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Appropriate feeding for early juvenile stages of eunicid polychaete *Marphysa sanguinea*

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Abstract

Survival rate (SR) and growth rate (GR) were tested with various feed sources to identify an appropriate feed to improve the productivity in the early life stage of the rock worm *Marphysa sanguinea* (Montagu, 1813) (Eunicidae: Polychaeta). In addition, feed supply rates were also examined. Three experiments were performed to identify the appropriate feed for the juvenile stages of *M. sanguinea*. Experiment 1 was done using seven different feed sources and without feed as well for the first 20 days of *M. sanguinea* culture. Decapsulated *Artemia* and extruded pellet for shrimp were showed with high SR and GR in the experiment 1. Experiment 2 was performed with five different feed sources. Two feeds were selected from experiment 1 in addition to eel feed, mixed micro-algae, and benthic diatom. Four different quantities of each feed were supplied to 3000 individuals of early juvenile stage of *M. sanguinea*. High quantity of decapsulated *Artemia* and shrimp feed resulted with a relatively good SR and GR. In experiment 3, we provided 20, 50, and 75 mg of decapsulated *Artemia* and shrimp feed to 3000 individuals of *M. sanguinea*. Our results demonstrated that after 3 months, decapsulated *Artemia* showed high survival rate and 75 mg/3000 inds provided the best quantity of feed in the earlier life stage culture of *M. sanguinea*.

Keywords: Polychaete, Feed, Feeding rate, *Marphysa sanguinea*, Decapsulated *Artemia*

Background

Polychaetes with a relatively short life cycle and a strong reproductivity not only play a role of secondary consumers in the ocean but also sometimes purify deposit by changing the organic ingredients through feeding behavior (Clark 1977; Paik 1989; Heo 2011). Polychaetes are considered as an indicator organism of marine pollution (Belan 2003; Giangrande et al. 2005; Samuelson 2001), as the major prey of benthic fish and as bait for angling becoming a target species of fishermen's sideline (Gambi et al. 1994; Olive 1994, 1999; Younsi et al. 2010). Among the polychaete species, especially, the rockworm *Marphysa sanguinea* (Montagu, 1813) (Eunicidae), is a commercially important species for aquaculture.

M. sanguinea lives in a rock block or between gravels mixed in tender deposit of upper and low intertidal region in the whole coast of South Korea and is well distributed around the world (Glasby and Hutchings 2010; Hutchings et al. 2012). The studies on the breeding of *M. sanguinea* were done by Imai (Imai 1975, 1976, 1981). Besides, the studies on feeding habit and inhabiting environment of adults (Prevedelli et al. 2007), on the early larval development (Imai 1982; Prevedelli et al. 2007), and on the salinity tolerance of juvenile (Garcês and Pereira 2011) were reported. However, the study on early nursery-stock cultivation for mass production of *M. sanguinea* was meager.

The aquaculture of *M. sanguinea* required the definite technologies for mass production and by supply of appropriated feed in each production stage. Unfortunately, no feed is placed on the market and only a pellet type of shrimp or finfish feed is used in the culture of *M. sanguinea*. It is not clear that efficiency of such feed is proper as a food source. It is uncertain how such feed affects growth and survival of *M. sanguinea*. Especially, survival of larva-

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juvenile until the third month is crucial to determine a seed production of *M. sanguinea*, and it is necessary to establish a reasonable food source and feeding rate to prevent a high mortality in early life stage. Hence, the purpose of this study was to examine survival rate and growth rate by testing various feeds to know appropriate feed, feeding rate, and supply rate in an early stage of nursery-stock production, the biggest fatal stage of *M. sanguinea* aquaculture.

Methods

To investigate an appropriate feed for early life stage of *M. sanguinea*, three different experiments were performed.

Experiment 1: preliminary survey with eight different feeds (20 days)

To investigate a suitable food source in early life stage of *M. sanguinea*, 15 ml of seawater filtered by membrane filter (GF/C) was put in 6-hole well plate (SPL Life Sciences Inc.), and 100 individuals of larvae were introduced in each hole. The larvae were produced in Fisheries Science and Technology Center of Pukyong National University. One microliter of diluted solution of 0.1 g of mud with 10 ml of filtered seawater was put in each hole as substrate to induce the planktonic larvae to do metamorphosis. Water temperature was maintained as 20 °C, and water was changed in 10 ml every 2 days. Without food source (control), *Chaetoceros* sp. (1×10^4 cells/ml), benthic diatom sp. (1×10^4 cells/ml), *Chlorella* powder (1 mg), *Tetraselmis suecica* (1×10^4 cells/ml), sea mustard (1 ml), decapsulated *Artemia* (1 mg), and extruded pellet (EP) for shrimp (1 mg) were used as food sources. Among them, decapsulated *Artemia* and EP for shrimp were started to supply after 6 days of introduction of larvae when they formed (Kim 2015). Feeds were supplied in every 2 days after renewal of seawater. The feeding tests were triplicated, and the experimental period was 20 days. The survival and growth rate were calculated at the end of the experiment. The living worms were counted, and the length was measured by taking 10 juveniles randomly in each experimental group for calculating the growth rate. The Dixi image program (Ver 2.89, Dixi optics) was used to measure the length of juveniles.

Experiment 2: investigation on the source and the amount of feed for early juvenile culture (for 2 months)

Five feed sources with four quantities in each (total 20 conditions) were tested. Decapsulated *Artemia* and shrimp feed showing the positive results in experiment 1 were also used in addition to eel feed. For microalgae, mixed microalgae solution sold on the market and benthic diatom species generally used for larva of invertebrate as a feed were selected (Table 1). For shrimp feed, eel feed, and

Table 1 Nutritional contents of feed used in this experiment and their ingredients

Ingredient	Protein	Lipid	Fiber	Moisture	Others
Shrimp feed	Min 52%	Min 14.5%	Max 3%	Max 10%	–
Decapsulated <i>Artemia</i>	Min 46%	Min 5%	Max 5.8%	Max 13%	–
Eel feed	Min 54%	Min 10%	Max 15%	–	Ca 1.5%, P 2.7%
Mixed microalgae	–	–	–	–	<i>Isochrysis Pavlova</i> , <i>Tetraselmis</i>
Benthic diatom	–	–	–	–	<i>Navicula</i> , <i>Nitzschia</i> , etc.

decapsulated *Artemia*, four different quantities such as 5 mg/3000 inds, 10 mg/3000 inds, 50 mg/3000 inds, and 100 mg/3000 inds were supplied. For mixed microalgae and benthic diatom, 1×10^4 cells/3000 inds, 1×10^5 cells/3000 inds, 1×10^6 cells/3000 inds, and 1×10^7 cells/3000 inds were supplied. Nutritional contents of used feed are presented in Table 1. The feeding was done for every 2 days after 7 days from installation of experiment. Filtered seawater was renewed constantly, and remained food was not removed. The experimental periods were 60 days by reflecting the high mortality in early juvenile period. Number of setigers and survival rate of worms were examined at the end of experiment. A rearing tank (Fig. 1) was constituted with three reserves of 45 cm × 9 cm × 5 cm which allowed three replicates of experiments. Mud was used as a substrate with 1-cm depth. Experimental installations are shown in Fig. 2. The conditions of seawater were maintained at 20 ± 2 °C, 32–33 psu, pH 8.0, and 6–8 ppm of DO during the experimental period.

Experiment 3: more detailed investigation on the source and the amount of feed for juvenile cultivation (3 months of experiment)

Decapsulated *Artemia* and shrimp feed could be considered as a good efficient feed through the results of experiment 2; hence, a closer feeding amount ranges were investigated in the third experiment for 3 months. Three quantities such as 25 mg/3000 inds, 50 mg/3000 inds, and 75 mg/3000 inds of two feeds were reinstalled. The experimental conditions were similar to experiment 2.

Statistical analysis

Growth rate (GR) was measured with growth of body length in experiment 1.

GR (growth rate) = [(final body length – initial body length)/initial body length] × 100

For experiments 2 and 3, GR was measured with number of setigers.

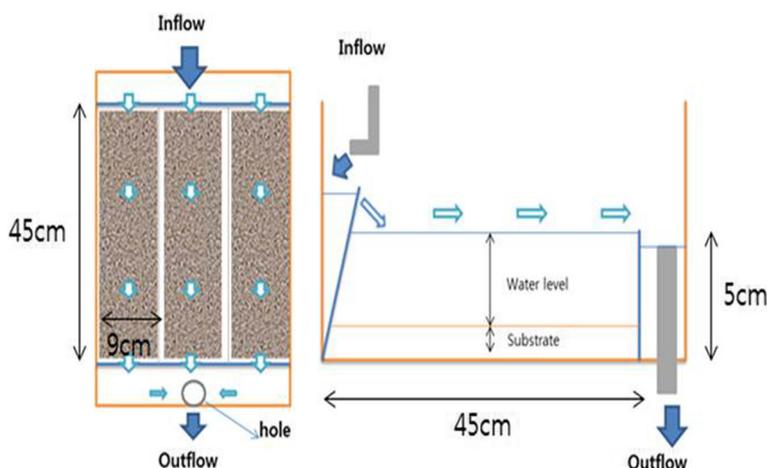


Fig. 1 Schematic diagram of rearing tank of *M. sanguinea* juveniles. Arrows indicate the flow of direction of running seawater

Survival rate was calculated as

$$SR \text{ (survival rate)} = \left(\frac{\text{final population number}}{\text{initial population number}} \right) \times 100$$

The validation of each study section was made by two-way ANOVA test with squared transformed data, and the significant test was conducted with a *p* value of 0.05. The statistical analysis was performed with the Systat v.9 package.

Results

Experiment 1: a preliminary survey about appropriate feed source of an early larval/juvenile cultivation for nursery-stock cultivation

The decapsulated *Artemia* and shrimp feed showed the high growth (Fig. 3) and survival rate (Fig. 4). The growth rate (GR) of shrimp feed group was $451.5 \pm 13.29\%$ which was the highest, and decapsulated *Artemia* was $411.3 \pm 6.94\%$ at the end of 20-day experiment. The other experimental groups were showed similar or lower growth rate than the control group (without feed).

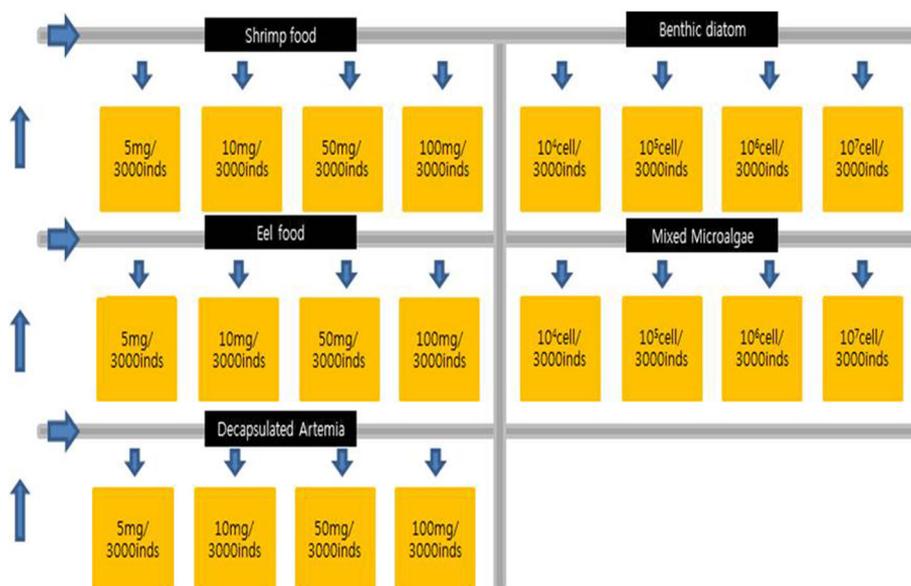


Fig. 2 Schematic diagram of experimental setup for experiments 2 and 3. Arrows indicate flow of direction of running seawater

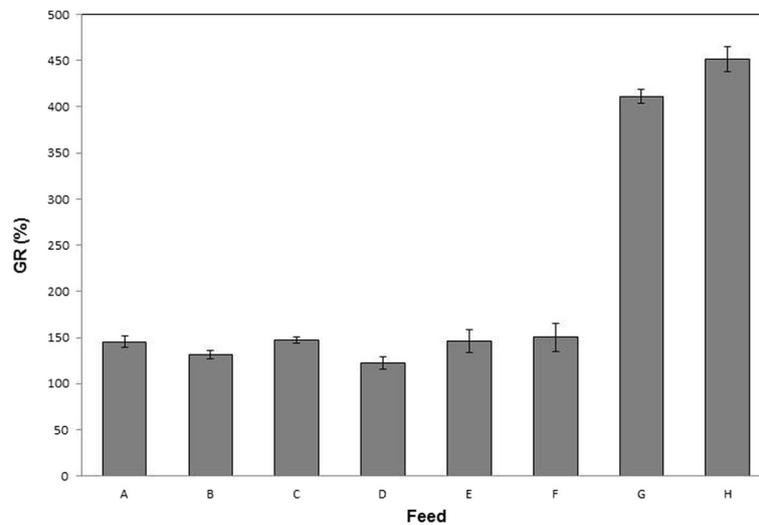


Fig. 3 Growth rate (GR) of early larval culture with eight different food sources after 20 days. A, without food source (control); B, *Chaetoceros* sp. (1×10^4 cells/ml); C, benthic diatom sp. (1×10^4 cells/ml); D, *Chlorella* powder (1 mg); E, *Tetraselmis suecica* (1×10 cells/ml); F, sea mustard (1 ml); G, decapsulated *Artemia* (1 mg); and H, extruded pellet (EP) for shrimp (1 mg)

The survival rate (SR) was the highest in decapsulated *Artemia* with $61 \pm 3.2\%$. Shrimp feed was followed with $51 \pm 3\%$. The control group was $35 \pm 4.6\%$. The lowest survival rate was $28 \pm 3\%$ of sea mustard which is lower than control (Fig. 4). The results of GR and SR were shown that decapsulated *Artemia* and shrimp feed were good candidate for feed of early juvenile stage.

Experiment 2: first survey for appropriate feed source of an early juvenile cultivation for nursery-stock cultivation (2 months of experiment)

Growth and survival rates of juveniles at the end of the experiment according to feeding amount with different feed types are shown in Table 2 and Figs. 5 and 6. In the survival rate of different feed sources, decapsulated

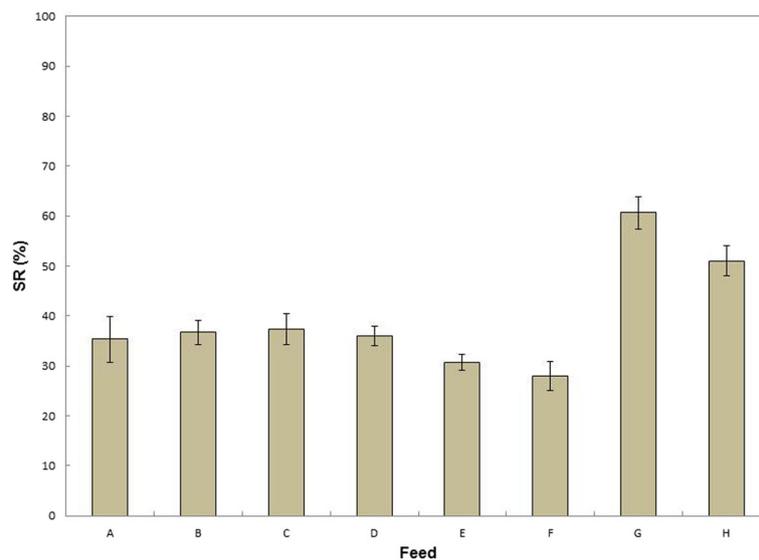


Fig. 4 Survival rates of early larval culture with eight different food sources after 20 days. A, without food source (control); B, *Chaetoceros* sp. (1×10^4 cells/1 ml); C, benthic diatom sp. (1×10^4 cells/1 ml); D, *Chlorella* powder (1 mg); E, *Tetraselmis suecica* (1×10 cells/1 ml); F, wakame (1 ml); G, decapsulated *Artemia* (1 mg); and H, extruded pellet (EP) for shrimp (1 mg)

Table 2 Survival rate (SR) and number of setigers of *M. sanguinea* juvenile by feeds (2 months)

Trial	Group	Amount of feed (per 3000 individuals)	SR (%)	Number of setigers
Shrimp feed	A	5 mg	1.00 ± 0.01	23.66 ± 1.25
	B	10 mg	0.45 ± 0.30	23.58 ± 2.58
	C	50 mg	11.81 ± 1.77	24.26 ± 3.78
	D	100 mg	3.65 ± 0.29	31.13 ± 4.95
Eel feed	A	5 mg	0.21 ± 0.01	26.10 ± 2.19
	B	10 mg	3.37 ± 1.40	20.33 ± 1.41
	C	50 mg	11.92 ± 3.28	21.80 ± 1.17
	D	100 mg	6.70 ± 1.82	22.46 ± 0.80
Decapsulated <i>Artemia</i>	A	5 mg	1.67 ± 0.54	29.66 ± 3.65
	B	10 mg	9.45 ± 1.33	23.10 ± 2.59
	C	50 mg	33.88 ± 2.54	25.03 ± 0.32
	D	100 mg	11.62 ± 2.98	33.43 ± 3.20
Benthic diatom	A	1 × 10 ⁴ cell	0	
	B	1 × 10 ⁵ cell	0	
	C	1 × 10 ⁶ cell	0	
	D	1 × 10 ⁷ cell	0.2 ± 0.1	22.00 ± 3.07
Mixed microalgae	A	1 × 10 ⁴ cell	0	
	B	1 × 10 ⁵ cell	0	
	C	1 × 10 ⁶ cell	0	
	D	1 × 10 ⁷ cell	0.03	18.67 ± 3.06

Artemia showed the highest survival rate, followed by shrimp feed and eel feed. They showed higher SR than other two feed sources such as benthic diatom and mixed microalgae. Fifty milligrams of decapsulated *Artemia* showed the highest average survival rate with 33.88 ± 2.54%. Followed

by 50 mg of eel feed 11.92 ± 3.28% and 50 mg of shrimp feed 11.81 ± 1.77%, 100 mg of decapsulated *Artemia* showed 11.62 ± 2.98%.

In feeding amount, 50 mg showed the highest survival rate in general. Benthic diatom and mixed microalgae

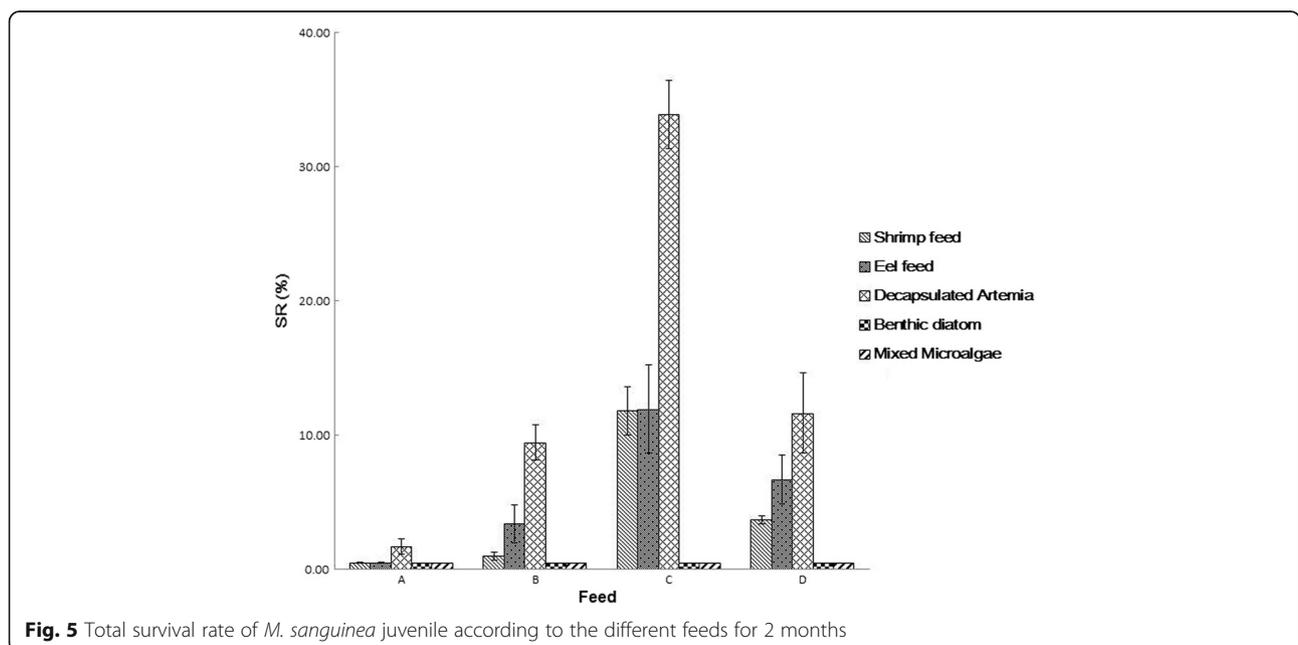
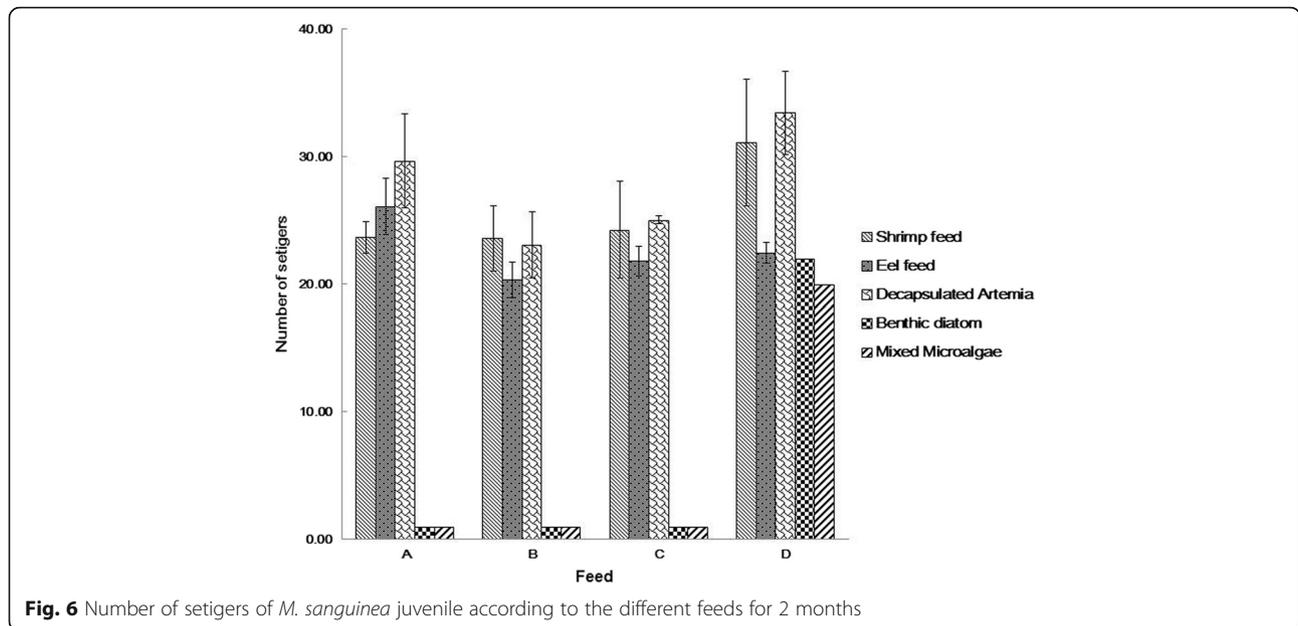


Fig. 5 Total survival rate of *M. sanguinea* juvenile according to the different feeds for 2 months



did not show survival individuals except in 1×10^7 cells (Fig. 5). Two-way ANOVA test showed the significant effect ($p < 0.05$) of feed and quantity of feed for survival rate of juvenile (Table 3). In growth presented with number of setigers, decapsulated *Artemia* and shrimp feed were higher than eel feed. Benthic diatom and mixed microalgae were showed 22.00 ± 3.07 setigers and 18.67 ± 3.06 setigers, respectively; however, they could not survive until 20 experimental days (Fig. 6). In decapsulated *Artemia* section with the highest growth rate, 100 mg/3000 inds section was 33.43 ± 3.2 setigers, and 5 mg/3000 inds section was 29.66 ± 3.65 setigers, but 50 mg/3000 inds section with the highest survival rate showed a low growth as 25.03 ± 0.32 setigers. In all sections, 100 mg/3000 inds section showed the highest growth and 50 mg/3000 inds section showed the highest survival rate (Table 2). Considering survival and growth, decapsulated *Artemia* 50 mg/3000 inds section showed good result.

Two-way ANOVA test showed the non-significant effect ($p < 0.05$) of feed and quantity of feed for increase of setiger numbers of juvenile (Table 4). So the effects of feed and feed quantity were significant for survival rate, not for juvenile's growth. Two-way ANOVA test was performed only with shrimp feed, eel feed, and decapsulated *Artemia*

which give statistically enough numbers at 20 experimental days.

Experiment 3: final survey for appropriate feed source of an early juvenile cultivation for nursery-stock cultivation (3 months of experiment)

Decapsulated *Artemia* group showed higher survival rate than shrimp feed group on average (Table 5); 75 mg/3000 inds of decapsulated *Artemia* section showed $7.91 \pm 1.28\%$ of survival rate. Next, decapsulated *Artemia* 50 mg/3000 inds showed $4.36 \pm 0.81\%$, and shrimp feed 75 mg/3000 inds section showed $3.99 \pm 1.19\%$ (Fig. 7). In feeding amount, 75 mg/3000 inds showed the highest survival rate in general. Two-way ANOVA test showed the non-significant effect ($p > 0.05$) of feed, significant effect ($p < 0.05$) of quantity of feed, and significant effect ($p < 0.05$) of interaction of two factors for survival rate of juvenile (Table 6).

In growth presented with number of setigers, decapsulated *Artemia* had more setigers than shrimp feed. Decapsulated *Artemia* showed the highest growth in 75 mg/3000 inds with 42.2 ± 8.31 setigers, followed by shrimp feed 75 mg/3000 inds section with 40.33 ± 5.94 setigers. In low amount of feed, growth was similar to shrimp

Table 3 Two-way ANOVA test results of survival rate (SR) cultured in different feeds for *M. sanguinea* juveniles

Source	Sum-of-squares	df	Mean square	F ratio	P
^a Feed	20.191	1	20.191	10.174	0.003
^b Qte of feed	30.851	1	30.851	18.463	0.000
Feed × Qte of feed	47.995	1	47.995	41.135	0.000

^aFeed: various kinds of feeds
^bQte of feed: various quantities of feeds

Table 4 Two-way ANOVA test results of setiger numbers cultured in different feeds for *Marphysa sanguinea* juveniles

Source	Sum-of-squares	df	Mean square	F ratio	P
^a Feed	0.145	1	0.145	0.576	0.456
^b Qte of feed	0.257	1	0.257	1.020	0.320
Feed × Qte of feed	0.275	1	0.275	1.092	0.303

^aFeed: various kinds of feeds
^bQte of feed: various quantities of feeds

Table 5 Survival rate (SR) and number of setigers of *M. sanguinea* juvenile after 3 months

Feed	Group	Amount of feed (per 3000 inds) (mg)	¹ SR (%)	Number of setigers (growth)
Shrimp feed	A	25	2.08 ± 0.29	35.36 ± 5.17
	B	50	2.37 ± 0.60	37.10 ± 4.98
	C	75	3.94 ± 1.19	40.33 ± 5.94
Decapsulated <i>Artemia</i>	A	25	2.12 ± 0.25	35.90 ± 7.98
	B	50	4.36 ± 0.81	39.56 ± 8.31
	C	75	7.91 ± 1.28	42.20 ± 5.23

feed and decapsulated *Artemia*. With high amount of feed, growth was higher in decapsulated *Artemia* than in shrimp feed (Fig. 8). Two-way ANOVA test showed that the non-significant effect ($p > 0.05$) of feed and significant effect ($p < 0.05$) of quantity of feed and significant effect ($p < 0.05$) of interaction of two factors for setiger numbers of juvenile (Table 7).

Discussion and conclusions

Imai (1975) reported that mucilage secreted after 10–13 days from fertilization, later it was well agreed to the report of Kim and Jang (2008). In this experiment, we made an attempt of food supply from the seventh day of larval release, as per the report of Kim (2015), and observed that larvae ate organic matter with jaw plate from the seventh day. In the larval stage, like other lecithotrophic larvae, the worm cannot feed and only looking for appropriate settling place (Thorson 1950; Jablonski and Lutz 1983). The other

Table 6 Two-way ANOVA test results of survival rate (SR) cultured in different feeds for *M. sanguinea* juveniles

Source	Sum-of-squares	df	Mean square	F ratio	P
^a Feed	0.987	1	0.987	4.232	0.056
^b Qte of feed	2.646	1	2.646	20.454	0.000
Feed × Qte of feed	4.026	1	4.026	93.244	0.000

^aFeed: various kinds of feeds
^bQte of feed: various quantities of feeds

polychaetes *Nereis pelagica* and *Nereis grubei* showed polyphagia, *Perinereis cultrifera* shows phytophagy feeding seaweed, and *Perinereis nuntia* showed polyphagia placing too much emphasizing on creophagy. *Pereneris nuntia* showed more than 80% of feeding efficiency with mixed feed of eel feed as powder in the Japanese aquaculture farm (Yoshida 1976). But only few reports are available on the early juvenile stage of *M. sanguinea* (Kim 2015).

In the pilot survey for 20 days, creophagy- and phytophagy-type feeds were tested. It was very clear that decapsulated *Artemia* and shrimp feed were reasonably good for early life stage of *M. sanguinea* culture and the other feed were shown similar level to the control group (without food) (Fig. 3). Similar result was reported by Nielsen et al. (1995), filter-feeding *Nereis diversicolor* grew on a diet of suspended algal cells, but the maximum specific growth rate was lower than the feeding experiments with shrimp meat. In the case of experiment 1, growth and survival rate of shrimp feed and decapsulated *Artemia* were higher than other feeding sources. Generally, 50 mg/3000 inds sections showed good survival rate in feeding amount of shrimp, eel, and

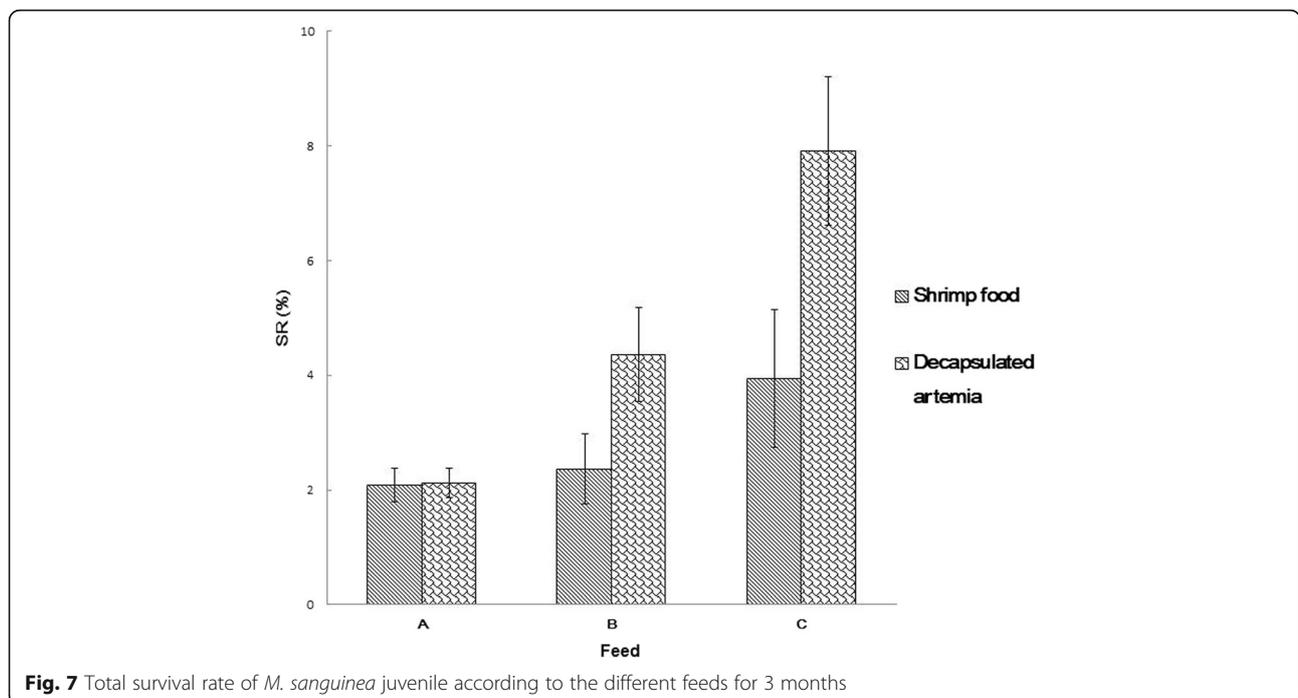
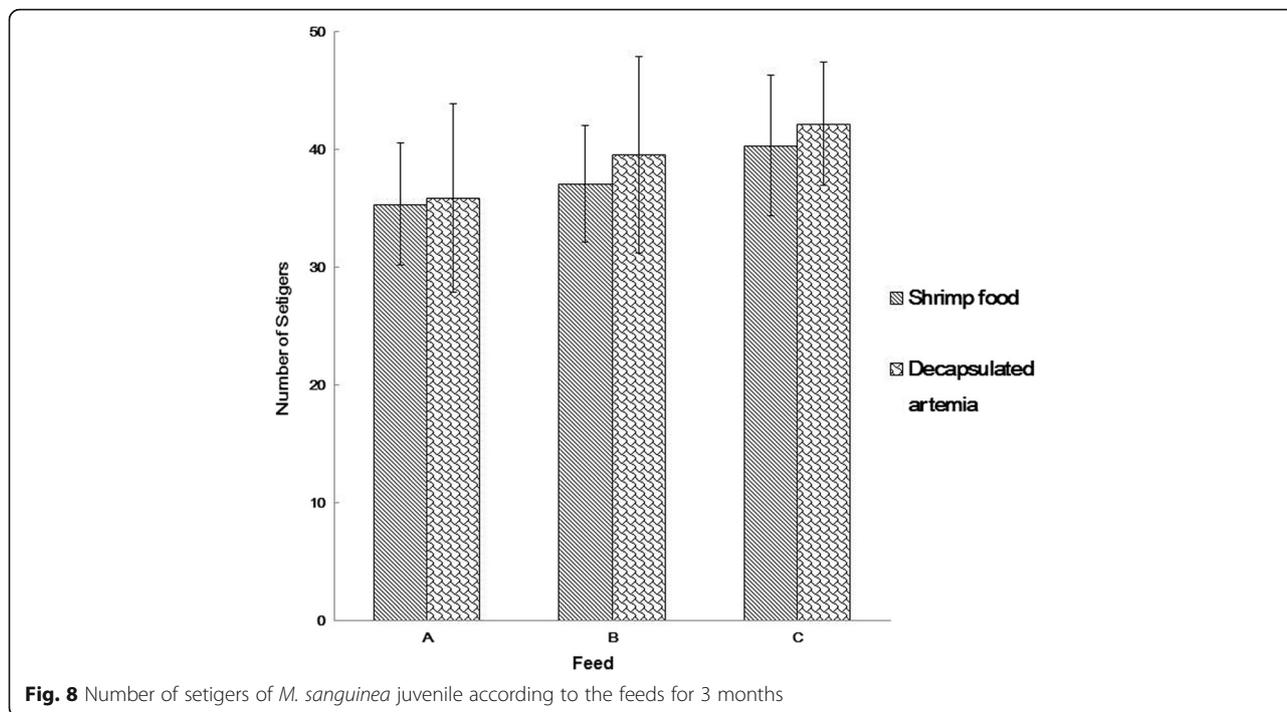


Fig. 7 Total survival rate of *M. sanguinea* juvenile according to the different feeds for 3 months



decapsulated *Artemia* feeds. Decapsulated *Artemia* showed the highest growth rate. Studies performed by Vedel and Riisgård (1993) showed that *N. diversicolor* fed algal cells (*Rhodomonas* sp.) obtained specific growth rates of 3.1% comparable to 3.9% measured in worms in glass tubes placed 15 cm above the bottom in the eutrophicated Odense Fjord. The maximum specific growth rate of the facultative *N. diversicolor* fed algae is lower than 9% found for the obligate suspension-feeding blue mussel *Mytilus edulis* grown in nature in net bags (Riisgård and Poulsen 1981).

However, 50 mg/3000 inds section with the highest survival rate showed lower growth than other feeding amount sections. Both 5 mg/3000 inds and 10 mg/3000 inds sections were showed very low survival rate by insufficient food sources. It seems that there was a cannibalization in the low feeding amount sections. The other section 100 mg/3000 inds was also found with relatively low survival rate due to the degradation of water quality by the accumulation of unused feeds. In addition, there was already no survival being in adhesive

diatom and microalgae sections, and it showed a very low survival rate below 0.2% of survival rate; thus, they could not play a role of food source for *M. sanguinea*. Hence, 50 mg/3000 inds of decapsulated *Artemia* was the best food and feeding amount for the first 2 months of nursery-stock cultivation.

Two-way ANOVA test showed significant effect of feed and feed quantity for survival rate. However, there was no significant difference in growth rate. The good survival rate was showed in decapsulated *Artemia*. Eel feed showed slightly higher survival rate than shrimp feed for *M. sanguinea* larvae. But growth rate was higher in shrimp feed and easier to control in water quality than in eel feed (Kim 2015). This is similar to the result of *N. diversicolor*, where the maximum specific growth rate was higher in shrimp feed (Nielsen et al. 1995). Hence, decapsulated *Artemia* and shrimp feed were chosen for experiment 3. The quantity of feed supplied was differentiated more and less 25 mg/3000 inds in the base of 50 mg/3000 inds. In the experiment 3, our results showed that enough amount of feed can help survival and growth rates, no matter what kind of feed is provided. However, decapsulated *Artemia* showed high survival rate and 75 mg/3000 inds section provided the best quantity of feed in the earlier life stage culture of *M. sanguinea*. Since there were no much studies on the larvae of *M. sanguinea* in Korea and other countries, our results were not extensively compared. However, further comparative studies are necessary to clarify the extent of feeding in the larvae of *M. sanguinea*.

Table 7 Two-way ANOVA test results of setiger numbers cultured in different feeds for *Marphysa sanguinea* juveniles

Source	Sum-of-squares	df	Mean square	F ratio	P
^a Feed	0.075	1	0.075	1.579	0.227
^b Qte of feed	0.618	1	0.618	45.722	0.000
Feed × Qte of feed	0.607	1	0.607	42.775	0.000

^aFeed: various kinds of feeds
^bQte of feed: various quantities of feeds

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Availability of data and materials

All datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KHK, BKK, and CHK manufactured the experimental feed and drafted the manuscript. KHK, BKK, SKK, and WWP conducted the feeding trial and performed the analyses. KHK and CHK conceived and designed the study and experimental facility and also revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Experimental protocols followed the guidelines of the Animal Care and Use Committee of Pukyong National University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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