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Abstract

Three experiments were designed to determine the appropriate size and density for optimum growth and survival of rockworm polychaete *Marphysa sanguinea* in the integrated culture with olive flounder *Paralichthys olivaceus* in the flow-through system under controlled laboratory condition over a 13-week period. The experimental design was that 200, 400, 800, 400 and 400 worms were in $T_1$, $T_2$, $T_3$ and $T_4$ for Experiment-1 (<0.5 g), 100, 200, 400, 200 and 200 worms were for Experiment-2 (<0.5-1.5 g) and 50, 100, 200, 100 and 100 worms were for Experiment-3 (1.5-2.5 g), respectively. The worm feed of $T_1$, $T_2$, and $T_3$ was fish feces and uneaten feed, and that of $T_4$ was controlled-no feed, and that of $T_5$ was commercial feed. The polychaete worms were kept in 15 boxes ($50 \times 40 \times 30$ cm), and bottoms of the boxes were filled with a 15-20 cm layer of substrate sediment of 50% gravel and 50% oyster shell. Thirty fishes were placed in each cubic tank ($70 \times 40 \times 20$ cm) with 55 L water. The weight gain of rockworms (<0.5 g) in $T_1$, $T_2$, and $T_3$ for Experiment-1 (<0.5 g) has shown 152.7%, 153.8%, and 143.0%, respectively. The weight gain was higher than the other two groups, as the weight gain of rockworms in $T_1$, $T_2$, and $T_3$ for Experiment-2 (0.5-1.5 g) was 51%, 30%, and 46%, respectively, and that of rockworms for Experiment-3 (1.5-2.5 g), that is, 75%, 73, and 62%, respectively. From this result, it can be concluded that in the flow through system a small size (< 0.5 g) group of rockworms can be one of the most suitable species at the density of 2000-4000 inds.m$^{-2}$ on fish faces and uneaten feed, as they can grow better than 0.5-1.5 g and 1.5-2.5 g rockworms. On the other hand, integrated results have indicated that in the flow through system around 8 g olive flounder fish was an excellent candidate to be associated with 0.5-1.5 g worms, which can grow better than a small size (< 0.5 g) group of rockworms and than 1.5-2.5 g worms at the optimum density 1000-2000 inds.m$^{-2}$ polychaetes.

Keywords: Polychaete rockworm; Growth; Integrated culture; Olive flounder; Flow-through system

Introduction

Among the polychaete species, especially, the rockworm *Marphysa sanguinea* (Montagu, 1813) is a commercially important species for aquaculture. Rockworm, *Marphysa sanguinea* belonging to the Eunicidae family is an important bait for fisheries and sport fishing in Korea [1]. *M. sanguinea* is an euryhaline polychaete species, which commonly lives in a rock block or between gravels mixed with tender deposit of upper and low intertidal region in the whole coast of South Korea, and which is also well distributed around the world [2], being used as bait in recreational fishing [3,4]. It is one of the most widespread polychaete species with a high economic value and increasing in demand day by day. It is used as bait organisms in fish angling industry with wide markets from Asian to European countries as well as U.S.A. [5]. Japan is the biggest importer in Asia, having imported 1000 tons of worms a year since 1969 with 25 types of live fishing bait worms including 19 species of polychaetes [6]. The polychaetes are commercially important because of using as bait for recreational fishing and as a food source for penaeid crustaceans and fish in aquaculture by Olive [7]. It is leading the development of small but economically viable aquaculture facilities providing a supply of different species for different purposes. The ecological role of polychaetes in marine benthic communities is very important [8]. It is of greater concern that the physical disturbance and the return of heavy metals to the surface, rendering them biologically available, are effects on the habitat, along with the release of ammonia and phosphorus compounds from the sediments leading to eutrophication. The polychaetes are known to be good indicators of species richness [9] and to be bio-indicators of the marine environment [8]. Rockworms help reduce nutrient loads of waste water in pol-y-aquaculture, when being simultaneously cultivated in aquaculture farms [10,11]. The production costs of polychaete worms in an intensive worm aquaculture system should be efficient enough to make profits as described by Nesto et al. [12].

Olive flounder, *Paralichthys olivaceus*, is one of the most economically important fish species farmed in Eastern Asia including the Republic of Korea, Japan and China. There is limited information available concerning the growth of polychaete rockworm culture associated with olive flounder in the flow through system. Therefore, the purpose of this study was to determine the appropriate size and density for optimum growth and survival of polychaete rockworm, *Marphysa sanguinea* in the integrated culture with olive flounder, *Paralichthys olivaceus* in the flow-through system under controlled laboratory condition.

Materials and Methods

Physical and chemical composition of experimental diet

Commercial pellet feed was used in the feeding trials supplied by Suhyp Feed Company Limited, Uiryeong, Gyeongsangnamdo, South Korea. Extruded pellets (EP) of the commercial diet were 2.4 to 2.6 mm, containing 46.61% crude protein, 11.06% crude lipid, 13.94% crude ash, and 8.60% moisture.

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Experimental design

Three experiments were designed as three different size groups of polychaete rockworms A1 (<0.5 g), A2 (0.5-1.5 g) and A3 (1.5-2.5 g) with 5 treatments in each group and three replicates as follows:

- **Experiment 1 (<0.5 g):** T1, 200 worms (1000 inds. m⁻²), T2, 400 worms (2000 inds. m⁻²), T3, 800 worms (4000 inds. m⁻²), T4, 400 worms (2000 inds. m⁻²) and T5, 400 worms (2000 inds. m⁻²).
- **Experiment 2 (0.5-1.5 g):** T1, 100 worms (500 inds. m⁻²), T2, 200 worms (1000 inds. m⁻²), T3, 400 worms (2000 inds. m⁻²), T4, 200 worms (1000 inds. m⁻²) and T5, 200 worms (1000 inds. m⁻²).
- **Experiment 3 (1.5-2.5 g):** T1, 50 worms (250 inds. m⁻²), T2, 100 worms (500 inds. m⁻²), T3, 200 worms (1000 inds. m⁻²), T4, 100 worms (500 inds. m⁻²) and T5, 100 worms (500 inds. m⁻²).

The experiments were conducted in three sets of flow through system. The worm feed of T₁, T₂ and T₃ was fish feces and uneaten feed; that of T₄ was controlled, that is, no feed; and that of T₅ was commercial feed. Boxes were arranged in 3 rows on 2 floors with 5 boxes each floor, of which only 3 boxes were with fish treatments in each row. All the fish boxes were connected to worm boxes (Figure 1).

The experiments were carried on for 13 weeks by using *Marphysa sanguinea* (Polychaeta Unicidae) obtained from the Fisheries Science and Technology Center of Pukyong National University, Goseong-gun, South Korea and juvenile olive flounder (*Paralichthys olivaceus*) fishes collected from a commercial marine fish hatchery named Sin Bi Co., Nam Hae, South Korea. Thirty fishes were placed in each cubic tank (L70 × W40 × H20 cm) with 55 L water. The total number of fishes and worms were determined after 24 h of starvation. The experimental rockworms were reared on flounder feces and uneaten feed that entered directly and 2 hours after feeding were removed by siphon from fish tanks. All the experimental tanks were under the condition of continuous darkness, except at feeding and siphon times. Water samples were taken from main water tank, outlet of fishes and worms' tanks to find out the concentration difference between the sampling points. Water temperature, salinity, pH and dissolved oxygen were always maintained carefully.

**Data collection and statistical analysis**

The normality and homogeneity of variance of data were confirmed by Kolmogorov-Smirnov test. Statistical significance differences of parameters were measured and computed using one-way ANOVA by SPSS 15 software for windows—SPSS Inc., Chicago, IL, USA [13]. Significant differences among treatments (p<0.05) were evaluated by the Duncan's Multiple Range Test [14]. Proximate composition analyses of experimental diets were performed by the standard methods of Association of Official Analytical Chemists-AOAC [15]. For determining moisture content, a number of samples of diets were dried to maintain constant weights at 105°C for 24 h. Ash content was determined using a muffle furnace (550°C for 4 h). Crude lipid content was determined by the soxhlet extraction using Soxtec system 1046 (Foss, Hoganas, Sweden) and crude protein content by kjeldahl method (N9 6.25) after acid digestion. Survival rate, growth performance and feed conversion ration were assessed by the following formulæ:

- **SR-Survival rate (%):** (Number of survivors at the end) × 100/Initial number of worms stocked.
- **WG-Weight gain (%):** (Final weight - initial weight) × 100/Initial weight.
- **SGR-Specific growth rate (%/day):** (Log of final weight - Log of initial weight) × 100/days.
- **FCR-Feed Conversion Ration:** Food weight/(Final weight – Initial weight).

**Results**

**Growth of rock worm in flow through system**

The weight gain, specific growth rate and survival rate in different groups of rock worms are shown in Table 1 and Figure 2. Weight gain of group 1 (<0.5 g) T₁, T₂, T₃, T₄ and T₅ was 152.7%, 153.8%, 140.3%, -18.4% and 321.7%, respectively. There was no significant difference in weight gain observed among T₁, T₂ and T₃. But these treatments were significantly lower in weight gain than commercial feeding treatment T₄ with 321.7% weight gain, and higher than the control group, T₅ (-18.4%) with no feeding treatment. The specific growth rates of group 1 (<0.5 g) were T₁ 0.96%, T₂ 0.99%, T₃ 0.94%, T₄ -0.22%, and T₅ 1.55%.
In group 3 (1.5-2.5 g), weight gain decreased with an increase in worm density from 250 to 1000 inds. m\(^{-2}\). However, no significant difference in WG was observed among treatments fed fish feces and uneaten feed, whereas the highest belonged to T\(_1\) with 75.2% and the lowest to T\(_5\) (73.04%) (Table 1 and Figure 2). But a high significant difference was observed between T\(_1\), commercial treatment 123.1% with other treatments fed fish feces and uneaten feed. The specific growth rate of group 3 of T\(_1\), T\(_2\), T\(_3\) and T\(_5\) was 0.60%, 0.59%, 0.52%, -0.33% and 0.86% respectively. The survival rates of T\(_1\) and T\(_2\) are the same as 94%. The lowest survival among treatments fed fish feces and uneaten feed was observed in T\(_5\) (85%). However, all of this treatment had a significant difference in survival from the control (T\(_4\), 77%).

**Discussion**

In this study, the weight gain of rockworms in T\(_1\), T\(_2\) and T\(_3\) of group 1 (<0.5 g) are comparatively higher than that of group 2 (0.5-1.5 g) and that of group 3 (<1.5-2.5 g). The specific growth rates of rockworms in T\(_1\), T\(_2\) and T\(_3\) of group 1 (<0.5 g) are higher than that of group 2 (0.5-1.5 g) and that of group 3 (<1.5-2.5 g). Control feeding T\(_5\) treatments of all groups have shown a negative weight gain due to lack of food. From the growth performance results, it was found that in the flow through system around 0.5 g rockworm could grow better than 0.5-1.5 g and 1.5-2.5 g rockworms, and that the appropriate density might be 2000-4000 inds. m\(^{-2}\) feed fish-feces and uneaten feed. On the other hand, in all of the T\(_5\) treatment supplied with commercial feed, WG and SGR were found to be lower than other treatments. The component of worm diet may give a good explanation as a high protein commercial diet determined a higher growth rate than low protein feed [12]. The specific growth rates were found in Honda et al. [16], being between 0.45 and 1.66% day\(^{-1}\) in P. nuntia vallata fed on flounder feces, and 3.23% day\(^{-1}\) in worms fed on the diet formulated for polychaetes over a 15-day period. Specific growth rates over 71 days were close to 3% day\(^{-1}\) when the polychaete worm, *Nereis virens*, fed on waste from a recirculating system with juvenile Atlantic halibut *Hippoglossus hippoglossus* [17]. It was also found that when *Nereis virens* of 0.37g initial weight was fed on a commercial worm diet and fish feces and uneaten feed, they reached the final mean weight of 2.42 to 2.33 g in 71 days. In most of our experiment, especially in group 1 and group 3 (Table 1), polychaete organisms kept at the highest density has showed the lower value of specific growth rates and weight gain, suggesting a negative influence of increasing intra-specific competition, as also observed in some nereid polychaetes by Zajac et al. [12,18].

In our results, the survival rates in T\(_1\), T\(_2\) and T\(_3\) of group 1 (<0.5 g)
were lower than that of group 2 (0.5-1.5 g) and that of group 3 (<1.5-2.5 g) rockworm. Among all the treatments, weight gain and survival rate demonstrated that WG was high whereas the survival rate was low. The density had negative effects on survival rates (Table 1). Growth rates also declined when individuals were reared at the highest density, as the negative influences were also observed in some nereid polychaetes [18].

This was probably due to the density as growth was significantly lower at the higher density [12]. In the present study, the weight gain of worms in T1, T2, and T3 of group 1 (<0.5 g) were relatively higher than that of group 2 (0.5-1.5 g) and that of group 3 (1.5-2.5 g), but the survival rate of the group 1 (<0.5) was relatively lower than that of group 2 and of group 3 (Table 1). The negative density effects on the growth of juvenile M. sanguinea were similarly found in other polychaete species [19].

Adverse effects on growth related to high rearing density were reported in other species of polychaetes such as Neanthes arenacodentata [20]. It was proved that polychaete M. sanguinea possessed the ability to grow better in a low density, but biomass production was not related because survival rate could be one of the main factors. In the present study, survival rate was negatively influenced by high rearing density, as also reported for N. arenacodentata [20], D. aciculata [12,19].

In the olive flounder fish study, the weight gain of fish in T1, T2 and T3 of group 2 (with 0.5-1.5 g size worm) was higher than that of group 1 (with <0.5 g size worm) and that of group 3 (1.5-2.5 g size worm). The specific growth rate of fish in T1, T2 and T3 of group 1 (with <0.5 g size worm) was higher than that of group 2 (with 0.5-1.5 g size worm) and that of group 3. The survival rate of fish in T1, T2 and T3 of group...
2 (with 0.5-1.5 g size worm) was comparatively higher than that of group 1 (with <0.5 g size worm) and that of group 3 (with 1.5-2.5 g size worm). From this result, it was be concluded that in the flow through system, around 8 g size olive flounder fish was an excellent candidate associated with 0.5-1.5 g size worm and they could grow better than those fish associated with small size rockworm(<0.5 g) and those fish associated with 1.5-2.5 g worms at the optimum density 1000-2000 inds.m⁻².

**Conclusion**

In this study, rockworms in T₅, T₆ and T₇ of group 1(<0.5 g) showed a higher weight gain than those of group 2 (0.5-1.5 g) and those of group 3 (<1.5-2.5 g). The specific growth rate of rockworms in T₅, T₆ and T₇ of group 1 (<0.5 g) was higher than that of group 2 (0.5-1.5 g) and group 3 (<1.5-2.5 g). The results showed that the weight gain and specific growth rate of rockworms were high whereas the survival rate was low, indicating that it might be due to increasing intra-specific competition and also that the density had negative effects on survival rates. From this result, it was be concluded that in the flow through system, around 0.5 g size rockworms could be one of the most suitable species as they grew better than 0.5-1.5 g and 1.5-2.5 g rockworms at the density of 2000-4000 inds.m⁻² feed fish-feces and uneaten feed. On the other hand, integrated results showed that in the flow through system, around 8 g size olive flounder fish was an excellent candidate associated with 0.5-1.5 g size worm and they could grow better than those fish associated with small size rockworms(<0.5 g) and those fish associated with 1.5-2.5 g worms at the optimum density 1000-2000 inds.m⁻² polychaete.

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**Table 1:** Growth and survival of the olive flounder (Paralichthys olivaceus) in the flow through system at 13-week.

<table>
<thead>
<tr>
<th>Fish size</th>
<th>Treatment (indv.m⁻²)</th>
<th>Initial wt¹ (g)</th>
<th>Final wt² (g)</th>
<th>Wt gain³ (g)</th>
<th>SGR⁴ (%/day)</th>
<th>Survival rate⁵ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp 1 &lt;0.5 g</td>
<td>T₁ 1000</td>
<td>8.39 ± 1.12</td>
<td>64.45 ± 0.76</td>
<td>668.7 ± 18.5</td>
<td>2.22 ± 0.03</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>T₂ 2000</td>
<td>8.33 ± 1.02</td>
<td>64.37 ± 1.13</td>
<td>673.4 ± 28.1</td>
<td>2.22 ± 0.04</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>T₃ 4000</td>
<td>8.32 ± 1.06</td>
<td>64.93 ± 0.59</td>
<td>680.8 ± 17.2</td>
<td>2.23 ± 0.02</td>
<td>98</td>
</tr>
<tr>
<td>Grp 2 0.5-1.5 g</td>
<td>T₄ 500</td>
<td>8.40 ± 1.12</td>
<td>70.11 ± 11.8</td>
<td>734.8 ± 28.3</td>
<td>2.31 ± 0.04</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>T₅ 1000</td>
<td>8.48 ± 1.04</td>
<td>66.06 ± 0.98</td>
<td>713.8 ± 8.6</td>
<td>2.28 ± 0.01</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>T₆ 2000</td>
<td>8.42 ± 1.04</td>
<td>70.95 ± 0.85</td>
<td>706.7 ± 13.1</td>
<td>2.37 ± 0.02</td>
<td>100</td>
</tr>
<tr>
<td>Grp 3 1.5-2.5 g</td>
<td>T₇ 250</td>
<td>8.38 ± 1.03</td>
<td>67.51 ± 0.78</td>
<td>705.7 ± 9.5</td>
<td>2.27 ± 0.01</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>T₈ 500</td>
<td>8.42 ± 1.02</td>
<td>65.44 ± 0.81</td>
<td>678.9 ± 12.1</td>
<td>2.23 ± 0.02</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>T₉ 1000</td>
<td>8.43 ± 1.08</td>
<td>68.52 ± 0.65</td>
<td>712.6 ± 4.4</td>
<td>2.28 ± 0.01</td>
<td>100</td>
</tr>
</tbody>
</table>

¹IW: Initial Weight. ²Final Weight. ³WGR (Weight Gain %): [final weight - initial weight]/initial weight] ×100. ⁴SGR (Specific Growth Rate %): [log final weight - log initial weight]/day] ×100. ⁵SR (Survival Rate %): (final individuals / initial individuals) ×100.
References


