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Salinity Effects on Growth and Survival of the Polychaete Rockworm *Marphysa sanguinea* (Montagu, 1813) Juveniles and Adults

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Abstract

Two experiments were conducted to investigate the effects of salinity on growth and survival of the polychaete rockworm *Marphysa sanguinea* (Montagu 1813) at the juveniles and adults stages under controlled laboratory conditions. The effects of different salinities 15, 20, 25, 30 and 35 psu on the survival and growth rates of *M. sanguinea* juveniles and adults was examined in two separate experiments in a semi-recirculating system. This study was examined over a three-month period in the Fisheries Science and Technology Center of Pukyong National University, South Korea. Experiments were conducted in plastic boxes of sizes L 40 cm × W 24 cm × H 28 cm has filled with sediment mixed with oyster shells 70% (5 mm) and sand 30% (2 mm). To identify the growth and survival rate monthly and end of the 3rd month, data was collected for juveniles and adults. In both experiments, the highest survival rate has found at 25 psu and lowest in 35 psu salinity. The juveniles and adults' polychaetes have shown the highest weight gain in 30 psu salinity. These results have obtained that *M. sanguinea* proved to be an excellent candidate for aquaculture under a suitable salinity range of 25-30 psu. The salinity showed a significant effect not only on the growth and survival rate, but also on the biochemical components of *M. sanguinea* adults. These results of optimum salinity ranges will support for mass production of this rockworm which is highly demands in the world for sport fishing and live food for aquaculture.

Keywords: Polychaete rockworm; *Marphysa sanguinea*; Salinity; Growth; Survival

Introduction

Salinity is one of the important factors which can affect all phases of life cycle of the marine invertebrates, particularly on species inhabiting in the intertidal, estuarine and coastal regions [1]. There are several studies have been demonstrated the effect of salinity on invertebrates in different stages of life cycle including crustaceans [2,3], barnacles [4], mollusks [5], gastropods [6] and other invertebrate species [7-9]. The rockworm, *Marphysa sanguinea* lives in deep borrows, 25-40 cm into the intertidal mudflats. This polychaete attaining a maximum length of about 40-50 cm and it is a gonochoric species with an annual iteroparous reproductive strategy [1].

Worldwide, it is increasing the commercial use of polychaetes in aquaculture farm. Polychaetes with commercial purposes are normally collected from the natural population. *M. sanguinea* is an euryhaline polychaete species with a wide distribution. Several kinds of researches have been conducted on this species such as on *M. sanguinea* Complex [10]; on the appropriate feeding rate [11]; on the effects of rearing density of *M. sanguinea* species [12]; on the reproductive cycle of *M. sanguinea* [13]; about the rearing conditions on the artificial seed production of *M sanguinea* [14,15]; on the diagnostic characters [16]; and also on the salinity effects of this polychaete *M. sanguinea* [1]. Eurihaline animals cannot immune to the effects of salinity changes and the altered salinity reduces their growth and survival rate [17] and also reduced their fecundity [18].

At the optimal range of salinity, polychaete *M. sanguinea* can represent the maximum growth and survival rate. There is limited information available concerning the optimum salinity ranges for maximum growth of this polychaete. Therefore, the objective of this study was to determine the effects of different salinities on growth and survival and biochemical components of *M. sanguinea* juveniles. In particularly, this is the first study about effect of salinity on the growth, survival rate and biochemical components of *M. sanguinea* adults. With this purpose in mind, a small-scale experiment was conducted in order to find out an important aspect of the role of salinity on the life cycle of *M. sanguinea.* This could be useful to identify a suitable salinity for culture and apply these results in the field at a commercial aquaculture.

Potential use of polychaetes

The rockworm *Marphysa sanguinea* (Montagu) that belongs to the Eunicidae family is important bait for fisheries and sport fishing in Korea [15]. It is one of the most widespread polychaete species with a high economic value and day by day increasing the demand. It is used as bait organisms in fish angling industry with wide markets from Asian to European countries as well as USA [19,20]. Japan is the biggest importer in Asia with 1000 tons a year since 1969 [21] with 25 types of live fishing bait worms including 19 species of polychaete [22]. The polychaetes are commercially important because of using as bait for recreational fishing and as food source for penaeid crustaceans and finfish in aquaculture [23]. It is leading to the development of small but economically viable aquaculture facilities providing a supply of different species [24] for the uses of different purposes. The ecological role of polychaetes in marine benthic communities is very important [25]. The physical disturbance and the return of heavy metals to the surface, rendering them biologically available, as well as the release of ammonia and phosphorus compounds from the sediments leading to eutrophication effects on the habitat that

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are of greater concern [26]. The polychaetes are known to be good indicators of species richness [27] and as bio-indicators of the marine environment [25]. The use of rockworms in poly-aquaculture to reduce waste water nutrient loads, while simultaneously cultivating them has been attempted [28,29].

Materials and Methods

Polychaetes collection

For the first experiment, juvenile polychaetes *M sanguineae* were collected from the artificial seed production hatchery of Fisheries Science and Technology Center of Pukyong National University, Goseong, South Korea. After raising, 4500 individuals of juvenile initial average weight of 0.0063 ± 0.004 g were subjected to the experimental conditions. In the second experiment, 525 adult polychaetes initial average weight of 2.005 ± 0.001 g was obtained from the same hatchery, where they had been cultured for the past 2 years.

Experimental design

Fifteen plastic boxes of sizes L 40 cm × W 24 cm × H 28 cm were prepared for both adult and juvenile worms. Same conditions were used in each box for both experiments**.** The outlet pipes were covered by 1 mm mesh size nets to prevent worms from escaping. On the other hand, the overflow pipes were kept without nets so that the rockworms live in the bottom layer of the sediment. Each prepared box was filled with sediment containing 70% oyster shells and 30% sand to a depth of 8 cm for juveniles and 15 cm adults. The plastic bottles for juveniles and adults both were filled with water to a depth of 20 cm. Five salinity levels of 15 psu, 20 psu, 25 psu, 30 psu and 35 psu were used in triplicate. Each box was filled with 300 individual worms for the juveniles' experiment and 35 individual worms for adults' experiment. Different water salinity was prepared by mixing appropriate portions of freshwater (tap water stored for not less than a week) and filtered seawater which was pumped from the Jaran Bay, adjacent to the Fisheries Science and Technology Research Center (FSTC). The rockworms were acclimatized for 48 hours at the selected salinity. The filtered sea water salinity 35 psu was gradually decreased to 15 psu within 8 days by adding the tap freshwater and salinity decreasing rate was 5 psu at every 2 days.

In the experiment, a semi-recirculation system was maintained which is described in Figure 1. Within all replicates, supplemental aerations were provided to maintain dissolved oxygen. The pH and water temperature are also maintained in the experimental plastic boxes. In juveniles' experiment dissolved oxygen, pH and water temperature was maintained 8.19 ± 0.07 mg L^{-1}, 8.31 ± 0.01 and 20~22°C respectively. On the other hand, in adults' experiment dissolved oxygen, pH, and water temperature was maintained 7.1~7.73 mg L^{-1}, 7.05~8.14 and 20°C respectively. Water was changed by weekly 30% and monthly 100% for juveniles and for adults it was weekly 50% and monthly 100%. Worms were kept under a 12:12 hour dark: light photo-period and a water flow rate of 2 L $minute^{-1}$. The significant effects of salinity on biochemical components of *M. sanguinea* adults also detected from the laboratory.

Composition of experimental diet and feeding trials

The rockworms were fed with a formulated diet (Table 1) once every three days. Juvenile rockworms were fed fish fry powder which was manufactured by INVE Company Limited, Thailand. The feed powder composition was crude protein 55%, crude fat 13%, calcium 1.6%, phosphorus 1.4%, crude fiber 1%, crude ash 10.8%, sodium 0.7%, and omega 3 was 35 mg g^{-1} dry wt basis. On the other hand, adult rockworms were fed shrimp feed pellet which was manufactured by Dong-A One Feed Company Limited, South Korea. The commercial shrimp feed diets extruded pellet (EP) size was 2.4 mm to 2.6 mm and the feed contained crude protein 38%, crude fat 5%, calcium 1.2%, phosphorus 2.7%, crude fiber 4% and crude ash was 17%.

Sample collection

Data was collected at the end of 1st and 3rd culturing month in both juveniles and adult's polychaete *M sanguinea* experiments. During data collection, worms in all replicate's salinity levels were counted and survival and weight gain of the individuals were measured by a Mettler Toledo analytical balance and a scale respectively.

Statistical Analyses and Calculations

The normality and homogeneity of variance of data was confirmed by Kolmogorov-Smirnov test. Statistical significance differences measured parameters computed using one-way ANOVA by SPSS 15 software for windows- SPSS Inc., Chicago, IL, USA [30]. Significant differences among treatments ($p<0.05$) were evaluated by the Duncan's Multiple Range Test [31]. Proximate composition analyses of experimental diets were performed by the standard methods of Association of Official Analytical Chemists-AOAC [32]. 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. For evaluation of the effects of salinity on growth rate in juveniles and adults, homogeneity of slope was measured, and covariance analysis was conducted. Data was transformed to logarithmic scale for significance testing of each slope using the growth rate slope. Regression analysis data for salinity was also transformed to a natural logarithmic scale in order to compare the slopes.

Growth performance and survival rate were assessed by the following formulae:

WG -Weight gain (%): (Final weight - initial weight) × 100/ Initial weight

SR-Survival rate (%): Number of survivors at the end) × 100/Initial number of worms stocked

Results

Growth and survival of juveniles

Weight gain (WG), specific growth rate (SGR) and survival rate (SR) of juvenile *M. sanguinea* rock worms are shown in Table 2 and Figure 2. Weight gain of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 0.7%, 8%, 40%, 43% and 44% respectively after 1 month of culture period. There was no significant difference of weight gain was observed between 25 psu, 30 psu and 35 psu. But significant difference was found between these groups and 15 psu and 25 psu groups. The weight gain of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was -20%, 9%, 59%, 70%, and 58% respectively after 3rd month of culture period. Height weight gain was found in 30 psu. There was no significant difference of weight gain which observed between 25 psu and 35 psu. But significant difference was found between these groups and 15 psu and 20 psu groups. The specific growth rate (SGR) of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 0.02%, 0.14%, 0.49%, 0.53% and 0.55% day^{-1} respectively after 1 month of culture period (Table 2). After the 3rd month of culture period specific growth rate of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was -0.10%, 0.05%, 0.23%, 0.26% and 0.22% day^{-1} respectively.

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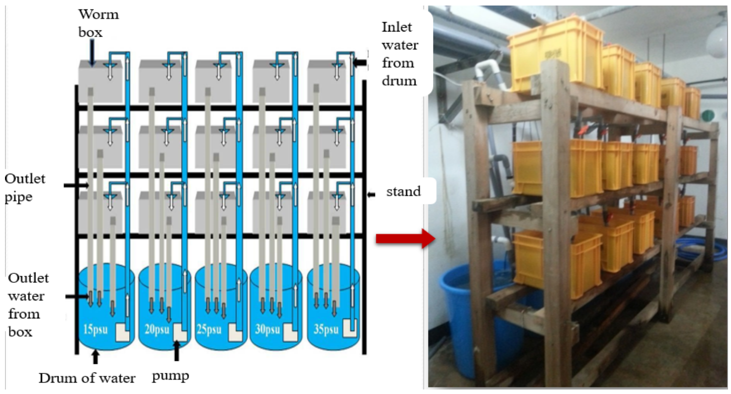

Figure 1: Schematic diagram of experimental semi-recirculation system at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu.

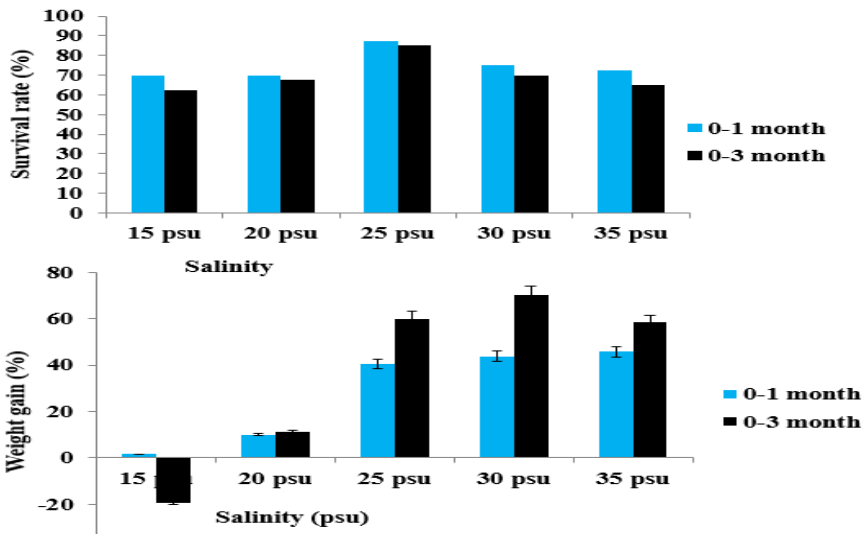

Figure 2: Weight gain and survival of *M. sanguinea* juveniles cultured at different salinities.

Survival rate (SR) of juvenile were detected from different salinities are shown in Figure 2 and Table 2. The survival rate of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 70%, 70%, 88%, 75% and 73% respectively after 1 month of culture period. There was no significant difference in survival rate between groups after the first month (*p>0.05*). It may be difficult to examine the effects of salinity for only one month. On the other hand, after 3rd month of culture period the survival rate of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 63%, 68%, 85%, 70% and 65% respectively. At the end of the experiment, the highest survival rate of 85% was exhibited at 25 psu, while the lowest survival rate of 62% was at 15 psu and 65% was at 35 psu (Table 2 and Figure 2).

Growth and survival rate of adult *M sanguinea*

Weight gain, specific growth rate and survival rate of adult *M. sanguinea* (2yrs) rock worms are shown in Table 2 and Figure 3. Weight gain of adults at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 38%, 100%, 130%, 160% and 132% respectively after 3rd month of culture period. Height weight gain was found in 30 psu which was significantly higher than other salinity groups. There was no significant difference of weight gain was observed between 25 psu and 35 psu. Lowest weight gain was found in 15 psu group of polychaete adults. The specific growth rate (SGR) of adults at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was -0.01%, 0.89%, 1.01%, 1.09% and 1.09% day^{-1} respectively after 1 month of culture period (Table 2). After the 3rd month of culture period specific growth rate of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 0.51%, 0.85%, 0.79%, 0.90% and 0.99% day^{-1} respectively. Height specific growth rate was found in 30 psu and 35 psu. After one month of culture period, the survival rate of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 34%, 40%, 71%, 43% and 31% respectively. The survival rate of juveniles at 15 psu, 20 psu, 25 psu, 30 psu and 35 psu was 31%, 43%, 60%, 54% and 26% respectively after 3rd month of culture period.

Biochemical studies of adult *M sanguinea*

At the end of experimental period, biochemical components of *M. sanguinea* at different salinities were analyzed for adults as shown in Figure 4. Polychaetes rockworm recorded the highest crude ash at 35 psu, lowest crude fat and protein at 15 psu and 25 psu. Result of analysis showed the highest water content in worms found at 15 psu. Crude fat, the major energy source was high at 30 psu and 35 psu. In 25 psu, the adults *M. sanguinea* showed lower crude fiber in the biochemical study.

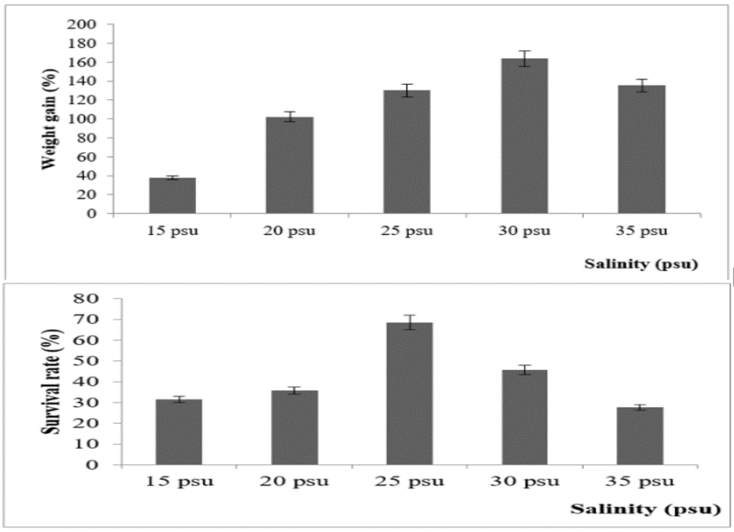

Figure 3: Weight gain and survival rate of *M. sanguinea* adults cultured at different salinities.

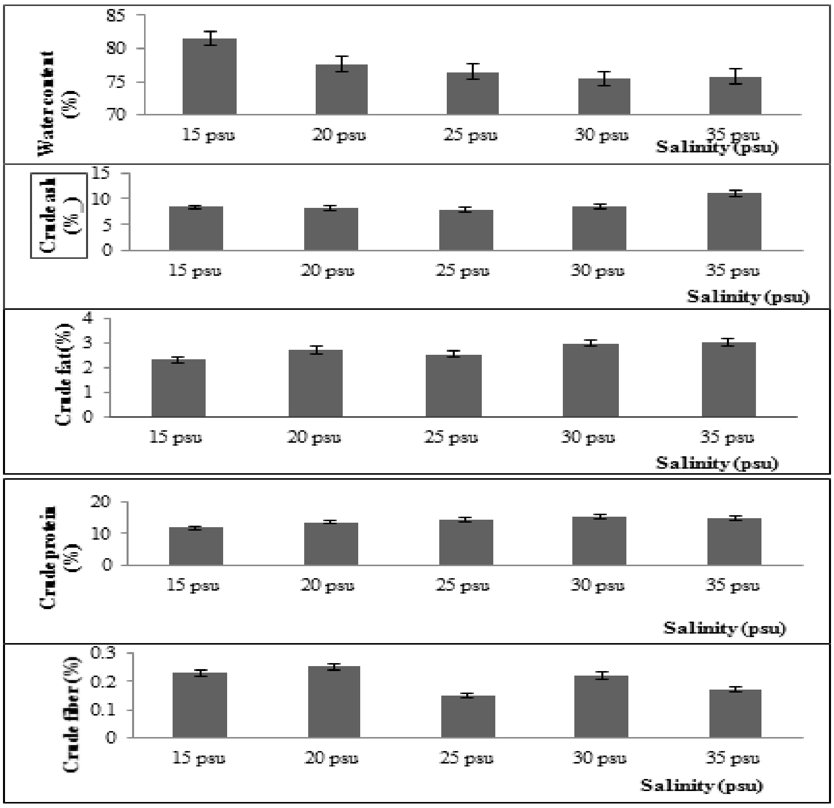

Figure 4: Biochemical components of *M. sanguinea* adults at different salinities level.

Discussion

Salinity effects on growth and survival of juveniles *M. sanguniea*

In the present study, the salinity has been affected in growth and survival rate of juvenile *M. sanguniea*. There was no significant difference in survival rate between groups after the first month ($p>0.05$). It may be difficult to examine the effects of salinity for only one month. At the end of the experiment, the height level of weight gain has been found in the salinity of 30 psu, because the juveniles have felt better at this salinity level. On the other hand, negative weight gain was found at 15 psu, because the juveniles cannot survive comfortably at this lower salinity as shown in Table 2 and Figure 2. Eurihaline animals cannot

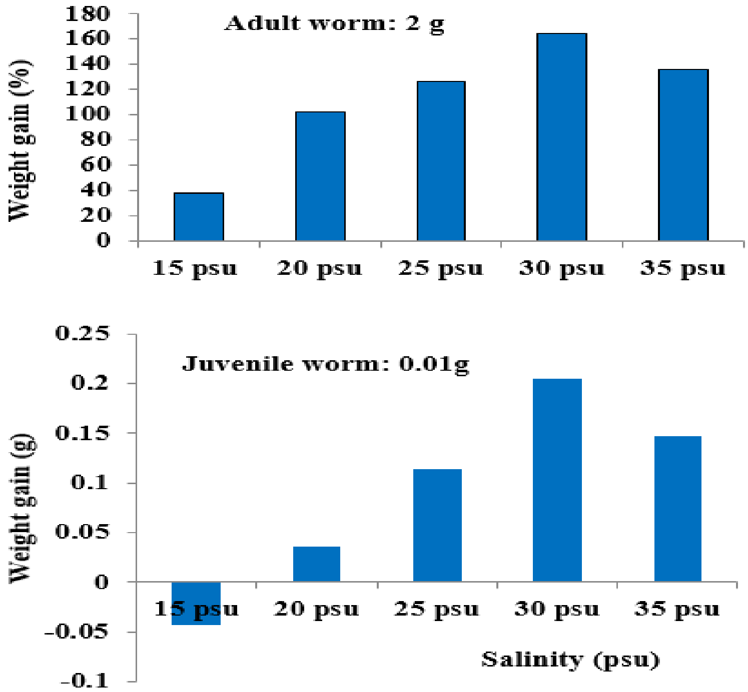

Figure 5: Weight gain of adults and juveniles polychaete *M. sanguinea* after 3rd month.

Ingredients	Feed for juvenile polychaete	Feed for adult polychaete
Feed	Fish fry powder*	Shrimp feed pellet**
Crude protein	55%	38%
Crude fat	13%	5%
Calcium	1.6%	1.2%
Phosphorus	1.4%	2.7%
Crude fibre	1%	4%
Crude ash	10.8%	17.00%
Sodium	0.7%	-
Omega 3	35mg g^{-1} dry wt basis	-

*****Fish fry powder:** Pellet Feed Manufactured by INVE Company Limited, Thailand.
******Shrimp feed pellet**: Manufactured by Dong-A One Feed Company Limited, South Korea.

Table 1: Dietary contents of pellet feed formulation of the experimental diets for *M. Sanguinea* juveniles and adults.

Variables	Month (Period)	15 psu	20 psu	25 psu	30 psu	35 psu
SGR (%/day)						
Juvenile	0-1 months	0.02	0.14	0.49	0.53	0.55
	0-3 months	-0.10	0.05	0.23	0.26	0.22
Adult	0-1 months	-0.10	0.89	1.01	1.09	1.09
	0-3 months	0.51	0.85	0.79	0.90	0.99
Survival rate (%)						
Juvenile	0-1 months	70.0	70.0	87.5	75.0	72.5
	0-3 months	62.5	67.5	85.0	70.0	65.0
Adult	0-1 months	34.3	40.0	71.4	42.9	31.4
	0-3 months	31.4	42.9	60.0	54.3	25.7

Table 2: Specific growth rate and survival rate of juvenile and adult stage of *M. sanguinea* at different salinity for 3 months.

immune to the effects of salinity changes and the altered salinity reduce their growth and survival rate [17]. There was no significant difference of weight gain has observed between 25 psu and 35 psu. But significant difference has been found between these groups and 15 psu and 25 psu groups due to the salinity tolerances. And after 3rd month of culture period height specific growth rate of juveniles has shown at 30 psu. So, in the case of weight gain and growth rate of juveniles 25-30 psu has shown the height growth and also the height specific growth rate. After 3rd month of the experiment, the highest survival rate has been exhibited at 25 psu, while the lowest survival rate has been detected at 15 psu and 35 psu as shown in Figure 2. Some researchers have found that the growth rate in the larvae and juvenile stages of polychaete *Capitella sp* has shown very slow growth in lower salinity at 12~15 psu and it has been happened especially for salinity affects and as well as poor feeding [33]. It has been declared that the polychaete *Perinereis rullieri* exhibited low growth and survival in low salinity [34]. The low salinity is considered as a fatal and growth-inhibiting factor for polychaete larvae has also been declared [1,35,36].

Tolerance of lower salinity is depending on different age groups of polychaete rockworms. Though the adult polychaete has the euryhaline salinity tolerance, but at the adult's stage of its life cycle can be different from that in the juvenile stage. In this research, *M. sanguinea* juveniles has shown the lower survival rate in high salinity as shown in Figure 3. Contrary to the low survival rate at high salinity, but weight gain has shown the height level, it might be cause of food monopoly exhibited by the few surviving heads.

Salinity effects on growth and survival of adults *M. sanguniea*

After 3rd month of the experimental period, height weight gain of adult polychaete was found in 30 psu which has significantly higher than other salinity groups. And the lowest weight gain has been found in 15 psu groups as shown in Figure 3. It might be happened due to salinity tolerances of polychaetes. It has been demonstrated that most of the adult polychaetes has shown more tolerance in the salinity fluctuations [37]. Adults rockworm generally lives in intertidal region and has the experience a variety of environmental changes like salinity and temperature. From our study, after 3rd month of culture period, the height specific growth rate of adults has found in 30 psu and 35 psu groups. It might be happened for the comfortable salinity and also for intra specific competition of food and space. It has been declared that rockworm *Nereis sp* has shown the lower gross energy demand for production and higher gross growth efficiency has shown in high salinity 30 psu rather than in low salinity which is similar to this experiment [38].

In the present study, after 3rd month of experimental period, the height survival rate has found at 25 psu groups and lowest has been found at 35 psu groups of polychaete. Altered salinity can reduce the growth and survival rate of polychaetes [17] and also can reduce their rate of fecundity [18]. Brackish-water polychaete, *Diopatra variabilis*, has shown the higher survival rate in relatively high salinity by Krishnamoorthi [37]. Tolerance to variation of salinity is higher in adults than in early life-historical stage of rockworms. In this study, for both juveniles and adults polychaete the highest survival rate has observed at 25 psu, while the highest growth has been observed at 30 psu as shown in Figure 5. This may suggest that high survival and growth rates has been exhibited in slightly lower salinity compared to the natural salinity conditions [38].

Conclusion

In conclusion, these studies demonstrate that both the juveniles and adults *M. sanguinea* has shown the highest survival rate at 25 psu and lowest in 15 psu and 35 psu salinity. The juveniles and adults also have shown the highest weight gain in 30 psu salinity. These results have obtained that *Marphasia sanguinea* proved to be an excellent candidate for aquaculture under a suitable salinity range of 25-30 psu. So, the salinity showed a significant effect not only on the growth and survival rate, but also on the biochemical components of *M. sanguinea* adults. These results of optimum salinity ranges will support for mass production of this rockworm which is highly demands in the world for sport fishing as well as live food for aquaculture.

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