

# Effects of Growth on Polychaete Rockworm, *Marphysa sanguinea* Integrated Culture with Olive Flounder, *Paralichthys olivaceus* in Flow Through System

Hossein Parandavar<sup>1</sup>, Mizanur Rahman<sup>2</sup>, War War Phoo<sup>1</sup> and Chang-Hoon Kim<sup>1,2\*</sup>

<sup>1</sup>Department of Marine Bio-materials and Aquaculture, Pukyong National University, Busan, Korea

<sup>2</sup>Fisheries Science and Technology Center, Pukyong National University, Korea

## 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<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> 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<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> was fish feces and uneaten feed, and that of T<sub>4</sub> was controlled-no feed, and that of T<sub>5</sub> was commercial feed. The polychaete worms were kept in 15 boxes (L50 × W40 × H30 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 (L70 × W40 × H20 cm) with 55 L water. The weight gain of rockworms (<0.5 g) in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> for Experiment-1 (<0.5 g) has shown 152.7%, 153.8%, and 140.3%, respectively. The weight gain was higher than in the other two groups, as the weight gain of rockworms in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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<sup>2</sup> on fish feces 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<sup>2</sup> 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 finfish 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 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 poly-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 Suhyup 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.

\*Corresponding author: Chang-Hoon Kim, Department of Marine Bio-materials and Aquaculture, Pukyong National University, Busan 48513, Korea, Tel: +82-10-2078-6892; Fax: +82-51-629-5908; E-mail: [chkpknu@hanmail.net](mailto:chkpknu@hanmail.net)

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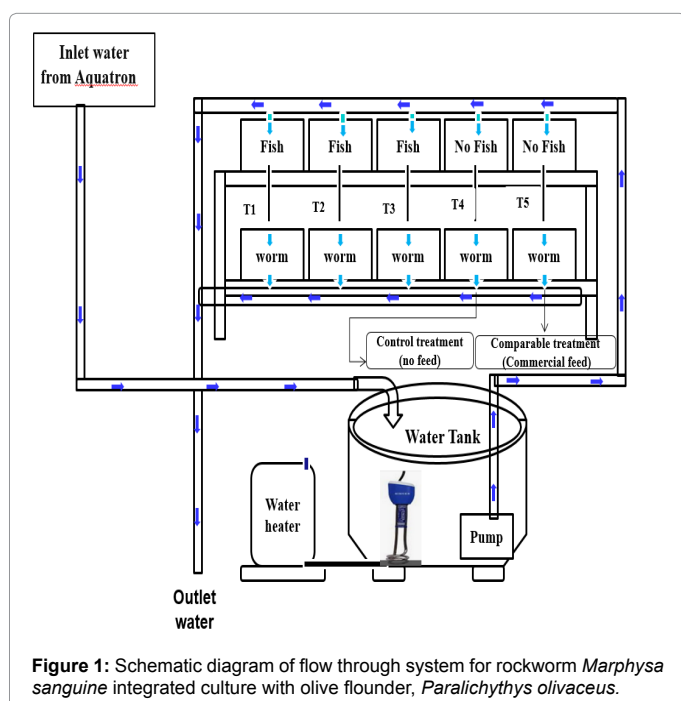
## Experimental design

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):** T<sub>1</sub> 200 worms (1000 inds. m<sup>-2</sup>), T<sub>2</sub> 400 worms (2000 inds. m<sup>-2</sup>), T<sub>3</sub> 800 worms (4000 inds. m<sup>-2</sup>), T<sub>4</sub> 400 worms (2000 inds. m<sup>-2</sup>) and T<sub>5</sub> 400 worms (2000 inds. m<sup>-2</sup>).
- **Experiment 2 (0.5-1.5 g):** T<sub>1</sub> 100 worms (500 inds. m<sup>-2</sup>), T<sub>2</sub> 200 worms (1000 inds. m<sup>-2</sup>), T<sub>3</sub> 400 worms (2000 inds. m<sup>-2</sup>), T<sub>4</sub> 200 worms (1000 inds. m<sup>-2</sup>) and T<sub>5</sub> 200 worms (1000 inds. m<sup>-2</sup>).
- **Experiment 3 (1.5-2.5 g):** T<sub>1</sub> 50 worms (250 inds. m<sup>-2</sup>), T<sub>2</sub> 100 worms (500 inds. m<sup>-2</sup>), T<sub>3</sub> 200 worms (1000 inds. m<sup>-2</sup>), T<sub>4</sub> 100 worms (500 inds. m<sup>-2</sup>) and T<sub>5</sub> 100 worms (500 inds. m<sup>-2</sup>).

The experiments were conducted in three sets of flow through system. The worm feed of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> was fish feces and uneaten feed; that of T<sub>4</sub> was controlled, that is, no feed; and that of T<sub>5</sub> 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* (Polychaete 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 x W40 x H20 cm) with 55 L water. The total number of fishes was 90 and the average weight was 8.3 g. Triplicate tanks were used for each treatment. To avoid the accumulation of catabolic production, water was changed every week. Sea water was filtered by aquatron unit and supplied to main water tank whose capacity was 2000 liters. Water temperature was controlled by a temperature control unit and was maintained at 20 ± 1°C. Water supply to the fish tanks was



**Figure 1:** Schematic diagram of flow through system for rockworm *Marphysa sanguinea* integrated culture with olive flounder, *Paralichthys olivaceus*.

maintained at the rate 1.5-1.8 L min<sup>-1</sup> for flow through system through the experiment period, and water was well aerated in each box. Fishes were fed commercial feed twice daily (09:00 and 17:00 h) on a ration equivalent to 3% of their body weight. The fish weight in each tank was determined every 2 weeks and the amount of diet was adjusted to the weight accordingly. Fish tanks were cleaned up to minimize algae and fungal growth while the fish were removed for weighing.

*M. sanguinea* polychaete worms were kept in the 15 cubic plastic tanks (L50 × W40 × H30 cm) and bottom of the tanks were covered with a 15-20 cm layer of substrate sediment of 50% gravel (150-500 µm) and 50% comminuted oyster shell, which was rinsed several times with fresh water and dried in the sun. The initial and final weight 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

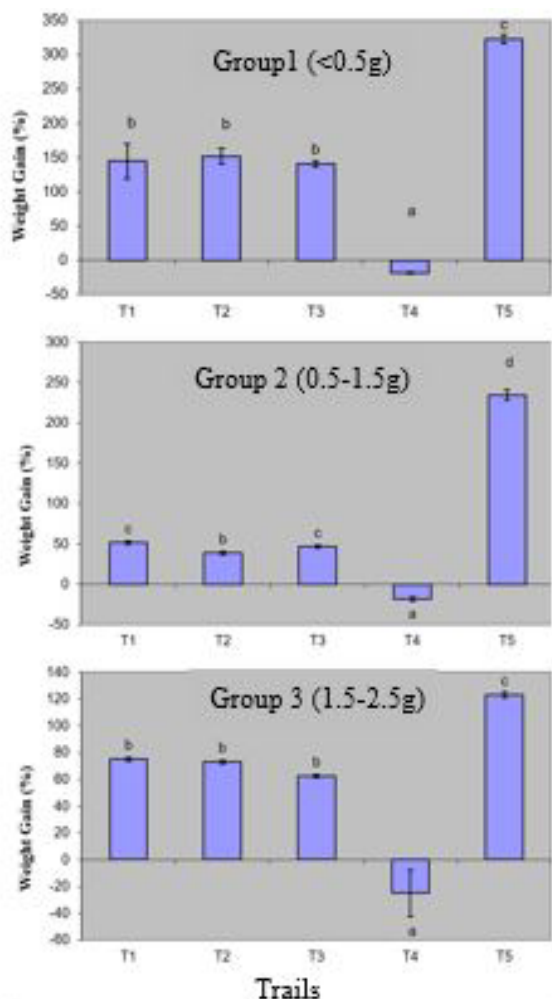
The normality and homogeneity of variance of data were confirmed by Kolmogorov-Smirnov test. Statistical significance differences of parameters were measured and computed using one-way ANOVA by SPSS 15 software for windows- SPSS Inc., Chicago, IL, USA [13]. Significant differences among treatments (p<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 formulae:

- 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 Ratio: 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) T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> was 152.7%, 153.8%, 140.3%, -18.4% and 321.7%, respectively. There was no significant difference in weight gain observed among T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. But these treatments were significantly lower in weight gain than commercial feeding treatment T<sub>5</sub> with 321.7% weight gain, and higher than the control group, T<sub>4</sub> (-18.4%) with no feeding treatment. The specific growth rates of group 1 (<0.5 g) were T<sub>1</sub> 0.96%, T<sub>2</sub> 0.99%, T<sub>3</sub> 0.94%, T<sub>4</sub> -0.22%, and T<sub>5</sub> 1.55%.



**Figure 2:** Weight gain of different sized polychaete worm group 1 (<0.5 g): (T<sub>1</sub> 1000 inds. m<sup>-2</sup>; T<sub>2</sub> 2000 inds. m<sup>-2</sup>; T<sub>3</sub> 4000 inds. m<sup>-2</sup>; T<sub>4</sub> 2000 inds. m<sup>-2</sup> and T<sub>5</sub> 2000 inds. m<sup>-2</sup>; worm group 2 (0.5 to 1.5 g): (T<sub>1</sub> 500 inds. m<sup>-2</sup>; T<sub>2</sub> 1000 inds. m<sup>-2</sup>; T<sub>3</sub> 2000 inds. m<sup>-2</sup>; T<sub>4</sub> 1000 inds. m<sup>-2</sup> and T<sub>5</sub> 1000 inds. m<sup>-2</sup>; and worm Group 3 (1.5 to 2.5 g): T<sub>1</sub> 250 inds. m<sup>-2</sup>; T<sub>2</sub> 500 inds. m<sup>-2</sup>; T<sub>3</sub> 1000 inds. m<sup>-2</sup>; T<sub>4</sub> 500 inds. m<sup>-2</sup> and T<sub>5</sub> 500 inds. m<sup>-2</sup> in the flow through system. (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> feed was feces of fish and uneaten feed; T<sub>4</sub> was controlled-no feed and T<sub>5</sub> was commercial feed).

Treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were fed on fish feces and uneaten feed of fish. There was no significant difference in survival rate between T<sub>1</sub> (73.5%) and T<sub>3</sub> (69.5%). The survival rates of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> in group 1 (<0.5 g) were 73.5%, 59.5% and 69.5%, respectively. Although, there was a significant difference in survival between T<sub>1</sub> (73.5%) and T<sub>2</sub> (59.5%) shown in Table 1 and Figure 3.

Group 2 (0.5-1.5 g) showed that there was no significant difference in weight gain between T<sub>1</sub> (51.7%) with T<sub>3</sub> (46.6%), but a significant difference observed between T<sub>1</sub> with T<sub>2</sub> (30.3%) fed fish feces and uneaten feed. The lowest WG was observed in control feeding T<sub>4</sub> with -19.1%, and the highest observed in commercial treatment T<sub>5</sub> with 236% (Table 1 and Figure 2). Specific growth rates of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> in group 2 were 0.45%, 0.35%, 0.41%, -0.22% and 1.30%, respectively. The survival rate of T<sub>1</sub> (95%) in group 2 was higher than that of T<sub>2</sub> (85%) and that of T<sub>3</sub> (66%) on fish feces and uneaten feed. No significant difference was found in survival between T<sub>2</sub> with T<sub>5</sub> (89%). Results in this group showed that there was no significant difference in survival rate between treatment T<sub>3</sub> 2000 inds. m<sup>-2</sup> with control treatment T<sub>4</sub> 68.5%, but there was a highly significant difference between T<sub>5</sub> and T<sub>3</sub> treatment shown in Table 1 and Figure 3.

In group 3 (1.5-2.5 g), weight gain decreased with an increase in worm density from 250 to 1000 inds. m<sup>-2</sup>. However, no significant difference in WG was observed among treatments fed fish feces and uneaten feed, whereas the highest belonged to T<sub>1</sub> with 75.2% and the lowest to T<sub>2</sub> (73.04%) (Table 1 and Figure 2). But a high significant difference was observed between T<sub>5</sub> commercial treatment 123.1% with other treatments fed fish feces and uneaten feed. The specific growth rate of group 3 of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> was 0.60%, 0.59%, 0.52%, -0.33% and 0.86% respectively. The survival rates of T<sub>1</sub> and T<sub>2</sub> are the same as 94%. The lowest survival among treatments fed fish feces and uneaten feed was observed in T<sub>3</sub> (85%). However, all of this treatment had a significant difference in survival from the control (T<sub>4</sub> 77%).

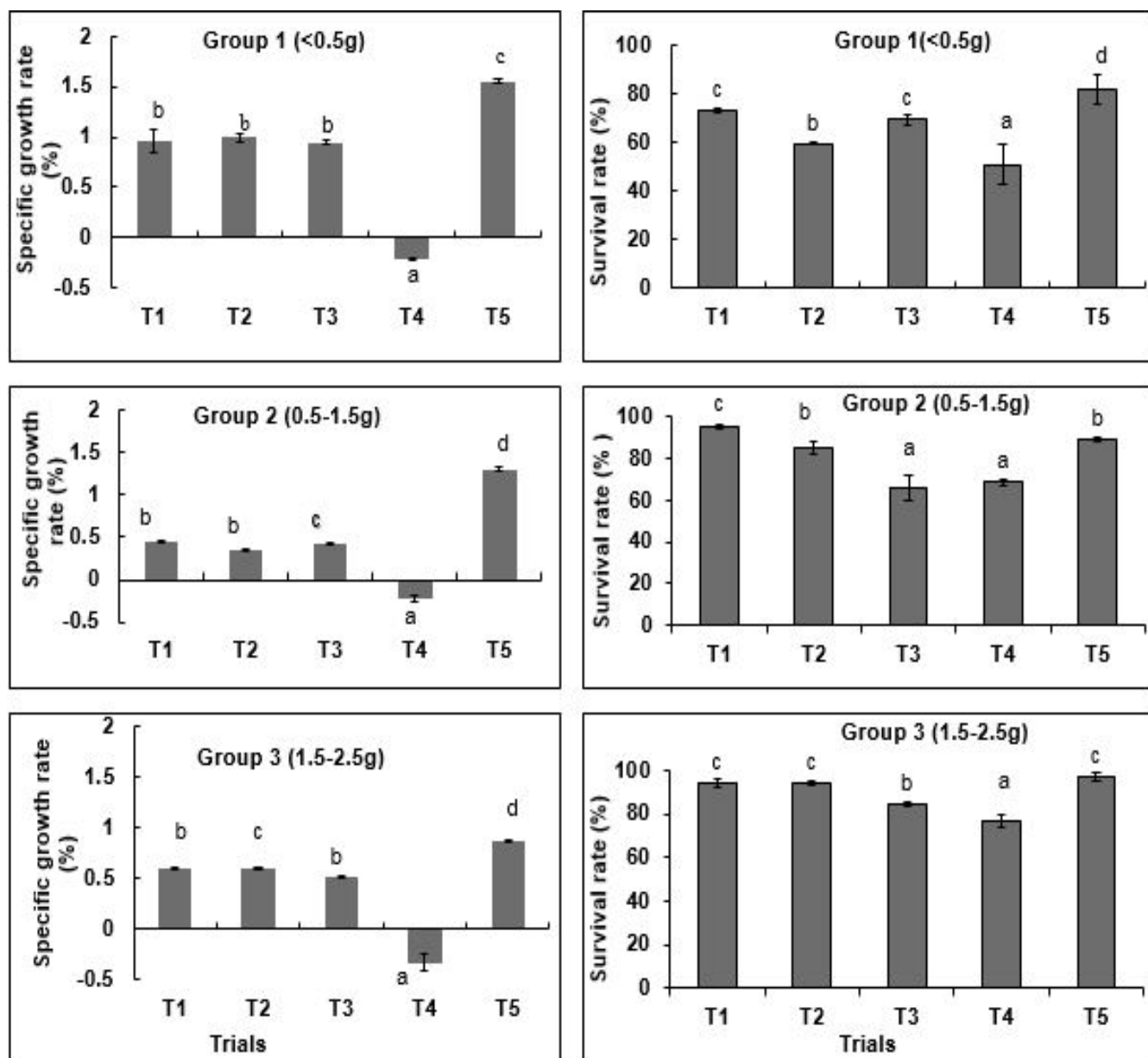
### Growth of olive flounder fish in flow through system

Weight gain (WG), specific growth rate (SGR) and survival rate (SR) of different groups and different sizes of olive flounder, *P. olivaceus* are shown in Table 2. In group 2, fish (with 0.5-1.5 g worm) weight gain of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 734.8%, 713.8% and 706.7%, respectively, and no significant difference was found in weight gain among of all fish treatments (p>0.05). But the average weight gain of fish (with 0.5-1.5 g size worm) of group 2 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 worm). The specific growth rate of fish in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> of group 1 (with <0.5g size worm) was 2.31%, 2.28% and 2.37%, respectively, which were higher than that of group 2 (with 0.5-1.5 g size worm) and that of group 3 fish. At the end of experiment, the highest survival rate (SR) 100% was observed in T<sub>1</sub> and T<sub>3</sub> treatment, and SR was 93% in T<sub>2</sub>. The highest survival rate was found in fish of group 2 (T<sub>1</sub> 100%, T<sub>2</sub> 96% and T<sub>3</sub> 100%), which was comparatively higher than the survival rate of T<sub>1</sub> and T<sub>3</sub> group of fish.

### Discussion

In this study, the weight gain of rockworms in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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<sub>4</sub> 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<sup>-2</sup> feed fish-feces and uneaten feed. On the other hand, in all the T<sub>5</sub> treatment supplied with commercial feed, WG and SGR were found to be higher 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 was found in Honda et al. [16], being between 0.45 and 1.66% day<sup>-1</sup> in *P. nuntia vallata* fed on flounder feces, and 3.23% day<sup>-1</sup> 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<sup>-1</sup> when the polychaete worm, *Nereis virens*, fed on waste from a recirculating system with juvenile Atlantic halibut *Hippoglossus hippiglossus* [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<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> of group 1 (<0.5 g)



**Figure 3:** Specific growth rate and survival rate of different sized polychaete worm group 1 (<0.5 g): (T<sub>1</sub> -1000 inds.m<sup>2</sup>; T<sub>2</sub> 2000 inds.m<sup>2</sup>; T<sub>3</sub> 4000 inds.m<sup>2</sup>; T<sub>4</sub> 2000 inds.m<sup>2</sup>; T<sub>5</sub> 1000 inds.m<sup>2</sup> and T<sub>5</sub> 1000 inds.m<sup>2</sup>; and worm Group 3 (1.5 to 2.5 g): T<sub>1</sub> 250 inds.m<sup>2</sup>; T<sub>2</sub> 500 inds.m<sup>2</sup>; T<sub>3</sub> 1000 inds.m<sup>2</sup>; T<sub>4</sub> 500 inds.m<sup>2</sup> and T<sub>5</sub> 500 inds.m<sup>2</sup> in flow through system. (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> feed was feces of fish and uneaten feed; T<sub>4</sub> was controlled-no feed and T<sub>5</sub> was commercial feed).

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). The density 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 T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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 arenaceodentata* [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. arenaceodentata* [20], *D. aciculata* [12,19].

In the olive flounder fish study, the weight gain of fish in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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 (with 1.5-2.5 g size worm). The specific growth rate of fish in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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 T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> of group

Worm size	Treatment (indv.m <sup>2</sup> )	Initial wt <sup>1</sup> (g)	Final wt <sup>2</sup> (g)	Wt gain <sup>3</sup> (%)	SGR <sup>4</sup> (%/day)	Survival rate <sup>5</sup> (%)
Grp 1 <0.5 g	T <sub>1</sub> 1000	0.44 ± 0.05 <sup>a</sup>	1.06 ± 0.13 <sup>c</sup>	152.7 ± 11.8 <sup>b</sup>	0.96 ± 0.11 <sup>b</sup>	73.5 ± 8 <sup>c</sup>
	T <sub>2</sub> 2000	0.34 ± 0.05 <sup>a</sup>	0.84 ± 0.13 <sup>b</sup>	153.8 ± 12.9 <sup>b</sup>	0.99 ± 0.05 <sup>b</sup>	59.5 ± 5 <sup>b</sup>
	T <sub>3</sub> 4000	0.34 ± 0.05 <sup>a</sup>	0.81 ± 0.11 <sup>b</sup>	140.3 ± 4.5 <sup>b</sup>	0.94 ± 0.02 <sup>b</sup>	69.5 ± 9 <sup>c</sup>
	T <sub>4</sub> 2000 <sup>x</sup>	0.34 ± 0.05 <sup>a</sup>	0.28 ± 0.05 <sup>a</sup>	-18.4 ± 0.9 <sup>a</sup>	-0.22 ± 0.01 <sup>a</sup>	51.2 ± 7 <sup>a</sup>
	T <sub>5</sub> 2000 <sup>y</sup>	0.35 ± 0.05 <sup>a</sup>	1.49 ± 0.23 <sup>d</sup>	321.7 ± 4.1 <sup>c</sup>	1.55 ± 0.02 <sup>c</sup>	82.2 ± 6 <sup>d</sup>
Grp 2 0.5-1.5 g	T <sub>1</sub> 500	1.08 ± 0.13 <sup>a</sup>	1.64 ± 0.12 <sup>c</sup>	51.7 ± 1.9 <sup>c</sup>	0.45 ± 0.014 <sup>c</sup>	95.1 ± 6 <sup>c</sup>
	T <sub>2</sub> 1000	1.13 ± 0.13 <sup>a</sup>	1.57 ± 0.12 <sup>b</sup>	30.2 ± 17 <sup>b</sup>	0.35 ± 0.017 <sup>b</sup>	85.5 ± 4 <sup>b</sup>
	T <sub>3</sub> 2000	1.08 ± 0.12 <sup>a</sup>	1.58 ± 0.09 <sup>b</sup>	46.6 ± 1.8 <sup>c</sup>	0.41 ± 0.013 <sup>c</sup>	66.1 ± 8 <sup>a</sup>
	T <sub>4</sub> 1000 <sup>x</sup>	1.06 ± 0.21 <sup>a</sup>	0.86 ± 0.12 <sup>a</sup>	-19.1 ± 2.8 <sup>a</sup>	-0.22 ± 0.34 <sup>a</sup>	68.5 ± 5 <sup>a</sup>
	T <sub>5</sub> 1000 <sup>y</sup>	1.09 ± 0.21 <sup>a</sup>	3.65 ± 0.37 <sup>d</sup>	236 ± 6 <sup>d</sup>	1.30 ± 0.020 <sup>d</sup>	89.3 ± 5 <sup>b</sup>
Grp 3 1.5-2.5 g	T <sub>1</sub> 250	2.16 ± 0.19 <sup>a</sup>	3.78 ± 0.21 <sup>c</sup>	75.2 ± 1.4 <sup>c</sup>	0.60 ± 0.007 <sup>c</sup>	94.2 ± 5 <sup>c</sup>
	T <sub>2</sub> 500	2.16 ± 0.2 <sup>a</sup>	3.74 ± 0.19 <sup>c</sup>	73.04 ± 1.3 <sup>c</sup>	0.59 ± 0.008 <sup>c</sup>	94.5 ± 7 <sup>c</sup>
	T <sub>3</sub> 1000	2.14 ± 0.2 <sup>a</sup>	3.48 ± 0.3 <sup>b</sup>	62.6 ± 1.1 <sup>b</sup>	0.52 ± 0.007 <sup>b</sup>	85.3 ± 9 <sup>b</sup>
	T <sub>4</sub> 500 <sup>x</sup>	2.14 ± 0.23 <sup>a</sup>	1.61 ± 0.13 <sup>a</sup>	-15.1 ± 0.9 <sup>a</sup>	-0.33 ± 0.270 <sup>a</sup>	77.1 ± 5 <sup>a</sup>
	T <sub>5</sub> 500 <sup>y</sup>	2.13 ± 0.24 <sup>a</sup>	4.76 ± 0.26 <sup>d</sup>	123.1 ± 2.2 <sup>d</sup>	0.86 ± 0.011 <sup>d</sup>	97.5 ± 4 <sup>c</sup>

<sup>1</sup>IW: Initial Weight.

<sup>2</sup>FW: Final Weight.

<sup>3</sup>WG (Weight Gain %): [(final weight. - initial weight.)/initial weight.] × 100.

<sup>4</sup>SGR (Specific Growth Rate %): [(log final weight - log initial weight.)/day] × 100.

<sup>5</sup>SR (Survival Rate %): (final individuals / initial individuals) × 100.

<sup>x</sup> T<sub>4</sub> : Control treatment-no feed.

<sup>y</sup> T<sub>5</sub> : Commercial feed treatment.

**Table 1:** Growth and survival of rockworm *M. sanguinea* in different groups in the flow through system fed on flounder fish-feces, uneaten fish feed and commercial feed at 13-week.

Fish size	Treatment (indv.m <sup>2</sup> )	Initial wt <sup>1</sup> (g)	Final wt <sup>2</sup> (g)	Wt gain <sup>3</sup> (%)	SGR <sup>4</sup> (%/day)	Survival rate <sup>5</sup> (%)
Grp 1 <0.5 g	T <sub>1</sub> 1000	8.39 ± 1.12	64.45 ± 0.78	668.7 ± 18.5	2.22 ± 0.03	93
	T <sub>2</sub> 2000	8.33 ± 1.02	64.37 ± 1.13	673.4 ± 28.1	2.22 ± 0.04	93
	T <sub>3</sub> 4000	8.32 ± 1.06	64.93 ± 0.59	680.8 ± 17.2	2.23 ± 0.02	98
Grp 2 0.5-1.5 g	T <sub>1</sub> 500	8.40 ± 1.12	70.11 ± 11.8	734.8 ± 28.3	2.31 ± 0.04	100
	T <sub>2</sub> 1000	8.48 ± 1.04	66.06 ± 8.98	713.8 ± 8.60	2.28 ± 0.01	96
	T <sub>3</sub> 2000	8.42 ± 1.04	70.95 ± 8.58	706.7 ± 13.1	2.37 ± 0.02	100
Grp 3 1.5-2.5 g	T <sub>1</sub> 250	8.38 ± 1.03	67.51 ± 0.78	705.7 ± 9.50	2.27 ± 0.01	100
	T <sub>2</sub> 500	8.42 ± 1.02	65.44 ± 8.21	678.9 ± 12.1	2.23 ± 0.02	90
	T <sub>3</sub> 1000	8.43 ± 1.08	68.52 ± 8.65	712.6 ± 4.4	2.28 ± 0.01	100

<sup>1</sup>IW: Initial Weight.

<sup>2</sup>FW: Final Weight.

<sup>3</sup>WG (Weight Gain) (%): [(final weight - initial weight)/initial weight] × 100.

<sup>4</sup>SGR (Specific Growth Rate) (%): [(log final weight - log initial weight)/day] × 100.

<sup>5</sup>SR (Survival Rate) (%): (final individuals / initial individuals) × 100.

**Table 2:** Growth and survival rate of the olive flounder (*Paralichthys olivaceus*) in the flow through system at 13-week.

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 of growth performance and survival rate, it was found that in the flow through system, around 8 g size olive flounder fish of group 2 (with 0.5-1.5 g size worm) could grow better than those of group 1 (with <0.5 g size worm) and those of group 3 (with 1.5-2.5 g size worm) at the optimum density of polychaete rockworm 1000-2000 indv.m<sup>2</sup>.

## Conclusion

In this study, rockworms in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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 T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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 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 indv.m<sup>2</sup> 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 indv.m<sup>2</sup> polychaete.

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