments exhibited a positive intrinsic rate of population increase (r) and showed no significant differences across diets, except for the 250 mg/L of MSW and MFB.

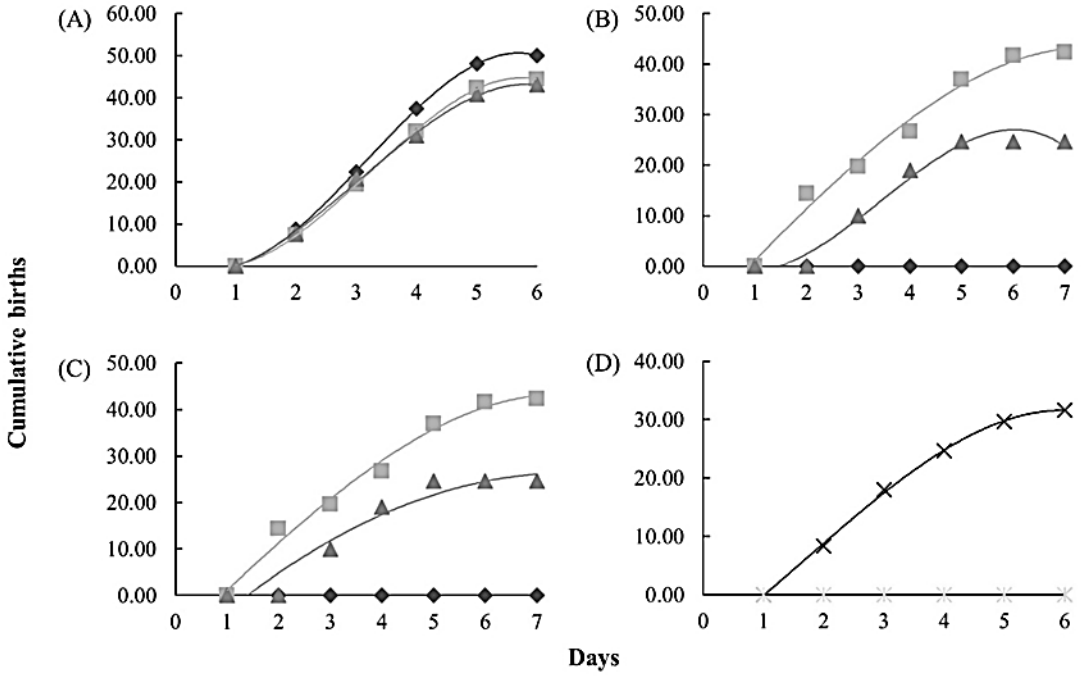

Figure 2. Cumulative births (polynomial curve) of *Moina micrura* fed with different diet (A) spent mushroom substrate, (B) mushroom stalk waste, (C) mushroom fruiting body, (D) positive control (baker's yeast) and negative control (no diet) at different concentration (◆) 1000 mg/L, (■) 500 mg/L, (▲) 250 mg/L, (×) 20 mg/L, (✳) 0 mg/L.

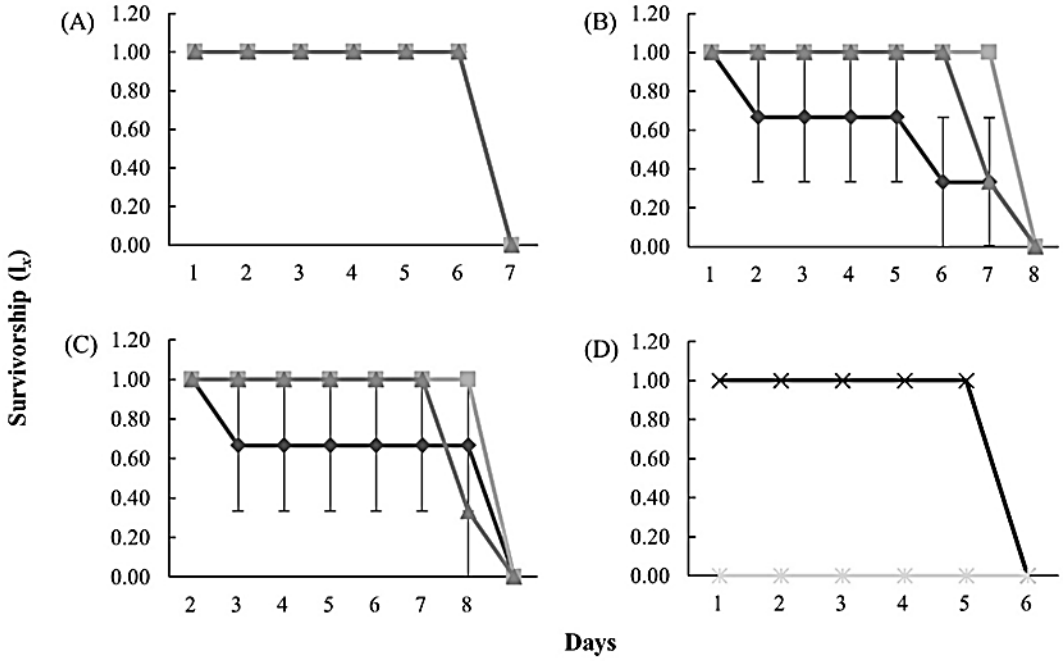

Figure 3. Survivorship of *Moina micrura* fed with different diets (A) spent mushroom substrate, (B) mushroom stalk waste, (C) mushroom fruiting body, (D) positive control (baker's yeast) and negative control (no diet) at different concentrations (◆) 1000 mg/L, (■) 500 mg/L, (▲) 250 mg/L, (×) 20 mg/L, (✳) 0 mg/L. Error bars indicate means ± standard error.

Effect on survivorship, life expectancy, longevity, and generation time: The survival patterns of *M. micrura* (Fig. 3), fed different diet types and concentrations, displayed a Type-I survivorship curve, where most organisms survived through early and middle life spans with minimal mortality. All experimental diets showed the longest survival compared to the (+) control. The same trend was also

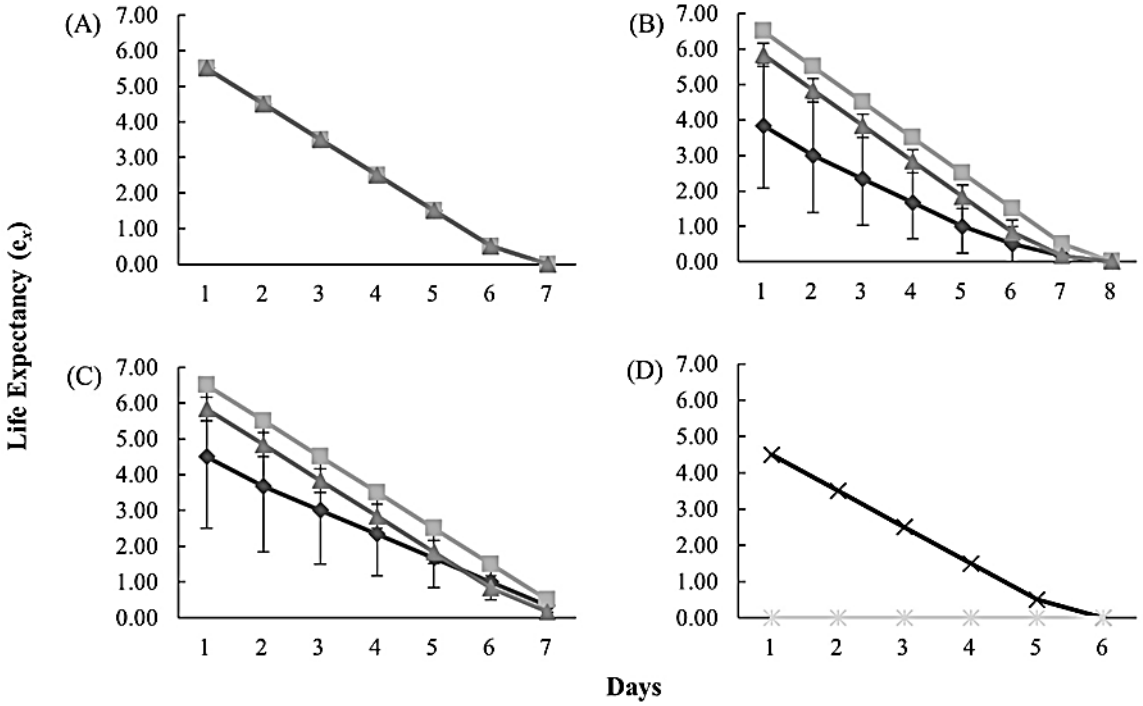

Figure 4. The life expectancy of *Moina micrura* fed with different diets (A) spent mushroom substrate, (B) mushroom stalk waste, (C) mushroom fruiting body, (D) positive control (baker's yeast) and negative control (no diet) at different concentrations (◆) 1000 mg/L, (■) 500 mg/L, (▲) 250 mg/L, (×) 20 mg/L, (✳) 0 mg/L. Error bars indicate means ± standard error.

observed in the life expectancy of *M. micrura* (Fig. 4), except for 1000 mg/L of MSW and MFB, where treatment diets increased life expectancy compared to the (+) control. Average longevity (Table 1) ranged from 4.33 to 7.00 days and showed no significant difference (P>0.05) across different diets and concentrations. Moreover, the generation time was significantly longer in MSW treatments, particularly at 250 mg/L (4.52 days), compared to other concentrations. This suggests a delayed reproductive cycle in this concentration compared to SMS and MFB, which had shorter generation times.

Discussions

Key factors influencing the population dynamics of *Moina* include water quality, food availability, and population density. However, food sources are the primary determinant of their growth and reproduction (Kabery et al., 2019). According to Mubarak et al. (2017), *Moina* population density can be enhanced by fecundity optimization and reducing the reproductive period, which can be achieved by regulating the quality and quantity of feed. Feed quality directly affects the increased population growth and improves the survival rate in *Moina*. Additionally, Rasdi et al. (2020) reported that the nutritional composition of diets provided to *Moina* is essential for sustaining their production in hatcheries. In this study, higher reproduction rates were observed at higher concentrations, except for MSW and MFB, where no reproduction was observed. According to Klüttgen et al. (1996), Repka (1997), and Rose et al. (2000), cladocerans exhibit higher growth rates when they were provided with sufficient food compared to those with limited food. However, overfeeding may result in adverse effects, such as a decline in population density, excessive uneaten food, and the accumulation of waste (Hena et al., 2025).

Phan and Sabaratnam (2012) reported that SMS comprises composted organic materials and fungal mycelium. The efficiency of SMS in the production of *M. micrura* may be due to its organic content, as *Moina* fed on various groups of bacteria, yeast, phytoplankton, and detritus or decaying organic matter (Rasdi et al., 2020). In addition, Jana and Pal (1983) reported that high fecundity and gross

reproduction rates of cladocerans largely depend on the high C/N ratio in the food source. Moreover, the significant production of MSW and MFB can be attributed to the nutritional content of mushrooms, as oyster mushrooms are known to contain high nutritional value, including proteins, carbohydrates, fiber, vitamins, and minerals (Raman et al., 2020).

The highest reproduction recorded at 1000 mg/L of SMS in this study was comparable with the results of Kabery et al. (2019), who reported that a 1000 ppm concentration of food waste produced better results. However, the present study observed that this concentration caused the culture water to be more turbid, making it unsuitable for *M. micrura* prolonged culture. On the other hand, the suppressed production observed at 1000 mg/L of MSW and MFB was possibly due to the high diet concentration, which caused a foul odor and the formation of a filamentous layer, which may have trapped *Moina.* Rottmann et al. (2003) stated that overfeeding *Moina* can rapidly degrade water quality. Additionally, Porter et al. (1982) and Burak (1997) described that high concentrations of particulate matter could result in the starvation of cladocerans. This happens because they cannot clear their thoracic limbs, which become clogged by excessive particles.

A similar trend was also observed in the intrinsic rate of population increase, where higher concentrations had the highest values. Stearns (1976) reported that higher *r* values may result from shorter generation times or increased fecundity. Moreover, the recorded positive *r* values in this study were within the range described by Nandini and Sarma (2003), where cladocerans have *r* values ranging from 0.01 to 1.5, depending on the species, type of food, and temperature conditions.

Moina micrura became sexually mature earlier at higher concentrations (2.00 days), except for those fed MSW and MFB, which inhibited growth. This indicates that diet concentration plays a significant role in influencing early reproduction. A similar observation was reported by Loh et al. (2013) in *M. macrocopa*, where a higher-concentration diet resulted in earlier reproduction compared to a lower-concentration diet. In contrast, Kabery et al. (2019) reported that the lower concentration showed an earlier reproductive ability, as they observed that Moina required more time to become sexually mature on a high-concentration diet. These observed differences in reproductive timing suggest that variations in diet composition and nutrient availability could influence *Moina* maturation.

The average longevity of *M. micrura* was not significantly influenced by diet concentration. However, the highest concentrations of MSW and MFB significantly reduced longevity, indicating that *M. micrura* showed better survival conditions when provided with a sufficient diet. The longevity recorded in this study fell within the range reported by Loh et al. (2013), who observed an average longevity of 2.50 to 11.25 days using fish feces, and Kabery et al. (2019), who reported a longevity of 1.00 to 9.82 days using organic waste. Additionally, the generation time observed in the current study aligns with the findings of the studies mentioned above. This suggests that mushroom byproducts support survival and production.

All concentrations (1000, 500, and 250 mg/L) of SMS, as well as 500 mg/L of MSW and MFB, showed significant reproductive effects. The successful use of *P. florida* byproducts (SMS and MSW) suggests that mushroom-derived agricultural byproducts have the potential to provide a sustainable and cost-effective alternative food source for *Moina* production. Using locally available mushroom byproducts can lower the cost of live food production, providing economic benefits for hatchery operations and reducing the dependence on expensive live food.

Conclusion

In conclusion, our findings suggest that mushroom byproducts have potential as an alternative dietary source for *M. micrura* cultivation. However, MSW requires concentration adjustments to prevent reproductive suppression in higher concentrations. Based on the study's results, 1000, 500, and 250 mg/L of SMS, as well as 500 mg/L of MSW and MFB, can be used in the production of *Moina*, which exhibited

better production and longevity. While MFB also supported favorable output at this level, its high cost makes it less practical for large-scale use. Mushroom byproducts, such as spent mushroom substrate and mushroom stalk waste, offer more viable and cost-effective alternatives. Further research is needed to improve the digestibility of these diet samples in *Moina*, especially the MSW. Studies should investigate the optimal concentration of these diets to assess their effectiveness in *Moina* production. Additionally, evaluating the biochemical composition of these diets is crucial to identifying their main effects on reproduction and optimizing their application as sustainable food alternatives. Moreover, the long-term effects of these diets and their application to large-scale culture should be investigated.

Acknowledgements

This study would like to acknowledge the support of the Department of Science and Technology Science Education Institute – Science and Technology Regional Alliance of Universities for National Development (DOST SEI-STRAND) program, Mindanao State University at Naawan – School of Marine Fisheries and Technology, and the Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (BFAR-NFFTC). Additionally, the authors would like to thank the Quisha's Mushroom Farm, located at Impao, Isulan, Sultan Kudarat, Philippines, for providing the mushroom byproducts used in this study.

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