Thesis for the Degree of Master of Fisheries Science

Growth and Survival Rate of *Marphysa sanguinea* (Polychaeta: Eunicidae) Juveniles by Tidal Water Drainage

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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바위털갯지렁이 치충의 성장과 생존율에 미치는 간출의 영향

Advisor: Prof. Chang-Hoon Kim

by

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Abstract

A study was conducted to evaluate the effect of different time exposures by tidal water drainage on growth and survival of *Marphysa sanguinea*, the abundance of copepods and relationship between copepod abundance and survival rate of *M. sanguinea*. Triplicate groups of worms (initial weight 0.19 ± 0.17g) were reared under five different time exposures (0hr, 1hr, 2hrs, 3hrs, 4hrs and 5hrs) for 120 days in a semi-recirculating system. Oyster shells (70%, 5 mm in diameter) and sand (30%, 2 mm in diameter) were used together as sediment. After the culture period, there were no significant differences in weight gain (WG) and specific growth rate (SGR) among juvenile *M. sanguinea* reared at 0hr, 1hr, 2hrs, 4hrs and 5hrs exposure to tidal water drainage. Survival rate of juvenile *M. sanguinea* reared at 3hrs and 4hrs was significantly higher than those of the control (0hr) ($p>0.05$). However, there were no significant differences among the survival rates of juvenile *M. sanguinea* reared at 0hr, 1hr, 2hrs and 5hrs and also among those of 1hr, 2hrs, 3hrs, 4hrs and 5hrs. The lowest copepod numbers were recorded at 5hr exposure and the lowest number was recorded at 0hr exposure of *M. sanguinea* juveniles to tidal water drainage. The copepod abundance reduced with increasing time exposure, however, at 5hrs time exposure, survival rate reduced. Therefore, it could be concluded that the optimum exposure time to tidal drainage for *M. sanguinea* could be between 3hrs to 4hrs for maximum survival.

Keywords: *Marphysa sanginea*, Time Exposure (tidal water drainage), Growth and Survival rate, Copepods.

1. Introduction

Polychaetes are amongst the most abundant of marine organisms. They are distinguished by linear series of external ringlike segments; the grooves between the segments coincide with internal compartments, often separated by transverse septa, containing serially repeated nervous, muscle and excretory systems. These worms are segmented annelids with a pair of leg-like appendages (parapodia, unique to polychaetes) (Fig. 1). They are known to be good indicators of species richness (Olsgart and Somerfield, 2000) and as bio-indicators of the marine environment (Pocklington and Wells, 1992; Giangrande et al., 2005). Polychaetes are distributed in all oceans of the world and at all depths of the water column. They are found in unique habitats, but are especially abundant in the benthos where they constitute 35-70% of macro invertebrate populations (Díaz-Castañeda and Reis, 2014). However, it is a diverse group with many species and they usually constitute a large proportion of the macro benthos.

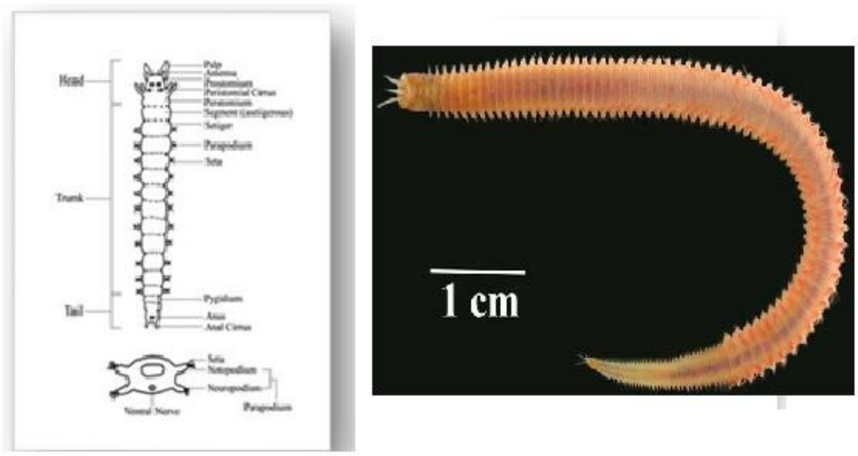

Fig. 1. General morphological features and structure of polychaetes.

The rockworm, *Marphysa sanguinea* is a large polychaete (order: Eunicida; family: Eunicidae) that can grow up to a length of 40 cm with a lifespan of approximately 90 days (Prevedelli et al. 2007). Juvenile worms develop two weeks after hatching and take approximately 45 days to reach adulthood. During this time, individuals feed, produce additional segments and structures, and grow to about 27 mm in length. It can be found in a variety of benthic environments, including soft muddy sediments around oyster reefs, sandy or muddy tidal flats, among other fouling organisms on dock pilings and buoys, and inside holes of calcareous rocks (Ruppert and Fox 1988). They are cosmopolitan species: distributed globally at temperate to tropical latitudes (Hutchings and Karageorgopoulos 2003; Prevedelli et al. 2007). *M. sanguinea* is widely distributed in the south coast of England, Eastern Scheldt in south-western Netherlands, and along the

Italian coasts (Prevedelli et al., 2007; Hutchings et al., 2011); along the Sado estuary, particularly within former oyster production areas in Portugal (Garcês and Pereira, 2011) and also in the lagoon of Tunis in Tunisia (Monia et al. 2013).

The demand for *M. sanguinea* has increased rapidly in recent years because they are important nutrient sources for stimulating gonad maturation and spawning of fish and crustaceans in hatcheries for aquaculture purposes (Olive, 1999). They have several advantageous features that may make it ideal for use in environmental studies (Jaramillo et al., 2001). They are also used for culture and commercial purposes to reduce the substrate harvesting disturbance, and the great biogeochemical and benthic community impact (Gambi et al., 1994). However, the production costs of *M. sanguinea* in an intensive aquaculture system should be efficient enough to make profits (Nesto et al., 2012).

The copepods are crustaceans, like shrimps or crabs, with the segmented body covered with a chitinous carapace similar to the pieces of armour. Their size ranges from some tenths of a millimetre to a couple of centimetres, although most commonly they are around one millimetre in length. In general, their body is semitransparent, but in occasions can accumulate pigments or droplets of reserve oil, and then have different colours, from reddish or green, to black or iridescent. They are the most frequent organisms of the zooplankton, can be found at all latitudes and depths, and are considered to be the most abundant metazoans in our planet, even more than insects.

As with other organisms, growth and lifespan of marine invertebrates varies with food availability, water temperature, salinity, predator abundance, and other environmental factors. The relationship between environmental conditions, such as time exposure to tidal water drainage, and growth of invertebrates has been well studied. Previous studies have also revealed that inter-specific competition may result in density dependent growth (Jensen and Kamerman; 1992). Therefore, this study was designed to evaluate the effects of tidal water drainage] at different time intervals on growth performance and survival rate of *Marphysa sanguinea* juveniles, the abundance of copepods and the relationship between copepod abundance and survival rate of *Marphysa sanguinea*.

2. Materials and Methods

2.1 Study Area

The study was conducted in Fisheries Science and Technology Centre of Pukyong National University (PKNU) located in Goseong-gun, Gyoungsang Nam-do (Fig. 2). It is adjacent to Geoje, Sacheon, Tongyeong, Masan, and Jinju and linked to the Daejeon-Tongyeong Expressway, National Roads No. 14 and No. 33.

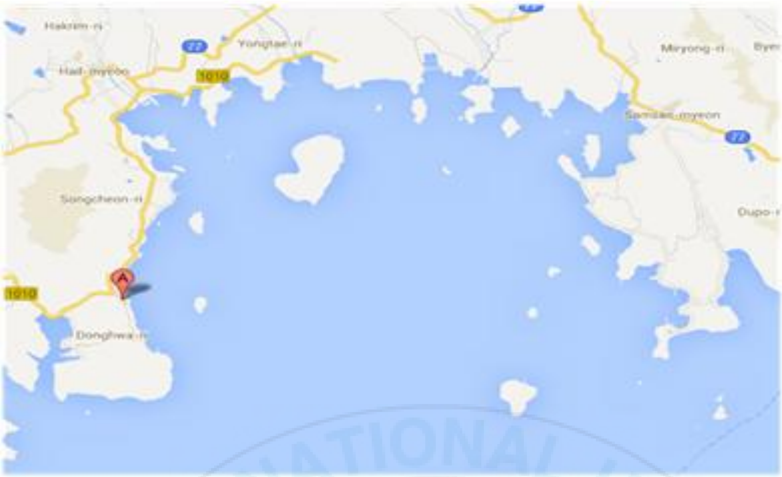

Fig. 2. Fisheries and science technology centre of Pukyong National University (PKNU) located in Goseong-gun, Gyoungsang Nam-do.

2.2 Juvenile Rock Worms and Experimental design

Approximately 360 (initial weight 0.19 ± 0.17g) juvenile rock worms were obtained from the Fisheries Science and Technology Centre of the Pukyong National University (PKNU) Goseong-gun, Gyoungsang Nam-do. The worms were collected by scooping the sediment siphoning with a pipette (Fig. 3). 20 individuals per box were collected and put in 28 litre (45cm × 25cm × 10cm) boxes where they were being raised after hatching. PVC pipes were fitted to the rearing tanks and used as water outlet and overflow. The outlet pipes were covered with nets (1mm mesh size). The containers were filled with treated oyster shells to about 15-16cm deep. The set-up is a semi-recirculating system fitted with reducers, making use of about 450 liters of seawater. The boxes were arranged in 3 rows on 3 floors with 6 boxes on each floor (Fig. 4). Seawater was pumped from the Jaran Bay adjacent to the Research Center, and tap water was the sources of water used in the experiment (Fig. 5). The water was constantly aerated and changed partially (30%) every week and totally on monthly basis throughout the experiment. The flow rate of the water was kept constant at 2L min^{-1}.

The rock worm juveniles were reared under different time exposure times to tidal water drainage; 0hr (control), 1hr, 2hrs, 3hrs, 4hrs, 5hrs in triplicate groups for 3 months. A timer was used to control the different time exposures. Nets were put under all reducers to collect copepods after the time exposure, and counted under the microscope. After the exposure, the tanks were filled with water again and the worms fed with a commercial shrimp feed at 3% of wet body weight once a day. Salinity and temperature were kept constant at 25 psu and 19 ℃ respectively. The set-up was kept under a 12 hrs dark and 12hrs of light photo-period. pH, NH_3 (Ammonia), NO_2 (Nitrite) and Alkalinity were measured weekly.

2.3 Data collection

At the end of the experimental period, rock worms in each box were weighed and counted to calculate weight gain (WG), specific growth rate (SGR) and survival rate. Worms were weighed after 30 days, 60 days, and finally after 120 days. During the collection process, boxes were emptied of their sediments and individual worms were collected.

2.4 Statistical Analysis

All data were subjected to one-way analysis variance (ANOVA) using SAS program Version 9.1(SAS Institute, Cary, NC, USA) to test the effects of the different exposure times. When a significant effect was observed, a least significant difference (LSD) test was used to compare the means. Treatment effects were considered significant at confidence level of $p>0.05$. The mean and SD of the mean was calculated for each treatment.

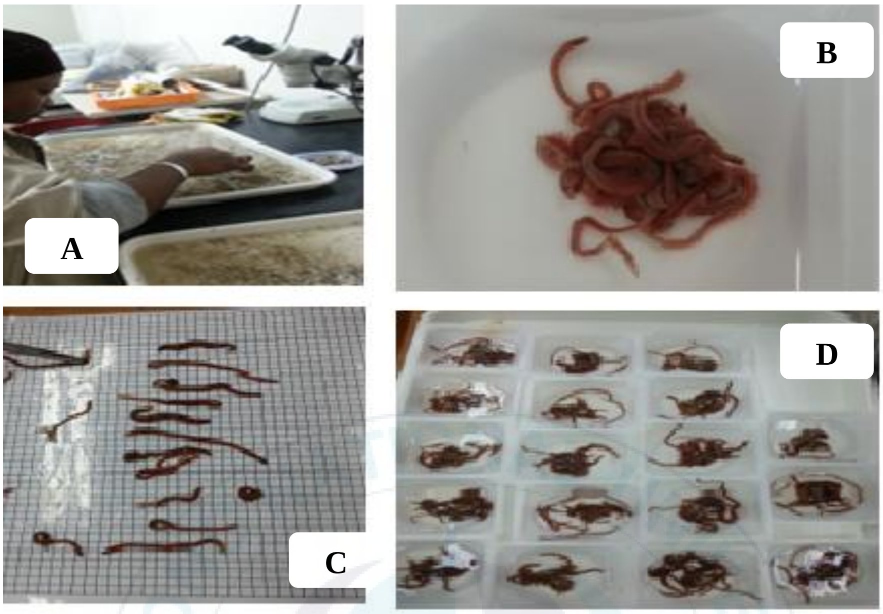

Fig. 3. Collection of worms (A) Specimen collection of individual worms siphoned out worms by pipette. (B) Juvenile worms collected Sediment from A spread on a tray; (C and D) Juvenile worms collected before measuring weight.

Fig. 4. Experimental set-up (A): Arrangement of boxes (B) nets used to collect copepods under the reducers and drums used as main recirculating tanks in the experiment. (C) Pump. (D) Drum of water with pump. (E) Inside of box, showing inlet water and aerator, (F) Timer for controlling time exposure.

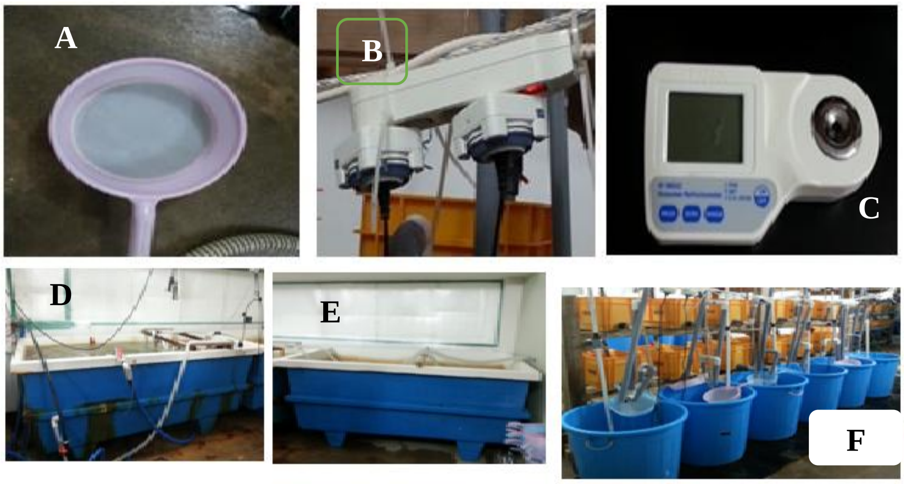

Fig. 5. Experimental water preparation. (A) Net for collecting copepod (B) The timer controlling the time of exposure connecting to the pump (C) H9682 Seawater Refractometer for checking the salinity (D) Tank for storing filtered sea water (E) Tank for storing fresh water (Tap water) (F) Net fitted under reducers for collecting copepods

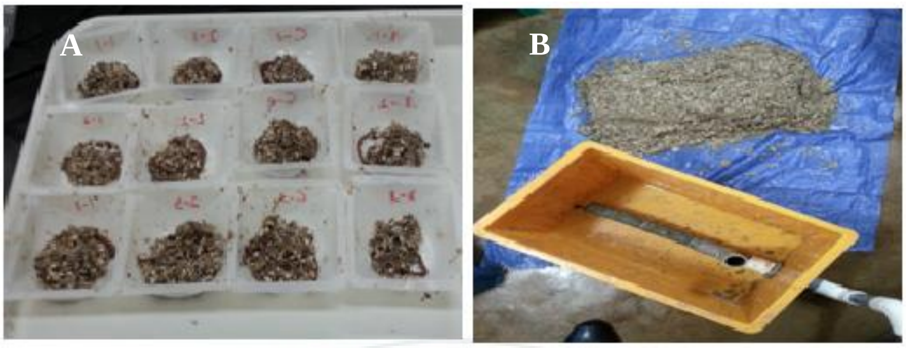

Fig. 6. Collection of juvenile rock worms for experiments (A) Scooping sediment bit by bit from box. (B) Collecting worms by hands.

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3. RESULTS

3.1 Growth performance and Survival rate

Growth performance of juvenile *Marphysa sanguinea* exposed to tidal water drainage is summarised in Table 1 and graphically presented in Fig. 7, 8 and 9. There were no significant differences in weight gain (WG) and specific growth rate (SGR) among juvenile *M. sanguinea* reared at 0hr, 1hr, 2hrs, 4hrs and 5hrs exposure to tidal water drainage. From Fig. 7and 8, the highest WG and SGR was recorded in the third month of the study. Survival rate of juvenile *M. sanguinea* reared at 3hrs and 4hrs was significantly higher than those of the control (0hr). However, there were no significant differences among the survival rates of juvenile *M. sanguinea* reared at 0hr, 1hr, 2hrs and 5hrs and also among those of 1hr, 2hrs, 3hrs, 4hrs and 5hrs.

3.2 Abundance of Copepods

The trend of copepod abundance recorded during the rearing of *M. sanguinea* juveniles exposed to tidal water drainage is shown in Fig. 10. Copepod abundance increased in all groups between day 21 and day 29 of rearing juvenile *M. sanguinea* juveniles. However, the lowest copepod numbers were recorded at 5hr exposure of *M. sanguinea* juveniles to tidal water drainage followed by 4hr, 3hrs, 2hrs, 1hrs and 0hrs in order of increasing numbers.

From the 41st to 48th days, copepod numbers were highest at 1h exposure to tidal water drain, of *M. sanguinea.*

3.3 Water Quality Parameters

Mean water quality measurements during the rearing of *M. sanguinea* juveniles exposed to tidal water drainage are presented in Fig. 11 to 14. There were no significant differences recorded in nitrates (NO_2), ammonia (NH_3), alkalinity and pH in the rearing water at 0hr, 1hr, 2hrs, 3hrs, 4hrs and 5hrs of *M sanguinea* exposure to tidal drainage over the study period.

Table 1 Weight gain, Specific growth rate and Survival rate of *Marphysa sanguinea* juveniles exposed to tidal water drainage for 120 days[1]

Time Exposure (h)	Initial weight (g)	Final weight (g)	Weight gain[2] (%)	SGR[3] (%/ day)	SR[4] (%)
0	0.162	0.65 [a]	410.90[a]	1.80 [a]	86.66 [b]
1	0.20	0.69 [a]	343.09[a]	1.65 [a]	90.00 [ab]
2	0.18	0.64 [a]	363.06[a]	1.68 [a]	96.66 [ab]
3	0.17	0.56 [a]	335.66[a]	1.63 [a]	100.00 [a]
4	0.19	0.58 [a]	310.32[a]	1.56 [a]	100.00 [a]
5	0.16	0.54 [a]	333.12[a]	1.63 [a]	90.00 [ab]

[1]Values are means from triplicate groups of worms (n = 3):

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

[3]Specific growth rate (%/day) = [(ln final weight of worm – ln initial weight of worm) / days] x100

[4]Survival rate (%): (final individuals / initial individuals) x 100

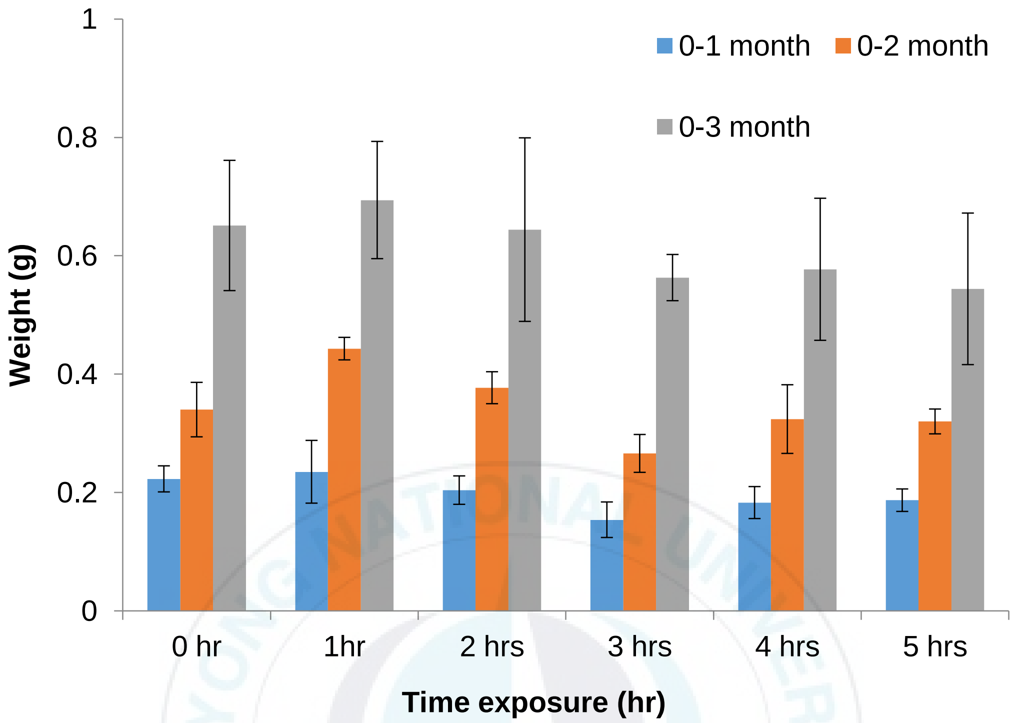

Fig. 7. Weight gain of *Marphysa sanguinea* juveniles exposed to tidal water drainage for 120 days.

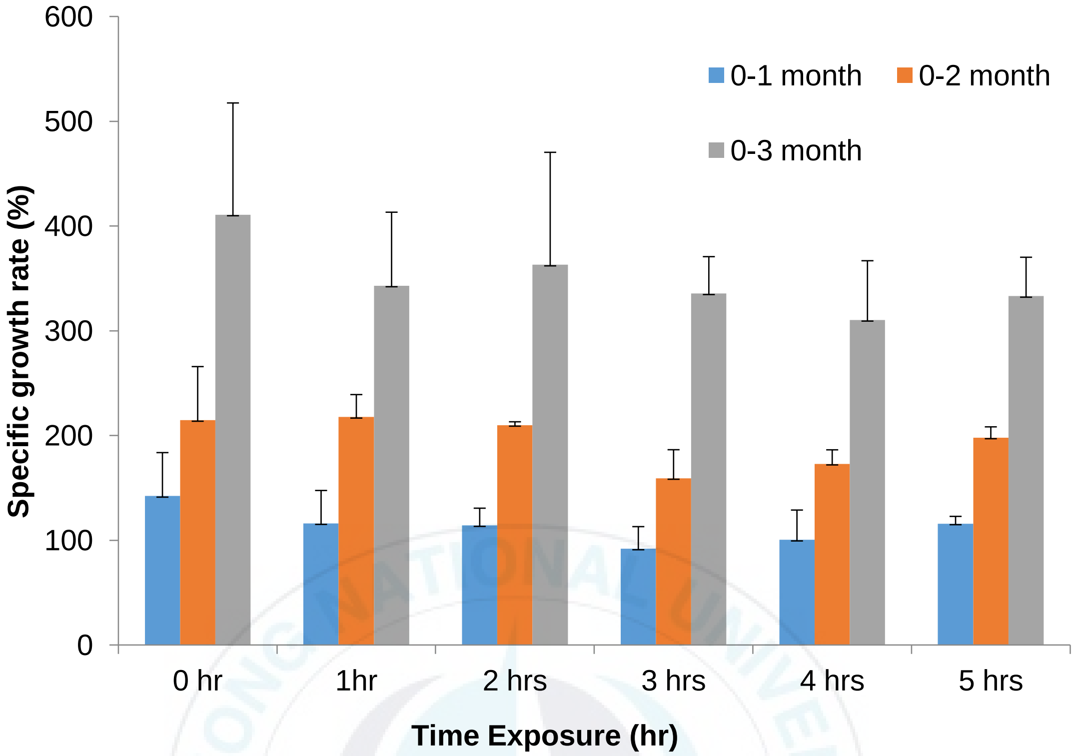

Fig. 8. Specific growth rate of *Marphysa sanguinea* juveniles exposed to tidal water drainage for 120 days.

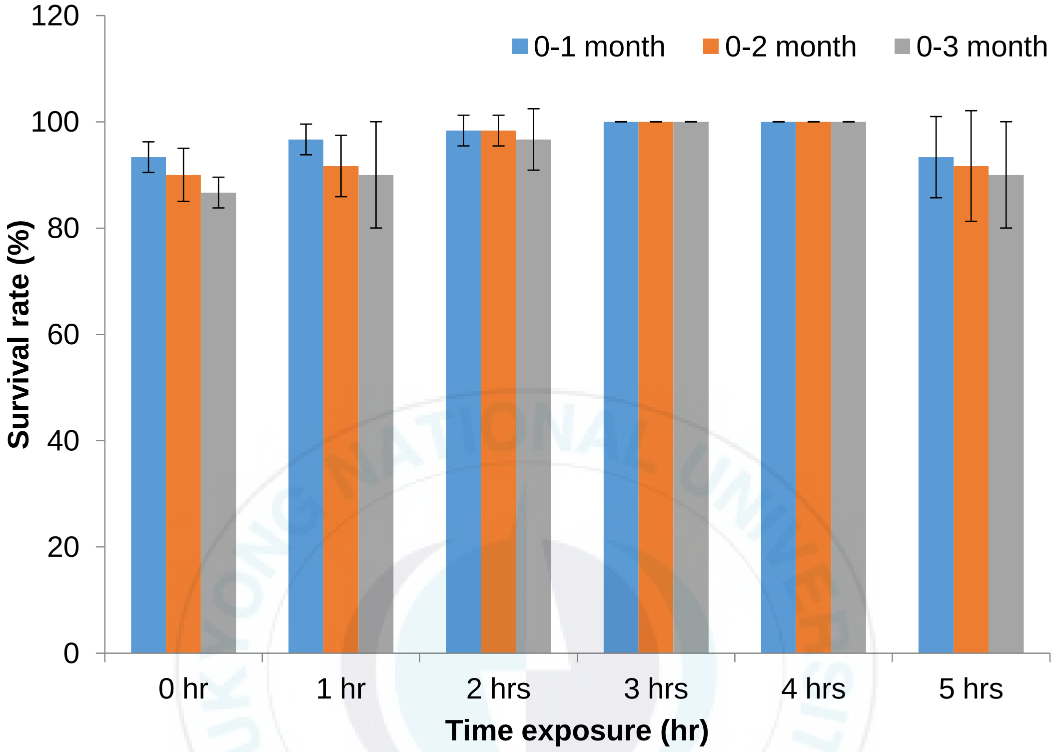

Fig. 9. Survival rate of *Marphysa sanguinea* juveniles exposed to tidal water drainage for120 days.

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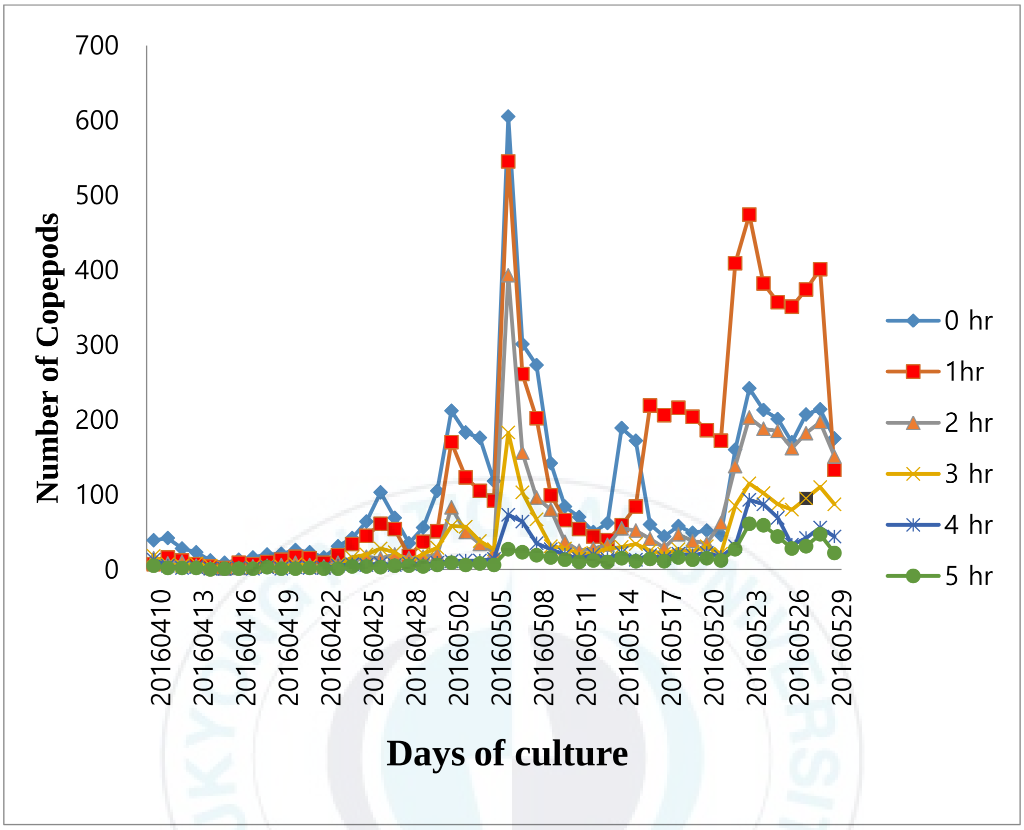

Fig 10. Trend of copepod abundance recorded during rearing of *Marphysa sanguinea* juveniles exposed to tidal water drainage for 120 days.

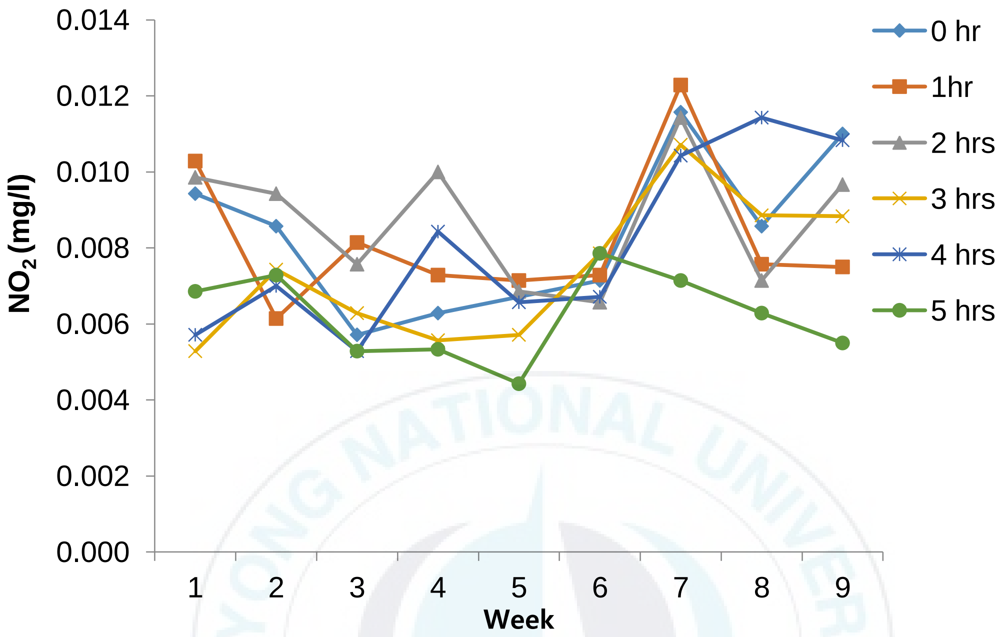

Fig. 11. Trend of nitrate (NO_2) concentration recorded in rearing water of *M. sanguinea* exposed to tidal water drainage for 120 days.

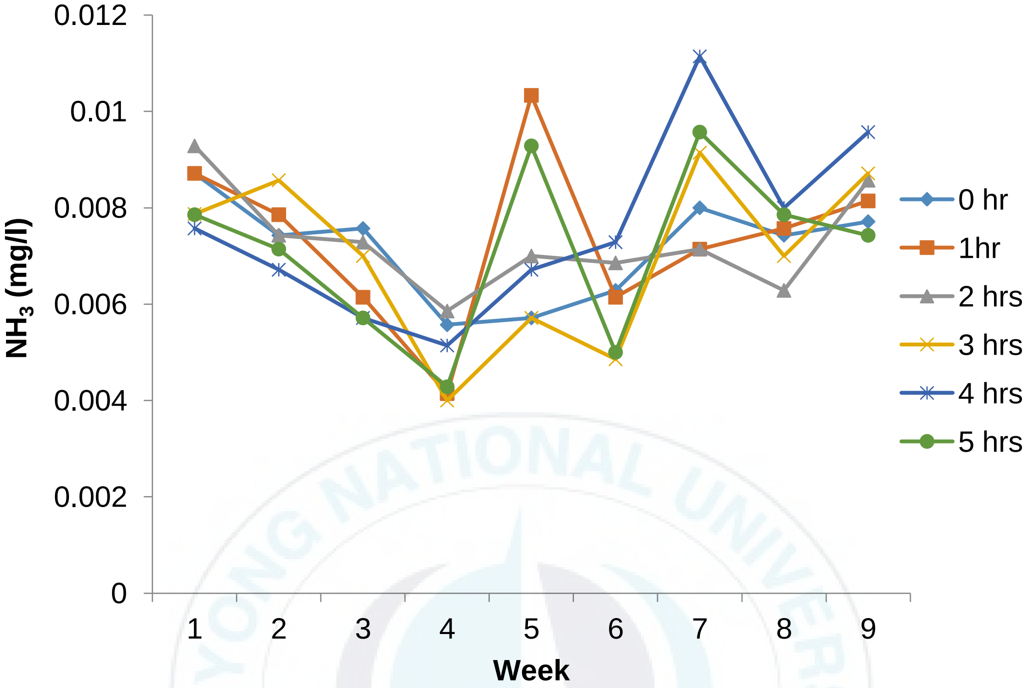

Fig. 12. Trend of ammonia (NH_3) concentration recorded in rearing water of *M. sanguinea* exposed to tidal water drainage for 120 days.

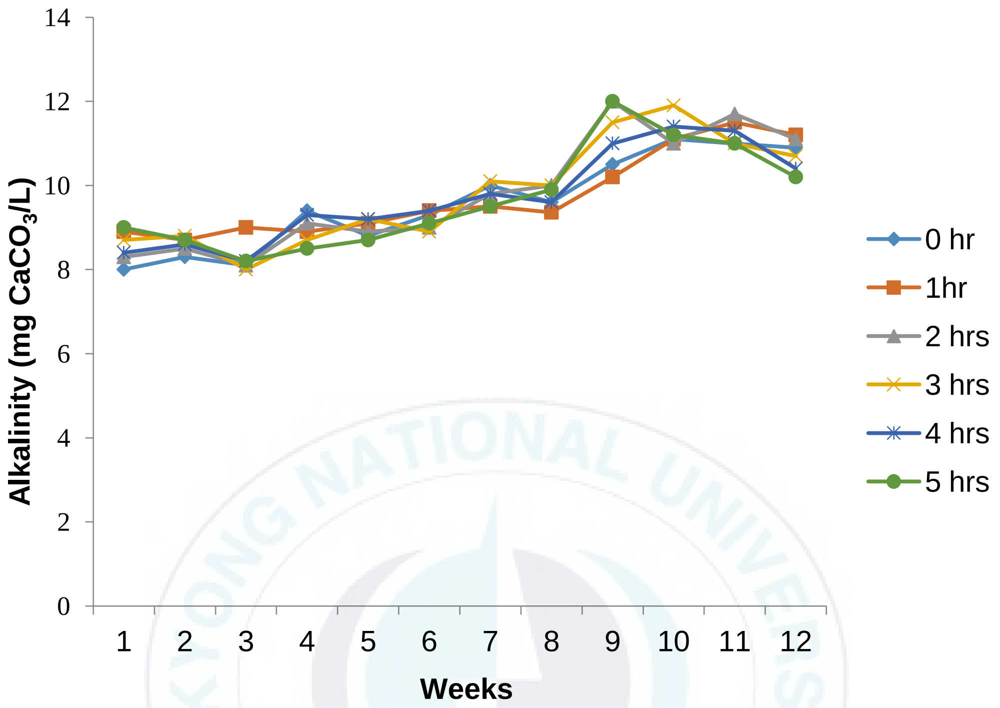

Fig. 13. Trend of alkalinity concentration recorded in rearing water of *M sanguinea* exposed to tidal water drainage for 120 days.

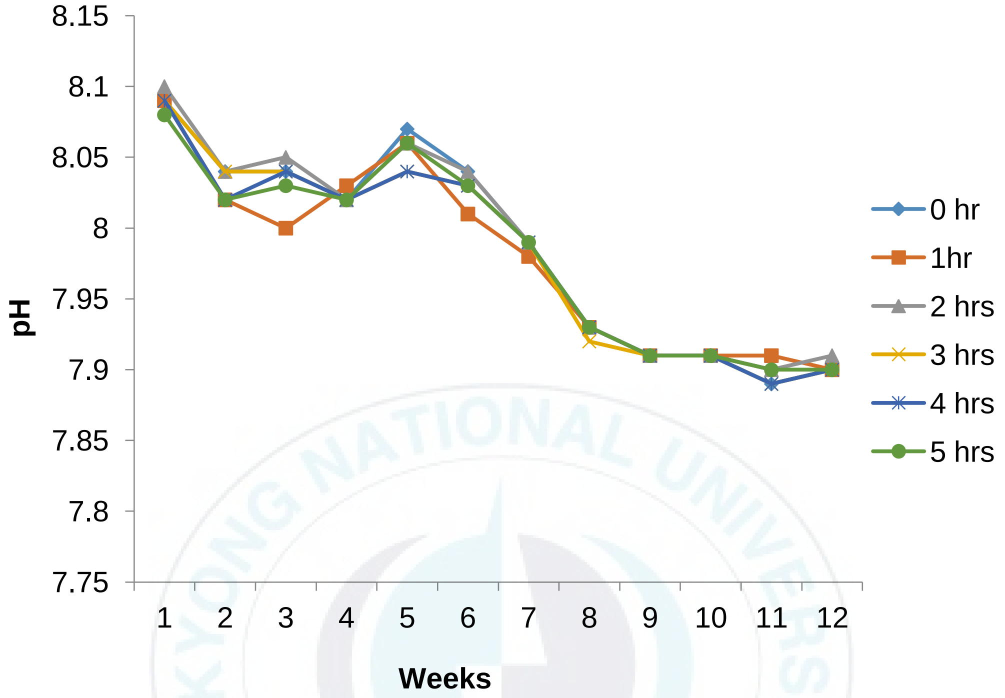

Fig. 14. Trend of pH concentration recorded in rearing water of *M sanguinea* exposed to tidal water drainage for 120 days.

4. Discussion and Conclusion

The results of this study demonstrate that exposure to tidal water drainage do not have significant effects on growth of *Marphysa sanguinea*. This is because no significant differences were found in the weight gain (WG) and specific growth rate (SGR) of juvenile *M. sanguinea* reared at the different time exposures to tidal water drainage. Some worms showed relatively small variations in growth under different environmental conditions, and this may also be attributed to their way of feeding. It is well known that the quality and quantity of available food can affect reproduction and growth in marine invertebrates (Minor and Scheibling 1997; Prevedelli and Vandini, 1997; Prevedelli and Simonini, 2000).

Contrary to growth performance, the time exposure to tidal drainage could affect the survival rate of *M. sanguinea*. The results demonstrate a more positive survival of juvenile *M. sanguinea* reared at 3hrs and 4hrs compared to those of 0hr, 1hr, 2hrs and 5hrs. Low survival rate during the time exposure to tidal water drainage could be attributed to tidal height and sediment type. Other factors such as salinity, light, current speed, exposure to high-energy environments, exposure to siphon-cropping, population density, and genotype have also been noted to influence the growth and survival of marine invertebrates. The interrelationships between many of these variables make it difficult to isolate the most important causal factors (Seed, 1980). Temperature, food quantity, composition (Rhoads and Young, 1970; Whitlatch, 1980, 1981) and the pressure of predation (Neill, 1975; Bell and Coull 1980; Zobrist and Coull 1992) are among the main factors influencing the growth and survival marine benthos.

Watzin (1983) showed that several groups of macro invertebrate larvae and juvenile were the prey of permanently meiofaunal turbellarians, nematodes and copepods. Copepods have been shown to be facultative predators of polychaete larvae in previous studies (Watzin 1983, Dahms et al., 2004). The abundance of copepods decreased with increasing time exposure during the study period. The negative relations between time exposure and growth have been found in majority of the species studied and predation was another factor which may obscure the relations between environmental conditions and growth of the invertebrates (Goss-Custard, 1977, Zwarts and Drent, 1981; Hulscher, 1982; Sutherland, 1982; Zwarts and Esselink, 1989). It is evident that the density of juvenile polychaetes is significantly affected by the increase in the density of meiofaunal predators (Dauvin, 1990; Fogarty et al., 1991; Coull, 1992). The abundance of different polychaete families (i.e. paranoids and spionids with dominant species, *P. caspersi*) was significantly affected when the number of predators (copepods) increased. Competition for food between deposit feeding macrofauna and deposit feeding meiofauna could also occur, as well as competition for space since both meiofauna and macrofauna juveniles occupy the uppermost of the sediments (Bell and Coull 1980).

The levels of dissolved ammonia were not significantly affected by the exposure time. In freshwater invertebrates, the main toxic action of nitrate is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen. Nitrate toxicity to aquatic animals increases with increasing nitrite concentrations and times exposure. In contrast, nitrite toxicity may decrease with increasing body size, water salinity and environmental adaptation. Freshwater animals appear to be more sensitive to nitrate than marine animals. Prolonged exposure to either high ammonia or nitrite levels can lead to poisoning.

Ammonia is extremely toxic and it can irritate and damage the sensitive tissue with prolonged exposure, which could affect their respiration as well as their ability to expel excess ammonia from the body. Nitrite poisoning is closely related to ammonia poisoning because high levels of one often accompany high levels of the other (Howe and Jeffrey, 2009). In *Penaeus chinensis* Juvenile, nitrite and ammonia concentration has been known to decrease with increasing exposure time (Colt and Armstrong 1981). It has been reported toxic to freshwater organisms at concentrations ranging from 0.53 to 22.8 mg/L. Excess ammonia may accumulate in the organism and cause alteration of metabolism or increases in body pH. At higher levels (>0.1 mg/liter NH_3) even relatively short exposures can lead to skin, eye, and gills damage. Slightly elevated ammonia levels falling within the acceptable range may adversely impact aquatic life (Brian, 2014).

In summary, the present study demonstrates that time exposure to tidal drainage does not affect the growth of *M. sanguinea* indicating the ability of the polychaetes community to respond to changes in their environment. However, the time exposure affects their survival and the density of copepods in the rearing environment. The copepod abundance reduced with increasing time exposure, however, at 5hrs time exposure, survival rate reduced. Therefore, it could be concluded that the optimum time exposure to tidal drainage from the results of this study for *M. sanguinea* could be between 3hrs to 4hrs for maximum survival.

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