



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

Thesis for the Degree of Master of Fisheries Science

Effect of salinity on survival and growth
of *Marphysa sanguinea* (Polychaeta:
Eunicidae) juveniles.

by

Grace Afumwaa Boamah

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

February, 2016

Effect of salinity on survival and growth
of *Marphysa sanguinea* (Polychaeta:
Eunicidae) juveniles

Marphysa sanguinea 의 성장률과
생존율에 미치는 염분의 영향

Advisor: Prof. Chang-Hoon Kim

by

Grace Afumwaa Boamah

A thesis submitted in partial fulfilment of the requirement for the degree of
Master of Fisheries Science

In KOICA-PKNU International Graduate Program of Fisheries Science

Pukyong National University

February, 2016

Effect of salinity on survival and growth of *Marphysa sanguinea*
(Polychaeta: Eunicidae) juveniles

A dissertation

by

Grace Afumwaa Boamah

Approved by:

(Chairman) Prof. Jong-Myoung Kim

(Member) Prof. Wongyu Park

(Member) Prof. Chang-Hoon Kim

February 26, 2016

Table of contents

Tables of contents	i
List of figures	iii
List of tables	v
Abstract	vi
1. Introduction	1
1.1 Background	1
1.2 Problem statement	5
1.3 Hypothesis	6
1.4 Objective	7
1.5 Justification	7
1.6 Significance of study	8
2. Materials and Methods	9
2.1 Design of experiment	9
2.1.1 Source of juveniles	9
2.1.2 Weighing of juveniles	11
2.1.3 Preparing experiment boxes	12

2.1.4 Sediment collection	13
2.1.5 System set-up	13
2.1.6 Feeding of worms	16
2.1.7 Experimental water preparation	17
2.2 Water quality analysis	19
2.3 Data collection	19
2.4 Statistical analysis	22
3. Results	23
3.1 Survival rate of worms	23
3.2 Growth performance of worms	24
3.3 Water quality analysis	28
4. Discussion and Conclusion	34
Reference	40
Acknowledgment	49

List of Figures

Figure 1. Major morphology features of a generalized polychaete	2
Figure 2. Photograph of specimen collection for the experiment	10
Figure 3. Photograph of apparatus used in weighing worms	11
Figure 4. Photograph of cubic boxes used in the experiment	12
Figure 5. A schematic diagram of experiment box	13
Figure 6. Photograph of the experiment set-up	15
Figure 7. A schematic diagram of the experiment set-up	16
Figure 8. Photograph of worm feed	17
Figure 9. Photograph of experimental water preparation apparatus	18
Figure 10. Photograph of data collection	20
Figure 11. Survival rate of <i>M. sanguinea</i> juveniles	24
Figure 12. Growth performance of <i>M. sanguinea</i> juveniles	25
Figure 13. Trend of pH at different salinity levels tested	28
Figure 14. Trend of DO at different salinity levels tested	29
Figure 15. Trend of Alkalinity at different salinity levels tested	30
Figure 16. Trend of Ammonia at different salinity levels tested	31

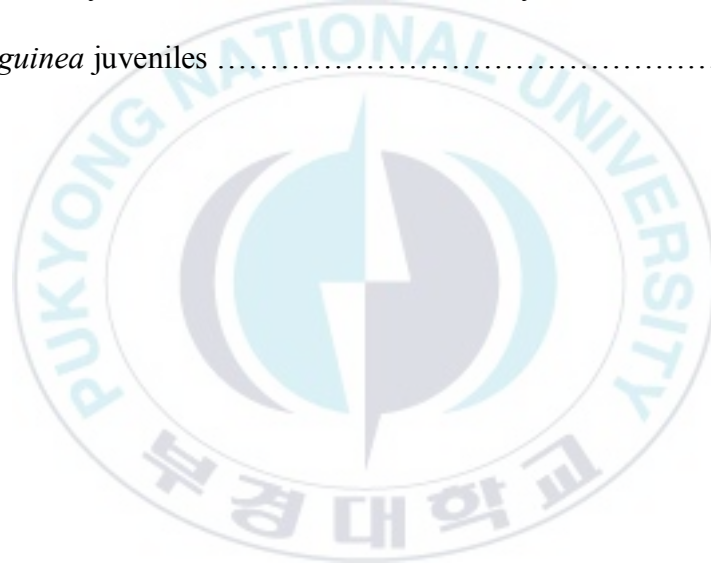
Figure 17. Trend of Nitrite at different salinity levels tested 32

Figure 18. Trend of Nitrate at different salinity levels tested 33



List of Tables

Table 1. Weight gain (%), specific growth rate and survival rate (%) of <i>M. sanguinea</i> juveniles	26
Table 2. One-way ANOVA test on effect of salinity on growth of <i>M. sanguinea</i> juveniles	27
Table 3. One-way ANOVA test on effect of salinity on survival of <i>M. sanguinea</i> juveniles	27



**Effect of salinity on survival and growth of *Marphysa sanguinea*
(Polychaeta: Eunicidae) juveniles**

Grace Afumwaa Boamah

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

Abstract

Today, integrated multi-trophic aquaculture (IMTA) is the way forward in managing waste from aquaculture farms. The identification of *Marphysa sanguinea* as a potential candidate for this purpose has aroused the need for its commercial artificial production. Meanwhile, there rarely exist studies on the environmental factors affecting its growth and survival. In order to test the hypothesis that salinity affects survival and growth of *M. sanguinea*, and to determine the range of salinity tolerance and their effect on the species' survival and growth, 4,500 juveniles of initial weight 0.006 ± 0.0003 g were cultured under controlled conditions. Triplicate groups of worms were reared under five different salinities (15, 20, 25, 30, and 35psu) for 120 days by semi-recirculation system. Oyster shells (70%, 5 mm in diameter) and sand (30%, 2 mm in diameter) were used together as sediment. Survival rate and growth rate were determined, both within and between groups. Both survival and growth were significantly affected by salinity ($p < 0.05$). Results of this research confirms

that *M. sanguinea* has high tolerance for salinity fluctuation and proves to be an excellent candidate of IMTA for fish species cultured within a salinity range of 20 – 35psu. For optimum survival and growth in commercial production of *M. sanguinea*, however, 25 psu is recommended.



1. INTRODUCTION

1.1 Background of study

According to Montagu (1815), *Marphysa sanguinea* can be classified into:

Kingdom; Animalia

Phylum; Annelida

Class; Polychaeta

Order; Eunicida

Family; Eunicidae

Genus; *Marphysa*

Species; *Marphysa sanguinea*

Common name; Rockworm

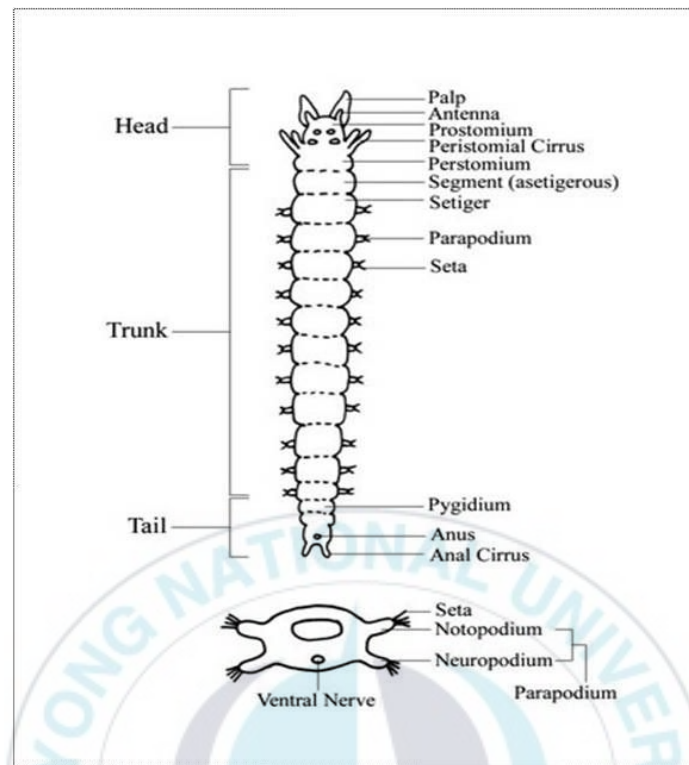


Fig. 1. Major morphology features of a generalized polychaete (Fauchald, 1977).

Hutchings and Karageorgopoulos (2003) claim it is one of over 55 species described in the genus *Marphysa*. Some of its life characteristics, as described by Prevedelli et al. (2007), include gonochorism and iteroparity. Detailed study done on its life cycle reveals that it is usually found in warm and temperate areas of coastal habitats, lives in burrows in the muddy

bottoms, can attain a maximum length of about 400 mm, and has a lifespan of approximately 90 days – not overemphasizing the fact that growth and lifespan may vary with food availability, water temperature, salinity, predator abundance among other environmental factors (Prevedelli et al., 2007). The species has an omnivorous and coprophagous feeding habit (Ruppert & Fox, 1988; Honda & Kikuchi, 2002). Prevedelli et al. (2007) concede that among the different species of *Marphysa* (*M. mulawa*, *M. fauchaldi*, *M. gravely*), the larval development of *M. sanguinea* has been studied in detail. Because the larvae of this species do not tolerate low temperature, the breeding season occurs in periods of the year where there are suitable conditions for larval development (Prevedelli, 1994). Kim and Jang (2008) also provide evidence that, trochophore larvae of *M. sanguinea* are released around the burrow when the water temperature is between 18°C to 22°C.

M. sanguinea is widely distributed in many locations globally, including the south coast of England, Eastern Scheldt in south-western Netherlands, and along the Italian coasts (Montagu, 1815; Hutchings et al., 2011; Prevedelli et al., 2007). As documented by Garcês and Pereira (2010), the species is

also present in Portugal, along the Sado estuary, particularly within former oyster production areas. Monia et al. (2003) also point out its presence on the coast of Tunisia, and in the lagoon of Tunis.

Generally, the demand for polychaetes is increasing rapidly in recent years due to their several uses (Olive, 1994, 1999; Henriksen et al., 1983; Hutchings, 1998; Ruppert & Fox, 1988; Ito et al., 2011). It was recounted that in Portugal, *M. sanguinea* in particular, is one of the most appreciated bait species among those collected and commercialized due to the high resistance it presents on the hook, and to its reputed ability to attract valuable fish species such as the seabreams – *Sparus aurata* and *Diplodus sargus* (Garcês & Pereira, 2010). Same writers emphasize its harvest activity as one of the most important socio-economic resources for local fishermen.

There is a cause for the rising interest in polychaete aquaculture – as it establishes an alternative source for its supply to the existing market, as well as minimizes collection impact from the natural environment (Olive, 1999; Garcês & Pereira, 2010). Aquaculture of any species, however, requires that both physiological and ecological conditions of the organism be studied in detail. Quite a number of researchers have argued out the limiting factors

affecting species distribution in marine and brackish-water habitats, and have concurred that primary attention should be given to salinity, temperature, nutrition and water quality (Kinne, 1970, 1971; Prevedelli & Vandini, 1997; Alderdice, 1972; Dorgelo, 1976).

In the quest to improve rearing techniques for *M. sanguinea*, it is rather prudent that the primary abiotic factors affecting its life cycle be understood in detail, as such documentation on the species is rare.

1.2 Problem statement

Coastal aquaculture is the most active and predominant type of mariculture in Korea. Most cage culture takes place in the embayed sites. However, the recent problem faced in this type of farming area is water pollution and red tide. Also, the issue of aquaculture waste water treatment has become of great concern. The future direction of Korean aquaculture thus lies in the integrated aquaculture management, most especially the multi-trophic aquaculture. This basically focuses on the ecosystem based aquaculture, where different trophic organisms are maintained in a given aquaculture

ground for the purpose of waste management (Han Kyu Lim. Korean Aquaculture: Status and Future Directions).

M. sanguinea has been identified as a potential candidate for aquaculture waste management in integrated multi-trophic aquaculture (Parandavar & Kim, 2014). It has thus become a necessity that commercial production of this species, for this purpose, among others, be encouraged. The intimidating challenge, however, has been with the high juvenile mortality associated with its life cycle.

1.3 Hypothesis

Kinne (1971) and Withers (1992) argue that even with the behavioural and physiological mechanisms adopted by euryhaline animals – in coping with fluctuating salinity – most of them are not immune from the effects of salinity change. Reduced growth rate and development (Tietjen & Lee, 1972; Lambert et al., 1994; Qiu & Qian, 1997; Anger et al., 1998; Rosas et al., 1999; Specker et al., 1999) as well as reduced fecundity (Morritt & Stevenson, 1993; Cheung, 1997; Remane & Schlieper, 1971) are some sub-lethal effects of salinity changes reported. These salinity effects have been

observed in other polychaete species (Pechenik et al., 2007; Qiu & Qian, 1997; Bogucki, 1963; Lyster, 1965; Smith, 1957, 1964).

Based on the findings of Garcês and Pereira (2010), I hypothesized that salinity significantly affects survival and growth of *M. sanguinea*.

1.4 Objective of study

This study investigated the range of salinity tolerance and its effect on the survival and growth of *M. sanguinea* juveniles.

1.5 Justification of study

As confirmed by Bochert et al. (1996), it is prudent to study the early embryonic or larval stage of marine invertebrates when investigating their physiological potential. It is for this reason that juveniles of this species were chosen for this experiment.

In the experiment conducted by Garcês and Pereira (2010), salinity effects on juveniles' survival and growth rates were observed under fine sediment.

According to Ushakova (2003), tolerance for abiotic factors of marine invertebrates depends mainly on conditions of their locality. In this experiment, we adopt two methodologies aimed at improving the organism's environment, while monitoring its response to the different salinity levels.

These are:

- The semi-recirculation system; an eco-friendly aquaculture technique believed to enable a more efficient use of water.
- The oyster shell as sediment, which is reported to permit a better circulation of water and oxygen, as well as allow easy access to food by the organism, compared to sand substrate.

1.6 Significance of study

Primarily, the scientific finding of this research can help our understanding of the salinity range the species can tolerate in IMTA system. Secondly, findings can help develop rearing techniques that improve survival and growth rates at the juvenile stage, consequently boosting commercial production of the species for the numerous aquaculture purposes.

2. MATERIALS AND METHODS

2.1 Design of experiment

The experiment adopted the following methodologies:

- A semi- recirculation system
- Two sediment types (70% Oyster shells – 5 mm diameter, and 30% sand – 2 mm diameter) were used together as culture sediment
- Initial weight of juveniles: 0.006 ± 0.0003 g
- Five different salinity levels (15, 20, 25, 30, 35 psu)
- Triplicates of each salinity level
- Experimental period of 120 days.

2.1.1 Source of juvenile worms

Juveniles used in the experiment were of those reproduced in the Fisheries Science and Technology Centre of the Pukyong National University (PKNU) in April, 2014. On the 9th of November, 2014, juvenile worms were collected from the boxes where they were being raised after hatching.

During collection, the sediment was scooped bit by bit unto plastic trays, spread evenly on the trays to expose the worms (which are often hidden in their tubes). Worms were then siphoned out using a pipette. Altogether, 4,500 juvenile worms were collected and placed in 15 separate bowls (300 individuals per bowl).

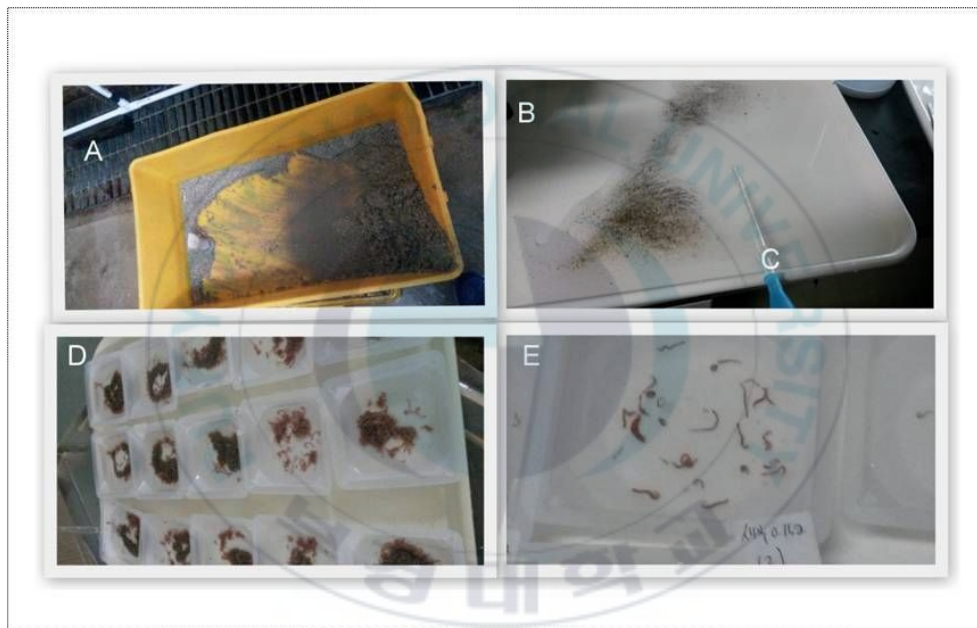


Fig. 2. Specimen collection. (A) Initial worm box. (B) Sediment from A spread on a tray to expose worms. (C) Pipette used for siphoning out worms. (D, E) Juvenile worms collected.

2.1.2 Weighing of juvenile worms

In the laboratory, 30 individuals were selected at random from each of the 15 bowls, and their initial weights were determined by a Mettler Toledo analytical balance as shown in Fig. 3 below. Individuals were placed on tissue paper to blot any water before weighing.

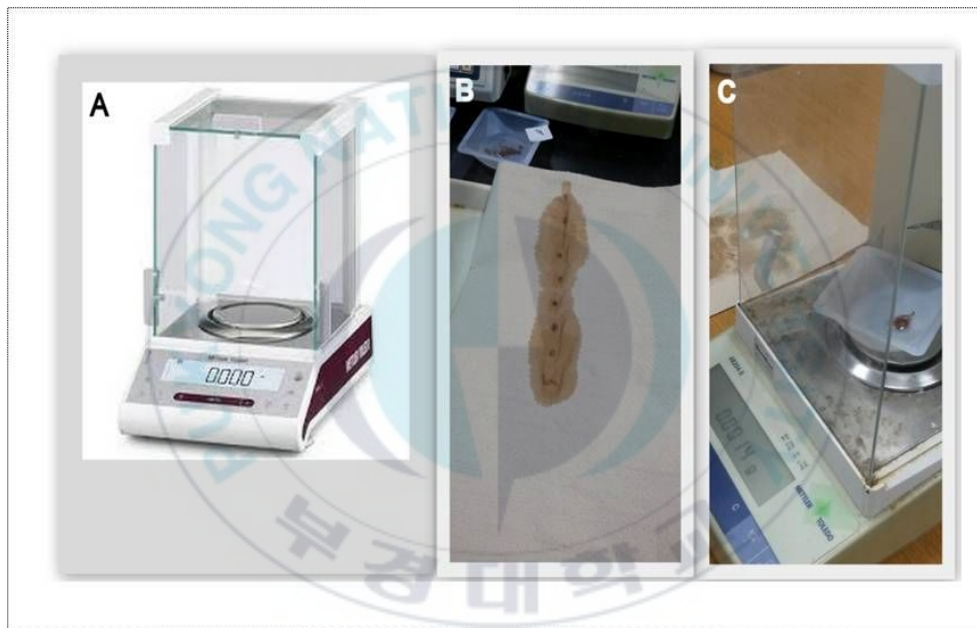


Fig. 3. Weighing of juvenile worms. (A) Mettler Toledo Analytical balance used in weighing. (B) Blotting of worms before weighing. (C) Weighing of individuals.

2.1.3 Preparing experiment boxes

Fifteen (15) boxes (see Fig. 4 and 5) of sizes L 40.5 cm x W 24 cm x H 28.5 cm (approximately 28 L each) were prepared to hold the worms. PVC pipes were used to create water outlet and an overflow. The outlet pipes were covered with nets (1mm mesh size) to prevent worms from escaping. Meanwhile, the overflow pipes were left without nets since the worms live in the bottom layer of the sediment.



Fig. 4. Cubic boxes used. (A) Box before it was prepared. (B) Box after it was prepared. (C) Overflow pipe. (D) Outlet pipe covered with net.

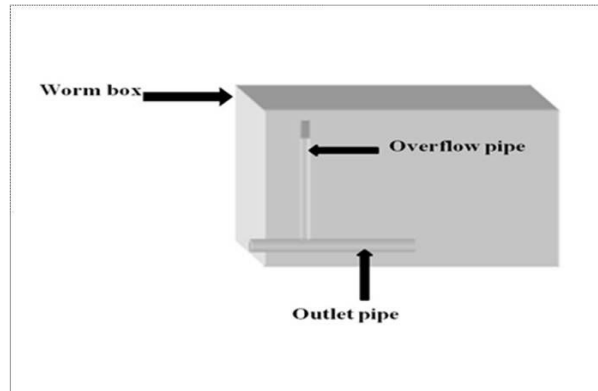


Fig. 5. A schematic diagram of box used in the experiment.

2.1.4 Sediment collection

Two sediment types were used: sand and oyster shells, which were collected from the research centre and an oyster farm near the centre, respectively. Both sand and oyster shells were filtered through 2 mm and 5 mm sieves, respectively. They were then treated by thoroughly washing with freshwater and sun-dried thereafter.

2.1.5 System set-up

Each prepared box was filled with sediment (70% oyster shells and 30% sand) to a depth of 8 ± 1 cm. In the experiment room, they were arranged in 3 rows and on 3 floors, with 5 boxes on each floor. To each column was

allocated a drum of 90-litre capacity, from which boxes in that column received inlet water (see Fig. 6 & 7). Each drum was served a pump, and later filled with filtered and aerated sea water (34-35psu, $16 \pm 1^{\circ}\text{C}$). Outlet pipes from boxes were directed into their respective drums separately. With the power source turned on, the boxes received water to a depth of about 20 cm. Each box was then stocked with 300 individual worms of initial weight 0.006 ± 0.0003 g. They were allowed a period of 48 hours to acclimate, burrow into the sediment bottom and make their tubes. All replicates received constant aeration. Dissolved oxygen (DO) averaged 8.19 ± 0.07 mg/L and pH, 8.31 ± 0.01 . The set-up was kept under a 12:12 hour dark:light photo-period and a flow rate of 2 litres per minute.



Fig. 6. Experiment set-up. (A) Arrangement of boxes and drums in the experiment room. (B) Pump. (C) Drum of water with pump. (D) Inside of box, showing inlet water and aerator.

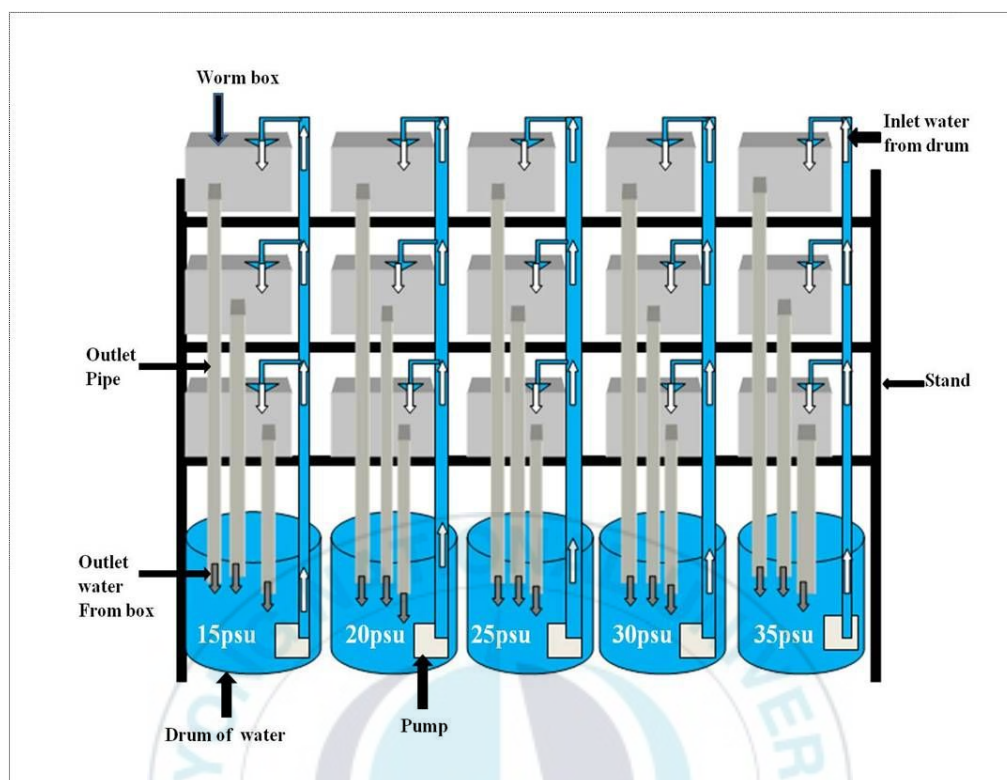


Fig. 7. A schematic diagram of the experiment set-up.

2.1.6 Feeding of worms

Worms were fed with equal amount of formulated diet, once a day in every three days, throughout the experiment period. Each replicate received 0.3 g, 0.4 g, and 0.5 g of feed in the first, the second, and the last two months, respectively (Kim, 2014. Personal comm.).



Fig. 8. NRD 3/5 larval diet used as feed in the experiment.

2.1.7 Experimental water preparation

Seawater (34-35psu), pumped from the Jaran Bay adjacent to the Research Centre, and tap water (stored for not less than a week before use) were the sources of water used in the experiment. A period of about one week was used to gradually vary the experimental water salinity, decreasing it by about 5 psu every two days to produce 30 psu, 25 psu, 20 psu and 15 psu, respectively. Varying of the salinity involved mixing appropriate portions of freshwater and sea water, both of which were stored in separate tanks (2,000-litre capacity each) in the experiment room (see Fig. 9). Both tanks

received constant aeration. Experimental water was changed partially (30%) every week, and by 100% monthly. Before changing water, the pumps were stopped by switching off the power source. The drums were emptied of their water, partially or fully, and new water mixed in the drums to the required salinity levels. Pumps were then turned on to distribute water to the boxes.



Fig. 9. Experimental water preparation. (A) Tank for storing filtered sea water. (B) Tank for storing fresh water (Tap water). (C, D) Filters used in filtering sea water. (E) Checking experimental water parameters. (F) Hydrolab Minisonde 5, together with Hydrotech Archer, used for checking water parameters.

2.2 Water quality analysis

Temperature, DO, pH, and salinity were measured daily, using Hydrolab Minisonde 5 together with Hydrotech Archer. Other water parameters analyzed by specific methods include: $\text{NO}_3\text{-N}$, tested by cadmium reduction method; $\text{NO}_2\text{-N}$, tested by diazotization method; and $\text{NH}_3\text{-N}$, tested by indophenols method.

2.3 Data collection

Data collected was basically on survival rate and growth rate of the worms. Data was collected after 31 days, 62 days, and finally after 120 days. During the collection process, boxes were emptied of their sediment and individual worms identified were siphoned out with a pipette, as described in 2.1.1 above. Worms from each replicate were placed in separate glass bowls. Counting of worms was done in all replicates at each salinity level to check for survival. Random selection of 30 individuals from each replicate was done and their weight measured in the lab. Weight measurement of

individuals was done by a Mettler Toledo analytical balance, as described in 2.1.2 above.



Fig. 10. Collection of juvenile worms at the end of experiment. (A) Scooping of sediment bit by bit from box. (B) Sediment spread on tray. (C) Siphoning out identified worm. (D) Collected worms kept in a glass bowl.

The equations below show how survival rate, weight gain, and specific growth rate were calculated:

$$Sr = \frac{Nf}{Ni} \times 100 \quad (1)$$

Where Sr denotes survival rate, Nf – number of survivors, and Ni – initial number of stocked worms.

$$WG = \frac{wf - wi}{wi} \times 100 \quad (2)$$

Where WG denotes weight gain, Wf – final body weight, and Wi – initial body weight.

$$SGR = \frac{\ln Wf - \ln Wi}{day} \times 100 \quad (3)$$

Where SGR denotes specific growth rate, $\ln Wf$ – natural log of final body weight, $\ln Wi$ – natural log of initial body weight and day represents the number of days of feeding.

2.4 Statistical analysis

Statistical analysis was performed using PASW (SPSS) 18 software for windows (SPSS Inc., Chicago, IL, USA). The effect of salinity on growth and survival was analyzed using a non-parametric one-way analysis of variance (ANOVA). The effects on growth rate were evaluated exclusively on intact specimens, whereas those on the survival rate were estimated on specimens both intact and fragmented by autotomy or cannibalism. Where there were significant mean differences, a Post Hoc multiple comparisons of means (LSD test), was used to determine the source of difference. A significant level of $\alpha = 0.05$ was chosen.

3. RESULTS

3.1 Survival rate of worms

There was a statistically significant difference between groups as determined by one-way ANOVA ($F= 8.143$, $p = 0.003$). Survival remained relatively high at all salinity levels until 62 days of culture. An unexpectedly high mortality occurred at 30 psu and 35 psu that resulted in a final survival rate of 51.8% and 23.8%, respectively, after 120 days. Though 25 psu recorded the highest survival of 84.9% at the end of the experiment, there was no statistically significant difference between 25 psu, 20 psu and 15 psu, as well as between 30 psu, 20 psu, and 15 psu; as described by Duncan's homogeneity of variance test (See Fig. 11 and Table 1).

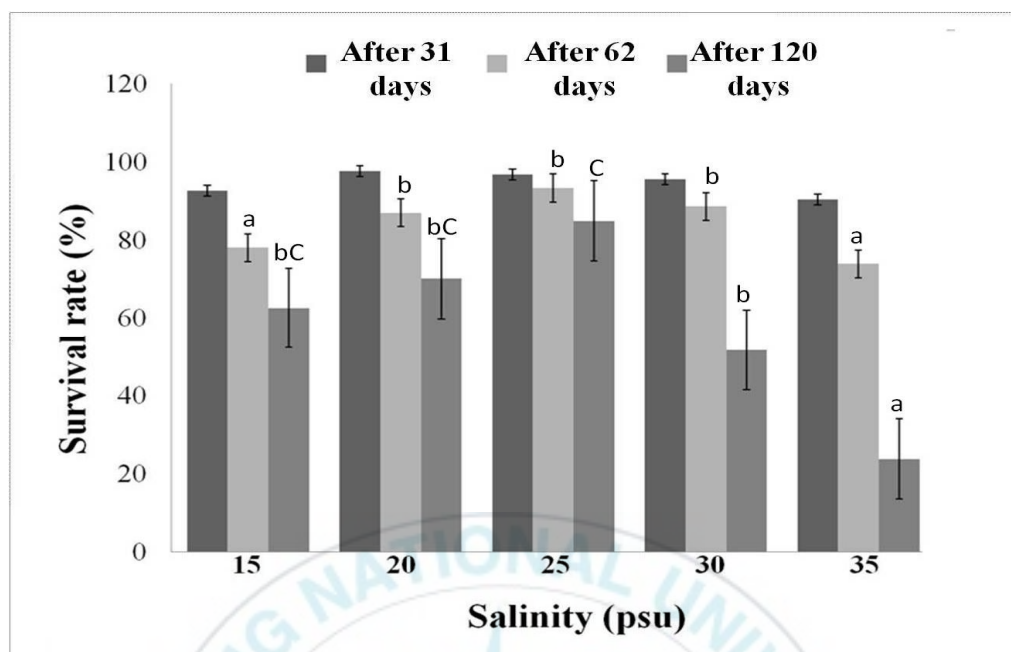


Fig. 11. Survival rate of *Marphysa sanguinea* juveniles cultured at different salinity levels. Different letters indicate significant difference at $p < 0.05$.

3.2 Growth performance of worms

Growth was also significantly affected by salinity as determined by one-way ANOVA ($F = 14.579$, $p = 0.000$). Contrary to survival, highest final weight was recorded at 35 psu (0.078 ± 0.015 g). There was no statistically

significant difference between 25 psu, 30 psu, and 35 psu, as described by Duncan's homogeneity of variance test. The lowest final weight of 0.028 ± 0.005 g was recorded at 15 psu. See Fig. 12 and Table 1 below.

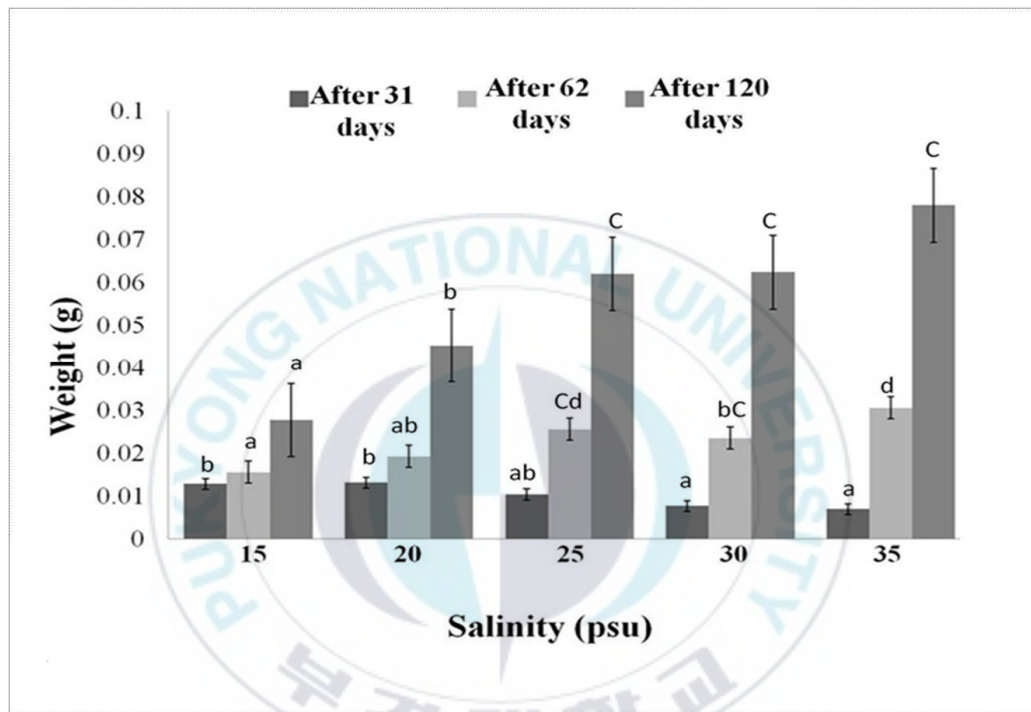


Fig. 12. Growth performance of *Marphysa sanguinea* juveniles cultured at different salinity levels. Different letters indicate significant differences at $p < 0.05$.

Table 1. Weight gain (%), specific growth rate (SGR) and survival rate (%) of *Marphysa sanguinea* juveniles cultured at different salinities for 120 days

Salinity	Initial weight of worm (g)	Final weight of worm (g)	Weight gain (%)	SGR ¹ (%/day)	SR ² (%)
15	0.007 ± 0.002	0.028 ± 0.005 ^a	320.5 ^a	1.19 ^a	62.6 ^{bc}
20	0.006 ± 0.002	0.045 ± 0.007 ^b	629.03 ^{ab}	1.66 ^{ab}	70.1 ^{bc}
25	0.007 ± 0.002	0.062 ± 0.007 ^c	838.9 ^{ab}	1.87 ^b	84.9 ^c
30	0.006 ± 0.001	0.062 ± 0.004 ^c	900.5 ^{ab}	1.92 ^b	51.8 ^b
35	0.006 ± 0.002	0.078 ± 0.015 ^c	1213.5 ^b	2.15 ^b	23.8 ^a

Values (mean ± SD of three replications) in the same column with different superscripts are significantly different.

Weight gain (%): [(final weight of worm – initial weight of worm) / initial weight of worm] x 100

¹Specific growth rate (%/day) = [(Ln final weight of worm – Ln initial weight of worm) / number of days of feeding] x 100

²Survival rate (%): (final individuals / initial individuals) x 100

Table 2. One- way ANOVA test on effect of salinity on growth performance of *Marphysa sanguinea* juveniles

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.004	4	.001	14.579	.000
Within Groups	.001	10	.000		
Total	.005	14			

Table 3. One- way ANOVA test on effect of salinity on survival of *Marphysa sanguinea* juveniles

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	56656.400	4	14164.100	8.143	.003
Within Groups	17393.333	10	1739.333		
Total	74049.733	14			

3.3 Water quality analysis

Trends of the various factors of experimental water quality tested are shown in Fig. 13 to 18 below.

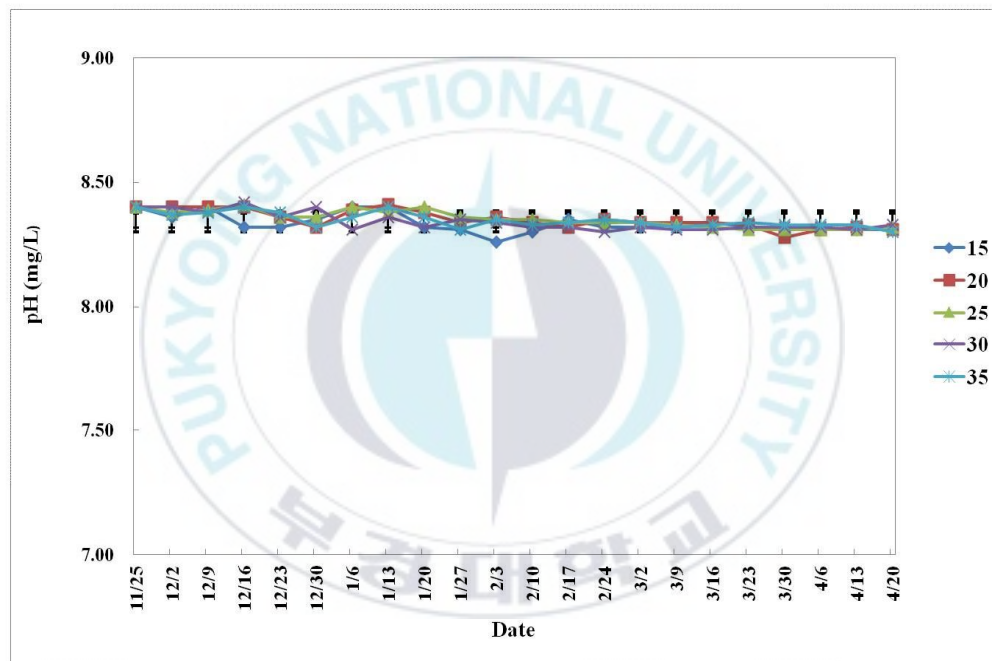


Fig. 13. Trend of pH at different salinity levels tested.

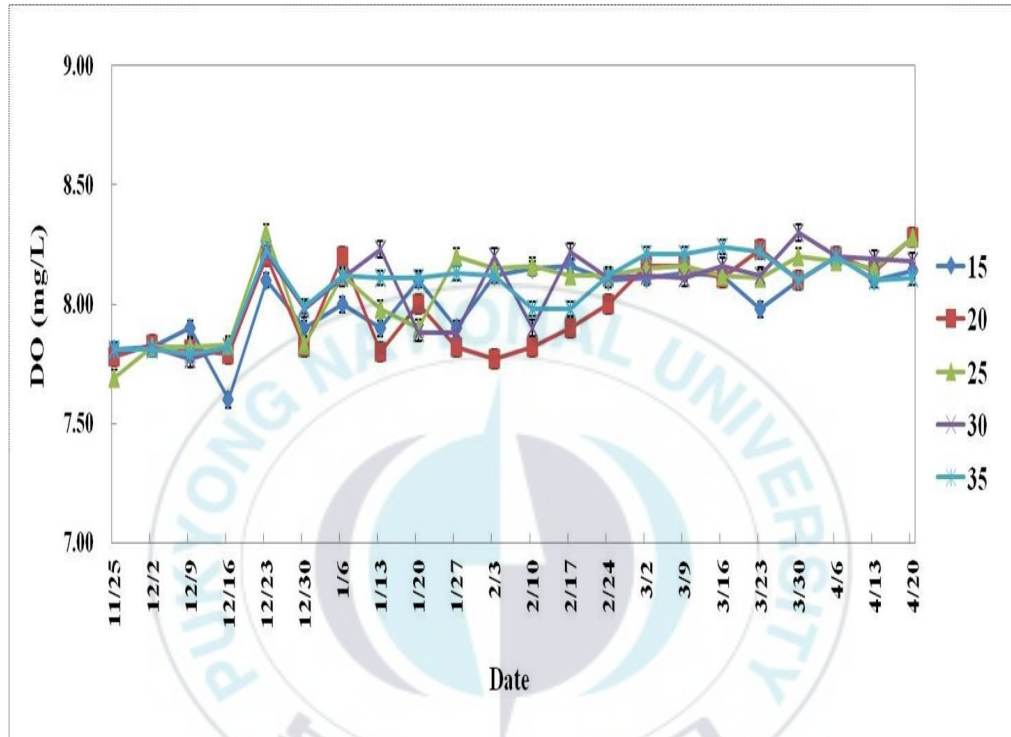


Fig. 14. Trend of dissolve oxygen at different salinity levels tested.

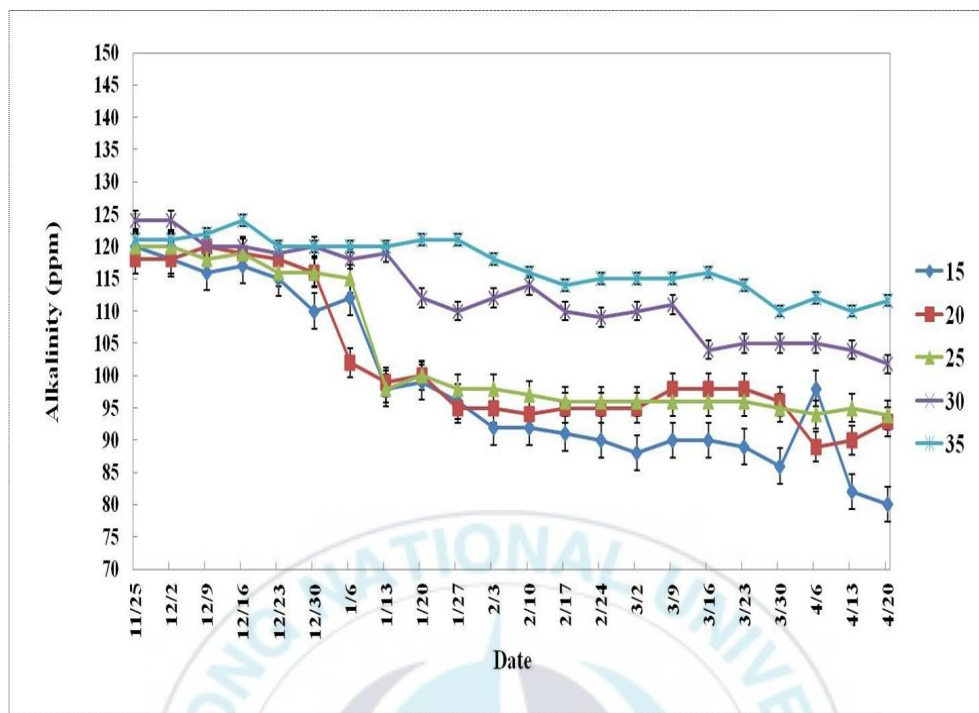


Fig. 15. Trend of alkalinity at different salinity levels tested.

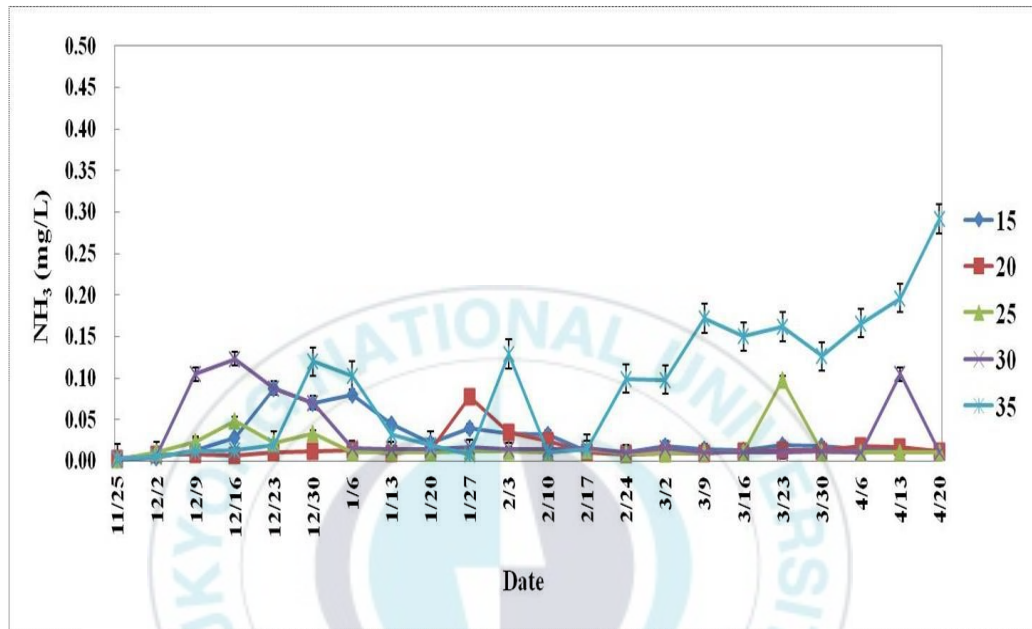


Fig. 16. Trend of ammonia at different salinity levels tested.

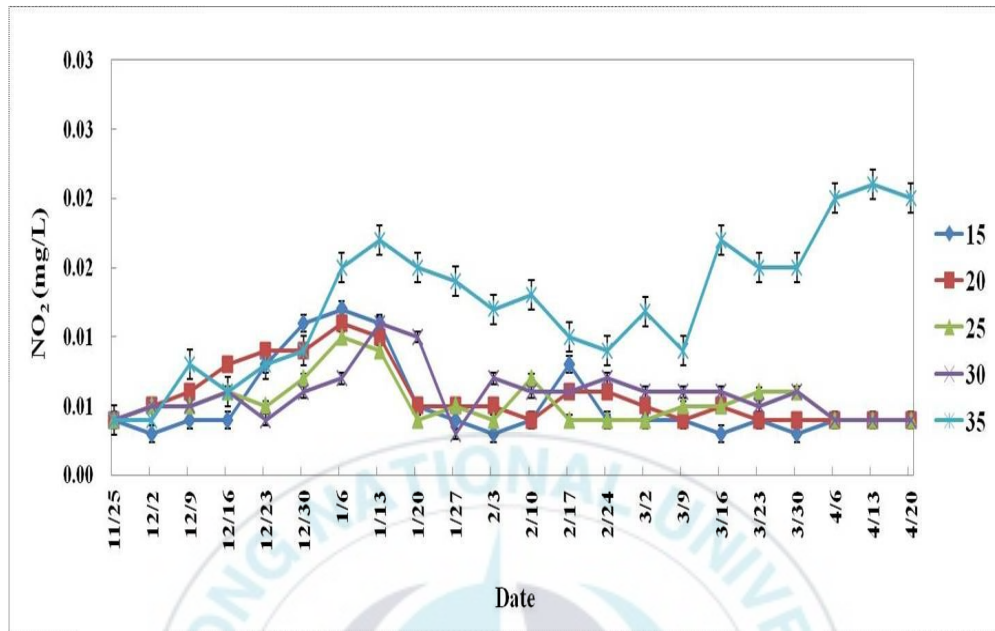


Fig. 17. Trend of nitrite at different salinity levels tested.

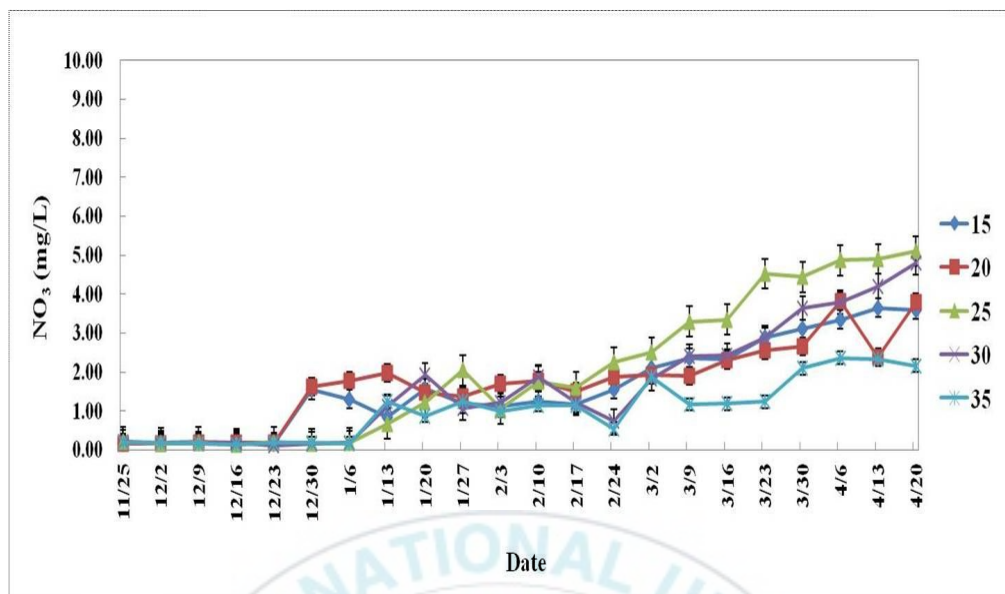


Fig. 18. Trend of nitrate at different salinity levels tested.

4. DISCUSSION AND CONCLUSION

Result from the present study support the hypothesis that salinity significantly affects this species' survival and growth. According to Garcês and Pereira (2010), survival rate of about 90% was recorded at 25 and 30psu, about 70% and 75% at 15 and 20 psu, respectively, after 2 months. This period represents approximately 62 days of the present study, which also recorded 78%, 87%, 93%, and 88.6% at 15, 20, 25, and 30psu, respectively. At 35psu, there was a contradictory outcome, which was also less expected. Highest growth of 0.031 ± 0.005 g and 0.078 ± 0.015 g, yet lowest survival rate of 73% and 23%; after 62 and 120 days of culture, respectively, were recorded. Inasmuch as it is not clear what exactly caused this low survival rate, it is believed to be a consequence of the worms' activity rather than an impact of the salinity level. Febry and Lutz (1987), Woo et al. (1997), among other researchers; offer good argument on the energetic cost of coping with salinity effects. According to them, under conditions requiring increased osmoregulatory or volume regulatory work, less energy may be available for growth, and organisms may reflect decreased rates of energy

intake (feeding). In the present study, juvenile worms used were hatched and raised to initial weight of 0.006 ± 0.0003 g at 35psu before subjecting them to reduced salinities of 30, 25, 20 and 15 psu. It could be that worms maintained at 35psu had quite adapted, hence, channelled their energy into active feeding and subsequent catabolism and growth; unlike those of the other salinity levels which probably channelled the bulk of their energy into coping with salinity effects. Though it is not clear the levels of dissolved ammonia and nitrite that are detrimental to the species' survival, a relatively high dissolved ammonia and nitrite of 0.3 mg/L and 0.02 mg/L, respectively were recorded at 35 psu compared with the relatively low values recorded in the others (see Fig. 16 and 17 above). In *Penaeus japonicus*, nitrite concentration has been known to increase directly with culture period (Chen et al., 1989, as cited in Edison, 2010). It is also reported that ammonia-N as low as 0.678 mg/L would cause an increase in oxygen consumption and ammonia-N excretion of *P. chinensis* juveniles (0.079 ± 0.004 g) in 20 h (Chen et al., 1991, as cited in Edison, 2010).

Ludwig (1957) in his quest to understand the quantitative laws in metabolism and growth noted that some group of organisms, including annelids, exhibit the second-type metabolism – where metabolic rate is

directly proportional to body weight. If increase in ammonia excretion reflects an increase in catabolism of amino acids, as opined by Chen and Nan (1993, as cited in Edison, 2010), then it suggests that the relatively high dissolved ammonia recorded at 35 psu could have been due to the worms' active feeding, which resulted in increased catabolism and growth, and the consequent increase in ammonia excretion into the ambient water. This might have had the reverse effect of ammonia toxicity – resulting in the low survival rate recorded at this salinity level. Diffusion of ammonia from blood to water is the main route for fish and crustaceans to excrete metabolic ammonia, since blood levels are normally much higher than ambient concentration (Kinne, 1976; Schmidt-Nielsen, 1997; as cited in Edison, 2010). Results of other studies exist that suggested that ammonia-N diffusion via hemolymph to ambient water was greater than that via ambient water to hemolymph (Chen & Kou, 1991; Chen et al., 1991; Chen and Nan, 1993; as cited in Edison, 2010).

In Garcês and Pereira's opinion, mortality was associated with high rates of cannibalism exhibited by *M. sanguinea* juveniles under disadvantageous environmental conditions.

Notwithstanding the above mentioned contradictory outcome, result of this experiment agrees with Garcês and Pereira's (2010) finding that juveniles of this species are very resistant to salinity fluctuation. A survival rate of 62% after 120 days at 15psu (see Table 1) is worth noting because low salinity, generally, reduces survivorship and growth in polychaetes – though tolerance to reduced salinity is species specific (Qiu & Qian, 1997; Pechnik et al., 1997; Prevedelli et al., 2009). At least, this species has proven to tolerate reduced salinities better than *H. elegans* adults which died at salinities ≤ 15 psu within 24 h (Qiu & Qian unpubl. data; as cited in Qiu & Qian, 1997). However, the mechanism which increases resistance to salinity range up to 15-35psu in *M. sanguinea* has not been described, like in other species (Ushakova et al., 2003),

As mentioned earlier, the primary significance of this study is to recommend this species as a potential candidate for IMTA, with respect to its salinity tolerance. Detailed documentation on IMTA system has been done by Barrington et al. (2009). These writers suggest that in IMTA system, site managers should consider some factors including temperature and salinity ranges, as these affect the performance of the species being grown. Lately, inland aquaculture of marine fish and shrimps in low salinity is on

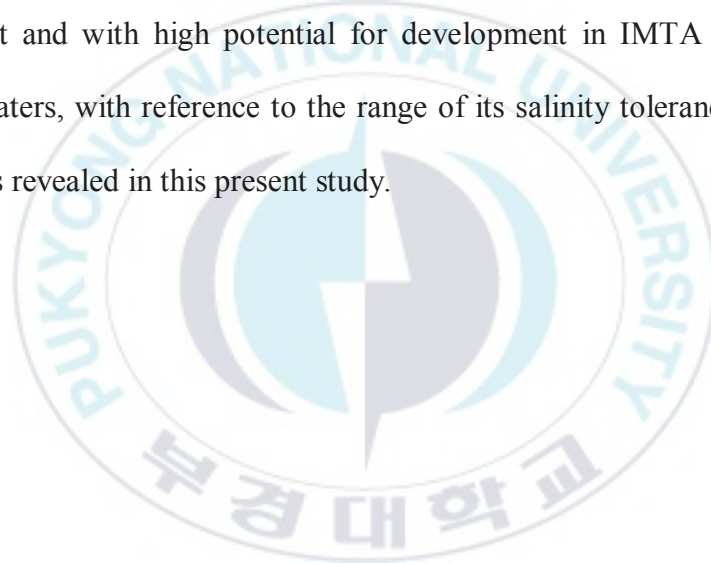
the rise (Riche et al., 2012; Roy et al., 2010); thus, results of this present study may offer a good argument that *M. sanguinea* has a good tolerance for reduced salinity (25 psu – 15 psu), hence, an excellent candidate for IMTA in such salinity range.

As opined by Barrington et al. (2009), in IMTA system, the co-cultured species should be more than just biofilters; they should also be harvestable, with economic value or potential. These writers also reveal that the present most advanced IMTA systems in open marine waters have three components (fish, suspension feeders such as shellfish, and seaweeds in cages and rafts), and that a more advanced systems will have several other components (e.g. crustaceans in mid-water reefs; deposit feeders such as sea cucumbers, sea urchins and polychaetes in bottom cages or suspended trays; and bottom-dwelling fish in bottom cages) for either different or similar functions but for different size brackets of particles, or selected for their presence at different times of the year.

Barrington et al. (2009) suggest *Nereis*, *Arenicola*, *Glycera* and *Sabella* as polychaete genera of particular interest and those with high potential for development in IMTA systems in marine temperate waters. Unfortunately, genus *Marphysa* is not mentioned, probably due to the lack of proper

documentation on this genus; because just like the other mentioned polychaetes, *M. sanguinea* has high market value as fishing bait, the potential as a food supply for fish brood stock, and their role as a sediment bioremediator (Garcês and Pereira, 2010; Parandavar & Kim, 2014).

Based on the initiation or continuity of further research into alternative species to ensure further expansion of IMTA, as suggested by Barrington et al. (2009); it is recommended that *M. sanguinea* be considered as a species of interest and with high potential for development in IMTA systems in marine waters, with reference to the range of its salinity tolerance (20 psu- 35 psu) as revealed in this present study.



REFERENCES

- Alderdice, D. E. (1972). Factor combinations: responses of marine poikilotherms to environmental factors acting in concert. *In*: Kinne, O (Ed.), *Marine ecology: a comprehensive, integrated treatise on life in oceans and coastal waters: 1. Environmental factors*, 3. (pp. 1659 - 1722).
- Anger, K., Spivak, E., & Luppi, T. (1998). Effects of reduced salinities on development and bioenergetics of early larval shore crab, *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology*, 220, 287 - 304.
- Barrington, K., Chopin, T., & Robinson, S. (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In D. Soto (Ed.), *Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper*, 529 (pp. 7–46). Rome, FAO.
- Bochert, R., Dirk F. & Roger B. (1996). Influence of salinity and temperature on growth and survival of the planktonic larvae of

- Marenzelleria viridis* (Polychaeta, Spionidae). *Journal of Plankton Research*, 18(7), 1239-1251.
- Bogucki, M. (1963). The influence of salinity on the maturation of gametes of *Nereis diversicolor* O. F. Müller. In P. W. Warszawa (Ed), *Polskie Archiwum Hydrobiologii*, 11, (343 - 347). Warsaw, Poland: Nencki Inst. Exp. Biol.
- Dorgelo, J. (1976). Salt tolerance in crustacea and the influence of temperature upon it. *Biological Reviews*, 51(3), 255 - 290.
- Edison, B. (2010). Acute toxicity of ammonia in white shrimp (*Litopenaeus schmitti*) (Burkenroad, 1936, Crustacea) at different salinity levels. *Aquaculture*, 306 (2010), 329 - 333.
- Fauchald, K. (1977). *The polychaete worms; definitions and keys to the orders, families and genera*. Los Angeles: Science series 28.
- Febry, R., Lutz, P. (1987). Energy partitioning in fish: the activity - related cost of osmoregulation in a euryhaline cichlid. *The Journal of Experimental Biology*, 128, 63 – 85.
- Garcês, J. P., & Pereira, J. (2010). Effect of salinity on survival and growth of *Marphysa sanguinea* Montagu (1813) juveniles. *Aquaculture International* (2011) 19, 523–530.

- Henriksen, K., Rasmussen, M. B., & Jensen, A. (1983). Effect of bioturbation on microbial nitrogen transformations in the sediment and fluxes of ammonium and nitrate to the overlaying water. *Ecological Bulletins*, 35, 193 - 205.
- Honda, H., & Kikuchi, K. (2002). Nitrogen budget of polychaete *Perinereis nuntia vallata* fed on the feces of Japanese flounder. *Fisheries Science*, 68(6), 1304-1308.
- Hutchings, P. (1998). Biodiversity and functioning of polychaetes in the benthic sediments. *Biodiversity & Conservation*, 7(9), 1133-1145.
- Hutchings, P. A., & Karageorgopoulos, P. (2003). Designation of a neotype of *Marphysa sanguinea* (Montagu, 1813) and a description of a new species of *Marphysa* from eastern Australia. *Hydrobiologia*, 496(1-3), 87 – 94.
- Hutchings, P., Christopher, J. G., & Sander, W. (2011). Note on additional diagnostic characters for *Marphysa sanguinea* (Montagu, 1813) (annelida: eunicida: eunicidae), a recently introduced species in the Netherlands. *Aquatic Invasions*, 7(2), 277–282.
- Ito K., Nozaki M., Kunihiro T. (2011). Study of sediment cleanup using polychaetes. In K. Omori, X. Guo, N. Yoshie, N. Fujii, I. C. Handoh,

- A. Isobe, and S. Tanabe (Eds.), *Interdisciplinary studies on environmental chemistry- marine environmental modelling and analysis* (pp.133-139).
- Kinne, O. (1970). Temperature: animals-invertebrates. In O. Kinne (Ed.), *Marine ecology, 1, Environmental factors, part 1* (pp. 407-514). London: Wiley.
- Kinne, O. (1971). Salinity: animals-invertebrates. In O. Kinne (Ed.), *Marine Ecology, 1, Environmental factors, part 2* (pp. 821 – 995). London: Wiley.
- Kim, C. H, & Jang, S. W. (2008). Effects of rearing conditions on the artificial seed production of a polychaete *Marphysa sanguinea*. *Journal of Aquaculture*, 21 (1), 34 - 40.
- Lambert, Y., Dutil, J. D., & Munro, J. (1994). Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, 51(7), 1569 - 1576.
- Lim H. K. *Korean Aquaculture: Status and Future Directions*. National Fisheries Research and Development Institute, Busan, Korea.

- Ludwig, V. B. (1957). Quantitative laws in metabolism and growth. *The Quarterly Review of Biology*, 32, 217 – 229.
- Lyster, I. H. J. (1965). The salinity tolerance of polychaete larvae. *Journal of Animal Ecology*, 34(3), 517-527.
- Monia, E. B., Patrick, S. & Fathia, Z. (2013). Reproductive Cycle of *Marphysa sanguinea* (Montagu, 1815) (Polychaeta: Eunicidae) in the Lagoon of Tunis. *The Scientific World Journal*, 2013, 7 pages.
- Montagu, G. (1815). Descriptions of several new or rare animals, principally marine, discovered on the South coast of Devonshire. *Transactions of the Linnean Society of London*, 11, 1–26.
- Morritt, D., Stevenson, T. D. I. (1993). Factors influencing breeding initiation in the beachflea *Orchestia gammarellus* (Pallas) (Crustacea: Amphipoda). *Journal of Experimental Marine Biology and Ecology*, 165(2), 191-208.
- Olive, P. J. W. (1994). Polychaeta as a world resource: a review of patterns of exploitation as sea angling baits and the potential for aquaculture based production. In Dauvin, J. C. et al. (Ed.), *Actes de la 4ème conférence internationale des Polychètes* (pp. 603–610). Angers, France.

- Olive, P. J. W. (1999). Polychaete aquaculture and polychaete science: a mutual synergism. *Hydrobiologia*, 402(0), 175-183.
- Parandavar, H. & Kim, C. H. (2014). Outlet water quality improvement in fish culture by rockworm *Marphysa sanguinea* in the semi-recirculating system. *Journal of Middle East Applied Science and Technology*, (7), 329-333.
- Pechnick, J. A, Richelle, B., & Lisa, K. (2000). Effects of reduced salinity on survival, growth, reproductive success, and energetics of the euryhaline polychaete *Capitella* sp. I. *Journal of Experimental Biology and Ecology*, 254(2000), 19- 35.
- Pechenik, J. A., Pearse, J. S., & Qian, P. Y. (2007). Effects of salinity on spawning and early development of the tube-building polychaete *Hydroides elegans* in Hong-Kong: not just the sperm's fault? *Biological Bulletin* 212(2), 151–160.
- Prevedelli, D. (1994). Influence of temperature and diet on the larval development and growth of juveniles *Marphysa sanguinea* (Montagu) (Polychaeta, Eunicidae). *National Marine Biological Library*, 162, 521- 526.

- Prevedelli D., & Vandini, R. Z. (1997). Survival and growth rate of *Perinereis rullieri* (Polychaeta, Nereididae) under different salinities and diets. *Italian Journal of Zoology*, 64, 135-139.
- Prevedelli, D., Gloria, M. N., Ivano, A., & Simonini R., (2007). Life cycle of *Marphysa sanguinea* (Polychaeta: Eunicidae) in the Venice Lagoon (Italy). *Marine Ecology*, 28, 384-393.
- Qiu, J. W. & Qian, P.Y. (1997). Combined effects of salinity, temperature, and food on early development of the polychaete *Hydroides elegans*. *Marine Ecology Progress Series*, 152, 79-88.
- Riche, M. A., Timothy, J. P., Paul, S. W., Jon, J. A., & Maria, S. S. (2012). Inland marine fish culture in low salinity recirculating aquaculture systems. *Bulletin of Fisheries Research Agency*, 35, 65-75.
- Rosas, C., Ocampo, L., Gaxiola, G., Sanchez, A., & Soto, L. A. (1999). Effect of salinity on survival, growth, and oxygen consumption of postlarvae (PL10-PL21) of *Litopenaeus setiferus*. *Journal of Crustacean Biology*, 19(2), 244-251.
- Roy, L. A., Davis, D. A., Saoud, I. P., Boyd, C. A., Pine, H. J., & Boyd, C. E. (2010). Shrimp culture in inland low salinity waters. *Reviews in Aquaculture*, 2, 191-208.

- Ruppert, E. E., & Fox, R. S. (1988). *Seashore animals of the Southeast: a guide to common shallow-water invertebrates of the south-eastern Atlantic Coast*. Columbia, SC.: University of South Carolina Press.
- Smith, R. I. (1957). A note on the tolerance of low salinities by Nereid polychaetes and its relation to temperature and reproductive habitat. *Ann. Biology*, 33(1957), 93-107.
- Smith, R. I. (1964). On the early development of *Nereis diversicolor* in different salinities. *Journal of Morphology*, 114(3), 437-463.
- Specker, J. L., Schreiber, A. M., McArdle, M. E., Poholek, A., Henderson, J., & Bengtson, D. A. (1999). Metamorphosis in summer flounder: effects of acclimation to low and high salinities. *Aquaculture*, 176(1-2), 145–154.
- Tietjen, J. H., & Lee, J. J. (1972). Life cycles of marine nematodes. *Oecologia*, 10(2), 167–176.
- Ushakova, O. O. (2003). Combined effect of salinity and temperature on *Spirorbis spirorbis* L. and *Circeus spirillum* L. larvae from the White Sea. *Journal of Experimental Marine Biology and Ecology* 296(1), 23 – 33.

- Withers, P. C. (1992). *Comparative Animal Physiology*. New York: Saunders College Publishing.
- Woo, N. Y. S., Ng, T. B., Leung, T. C., & Chow, C. Y. (1997). Enhancement of growth of tilapia *Oreochromis niloticus* in iso osmotic medium. *Journal of Applied Ichthyology*, 13(2), 67–71.



ACKNOWLEDGMENT

My deepest appreciation goes to Prof. Chang-Hoon Kim, my able supervisor.

Secondly, I am very grateful to all my laboratory mates, especially, to Em Thu and Kyeon Hun Kim, for their diligent assistance in designing and carrying out this experiment.

Thanks to my thesis advisory committee members – Prof. Jong Myoung Kim and Prof. Wongyu Park.

Thanks to KOICA- PKNNU, especially, to Prof. Kang Kyoungmi (Program coordinator) and Ms. Seulki Kim (Student coordinator).

I thank all loved ones who spent their quality time to proof-read my manuscript, especially, Mr. Daniel Asante Boamah of Queen's University, Kingston, Canada.

I am ever grateful to the Ministry of Fisheries and Aquaculture Development (MoFAD) – Ghana.