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PRELIMINARY INVESTIGATION ON ROTIFER (*Brachionus plicatilis*) AS NATURAL FOOD FOR POLYCHAETE WORM (*Marphysa iloiloensis*): GROWTH, SETIGER, AND SURVIVAL PERFORMANCE

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Abstract

Polychaete *Marphysa iloiloensis* is one of the most common live bait species targeted by bait diggers in Tawi-Tawi, Philippines. It is considered a nutritious and high-protein source of food for aquaculture, either in fresh form or incorporated in feeds. However, as wild populations of polychaete are subject to ecological and animal welfare concerns, along with resource depletion, the literature on feeding polychaete *M. iloiloensis* larvae is inadequate and scarce, making polychaete aquaculture an ideal alternative. The present study aimed to investigate the rotifer (*Brachionus plicatilis*) as natural food for the cultured polychaete *M. iloiloensis* on the growth, setigers, and survival performance. Polychaete *M. iloiloensis* larvae were fed with three different concentrations of rotifer, namely, Treatment 1 (T1) = 5 mL, Treatment 2 (T2) = 10 mL, and Treatment 3 (T3) = 15 mL cultured for 45 days. Results showed that 5 mL and 10 mL of rotifer stimulated improvement in growth and an increase in setiger count after 45 days of the culture period, although the differences were not significant ($p>0.05$) compared to 15 mL concentration of rotifer. Moreover, a 5 mL and 10 mL concentration of rotifer significantly increased ($p<0.05$) polychaete *M. iloiloensis* survival compared to a 15 mL concentration.

Hence, this study suggests that rotifer (*B. plicatilis*) can effectively alter the diet of the polychaete *M. iloiloensis* to promote growth, setiger formation segments, and enhance survival.

Keywords: *Brachionus plicatilis*, growth, *Marphysa iloiloensis*, polychaete, setigers, survival

Introduction

Polychaetes have a dominant role in marine ecologies throughout temperate and tropical intertidal zones (Fauchald & Jumars, 1979; Jumars et al., 2014; Kihia et al., 2017). One key difference between polychaetes and annelids is that polychaetes typically have well-defined, distinct body segments with numerous paired appendages called parapodia, whereas annelids, such as earthworms, typically have fewer segments and lack parapodia (Rouse & Fauchald, 1998; Zanol et al., 2007; Budaeva & Fauchald, 2011; Purschke et al., 2014). There are a variety of lifestyles among polychaetes, including feeding on the organic matter on the seafloor, active predators, and filtering nutrients from the ocean (Parry et al., 2014). In addition, researchers stated that aquaculture polychaete had been used as one of the ingredients in feed supplements (Olive, 1994; Naessens et al., 1997; Olive, 1999; Alava et al., 2017), live fishing bait (Cunha et al., 2005; Sypitkowski et al., 2010; Cole et al., 2018) and others for commercial applications. It contains polyunsaturated fatty acids (Wouters et al., 2001; Meunpol et al., 2005; Limsuwatthanathamrong et al., 2012) and reproductive hormones (Meunpol et al., 2007; Meunpol et al., 2010; Nguyen et al., 2012) that are beneficial to crustaceans broodstock during maturation. For instance, research on polychaetes *Namalycastis* sp. and *Eunice reticulata* as potential mangrove crab feed to improve growth, survival, and fatty acid composition (Lim et al., 2021). Additionally, the black tiger prawn hatcheries in Asian countries rely heavily on sandworms (*Perinereis* sp.) and mudworms (*Marphysa* sp.) as maturation diets (Meunpol et al., 2005).

Polychaete *Marphysa* sp. is one of the most common bait species targeted by bait diggers (Cabral et al., 2019). Polychaetes from the wild are associated with a variety of ecological and animal health issues, including habitat loss and resource depletion (Pires et al., 2012; Watson et al., 2017; Cabral et al., 2019) and transboundary disease transmission risk when used as culture feed (Vijayan et al., 2005). Moreover, as polychaetes are in high demand on the international market, the introduction of non-native species is at risk as a result of importation (Fidalgo e Costa et al., 2006). Consequently, artificial polychaete culture is recommended as an alternative to wild harvesting, and another benefit of cultivating and using this bait is to reduce substrate harvesting disturbances and the impact on the biochemical and benthic communities (Gambi et al., 1994; Olive, 1999).

Growing demand for polychaetes both from aquaculture and recreational fishing has led to the development of sustainable polychaete culture (Pombo et al., 2018). Sandworms (*Perinereis* sp.) and mudworms (*Marphysa* sp.) are a couple of polychaetes that have been well-studied as culture species. (Scaps, 2003; Meunpol et al., 2005; Panakorn, 2015). Furthermore, the consumption of food is one of the factors that contribute to a species' growth and survival. There have been many studies on fish meals used as a feed for polychaetes (Palmer et al., 2014). Additionally, seabream sludge (Bischoff et al., 2009), eel sludge (García-Alonso et al., 2008), shrimp feed and decapsulated *Artemia* (Kim et al., 2017), as well as waste from prawn ponds (Palmer, 2010) are also used as food for polychaetes. However, some of these feeds are unsustainable, expensive, and contaminated with pathogens (Brummett, 2008; Evans, 2019; Desrina et al., 2018; Vijayan et al., 2005). It is still unknown whether rotifer has been used as a feeding medium for polychaetes; therefore, this study investigated the effect of rotifer

(*Brachionus plicatilis*) on the growth, setigers, and survival performance of polychaete (*M. iloiloensis*).

Materials and Methods

Study Site and Duration

The study was conducted in the Multi-Species Hatchery, College of Fisheries (COF), Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, Philippines (Figure 1) and lasted for 45 days.

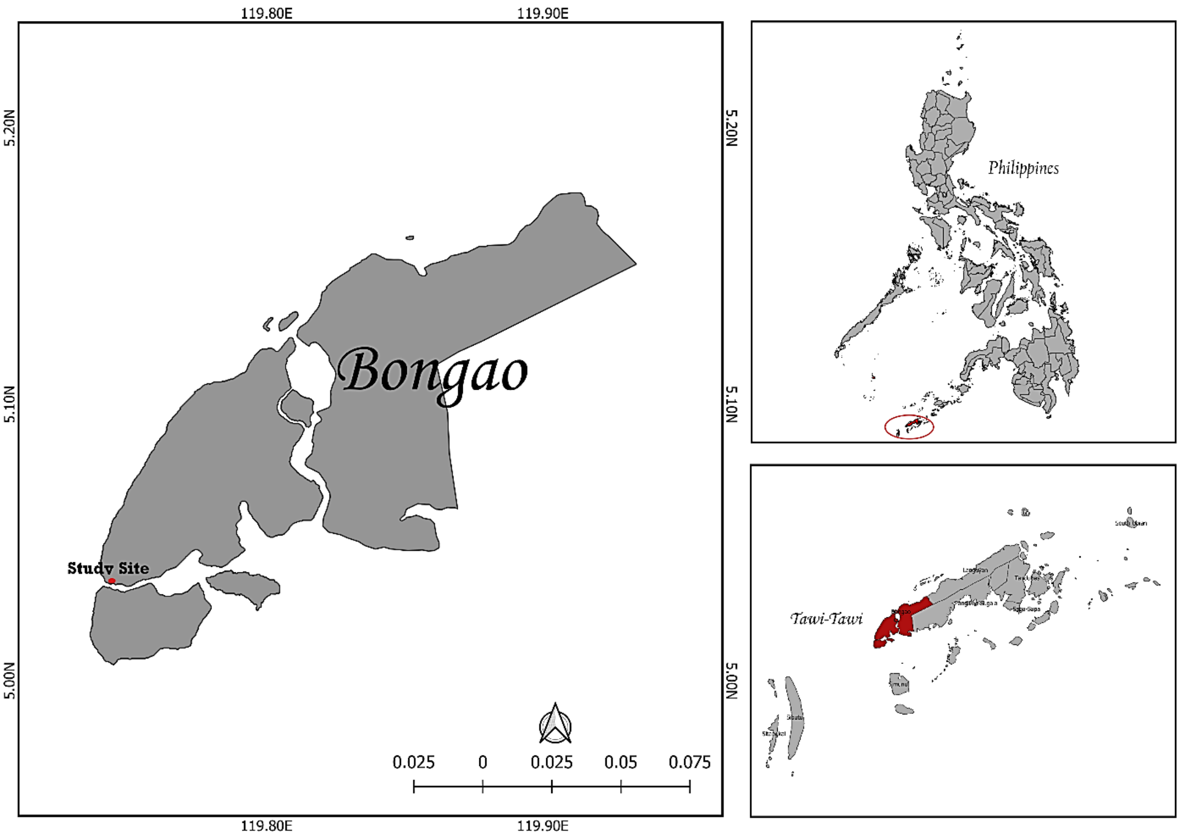

Figure 1. Map of the study site

Experimental Species

The jelly cocoon of *M. iloiloensis* was collected bare hands in Sunkist, Sanga-Sanga, Bongao, Tawi-Tawi with muddy substrate and carefully placed in a plastic cup with water from the area. After six days, the jelly mass disintegrated, and the nectochaetes settled at the bottom of the plastic cup. The polychaete was transferred into the experimental cup prior to stocking and examined the polychaete under the microscope, then stocked in a plastic cup by pipetting individually.

Source and Preparation of Rotifer

The source of rotifer (*B. plicatilis*) is from the Phycology Laboratory of Multi-Species Hatchery, COF, MSU TCTO, and was propagated and maintained in the same area. Rotifers were first produced by feeding with microalga *Nannochloropsis* sp. before becoming food for polychaetes.

Experimental Design

The experiment was conducted in 9 plastic cups (300 mL capacity) containing treated seawater. Filtered seawater was treated with 10 ppm of chlorine and neutralized with sodium thiosulfate (10 ppm) after 24 hours. Three replicates were assigned to each treatment following a Completely Randomized Design (CRD). Thirty nectochaetes were stocked in every culture plastic cup. The average initial density of rotifers was 60 - 80 per mL. Feeding of polychaetes was done according to the treatment of different concentrations of rotifer, namely, Treatment 1 (T1) = 5 mL, Treatment 2 (T2) = 10 mL, and Treatment 3 (T3) = 15 mL every after changing 50% of the water in the cup every two days.

Sampling

Each polychaete in the container was observed and examined through a compound microscope by pipetting from the cup into the petri dish. Each number of setigers was counted based on Figure 2, and all polychaetes per cup were recorded for survival. Sampling was done every 15 days. Final setigers, setigers gain, specific growth rate (SGR) (Ricker, 1979), and survival rate (Bautista-Teruel, 2016) formula are presented below.

Final setigers = Total number of setigers present in the polychaete
Setigers gain = Final setigers – Initial setigers

$$\text{SGR (\% day)} = \frac{\ln(\text{Wf}) - \ln(\text{Wi})}{\text{Days of culture}} \text{X } 100$$

Where:

Wi = Initial weight
Wf = Final weight

$$\text{Survival Rate} = \frac{\text{Total number of polychaete}}{\text{Initial number of polychaete stock}} \text{x}100$$

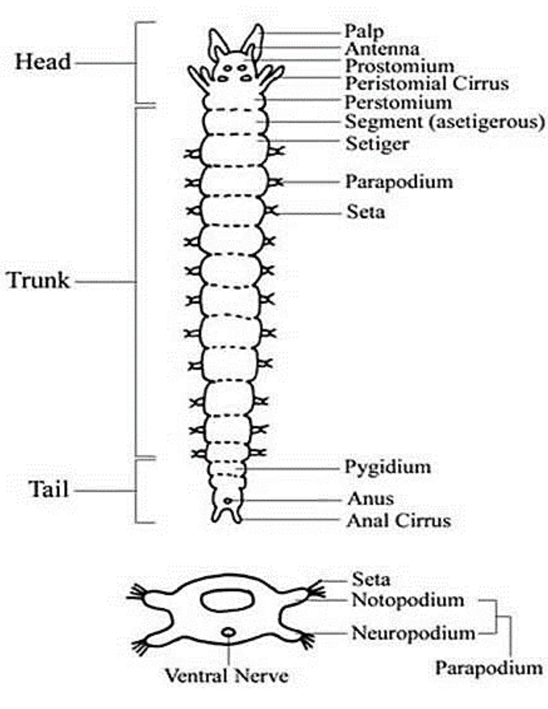

Figure 2. Major morphology features of a generalized polychaete (Fauchald, 1977).

Statistical Analysis
Data were presented as mean ± standard error of the mean (SEM) and were tested for normality and variance homogeneity. The one-way analysis of variance (ANOVA) was used on data on the total setigers, setigers gain, and survival rate at $p<0.05$. Post hoc analysis was done to rank the means using Duncan's Multiple Range Test.

Results
The results on the effects of different concentrations of rotifer in *M. iloiloensis* is presented in Figures 2-5. One-way ANOVA manifested that there is no significant difference ($p>0.05$) in the SGR fed with different concentrations of rotifer for 45 days of culture (Figure 3). On day 15, T2 obtained the highest specific growth rate of 8.6 ± 0.23 % day^{-1}, followed by 7.8 ± 0.28 % day^{-1} of T1, and the lowest was 3.6 ± 3.58 % day^{-1} in T3. On day 30, the highest SGR was in T1 with 6.2 ± 0.04 % day^{-1}, followed by 6.2 ± 0.370 % day^{-1}, and the lowest was in T3 with 2.6 ± 2.63 % day^{-1}. Last sampling, which is 45 days, the highest SGR was tied with 5.2 ± 0.19 % day^{-1} in T1 and T2, while the least among the treatments was T3 with 2.0 ± 2.01 % day^{-1}.

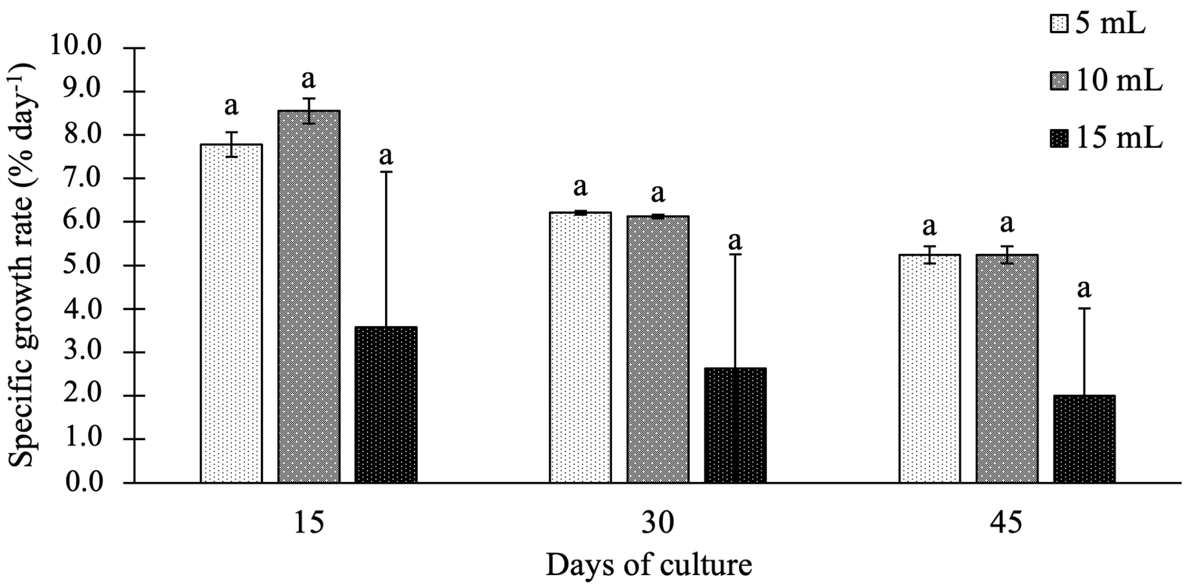

Figure 3. Specific growth rate of *M. iloiloensis* in every sampling fed with different concentrations of rotifer; T1=5 mL, T2=10 mL, T3=15 mL. Bars with the same letters are not significantly different ($p>0.05$). Error bars in SEM (standard error mean), n=30.

In total setigers, one-way ANOVA showed no significant difference ($p>0.05$) among the treatments for 45 days of culture (Figure 4). On the 15 days, the highest total setigers were 10.8 ± 0.38 from T2, followed by 9.7 ± 0.42 from T1, and the lowest was 5.0 ± 5.0 from T3. On the 30 days of culture, the highest total setigers were from T1 with 19.4 ± 0.25, followed by T2 at 19.1 ± 2.02, and the least among the treatments was 10.7 ± 10.67 of T3. On the last sampling (45 days), T2 enormously increased the total setigers up to 31.9 ± 2.749 and followed by 27.2 ± 1.88 from T2, and the least in total setigers is 15.0 ± 15 of T3.

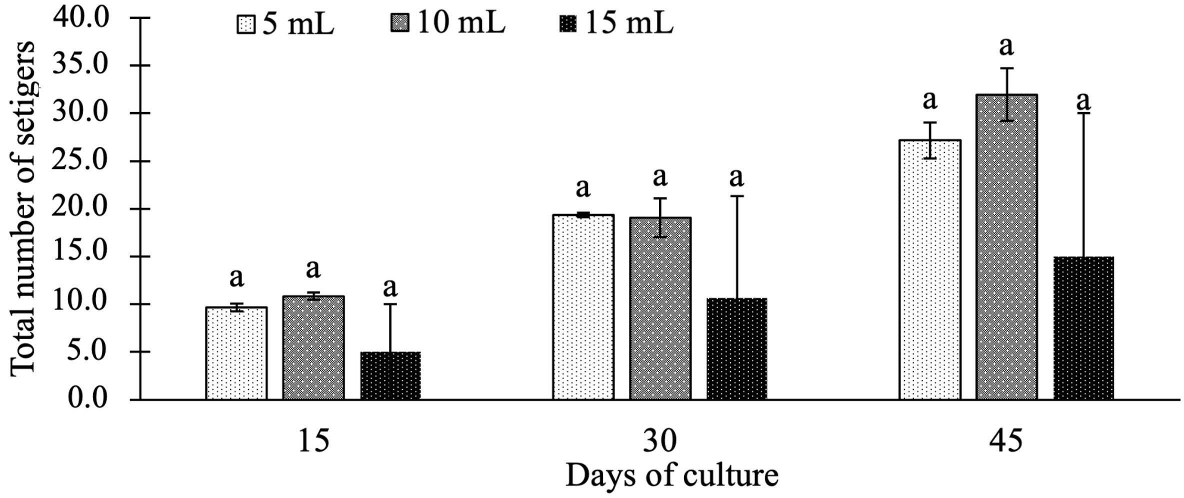

Figure 4. Total setigers of *M. iloiloensis* in every sampling fed with different concentrations of rotifer; T1=5 mL, T2=10 mL, T3=15 mL. Bars with the same letters are not significantly different (p>0.05). Error bars in SEM (standard error mean), n=30.

One-way ANOVA shows no significant difference (p>0.05) among the treatments on setigers gain (Figure 5). On the 15 days, T2 has the highest setigers gain with 7.8 ± 0.39, followed by T1 with 6.7 ± 0.47, and the lowest is T3, garnered only 4 ± 4. On the 30 days of culture, the highest was from T1, having 16.4 ± 0.25 setigers gain; after that, T2 was 16.1 ± 2.02, and the lowest was T3, having only 9.7 ± 9.67. On the 45 days of final sampling, the T2 performed considerably in setigers gain, having 28.9 ± 2.75, then T1 with 24.2 ± 1.88, and the least in setigers is T3, with 14 ± 14.

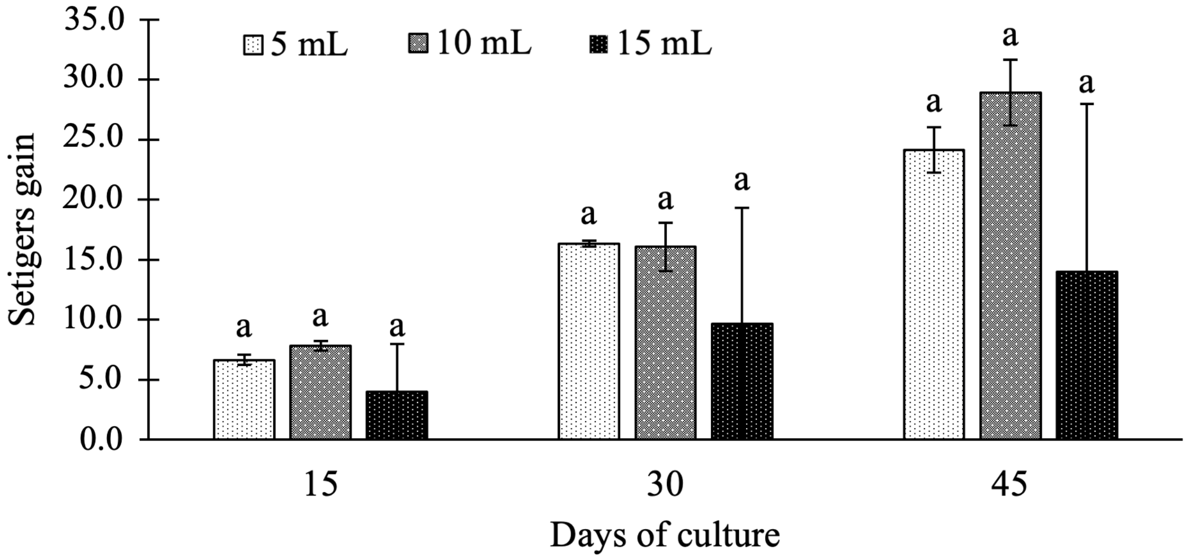

Figure 5. Setigers gain of *M. iloiloensis* in every sampling fed with different concentrations of rotifer; T1=5 mL, T2=10 mL, T3=15 mL. Bars with the same letters are not significantly different (p>0.05). Error bars in SEM (standard error mean), n=30.

The survival performance of polychaete is shown in Figure 6. On the 15 days, one-way ANOVA showed that T1 has the highest survival rate with 34.4 ± 7.778 % and is highly significant (p<0.01) to 13.3 ± 3.333 % and 1.1 ± 1.11 % for T2 and T3, respectively. For the 30 days, one-way ANOVA presents no significant difference (p>0.05), and the highest is in T1, then T2 and the lowest is T3 with a survival rate value of 13.3 ± 3.849 %, 10 ± 3.33 %, and 1.1 + 1.11%, respectively. On 45 days of culture, the highest survival rate in T1 is 8.9 ± 2.22 %, then in T2 has 7.8 ± 1.11%, which is significantly higher (p<0.05) to 1.1 ± 1.1% of T3.

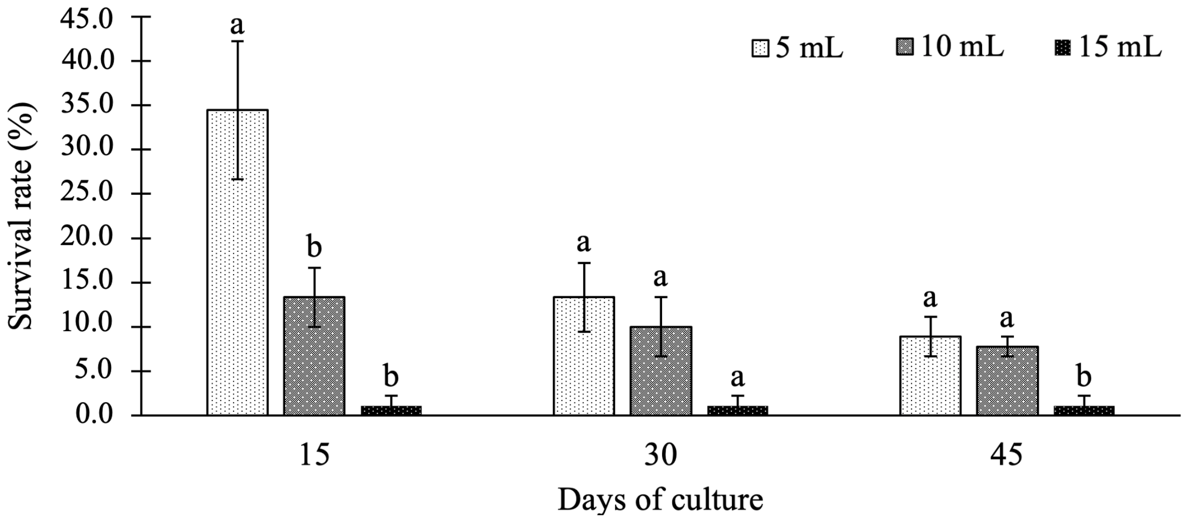

Figure 6. Survival rate of *M. iloiloensis* in every sampling fed with different concentrations of rotifer; T1 = 5 mL, T2 = 10 mL, T3 = 15 mL. Bars with different letters are significantly different (p<0.05 and p<0.01). Error bars in SEM (standard error mean), n=30.

Discussion

Polychaetes fed with 10 mL of rotifer had better growth and a higher number of setigers, although they did not significantly differ from other concentrations of rotifer. In addition, both 5 mL and 10 mL concentrations of rotifers showed significantly higher survival performance of polychaete *M. iloiloensis* compared to 15 mL concentrations of rotifers after 45 days of the culture period. An experiment using 1-day-old trochophore larvae polychaete *M. iloiloensis* obtained a survival rate of 6.43 % and a final body weight of 0.52 g after feeding them with bioflocs in a nursery tank for 30 days, then transferring them into sediment tanks to be fed with feed mill sweepings for 150 days (Mandario, 2020). In the present study, a 6-day-old of polychaete *M. iloiloensis* having a 3-setiger count called "nectochaete" fed with rotifer obtained a higher specific growth performance of 5.2 % day^{-1}. Additionally, a higher setigers gain of 28.9 after 45 days cultured in a 300 mL cup, while the survival performance of polychaete *M. iloiloensis* obtained at the highest at 34.40 % as early as 15 days, but superiority decreased after 45 days of the culture period. In contrast, many researchers have examined the growth and survival performance of juvenile polychaete *M. sanguinea* cultured for 2 to 3 months in the laboratory (Garcês & Pereira, 2011; Kim et al., 2017; Thi Thu et al., 2019). According to Garcês and Pereira (2011), polychaete *M. sanguinea* had the highest survival rate of 85 % after 2 months of culture in the natural sediments when fed with seaweed *Ulva lactuca*. In addition, An average survival rate of 34 % was achieved by polychaete *M. sanguinea* larvae fed with decapsulated zooplankton *Artemia* cultured with mud sediments for two months. However, following 3 months of culture, the survival rate of juveniles dropped to 8 % with 42 setigerous segments (Kim et al., 2017). According to our study, polychaete *M. iloiloensis* cultured in 10 mL of rotifer *B. plicatilis* after 45 days obtained 32 setigerous segments with an 8 % survival rate. It is believed that the polychaete *Marphysa* sp. prefers sand or mud since they are burrower organisms (Du Clos, 2012; Mandario et al., 2019; Mandario et al., 2022). In the present study, one of the challenges encountered by polychaetes aquaculture is that they were cultivated without sediments of sand or mud, which made it necessary for them to dig holes for their place, which may have contributed to the decrease in survival.

Conclusion

The present study suggests that zooplankton rotifer *Brachionus plicatilis* can efficiently alter the diet of polychaete *Marphysa iloiloensis,* thereby improving growth, creating setigerous segments, and enhancing survival. However, the presence of sediments such as sandy or muddy soil must be considered when cultivating polychaetes.

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Ethical approval

The author declares that this study complies with research and publication ethics

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Funding organizations

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Contribution of authors

All authors in this study have equally contributed in terms of conceptualization, data curation and evaluation, writing the original draft, investigation, methodology, resources, validation, and visualization, and finalizing the paper.

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