Thesis for the Degree of Master of Fisheries Science

# EFFECT OF CULTURE SUBSTRATES ON THE GROWTH AND SURVIVAL OF ROCKWORM (*Marphysa sanguinea*)

by

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KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

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# EFFECT OF CULTURE SUBSTRATES ON THE GROWTH AND SURVIVAL OF ROCKWORM (*Marphysa sanguinea*)

# 바위털갯지렁이 치충의 성장과 생존에 미치는 사육기질의 영향


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by

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ON THE GROWTH AND SURVIVAL OF

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**EFFECT OF CULTURE SUBSTRATES ON THE GROWTH AND SURVIVAL OF ROCKWORM (*Marphysa sanguinea*)**

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**Abstract**

Rockworm *Marphysa sanguinea* is a new and high market value animal in aquaculture. It is used in medicines; animal feed and fish bait industry and thus calling for mass production through intensive eco-friendly aquaculture. In this study, individual worms were cultured under controlled conditions in a semi re-circulating aquaculture system in oyster shells and sand culture substrate at different concentrations (%) with a constant water flow and aeration. Three sets of experiment were conducted: (1). Juvenile rockworm individuals, single water supply system and commercial pellet feed, (2). Adult worms, independent water supply system and waste water, (3). Comparison of water quality in culture units with and without worms. In the first set, 6 trials were designed at the following concentrations of substrates; A, 100% sand; B, 80% sand + 20% oyster shells; C, 60% sand + 40% oyster shells; D, 40% sand + 60% oyster shells; E, 20% sand + 80% oyster shells and F, 100% oyster shells. Growth rate increased in the increase in oyster shells concentrations (%) in culture units for each trial as outlined; A, 40±5.2; B, 55±9.3 ; C, 53±0.5; D, 62±8; and E,

68±10; however, the trend dropped sharply in treatment (F) to 15.1±6.8; however, treatment F had higher concentrations of phosphate, nitrates and nitrites than other trials. Therefore growth performance increased significantly ($P<0.05$) with increasing concentration (%) of oyster shells. The trend for survival rate did not correspond directly with the concentration (%) of the substrates as outlined; A, 68±7; B, 40±4; C, 58.5±12; D, 46.5±5; E, 83.5±19, and F, 60±24 and thus showed no statistical difference ($P>0.05$). The second set was conducted to investigate the cause of the drop in treatment F below the control experiment; 4 trials were designed as follows; A: 0% oyster shells, 100% sand (control); B: 25% oyster shells, 75% sand; C: 50% oyster shells, 50% sand and D: 100% oyster shells and growth rate (%) for each trial was as follows; A, -17; B, -12; C, -6, and D, 1 while survival was; A, 91%; B, 90%; C, 91% and D, 96%. In this experiment water quality was well controlled and the trends of fluctuations of different components were almost similar in all trials. Water quality parameters which included; temperature, salinity, DO, pH, TSS, COD, $NO_3$-N, $NO_2$-N, $NH_3$-N and $PO_4$-P were analysed. From the analyses of these physical-chemical components, $NH_3$-N was generated in culture units in the first week of rearing and then $NO_3$-N and $NO_2$-N appeared in higher concentrations after 2 weeks and disappeared in the third week, which might have been due to different growth patterns of nitrifying bacteria. Treatments which had higher concentrations nitrogenous compounds especially nitrite for a longer time showed poor growth rate of the worms. *M.sanguinea* also showed higher and positive growth rates when feeding on commercial pellet feeds and low growth was recorded in the experiment where rotifer farm waste water was used as feed. However, the trend of growth generally increased with an increasing concentration of oyster shells in the culture medium regardless of the feed eaten. The trend of water quality components was almost similar in all treatments with and without worm samples, which was an indication that the difference in growth or survival was not due to water quality but the type of substrate.

# 1. INTRODUCTION

## 1.1. Background

Rockworm *Marphysa sanguinea* is among more than 55 species described in the genus *Marphysa* (Hutchings and Karageorgopoulos, 2003). It is recorded as a cosmopolitan species, distributed globally from temperate and tropical latitudes, and lives in brackish and marine water (Hartmann-Shroder and George 1971; Hutchings and Karageorgopoulos, 2003; Previdelli et al., 2007).

Rockworm is also one of new animals in aquaculture. Its high market value and numerous uses in medicines, animal feed and fish bait industry call for intensive production through eco-friendly aquaculture, and it is mainly produced in South Korea (Olive, 1994). The commercial interest in polychaetes arises from their value as bait for recreational fishing and as feed for penaeid crustaceans and finfish in aquaculture (Olive, 1994). *M. sanguinea* and other *Marphysa* species are often used as bait by fisherman in regions where they are abundant (Pettibone, 1963; Hutchings and Karageorgopoulos, 2003; Skilleter et al., 2006).

In Portugal, rockworm is locally known as “ganso” and “minhocao″ and is one of the most appreciated bait species due to its high endurance on the hook and ability to attract valuable fish species; its price per liter ranged between €150-200 in 2007 to €200-300 in 2008 (Garces and Pereira, 2010).

*M. sanguinea* is a gonochoric species being either male or female, and its population reproduces sexually, and is capable of producing multiple eggs clutches throughout the lifetime, a process called iteroparity (Previdelli et al., 2007). It is also a large benthic polychaete attaining a maximum length of 40-50 cm (Previdelli et al., 2007; Garces and Pereira, 2010). Like other worms belonging to the phylum Annelida, the body of *M. sanguinea* is divided into numerous segments which are known as setigers and small scarlet-red hairs located on either side of the segment called setae (Ruppert and Fox, 1988). This worm is also an omnivore feeding on a wide range of food (Ruppert and Fox, 1988). All species of *Marphysa* genus that colonize soft substrates are burrowers and this ecological strategy is enhanced by the strong muscular anterior region (Previdelli et al., 2007). The burrowing and pumping activities in the sediment helps them to establish a suitable microhabitat for its survival, and enables it to avoid predators and competition. The worms make tunnels in the sediment that are kept open and oxygenation is increased by their vertical movements that allow a water circulation (Previdelli et al., 2007).

In near shore coastal marine environments, complex benthic habitats such as sea grass and oyster reefs possess a suite of spatial and ecological characteristics which makes assessment of the effects of habitat variability on animal abundance possible (Bell et al., 1995; Eggleston et al., 1999). For estuarine macro-fauna (for example; post larval and early juvenile stages of shrimp and crabs, amphipods, polychaetes, bivalves, gastropods, etc.), the spacing of sea grass and oyster shell among unstructured, soft-bottom habitat is relatively large (1–100 m) compared to their body size (0.5–10 mm), thus mixtures of sediment, sea grass and oyster reefs may directly or indirectly impact on its population through a variety of mechanisms such as alteration of predators (for example; shrimps and fish) distribution, abundance, and foraging behavior (Bell and Hicks, 1991; Irlandi, 1994). *M. sanguinea* like other polychaetes lives in a variety of benthic environments, including soft muddy sediments around oyster reefs, sandy or muddy tidal flats, on other fouling organisms on dock pilings and buoys, and inside holes of calcareous rocks (Ruppert and Fox, 1988; Fauchald, 1992a). It is also common in a mixture of both clay and sand, under stones, in crevices of rocks, oyster beds, roots of sea grass (Zostera), sponges and on wooden pilings from intertidal zone to a depth of about 200 meters, and lives in well-defined burrows lined with mucus, mud and sand (Hartmann-Shroder and George, 1971).

*M. sanguinea* is among the key species in Sado estuary (Portugal) and functions as a major constituent of the benthic biomass of mudflats as well as an important food item for crustaceans, fishes and waders (Castro, 1993).

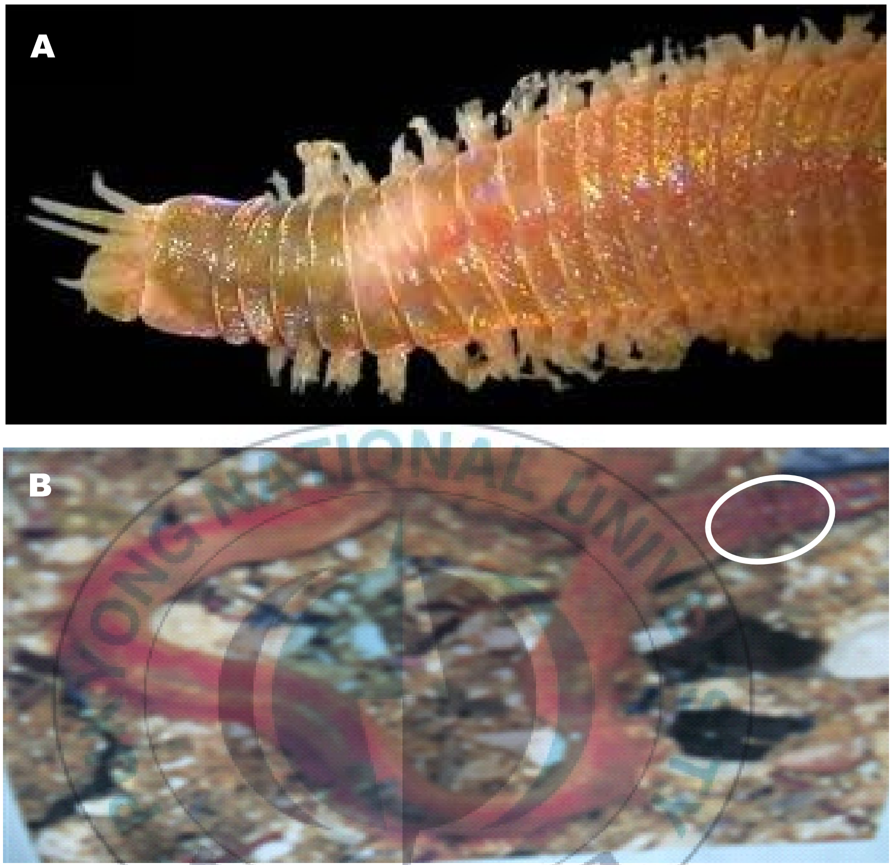


Fig. 1. External body features of rockworm. (A), Micrograph showing **s**egments which are known as setigers and small hairs on either side of the segment called setae. (B), White circle on the photograph shows muscular anterior region, it is useful for movement and burrowing in sediment. (Source of pictures: http://researcharchive.calacademy.org).

## 1.2. Hypothesis

The growth and survival rate of the Rockworm *Marphysa sanguinea* is not different in oyster shells and sand culture substrates, and the oyster concentration in the trials does not impact the growth and survival of the worm.

## 1.3. Problem statement

Studies on the lifecycle, habitats, effects of temperature and salinity on growth and the distribution of *M. sanguinea* have been conducted and documented (Eggleston et al., 1999; Previdelli et al., 2007; Garces and Pereira, 2010), but few studies on the effect of culture substrates particularly oyster shells and sand substrates on the growth and survival of rockworm have been done. As it is one of the new animals in aquaculture, more literature and information is required to enhance its cultivation and management.

## 1.4. Objectives

I. To compare the growth and survival rate of rockworm *M. sanguinea* in oyster shells and sand culture substrates.

II. To analyze some water quality parameters which affect the growth and survival of the rockworm in sand and oyster shells culture medium.

## 2. METHODS AND MATERIALS

Culture experiments were carried out at the Fisheries Science and Technology Center, Pukyong National University at Goseong-gun, while water quality analyses were conducted at Marine Environmental Biology laboratory in Department of Marine Bio-materials and Aquaculture at Pukyong National University, Busan, Korea.

Three sets of experimentation were conducted:

i. Juvenile worms - single water supply – pellet feed

   The worms were fed on commercial pellet feed, with replicates in the same treatment sharing water supply in a semi re-circulating aquaculture system design.

ii. Adult worms – Independent water supply – waste water

   The worms were fed on waste water from rotifer farm with each replicate having an independent water supply, and also designed in semi re-circulating system.

iii. Adult worms / without worms – waste water

Water quality effect confirmatory experiment was carried out to verify whether the differences in the growth and survival of the worms resulted from water quality or the substrates where they were raised.

2.1. Juvenile worms - pellet feed - single water supply system

This experiment constituted six treatments with two replicates for each treatment, thus making a total of 12 replicates. This experiment was designed in a semi re-circulating aquaculture system with a single water supply system for each treatment. It was designed in this way to enhance the analysis of the cause of either the differences or similarities in the growth and survival of the juvenile worms among the six treatments. The experiment was conducted for 40 days, 5 kg of sand and oyster shells of 1-2 mm diameter were used as the culture substrates and were mixed at different concentrations (%) in four treatments, and two other treatments were not mixed, that is; the one containing 100% sand which was the control experiment and the one of 100% oyster shells were not mixed. The substrates were put in culturing boxes of 27 cm width, 45 cm length and 11 cm height for each replicate. A total 30 individual juvenile worms weighing between 4.5 to 10 grams were stocked in each box. Continuous aeration and constant water flow was maintained, and submersible water pumps and heaters were installed in the water tanks of each treatment to enhance water recirculation and temperature control, respectively.

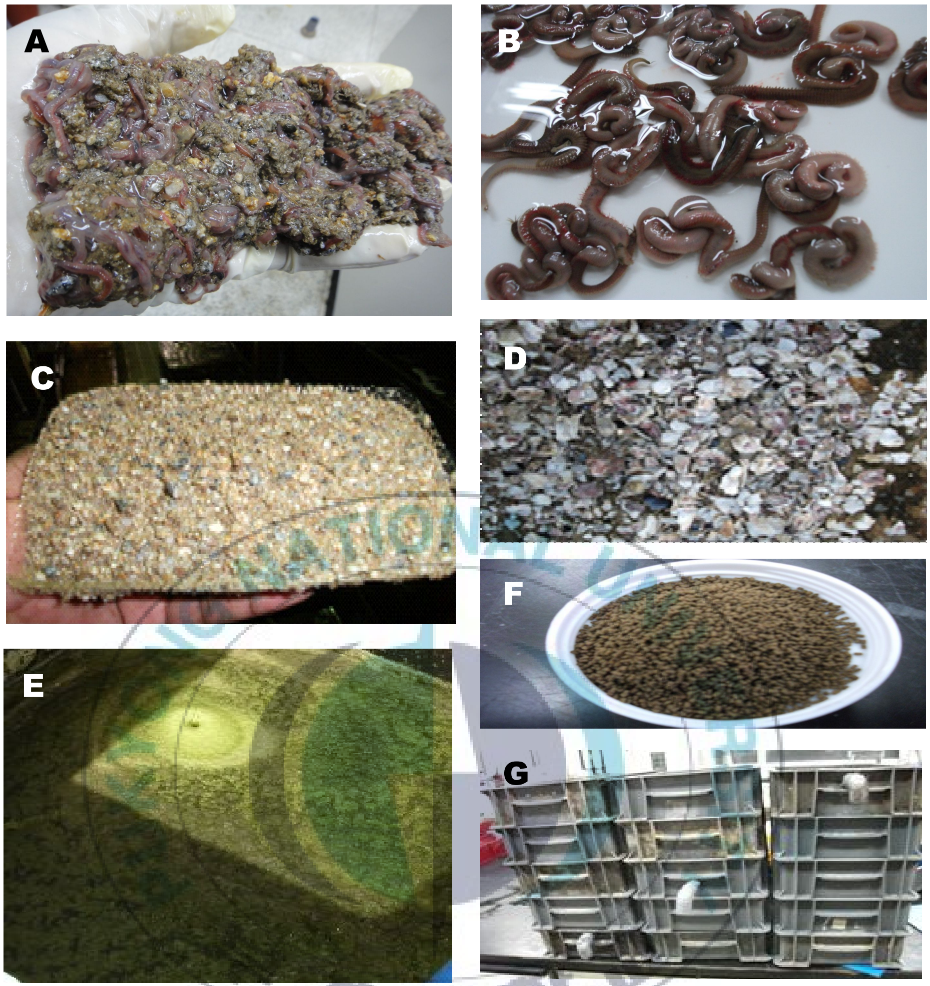


Fig. 2. Photographs of materials used. Rockworm samples (A-B), substrates (C-D), feeds (E-F) and culturing units (G). (A), Juvenile worms. (B), Adult worms. (C), Sand. (D), Oyster shells. (E), Waste water. (F), Commercial pellet feed. (G), Culture boxes.

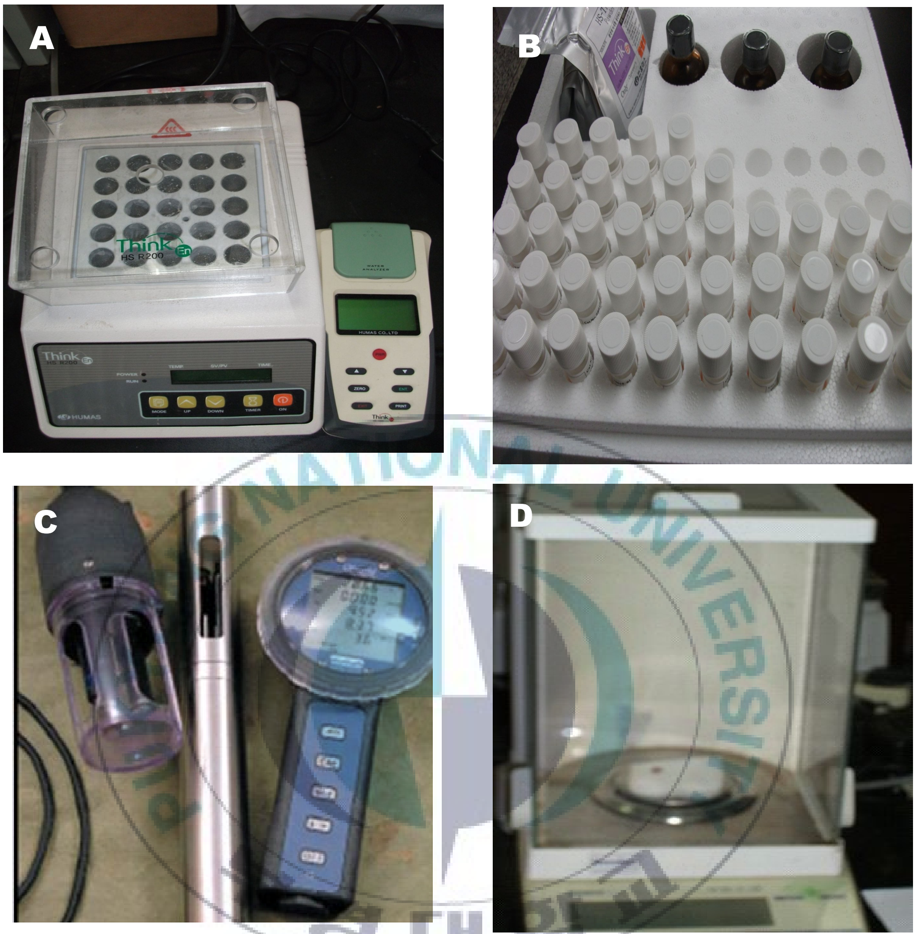


Fig. 3. Photographs of some apparatus used. Water quality analysis; (A-C) and body weight measurement; (D). (A), Water test kit. (B), Water testing reagents. (C), Multi-parameter analyzer. (D), weighing scale.

2.1.1. Design of experiment

Six treatments were designed in accordance with Heo (2011) and the concentrations of the substrates were as follows; A: 100% sand (control experiment), B: 80% sand + 20% oyster shells, C: 60% sand + 40% oyster shells, D: 40% sand + 60% oyster shell, E: 20% sand + 80% oyster shells and F: 100% Oyster shells. The experiment was subjected to continuous water flow and aeration, and water quality parameters including dissolved oxygen, salinity, temperature, pH, COD, TSS, ammonia, nitrate, nitrite and phosphates were sampled, analyzed and recorded on a weekly routine. The water temperate ranged between 19℃ and 20.5℃, and controlled by an adjustable water heater and there was continuously aeration in all treatments with dissolved oxygen (DO) ranging from 6.5 to 8.5 mg/l and pH ranged between 7.5 and 8.5, while salinity ranged from 32 to 39 psu and worms were fed at 5% of their initial body weight using commercial pellet feeds

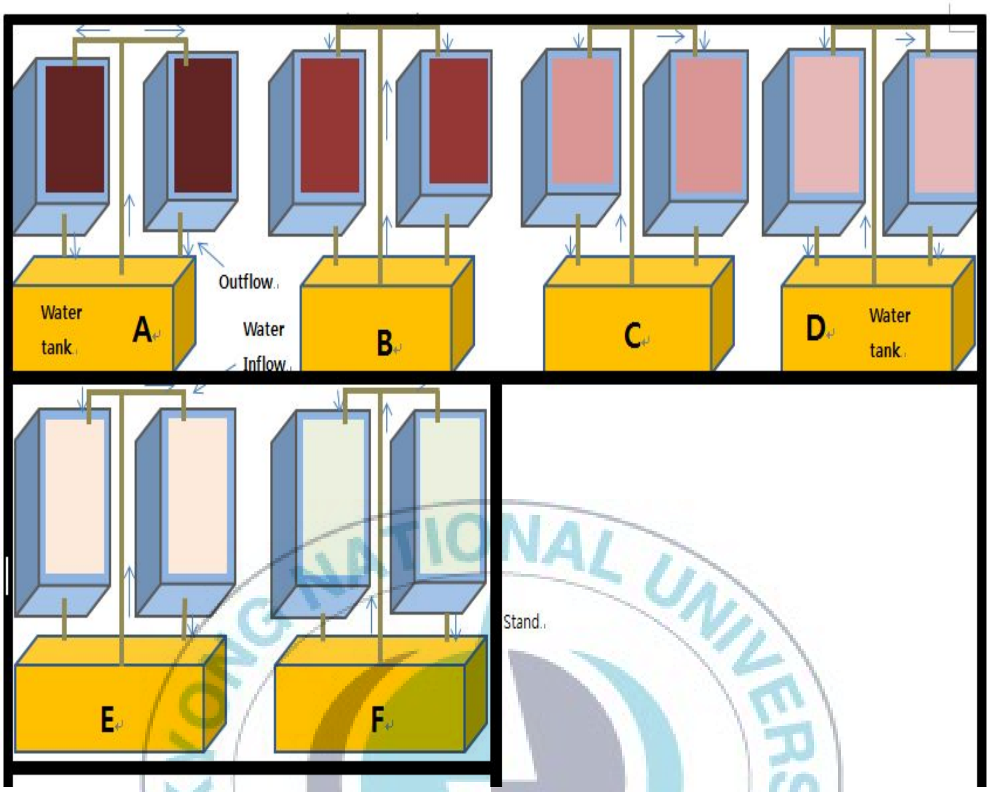


Fig. 4. Design and layout of the single water supply systems. Replicates in each treatment used the same water tank to feed it with water in a semi-recirculating flow system. The decreasing intensity of brown color in culture boxes from treatments A-F indicates decreasing concentration (%) of sand and increasing oyster shells substrate. A, 100% sand; B, 80% sand + 20% oyster shells; C, 60% sand + 40% oyster shells; D, 40% sand + 60% oyster shells; E, 20% sand + 80% oyster shells, and F, 100% oyster shells.

Fig. 5. Photograph of the design of the single water supply system for each treatment. Two replications shared the same water supply system as shown in the diagram of the experiment layout in Fig. 4.

2.1.2. Water quality analysis

A water test kit and reagents from Humas company were used to analyze chemical oxygen demand (COD), total suspended solids (TSS), ammonia nitrogen ($NH_3$-N), nitrite nitrogen ($NO_2$-N), nitrate nitrogen ($NO_3$-N), and phosphate phosphorus ($PO_4$-P) and a multi - parameter instrument (Quanta–G) of Hach-Hydrolab from Shinhantech company in Korea was used for measuring temperature, salinity, pH and dissolved oxygen; these apparatus are showed in Fig. 3. Water components were analyzed using specific methods as follows; TN and $NO_3$-N were tested using cadmium reduction method, N-(1-napthyl)-ethylenediamine (NED) was used for testing $NO_2$-N; $NH_3$-N was tested using cyanuric acid and Nessler method, and TP and $PO_4$-P were tested using ascorbic acid and molybdo-vanadate, and the chemical oxygen demand was analyzed using reduction digestion method.

2.2. Adult worms - waste water - independent water supply system

Waste water was collected from Aquanet rotifer farm at Tongyeong located in southern part of Korea. The water was used as a food for the worms, and 8 L of waste water were mixed with 40 L of seawater in culture boxes.

2.2.1. System design

Treatments were aligned in a completely randomized design. This experiment was composed of four treatments; each had three replicates, hence making a total of 12 replicates. Treatments also contained different concentrations of culture substrates, as follows; A, 100% sand (control experiment); B, 25% oyster shells + 75% sand, C, 50% oyster shells + 50% sand and D, 100% oyster shells.

Thirty individual adult worms weighing between 119 and 149 g were stocked into culture boxes of 30 cm length, 15 cm width and 48 cm height. The boxes were fitted with a sieved outlet pipe at the bottom and continuous water flow and aeration in a semi re-circulating aquaculture system.

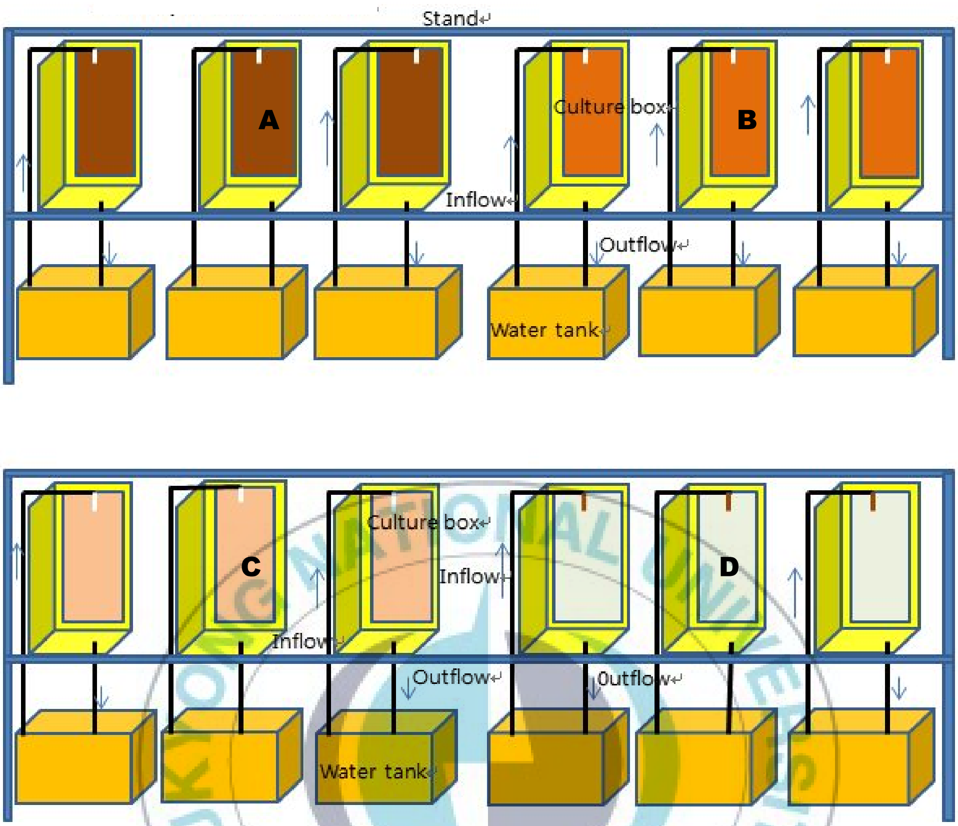


Fig. 6. Design and layout of independent water supply system. Each replication in each of the four treatments used an independent water tank to feed it with water in a semi-recirculating flow system. The trend of intensity of brown color in culture boxes from treatment A to D indicates decreasing amount of sand and increasing oyster shells composition. (A), 100% sand; (B), 75% sand + 25% oyster shells; (C), 50% sand + 50% oyster shells.

Fig. 7. Photograph of experiment of independent water supply system. Each replication separately re-circulated its water supply in each treatment as shown in the diagram of the experiment layout in Fig. 6.

2.2.2. Water quality analysis of trials with and without worms

In each treatment of three replicates, thirty (30) individual adult worms were put in each of the two replicates and the third replicate was not stocked with worms.

Water quality parameters which were analyzed included; TN, $PO_4$-P, COD, TSS, temperature, salinity, pH, and DO. The same equipment was used in this test as those in section 2.1.2.

2.3. Data collection and processing

Rockworm juveniles were counted and weighed to get the initial number and weight for each treatment before stocking the juvenile worms in the culture system. After the growing period, the worms were again counted and weighed to get the number of survivors and the final body weight respectively; and data was computed into survival and growth rate. The survival rate was got dividing the number of survivors by the initial number of worms stocked while growth rate was got by subtracting the initial weight from the final body weight and divided by the initial weight as showed in the equations below;

$$Sr = \frac{Ni}{No}\ X\ 100 \qquad (1)$$

Where *Sr* denotes survival rate, *Ni* - for number of survivors and *No* – for initial number of worms stocked,

$$Gr = \frac{Wi - Wo}{Wo}\ X\ 100 \qquad (2)$$

Where *Gr* denotes growth rate, *Wi* - final body weight and *Wo* - initial body weight.

Water quality sampling was also carried out daily and weekly for some physical-chemical components in all treatments, this helped to analyze and quantify these parameters and associated effect on growth and survivorship of rockworm.

### 2.4. Data analysis

An analysis of data was conducted using SAS software, version 9.1.3 for one-way ANOVA test for the mean difference between treatments, and multiple comparison analysis was also carried out using Duncan's multiple range tests for comparisonwise error test between means of all treatments.

# 3. RESULTS

## 3.1. Juvenile worms – pellet feed – single water supply system

### 3.1.1. Growth and survival rate of rockworm juveniles

Growth and survival rates were calculated for each replicate in accordance with equations (1) and (2) respectively, and the average weight gain and the number of survivors for each treatment were the final growth and survival rates for the trials; A, 100% sand; B, 80% sand + 20% oyster shells; C, 60% sand + 40% oyster shells; D, 40% sand + 60% oyster shells; E, 20% sand + 80% oyster shells and F, 100% oyster shells and water quality sampling was conducted once or twice a week. Five treatments; (A-E), showed an increase in growth with increasing concentration (%) of oyster shells in the culture substrates, as follows; A, 40±5.2; B, 55±9.3; C, 53±0.5; D, 62±8; and E, 68±10; however, the trend dropped sharply in treatment (F) to 15.1±6.8, which had the highest concentration (100%) of oyster shells, and one-way ANOVA showed a statistical difference ($P<0.05$) between the means of the treatments. The growth rate in treatment (C), which contained 60% sand + 40% oyster shells, was slightly lower than that in treatment (B), which consisted of 80% sand and 20% oyster shells; however, there was a big difference in the standard deviation among replicates of the two treatments; that is, trial (B) had a standard deviation of 9.3 while trial (C) had 0.5; nonetheless, ANOVA comparisonwise analysis using Duncan's multiple range tests showed the two treatments not significantly different.

Survival rate did not increase with the increasing concentration of oyster shells in the culture units; treatments had the following rates; A, 68±7; B, 40±4; C, 58.5±12; D, 46.5±5; E, 83.5±19, and F, 60±24. The means of survival rate between treatments were not significantly different ($P>0.05$), according to one-way ANOVA test and Duncan's multiple range test showed significant difference ($\alpha<0.05$) among replicates of some treatments but the difference was not related to trend of increment of either sand or oyster shells composition. Details of statistical analysis for growth and survival of the rockworm using one-way ANOVA test and Duncan's multiple range tests are shown in, tables; 1-3, and Fig.8 and 9.

Further investigations were conducted to find out the causes of the sharp drop in growth rate of worms in treatment, F (100% of oyster shells) lower than the rate in control experiment (treatment A, 100% sand) and far below treatment E, (80% oyster shells) and two more experiments were designed and adult worms were used as culturing samples as explained in section 2.2 and 3.2.

Table 1. Growth and survival of rockworm juveniles in sand and oyster shell substrates

| Trial | Initial stock | Survivors | Initial weight | Final body wt. | Growth rate (%) | Survival rate (%) |
|---|---|---|---|---|---|---|
| A | 30 | 20.5 | 5.15 | 4.8798 | 39.9±5.2 | 68±7 |
| B | 30 | 12 | 7.5 | 4.3121 | 55.1±9.3 | 40±4 |
| C | 30 | 17.5 | 7 | 5.9692 | 52.7±0.5 | 58.5±12 |
| D | 30 | 14 | 7.65 | 5.7948 | 61.8±8.0 | 46.5±5 |
| E | 30 | 25 | 5.8 | 8.2073 | 68.2±10.0 | 83.5±19 |
| F | 30 | 18 | 7.5 | 5.1538 | 15.1±6.8 | 60±24 |

Means of six trials; Initial number of juvenile worms, number of survivors after 40 days, initial body weight and final body weight, and mean±standard deviation of growth and survival rate. Trial categories; A, 100% sand (control); B, 80% sand + 20% oyster shells; C, 60% sand + 40% oyster shells; D, 40% sand + 60% oyster shells; E, 20% sand + 80% oyster shells and F, 100% oyster shells.

Table 2. Results of one-way random effects ANOVA test for growth rate of rockworm juveniles in sand and oyster substrates

| Dependent variable: Growth rate (%) | | | | | |
|---|---|---|---|---|---|
| Source | DF | Sum of squares | Mean squares | F value | P |
| Model | 5 | 3627.504167 | 725.500833 | 13.42 | 0.0033 |
| Error | 6 | 324.405000 | 54.067500 | | |
| Corrected total | 11 | 3951.909167 | | | |

ANOVA, analysis of variance; showed a significant difference (P<0.05) in the growth rate of rockworm at different composition of sand and oyster shells substrates.

Table 3. Results of one-way random effects ANOVA test for survival rate of rockworm juveniles in sand and oyster substrates

| Dependent variable: Survival rate (%) | | | | | |
|---|---|---|---|---|---|
| Source | DF | Sum of squares | Mean squares | F value | P |
| Model | 5 | 2397.416667 | 479.483333 | 244 | 0.1541 |
| Error | 6 | 1179.500000 | 196.583333 | | |
| Corrected total | 11 | 3576.916667 | | | |

ANOVA, analysis of variance; showed no significant difference (P>0.05) in the survival rate of rockworm at different concentrations of sand and oyster shells substrates.

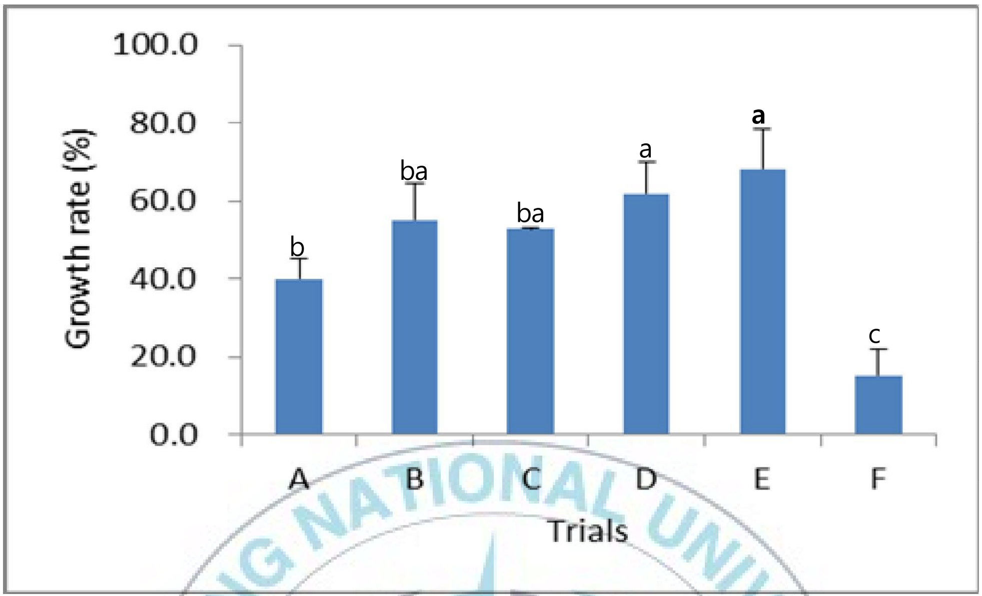


Fig. 8. Growth rates of rockworm juveniles in sand and oyster shell substrates. There was a general increment of growth in worms with increasing oyster shells content in trials (A-E), and a sharp drop in trial (F), which was attributed to poor water quality. Different letters on the bar indicate a statistical significant difference ($\alpha<0.05$) between treatments using Duncan's multiple range test, which controls type 1 comparisonwise error rate. Means±standard deviation of treatments are also shown in table 1 above. Trial categories (A), 100% sand (control); (B), 80% sand + 20% oyster shells; (C), 60% sand + 40% oyster shells; (D), 40% sand + 60% oyster shells; (E), 20% sand + 80% oyster shells and (F), 100% oyster shells.

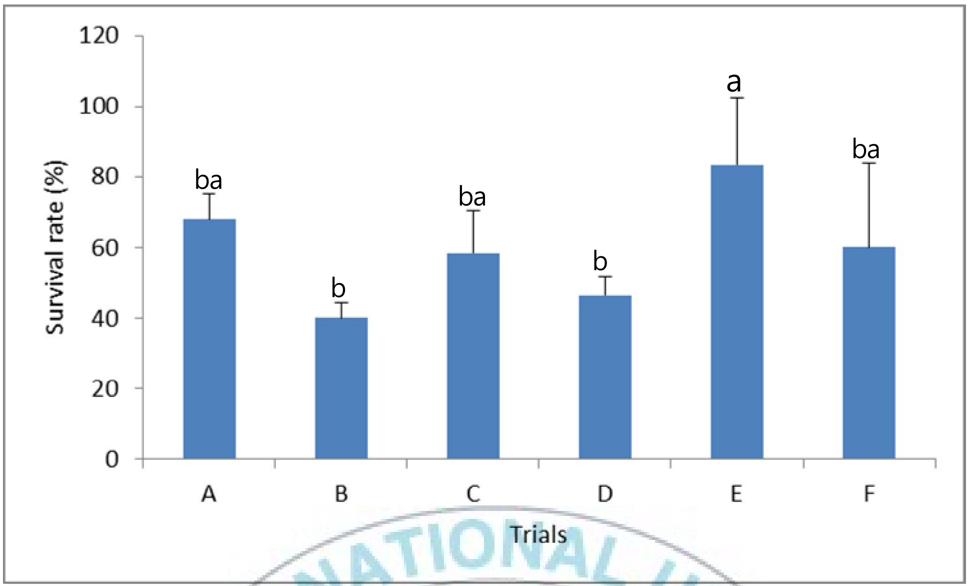


Fig. 9. Survival rates of rockworm juveniles in sand and oyster shell substrates. One-way ANOVA test showed no significant difference (*P>0.05*) between the means of all treatments and different letters on the bar indicate a significant difference (α<0.05) between means of treatments using Duncan's multiple range test. Trial categories (A), 100% sand (control); (B), 80% sand + 20% oyster shells; (C), 60% sand + 40% oyster shells; (D), 40% sand + 60% oyster shells; (E), 20% sand + 80% oyster shells and (F), 100% oyster shells.

3.1.2. Water quality analysis

Analysis of some physical–chemical components was conducted using apparatus shown in Fig. 3 (A-C) and water components including; nitrites, nitrates, phosphates, chemical oxygen demand (COD), total suspended solids (TSS), salinity, temperature, dissolved oxygen (DO) and pH were analyzed and quantified as showed in table 4.

Table 4. Concentration of water quality components in juvenile rockworm culture units

| Trial | Water quality parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Temp | pH | Sa | DO | TSS | COD | $NH_3$ | $NO_2$ | $NO_3^-$ | $PO_4^-$ |
| A | 20.1±1.0 | 8.14±0.03 | 34.9±1.0 | 6.58±0.4 | 0.96±1.0 | 3.96±1.0 | 0.16±0.2 | 0.30±0.4 | 1.02±0.8 | 0.08±0.1 |
| B | 19.9±0.9 | 8.17±0.03 | 34.8±2.3 | 6.58±0.5 | 0.98±0.9 | 3.09±1.6 | 0.33±0.4 | 0.20±0.4 | 1.12±0.8 | 0.27±0.1 |
| C | 18.8±0.8 | 8.16±004 | 34.3±2.5 | 6.18±1.6 | 0.93±1.1 | 3.24±0.8 | 0.28±0.3 | 0.25±0.5 | 1.78±1.5 | 0.37±0.1 |
| D | 19.3±0.8 | 8.16±0.03 | 34.8±2.5 | 6.4±0.4 | 0.98±0.8 | 3.70±1.4 | 0.32±0.4 | 0.28±0.7 | 1.47±1.3 | 0.35±0.1 |
| E | 19.7±1.0 | 8.19±2.07 | 34.8±2.8 | 6.6±0.4 | 1.18±1.1 | 4.61±1.5 | 0.28±0.3 | 0.15±0.2 | 1.66±1.3 | 0.52±0.1 |
| F | 19.8±1.0 | 8.19±0.05 | 35.0±2.02 | 6.6±0.3 | 1.49±0.6 | 3.96±1.5 | 0.32±0.4 | 0.50±0.6 | 2.02±1.8 | 0.66±0.2 |

Mean±STD of the concentration of water quality parameters analyzed. Trial categories (A), 100% sand (control); (B), 80% sand + 20% oyster shells; (C), 60% sand + 40% oyster shells; (D), 40% sand + 60% oyster shells; (E), 20% sand + 80% oyster shells and (F), 100% oyster shells.

Trends of water components were as described; the concentration of $NO_3$-N ranged between 1 to 7mg/l and increased sharply after the first week of culture and dropped sharply to almost zero in 2 days and then increased sharply again in the second week and stabilized in all treatments except in treatment F where it persisted for more than 2 weeks and had the highest increment from 0.1 mg/l to 6 mg/l. . Except in treatment F, $NO_2$-N concentration ranged between 0.0 to 2.5 mg/l and increased gradually in the rest of the treatments and was low between first and second week and later stabilized, which was probably due to the bacteria that were mainly converting $NH_3$-N to $NO_3$-N because $NH_3$-N was generated from the onset of culturing period as shown in Fig. 13. $NO_2$-N was higher and persisted for a longer in treatment F than other treatments (A-E). $NH_3$-N ranged between 0.0 to 1.2 mg/l and was generated from the start of culturing period, stabilized and later dropped after one week in the same pattern in all treatments this was probably because it was converted to $NO_3$-N and $NO_2$-N after first and second week, except in treatment F. Trends of $PO_4$-P concentration fluctuated throughout the culturing period in all treatments and it ranged from 0.01 to 1.2 mg/l. Higher concentration and abrupt increments and reductions occurred in treatment F where its concentration ranged from 0.4 to 1.2 mg/l. Concentration of TSS and COD fluctuated almost uniformly throughout the culturing period in all treatments (A-F). Temperature ranged between 18 to 21℃ among all treatments and was maintained by an adjustable water heater which was inserted in the water supply tank for every pair of replications in for each treatment. Levels of pH ranged between 8 and 8.5, and the trend of fluctuations was almost similar among all treatments. The salt composition in all treatments was slightly higher (34 to 40 psu) in the first 5 days because there was evaporation since the experiment was designed in a closed re-circulating system. Dilution of water was carried out throughout the experiment life in order to maintain favorable salinity levels. DO ranged between 6 and 8 mg/l and was maintained by continuous water re-circulation and aeration.

Generally $NH_3$-N decreased gradually after 2 weeks in all treatments except B; and $PO_4$-P, TSS and COD fluctuated throughout the culture period while DO, temperature pH and salinity were almost stable in all treatments throughout the culturing period, as shown in Figs. 10-19.

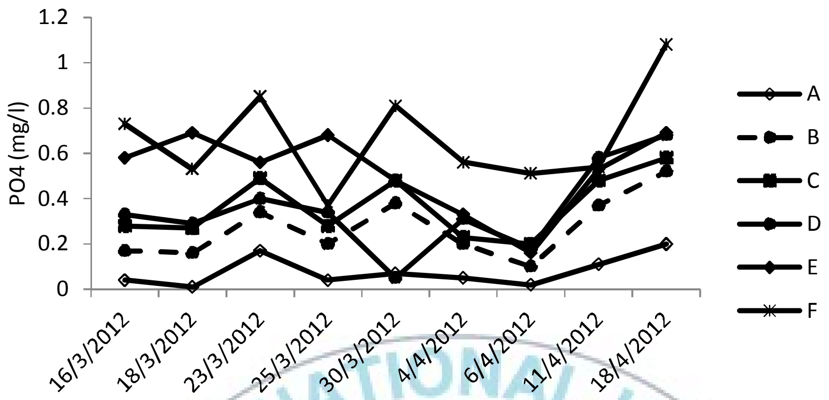


Fig. 10. Trend of the concentration of phosphate among six treatments. $PO_4$-P concentration fluctuated throughout the culturing period in all treatments. However, higher concentration and abrupt increments and reductions occurred in treatment F.

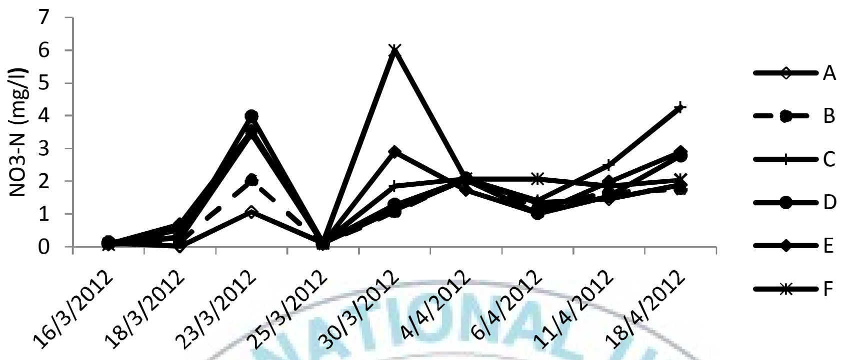


Fig. 11. Trend of the concentration of nitrate. $NO_3$-N concentration increased sharply after the first week of culture and dropped sharply to almost zero in 2days and then increased sharply again in the second week in all treatments; however, treatment F had the highest increment from 0.1 mg/l to 6 mg/l between 25$^{th}$ and 30$^{th}$ March. Between 2-3 weeks, $NO_3$-N concentration stabilized in all treatments which was possibly due to growth of nitrifying bacteria which converted $NH_3$ in the first week to $NO_3$-N and then to $NO_2$-N, as shown in Figs.12and 13.

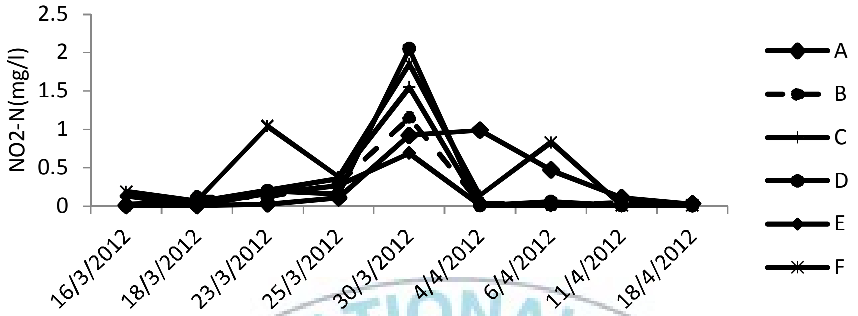


Fig. 12. Trend of the concentration of nitrite. $NO_2$-N concentration was low between first and second week, except in treatment F and this was possibly due to the bacteria that were mainly converting $NH_3$-N to $NO_3$-N because $NH_3$-N was generated from the onset of culturing period.

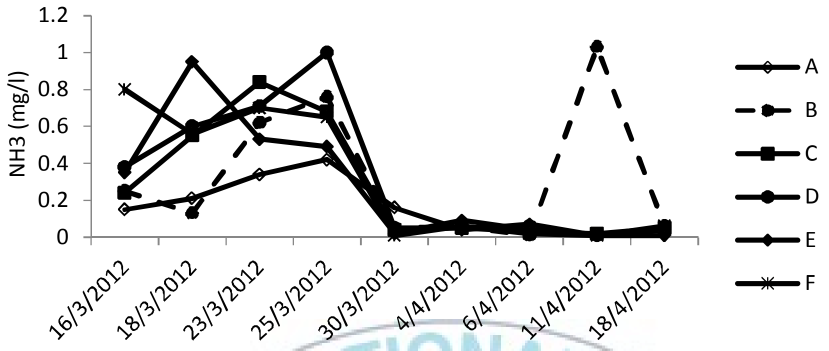


Fig. 13. Trend of the concentration of ammonia. $NH_3$-N ranged between 0.0 to 1.2 mg/l and was generated from the onset of culturing period and dropped after one week and $NO_3$-N and $NO_2$-N concentration increased after first and second week, except in treatment F (see Fig. 11 and 12).

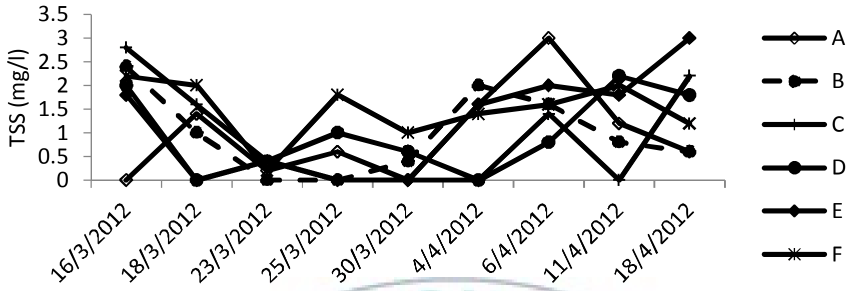


Fig. 14. Trend of the amount of total suspended solids (TSS). The concentration of TSS fluctuated almost uniformly throughout the culturing period in all treatments (A-F).

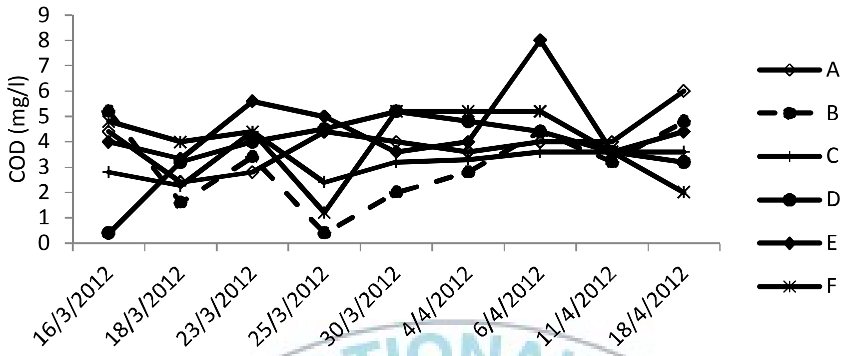


Fig. 15. Trend of chemical oxygen demand (COD). The concentration of COD in all treatments (A-F) fluctuated almost in the same manner.

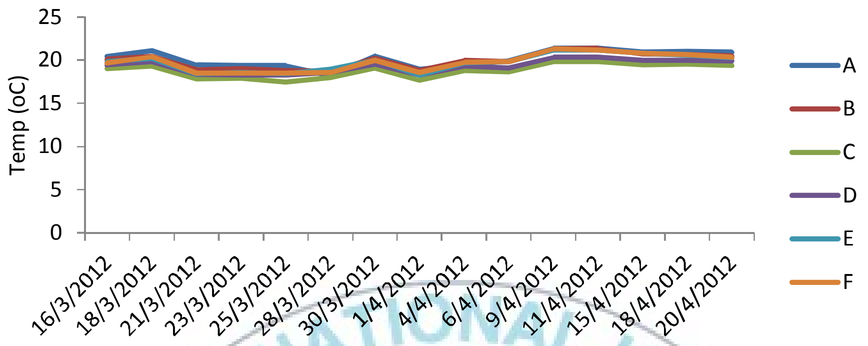


Fig. 16. Trend of temperature fluctuations. Temperature ranged between 18 to 21℃ among all treatments and was maintained by an adjustable water heater which was inserted in the water supply tank for every pair of replications in for each treatment.

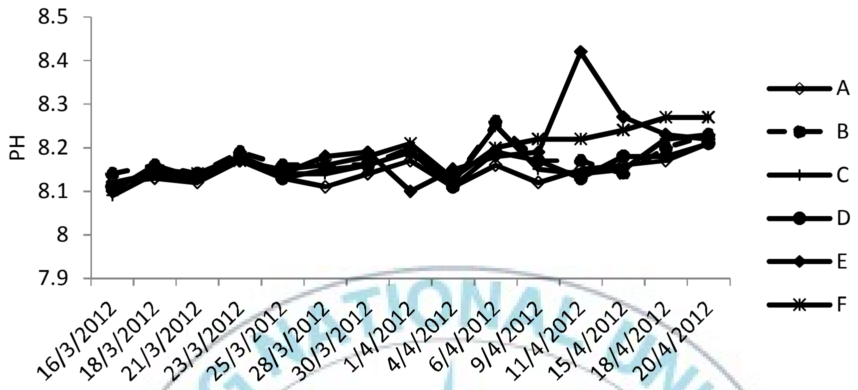


Fig. 17. Trend of pH variations. Generally pH ranged between 8 and 8.5, and the trend of fluctuations was almost similar among all treatments.

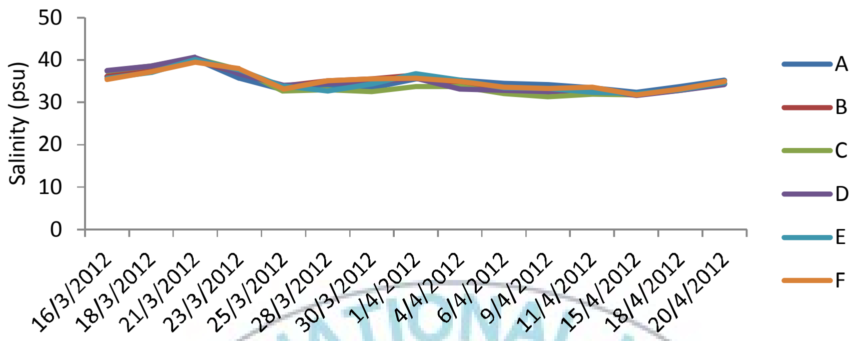


Fig. 18. Trend of variations in salinity. The salt composition in all treatments was slightly higher (34 to 40 psu) in the first 5 days because there was evaporation since the experiment was designed in a closed re-circulating system. Dilution of water was carried out throughout the experiment life in order to maintain favorable salinity levels.

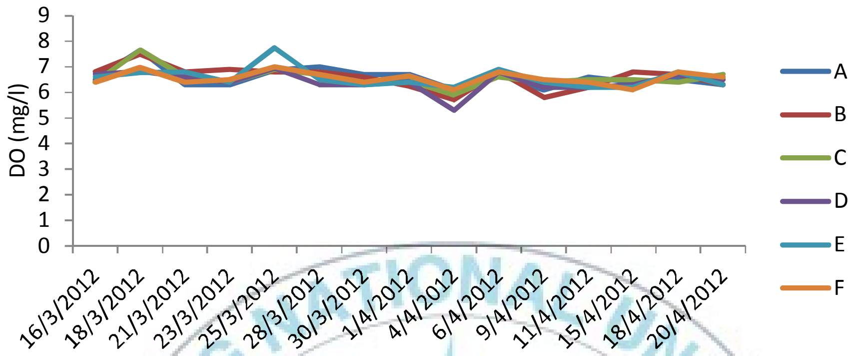


Fig. 19. Trend of the concentration of dissolved oxygen. DO ranged between 6 and 8 mg/l and was maintained by continuous water re-circulation and aeration.

3.2. waste water – adult worms – independent water supply system.

3.2.1. Growth and survival of the adult rockworm individuals.

The drop in the growth and survival of the juvenile worms in treatment F (shown in Figs. 8 and 20), necessitated further investigations and two more experiments which were conducted to investigate the trend, and these included; (1), Culturing worms using wastewater from rotifer farm as food for the worms; (2), Analyzing water quality in which two replications were containing worms and the third replication of the same treatment was without worms.

Adult worms of 6 - 14 g were stocked in culture boxes and fed on rotifer farm waste water. This experiment was carried out for only 10 days because it was meant to justify the trend in the first experiment. Unlike the first experiment which had 6 treatments, this one had four and they were as follows; A: 0% oyster shells, 100% sand (control); B: 25% oyster shells, 75% sand; C: 50% oyster shells, 50% sand and D: 100% oyster shells. Growth rate increased with the increasing concentration (%) of oyster shells as outlined; A, -17; B, -12; C, -6, and D, 1; while the pattern survival rate did not increase with increasing concentration of oyster shells, as outlined;

A, 91%; B, 90%; C, 91% and D, 96%. In this experiment, treatment D (100% oyster shells) had the highest survivorship and negative growth occurred in treatments A-C. Since this experiment was carried out to investigate the drop in trend of growth of worms which occurred in the first experiment (see section 2.1 and 3.1), and the trend of increasing growth rate with oyster concentration was confirmed by this experiment.

Table 5. Growth and survival rate of rockworm in sand and oyster shells using waste water as food

| Trial | Body weight | Survival |
|---|---|---|
| A | -17 | 91 |
| B | -12 | 90 |
| C | -6 | 91 |
| D | 1 | 96 |

Mean body weight and survivors (mean±STD). Trial categories: A, 100% sand (control); B, 25% oyster shells, 75% sand; C, 50% oyster shells, 50% sand and D, 100% oyster shells.

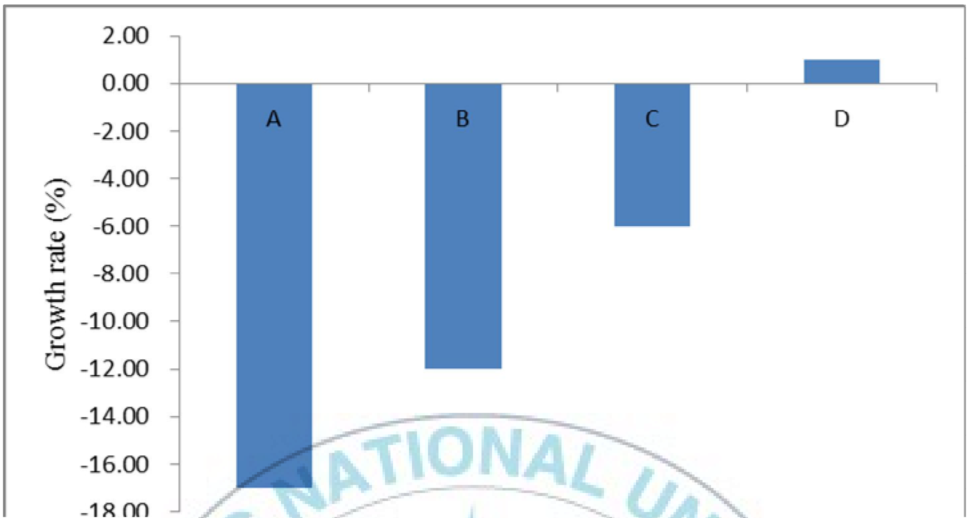


Fig. 20. Growth of the rockworm in sand and oyster shells using waste water as food. Generally, growth rate increased with the increasing concentration (%) of oyster shells. Negative growth occurred because worms were fed on waste water from a rotifer farm, and probably they could not filter food from the water; however, this experiment was carried out to examine the drop in growth of worms which occurred in the first experiment (see section 2.1 and 3.1), therefore the trend of increasing growth rate with oyster concentration was confirmed by this experiment. Trial categories: A: 0% oyster shells, 100% sand (control); B: 25% oyster shells, 75% sand; C: 50% oyster shells, 50% sand and D: 100% oyster shells, 0% sand.

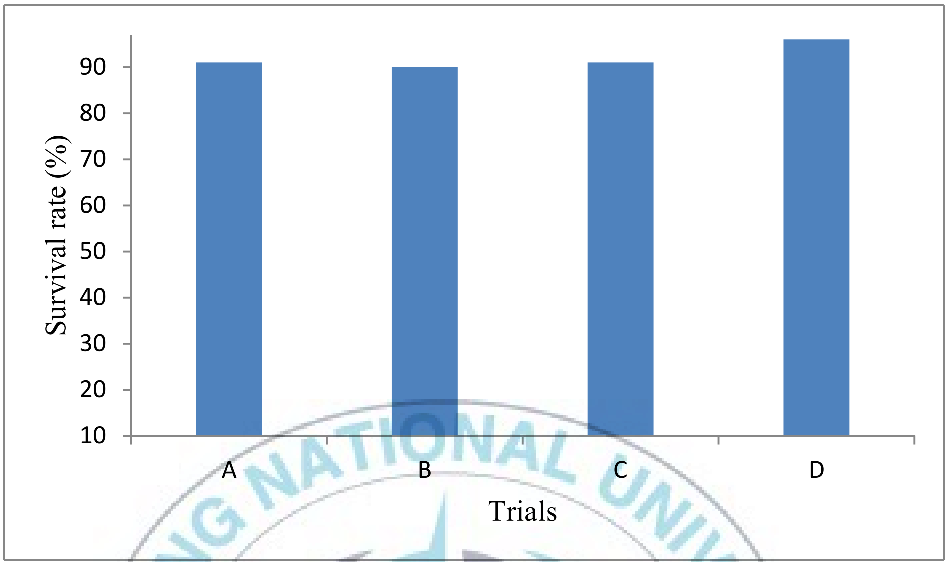


Fig. 21. Survival of rockworm using waste water as food. There was a slight increment in the number of survivors with increasing concentration of oyster shells as opposed to the results in fig. 9, section (3.1). Trial categories: A: 0% oyster shells, 100% sand (control); B: 25% oyster shells, 75% sand; C: 50% oyster shells, 50% sand and D: 100% oyster shells, 0% sand.

3.2.2. Water quality analysis for adult rockworm – waste water eexper iment

Water components were tested using apparatus shown in Fig. 4, (same as for the first experiment) and the parameters that were analyzed included; total nitrogen (TN), total phosphate (TP), chemical oxygen demand (COD) total suspended solids (TSS), temperature, salinity, pH and dissolved oxygen (DO), and their trends and fluctuations over the culturing period were as shown in Figs. 23-29.

The concentration of TN fluctuated in the same manner in all treatments; nonetheless, treatment (A) had the highest concentration than treatments (B-D). This shows that nitrogenous compounds have adverse effects on the growth performance of rockworm; as the same trend occurred in treatment F of the first experiment in section 2.1. After 2-3 weeks, TN reduced probably because nitrifying bacteria grow and convert more toxic nitrogenous compounds to less toxic ones. Variations in the level of COD were minimal as the trend of fluctuations was almost similar in all treatments. The maximum level of TSS in this experiment in one week was below 0.1 mg/l as compared to that in fig. 14 of the first experiment, which was as high as

3.5 mg/l. The concentration of $PO_4$-P was low and ranged between 0.001-0.12 mg/l and good for growth and survival of aquatic organisms as compared to the previous experiment in section 3.1 and Fig 10, in which $PO_4$-P concentration ranged between 0.1-1.2 mg/l. The range of 19℃ to 20℃ and was maintained using an adjustable water heater as mentioned earlier in section 3.1 and in Fig. 16. The pH levels ranged between 7.7 and 8.1, and some increment in pH occurred after 3 days of stocking adult worms and later fluctuations were almost similar in all treatments. Having maintained pH levels, the difference in growth or survival of rockworm among culture units of this experiment might have not been as a result of pH or other factors related to pH. Salinity in culture units was maintained through regulation, monitoring and dilution because the concentration increased with time due to evaporation. Salinity ranged between 35-36.5 psu. DO concentrations were between 5.5-6.8 mg/l and was maintained by continuous water flow and aeration.

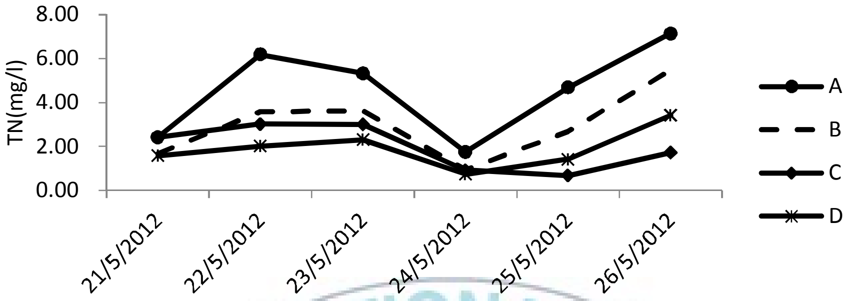


Fig. 22. Trend of the concentration of total nitrogen (TN). The concentration of TN fluctuated in the same manner in all treatments; nonetheless, treatment (A) had the highest concentration than treatments (B-D). This shows that nitrogenous compounds have adverse effects on the growth performance of rockworm; as the same trend occurred in treatment F of the first experiment in section 2.1. After 2-3 weeks, TN reduced probably because nitrifying bacteria grow and convert more toxic nitrogenous compounds to less toxic ones.

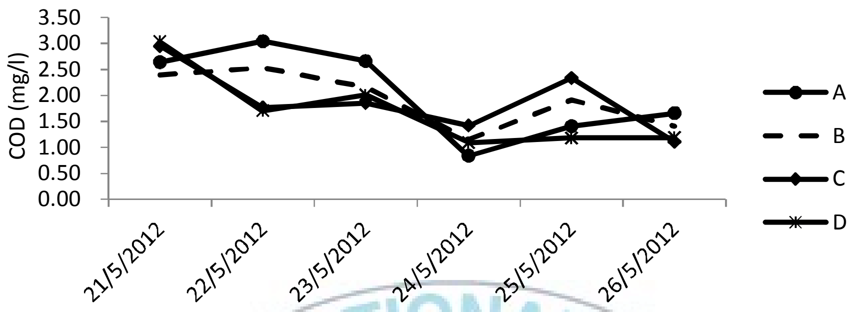


Fig. 23. Trend of chemical oxygen demand (COD). Variations in the level of COD were minimal as the trend of fluctuations was almost similar in all treatments.


Fig. 24. Trend of total suspended solids (TSS). The maximum level of TSS in this experiment in one week was below 0.1 mg/l as compared to that in Fig. 14 of the first experiment, which was as high as 3.5 mg/l.

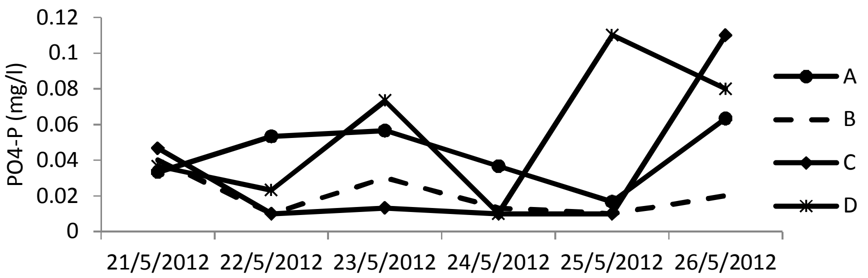


Fig. 25. Trend of the concentration of phosphate. The concentration of $PO_4$-P was low between 0.001-0.12 mg/l compared to the previous experiment in which $PO_4$-P concentration ranged between 0.1-1.2 mg/l, as shown in Fig.10. This level of $PO_4$-P may not have had an adverse effect on rockworm performance in a short period of the second experiment and therefore any changes which occurred in worm growth or survival in this experiment was not be attributed to $PO_4$-P concentration but possibly substrates type or other water quality components.

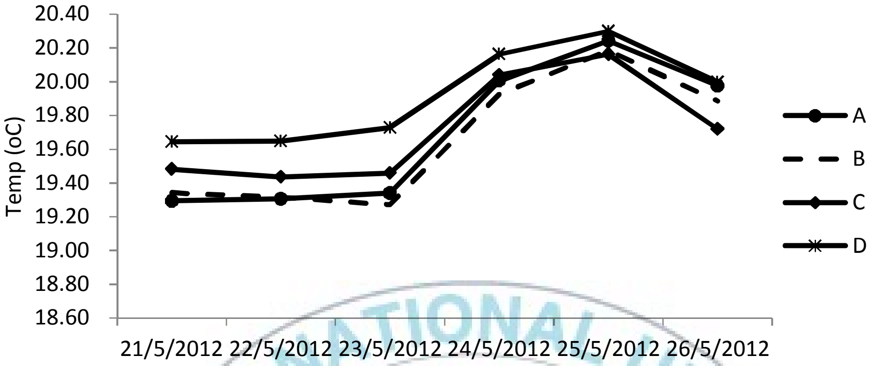


Fig. 26. Trend of temperature fluctuations. The range of 19℃ to 20℃ was maintained using an adjustable water heater as mentioned earlier in Fig. 16 and fluctuations in all treatments were almost similar.


Fig. 27. Trend of salinity variations. Salt content in culture units was maintained through regulation, monitoring and dilution because the concentration increased with time due to evaporation. Salt concentration ranged between 35-36.5 psu.

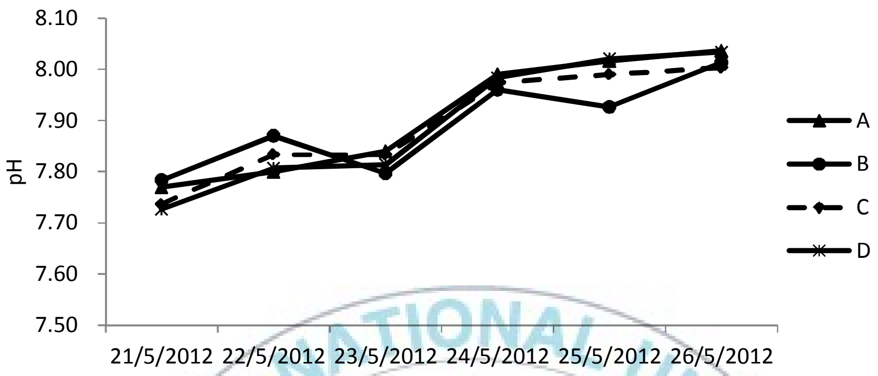


Fig. 28. Trend of pH variations. The pH level was between 7.7 and 8.1. The trend of some increment in pH was also similar in all treatments and therefore the difference in growth or survival of rockworm among culture units might have not been as a result of pH or other factors related to pH.

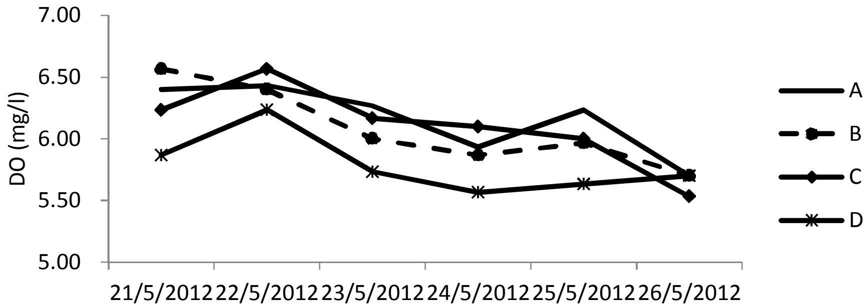


Fig. 29. Trend of the concentration of dissolved oxygen (DO). Oxygen concentration was between 5.5-6.8 mg/l and was maintained by continuous water flow and aeration. Decrease in oxygen level as time went on may have been due to the increasing number micro-organisms especially nitrifying bacteria which consume oxygen. Sufficient aeration was provided to maintain a good bacterial biomass for nitrification process.

3.3. An experiment with and without worms

In order to investigate whether water quality was the leading factor in determining growth and survival performance of rockworm at different substrate concentrations, an experiment with the same design as in Fig. 5 was conducted and adult worms were used as specimens. Out of three replications in each treatment, one did not contain worms, as a control experiment and the two other replications had adult worms.

3.3.1. Water quality analysis

What quality components which were analyzed in this experiment were the same as those in sections; 3.1 and 3.2. Trends of water quality were as follows; temperature ranged between 19℃ and 20.5℃, and fluctuations were almost the same in all treatments, regardless of whether the culture unit contained worms or not. Adjustable water heaters were used to regulate temperature. Levels of salinity in all culture units also fluctuated in the same manner in all culture units ranging between 35 and 37 psu. The trend of pH was ranged between 7.7 and 8.1 and fluctuations were also almost the same in both categories of culture units, with and without worm samples. The level of DO was between 5.5 and 7 mg/l except for culture unit C2, where it increased up to 8 mg/l. As mentioned earlier in section; 3.2, DO is important for survival of nitrifying bacteria and worms. TN was higher in the first 3 days after starting the experiment, and later reduced in almost the same mode in all culture units on the fourth day, which might have been caused by increase in number and activity of nitrifying bacteria and reduced after 4 days in treatments which did not contain worms than those which contained worms. Levels of COD increased after one day of starting the experiment and reduced gradually on from the third day and fluctuated almost in a similar trend among all treatments and ranged between 1.0-5.0 mg/l.

The trends of fluctuations in the concentrations of water components in treatments that contained worms were almost similar with treatments that did not contain worms as shown in Figs. 31-37. This was an implication that the difference in growth rates which occurred in the second experiment (see Fig. 21), was not due to water quality but the type of substrate used.

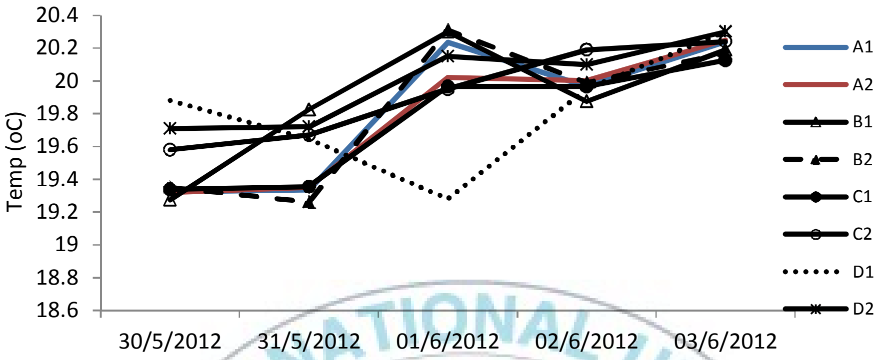


Fig. 30. Trend of temperature variations. The range of temperature was between 19℃ and 20.5℃ and fluctuations were almost the same, irrespective of whether the culture unit contained worms or not. Adjustable water heaters were used to regulate temperature.

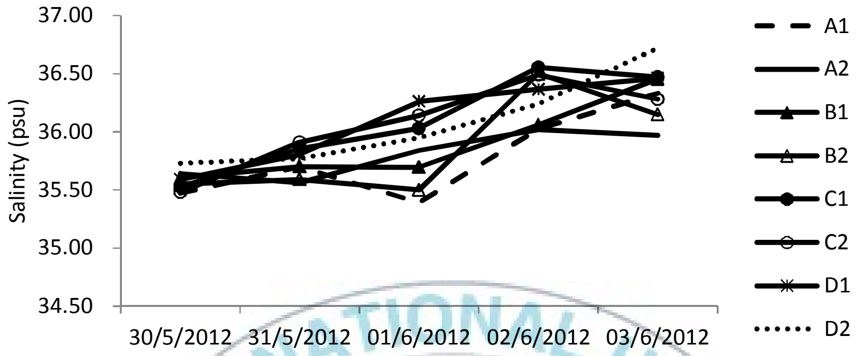


Fig. 31. Trend of variations in salinity. Levels of salinity in all culture units fluctuated in the same manner in all culture units. The concentration ranged between 35 and 37 psu.

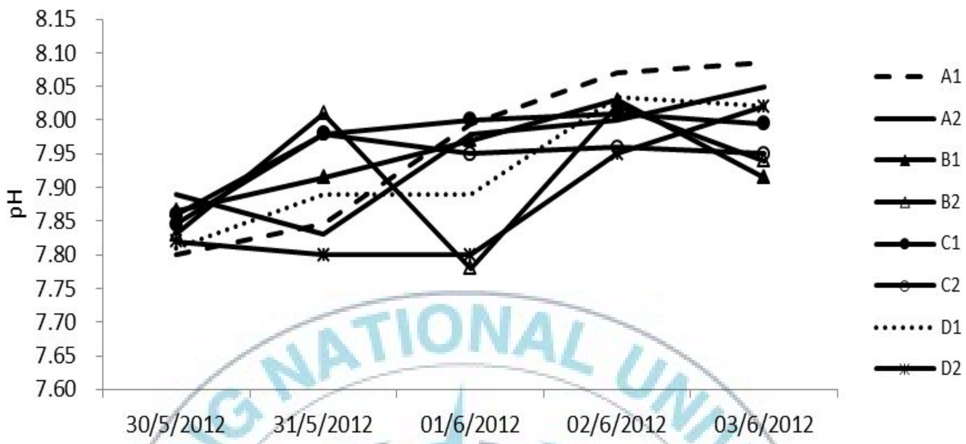


Fig. 32. Trend of pH variations. The range of pH was between 7.7 and 8.1 and the trend and fluctuations were almost the same in both categories of culture units, with and without worm samples.

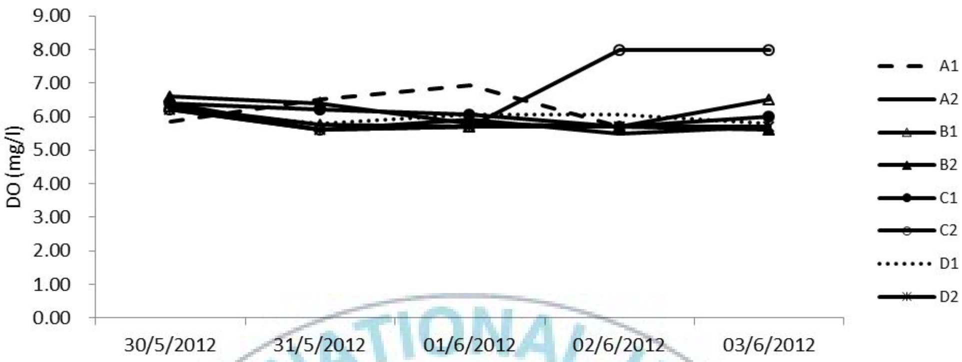


Fig. 33. Trend of the concentration of dissolved oxygen (DO). The level of DO ranged between 5.5 and 7 mg/l except in culture unit C2, where it increased up to 8 mg/l. As mentioned earlier in section; 3.2, DO is important for nitrifying bacteria growth and also for the physiological balance of the worm.

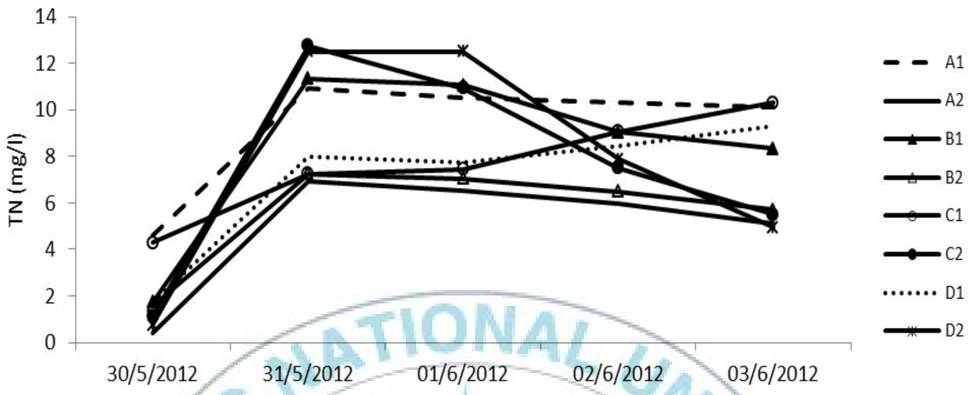


Fig. 34. Trend in the concentration of total nitrogen (TN). Within 3 days after starting the experiment, TN was higher and later reduced in almost the same manner in all culture units on the fourth day, which might have been caused by increase in number and activity of nitrifying bacteria. TN levels reduced more in treatments which did not contain worms than those which contained worms.

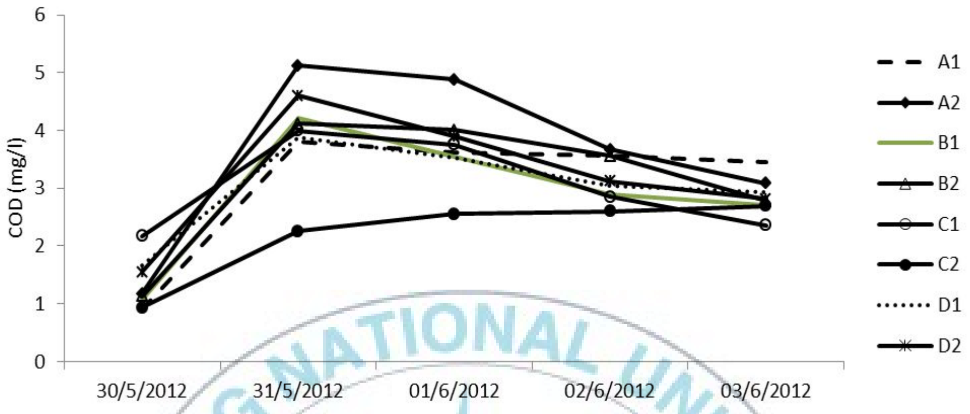


Fig. 35. Trend of chemical oxygen demand (COD). Levels of COD increased after one day of starting the experiment and reduced gradually on from the third day. It fluctuated almost in a similar trend among all treatments and ranged between 1-5 mg/l.


Fig. 36. Trend of Total Suspended Solids (TSS). The concentration of TSS increased from 0.01 the first day to 0.1 mg/l on the second day and later reduced gradually until the final day of the experiment. Like in most of the parameters in this experiment, the trend of fluctuations in TSS levels was also similar in all culture units regardless of whether they contained worms of not.

## 4. DISCUSSION

Experiments carried out showed an increase in growth rate in terms of body weight in rockworm *M.sanguinea* juveniles and adults with the increase in the percentage of oyster shells but the survival rate was not much affected by the concentration (%) of either sand or oyster shells in the culture vessel.

Three experiments were conducted to investigate growth and survival, and third one was the analyze water quality for culture units which contained worms and those without. The first two experiments were distinguished by the type of specimen, food, and design as follows; (1), Juvenile worms, commercial pellet feed and single water supply system; (2), Adult worms, waste water and independent water supply system; (3), Water quality analysis of culture units with and without worms.. All the three experiments were conducted in a closed re-circulation system. The first experiment was composed of six treatments, and these were; (A), 100% sand (control); (B), 80% sand + 20% oyster shells; (C), 60% sand + 40% oyster shells; (D), 40% sand + 60% oyster shells; (E), 20% sand + 80% oyster shells and (F), 100% oyster shells. And the second experiment composed of; A: 100% sand (control); B: 25% oyster shells, 75% sand; C: 50% oyster shells, 50% sand and D: 100% oyster shells.

General growth rates in the first experiment increased with increasing levels of oyster shell except in treatment F and the rates were as follows; A, 40%; B, 55%; C, 53%; D, 62%; E, 68% and F, 15% and the survival rate per treatment was as follows; A, 68%; B, 4O%; C, 58.5%; D, 46.5%; E, 83.5%; 60%. The second and third experiments were carried out after first one experiment gave different results from the ones reported by Heo, (2011). There was a big difference between 200% increment in growth rate of rockworm juveniles in culture units containing 100% of oyster shells reported by Heo (2011) compared to 15% increment in this study and also below that of control experiment (A) in which the growth rate was 40%. A sharp drop in both survival and growth in treatment F, (see Fig. 10) as opposed to the reported trend, prompted further investigations and tests were conducted to find out the possible causes of the drop in body weight with increased concentration of oyster shells; from 68% in treatment E (80% oyster shells+ 20% sand) to 15% in treatment F (100% of oyster shells). One-way ANOVA test of the means of all treatments showed a *P-value* of

0.0033 which was significantly different (*P<0.05*) and no significant difference (*P>0.05*) occurred in the survival rate. Duncan's multiple range test showed no significant difference (*α>0.05*) between treatment B and C even though B had a slightly growth rate (%). The second experiment was carried out using waste water as feed of adult worms, and critically controlled water quality, which afterwards showed an increase in growth and survival rate with increasing concentration of oyster shells in culture media. From the results of second the trend of growth and survival increased with the increasing concentrations of oyster shells, in the same way as reported by Heo (2011); however, the juvenile growth rates of 90% to 200% in culture units of 100% sand and 100% oyster shells respectively, were not observed in this study. Nonetheless, he used a flow through system, big culturing boxes, and larger size of sediment particles.

Water quality components fluctuated almost uniformly in all treatments of both experiments apart from nitrite and nitrate in treatment F (see Fig. 12 and 13) which might have occurred due to abrupt adjustment in water quality when diluting the salinity of this treatment. However, the second experiment confirmed the steady increment in growth and survival with increasing concentration of oyster shells and decreasing amount of sand.

From the first experiment, water quality showed to have a binding effect on the worm performance and was more controlled in the second experiment. Water chemical components like nitrite can result in high toxicity to aquatic animals (Alvaro & Camargo, 2006). From table 4, nitrogenous compounds especially $NO_2$-N and $NO_3$-N were higher in treatment F, where there was lowest growth rate of rockworm. $NO_2$-N concentration was at 0.5 mg/l in treatment F where the lowest growth rate occurred and 0.15 mg/ in treatment E where growth rate was the highest. Results of treatment E and F were expected to be closely related because of the closeness of oyster shells concentration in culture substrates of both treatments but the results were too different and this was attributed to water quality differences. According to Chen and Cheng (1999), $NO_2$-N can be lethal to aquatic animals at 0.5 mg/l upon a long time of exposure. Total suspended solids and $PO_4$-P were also higher in this treatment than the rest of the treatments.

In treatments (A-E), NO2-N increased gradually in the first week and then increased rapidly in the second week for 5 days and sharply reduced;

however, in treatment F, it increased after 2 days of culture and persisted for 3 weeks which was longer time than the rest of treatments. Nitrite generation was looked at as a critical control parameter because of its high levels and toxicity compared to other water components which were analyzed. The trend of $NO_3$-N was almost similar to that of $NO_2$-N but it increased more sharply in the first week, dropped and again increased in the second week; however, treatment F still had the highest concentration with maximum of 7 mg/l compared to other treatments which had a range of 0.0 to 4.5 mg/l. $PO_4$-P concentration fluctuated throughout the culturing period in all treatments but the concentration in treatment F ranged between 0.45 to 1.2 mg/l as compared to 0.02 to 0.6 mg/l in other treatments. According to (Stephanie), the concentration of $PO_4$-P above 0.45 is very high for aquatic organisms, in this case, higher concentration and abrupt increments and reductions which occurred in treatment F might have contributed to low growth rate of rockworm. $NO_3$-N concentration increased sharply after the first week of culture and dropped sharply to almost zero in 2days and then increased sharply again in the second week in all treatments; nonetheless, treatment F had the highest increment from 0.1 mg/l to 6 mg/l, between 25th and 30th March. Between 2-3 weeks, NO3-N concentration stabilized in all treatments which were probably due to growth of nitrifying bacteria which converted $NH_3$-N in the first week to $NO_3$-N and later to $NO_2$-N, as shown in Figs.12 and 13. The presence of $NO_2$-N in treatment F from the beginning to the second last week of the experiment might have had an adverse effect on worm growth performance and thus one of the reasons for lowest weight gain among all treatments. Though nitrate is considerably less toxic than ammonia or nitrite, it can also be lethal because it can produce toxic effects (Colt and Armstrong, 1981). These effects can be methaemoglobin formation, resulting in a reduction in the oxygen carrying capacity of blood and inability of the organisms to maintain proper osmoregulation under high salt concentration associated with elevated nitrate levels (Colt and Armstrong, 1981). In contrast, nitrate toxicity may decrease with increasing body size, water salinity, and environmental adaptation (Camargo, et al., 2005). Other water quality parameters such as pH, salinity, temperature and dissolved oxygen can influence the conversion of nitrate to other forms of nitrogen, or vice versa and low pH can lead to conversion of nitrite to more poisonous nitrous acid (Chen and Cheng, 1999); however, in this experiment, concentrations of components fluctuated in the pattern in all treatments, this suggests that these parameters may not have affect growth performance of rockworm.

In the second experiment, water these parameters were kept almost uniform in all treatments in the second experiment to avoid water quality related stress to the worm. ; A, -17; B, -12; C, -6, and D, 1; and survival rates were as outlined: A, 91%; B, 90%; C, 91% and D, 96%. In this experiment, treatment D (100% oyster shells) had the highest survivorship and negative growth occurred in treatments A-C because worms were fed on rotifer farm waste water, and probably they could not filter food from the water hence they lost weight, nonetheless, weight loss decreased with an increasing concentration of oyster shells in the culture medium.

Water quality parameters were controlled and fluctuations were not as intense as they were in the first experiment. The concentration of TN fluctuated in the same pattern in all treatments; except for treatment (A) that had the highest concentration than treatments (B-D) and the lowest growth rate (see. Fig.22), this implied that under well controlled water quality, nitrogenous compounds can be more generated in sand substrate compared to oyster shells substrate and have adverse effects on the growth performance of rockworm; as the same trend occurred in treatment F of the first experiment in section 2.1. After 2-3 weeks, TN reduced probably because nitrifying bacteria grow and convert more toxic nitrogenous compounds to less toxic ones. Salinity was maintained between 30-36 psu and at went up to 37-39 psu in few cases of the first experiment, however it was maintained between 30-36 psu in the second experiment which according to Garces and Pereira (2011), salinity of 30-35 psu is best recommended for juvenile rockworm culture. . The concentration of PO4-P was low and ranged between 0.001-0.12 mg/l and good for growth and survival of aquatic organisms as compared to the the first experiment in section 3.1 and Fig 10, in which $PO_4$-P concentration ranged between 0.1-1.2 mg/l, which is considered to be high for most aquatic organisms (Stephanie). The level of $PO_4$-P in this experiment may therefore not have had an adverse effect on rockworm performance in this culturing which implies that the changes which occurred in worm growth or survival in this experiment was not be attributed to $PO_4$-P concentration but probably because of the quality of substrates or other water quality components. The range of 19℃ to 20℃ and was maintained to avoid temperature shock to the worms using an adjustable water heater as mentioned earlier in section 3.1 and in Fig. 16. Increasing water temperature reduces the amount of DO, at 0℃ water holds up to 14.6 mg/l of DO while at 30℃, it holds only up to 7.6 mg/L (Stephanie). The level of pH ranged between 7.7 and 8.1, and some increment in pH occurred after 3 days of stocking adult worms and later fluctuations were almost similar in all treatments. pH level of 6.5-8.0 is recommended for freshwater and 8-9 for estuarine and seawater organisms (Stephanie). DO concentration was maintained by continuous water flow and aeration and ranged between 5.5-6.8 mg/l. It slightly decreased in the same pattern in all treatments as time went on, and this might have been due to the increased number of micro-organisms especially nitrifying bacteria which consume oxygen in nitrification process. Other properties of the sediment such as texture and porosity have an effect on growth and distribution of polychaetes (Sukumaran, et al., 2010). Water quality was maintained almost similar all treatments in the second experiment (using waste water and adult worms) and the third experiment (with and without worms) and the better performance of the worms with increasing concentration of oyster shell may be attributed to the ability to access food, avoid predators and aeration as compared to the sand substrate.

## 5. CONCLUSION

According to this study, the growth rate of rockworm *M. sanguinea* increased with increasing concentration of oyster shells in the culture units while survival rate did not show the relation between the concentration of culture substrates and number of survivors. Nonetheless, rockworm showed higher growth and survival rate in culture units containing at least 80% of oyster shells. Therefore this concentration (80% oyster shells) is recommended for faster growth and increase survivorship; however, in areas where oyster shells are not abundant, at least 50% oyster shells and 50% of sand material can be used. Management and control of physical-chemical water quality components in rockworm cultivation is paramount because some parameters such as pH, salinity, temperature and dissolved oxygen can influence the conversion of nitrate to other forms of nitrogen, or vice versa and low pH can lead to conversion of nitrite to more poisonous nitrous acid; some components such as nitrite and phosphate are highly toxic at low concentrations (0.5 mg/l) after a long time of exposure. Nitrogenous compounds were sequentially generated; that is, from ammonia to nitrate and lastly nitrite, therefore a well-designed bio-filter system is important to enhance nitrification process and control toxic effects of these compounds. Generally all water quality parameters have adverse effects on rockworm survival and growth performance therefore system management is important for maximizing production.

Under controlled water quality rockworm growth and survival performed better in oyster shells substrate than in sand, which was probably due to the ability to move and access adequate food, avoid predators and oxygen compared to culture units containing much sand substrate.

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