DISTRIBUTION OF SIPUNCULA IN RELATION TO THE ENVIRONMENTAL CHARACTERISTICS AT SELECTED SITES IN KUCHING DIVISION, SARAWAK.

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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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List of Abbreviations

%	percent
cm	centimetre
Kg.	Kampung
m	metre
ml	millilitre
PSU	Practical Salinity Unit
s	second

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Abstract

This study focus on soft substrate. Rectangular plot (20, 000 m^2) was made parallel to the beach. The distribution of peanut worm were based on burrows counting and actual extraction. The distance between burrows and distance of burrows from high tide and low tide water marks were recorded. Simultaneously, pH, salinity and temperature of pore water were recorded while sediments cores were taken for sediment moisture and particle size analysis. Burrowing behaviour in the laboratory was carried out using life specimen. The size of peanut worm also were recorded. Only *Sipunculus nudus* were found in this study and the highest density of adult was at Kg Pugu with 270 burrows per 20, 000 m^2 while the highest juvenile density was found at Trombol with172 individuals per 15 m^2. They were found along in middle watermarks along intertidal area. It was observed that sipunculans inhabit habitat which has these characteristics: (i) pH: 6.72 - 7.28 (ii) salinity: 29.44 - 35.6 PSU (iii) percentage of sediment moisture: 72.11 -79.80 % (iv) fraction of sediment: sand. Six patterns of burrow show were observed on the surface of sediment. Size of adult *S. nudus* ranged from 15.8 - 30.0 cm and their burrow range from 47 - 64 cm while the size of juvenile *S. nudus* were about 4.0- 5.0 cm and they were found just few cm beneath the sediment. The burrowing ability of the juvenile depending on the water level covering the sediment. The time for burrowing was about 31 s until 507 s.

Key words: *Sipunculus nudus*, soft-substrate, environmental characteristic, burrowing

Abstrak

Fokus kajian ini ialah pada substrat lembut. Plot segi empat tepat (20, 000 m^2) dibuat selari dengan pantai. Taburan Wak-wak adalah dengan mengira lubang dan pengekstrakan sebenar. Jarak antara lubang dan jarak dari lubang air pasang dan tanda air surut telah direkodkan. Pada masa yang sama, pH, saliniti dan suhu air liang direkodkan manakala sedimen telah diambil untuk kelembapan sedimen dan analisis saiz zarah. Tingkah laku Wak-wak mengorek lubang di dalam makmal telah dijalankan dengan menggunakan spesimen hidup. Saiz Wak-wak juga telah direkodkan. Hanya *Sipunculus nudus* ditemui dalam kajian ini dan kepadatan tertinggi dewasa adalah di Kg. Pugu dengan 270 lubang per 20, 000 m^2 manakala kepadatan juvenil yang tertinggi ditemui di Trombol dengan 172 individu per 15 m^2. Mereka didapati berada di kawasan tengah di sepanjang kawasan pasang surut. Diperhatikan bahawa sipuncula mendiami habitat yang mempunyai ciri-ciri ini: (i) pH: 6.72-7.28 (ii) saliniti: 29.44- 35.6 PSU (iii) peratusan kelembapan sedimen: 68-75,71% (iv) ciri-ciri sedimen: pasir. Enam corak lubang telah diperhatikan pada permukaan sedimen. Saiz dewasa *S. nudus* antara 15.8 - 30.0 cm dan lubang mereka menggali antara 47- 64 cm manakala juvenil *S. nudus* kira-kira 4.0 - 5.0 cm dan mereka didapati hanya beberapa cm di bawah sedimen. Keupayaan mengorek lubang bagi juvenil *S. nudus* bergantung kepada paras air yang meliputi sedimen. Masa untuk mengorek lubang adalah kira-kira 31 hingga 507 s.

Kata kunci: *Sipunculus nudus*, substrat lembut, ciri-ciri alam sekitar, mengorek lubang

1.0 Introduction

Phylum Sipuncula is also known as sipunculans that has unsegmented body and can be found at estuaries as well as from intertidal to abyssal depth of marine habitats (Vargas and Dean, 2009). These organisms are well distributed in marine water worldwide including China and Taiwan (Adrianov and Maiorova, 2012). In Vietnam, their distributions were influences by some environmental characteristics such as sediment grain size, hydrological status, pH and salinity (Ha et al., 2007).

The body of sipunculans is divided by two parts, the thinner retractile introvert and the thicker trunk (Cutler et al., 1992). Their tentacles are located at the end of introvert which is function in gas exchange, feeding process and water transport (Adrianov et al., 2005). They are known as sipunculans as they take on the shape of peanut when their introvert is retracted (Johnson, 2001).

Most of the sipunculans are deposit feeders while others are filter feeders (Maiorova, 2010). They can be found in sandy and muddy substrate along the intertidal area as well as inside corals and rocks (Schulze, 2005).

This study is only focus on soft-substrate Sipuncula. According to Maiorova and Adrianov (2013), in soft substrate, some species live just a few centimetres beneath the surface of sediment while the other large species burrow down a meter deep in coarse or silty sands. They may gain a substantial part of their food by the ingestion of sediment (Vicente, 2011).

Although sipunculans are distributed worldwide, information on the distribution in Sarawak are still lacking. Salazar (2013) reported that there are a few factors that contribute to the lack of expertise studying the sipunculans including poor of extensive records and complex anatomy as well as problems with their taxonomy. Therefore, this study is important to provide baseline data for future research especially in Sarawak.

The aims of this study are (i) to determine the distribution of Sipunculans, (ii) to identify the characteristics of sipunculans habitats (sediment grain size, pH and salinity) and (iii) to document the sipunculans burrowing behaviour.

2.0 Literature Review

2.1 Taxonomy Classification

There are two classes under phylum Sipuncula which are Phascolosomatidea and Sipunculidea (Adrianov and Maiorova, 2012). According to Cutler and Cutler (1984), there were altogether 59 species of known Japanese Sipuncula representing 14 genera described namely *Sipunculus, Siphonosoma, Golfingia, Nephasoma, Phascolion, Onchnesoma, Themiste* and *Thysanocardia* which are under class of Sipunculidea, while *Antillesoma, Phascolosoma, Apionsoma, Aspidosiphon, Cloeosiphon* and *Lithacrosiphon* are under class of Phascolosomatidea.

Kingdom: Animalia

Phylum: Sipuncula

Class: Sipunculidea

Order: Sipunculiformes

Family: Sipunculidae

Class: Phascolosomatidea

Order: Phascolosomaformes

Family: Phasoclosomatidae

2.2 Morphology of Sipunculans

Sipunculans are an unsegmented worm which has thick trunk and retractable introvert accompanied with tentacles at its distal end as shown in Figure 1 (Schulze, 2005). Maiorova and Adrianov (2010) reported that the size of sipunculans varies for example: 2-3 mm long in *Phascolion psammophilum*; 3 mm in *Onchnesoma steenstrupii* to 500 mm in *Siphonosoma ingens*; 550 mm in *Sipunculus indicus* and 600 mm in *Sipunculus nudus.*

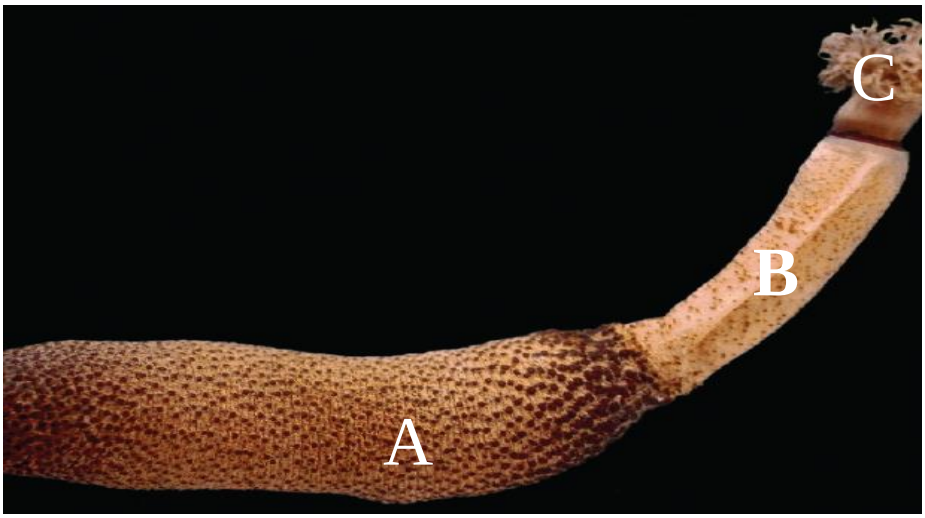

Figure 1: Morphology of Sipuncula: (A) Thicker trunk (B) Thinner retractable introvert (C) Tentacle. Adopted from: Google Image

2.3 Distribution of Sipunculans

According to Maiorova and Adrianov (2010), sipunculans are distributed worldwide and inhabit variety of marine habitats. There are 150 documented species (Vicente et al., 2011) including about 61 species found in West Pacific which are from Taiwan, Vietnam and China. In Taiwan sipunculans inhabit seagrass bed and wetland (Hsieh, 2012) while in Sepangar bay, sipunculans that inhabit seagrass are being collected for fish baits (Bujang et al., 2006).

Schulze (2005) reported that in Panama, some species including *Sipuncul*us sp. were found in the soft mud and were collected by trawling while in Costa Rica while some

of them were found in shallow waters in temperate, subtropical and tropical region as well as in the sediment containing 65% sand and 32% silt and clay (Cutler et al., 1992).

2.4 Habitat of Sipunculans

Schulze (2005) reported that Sipunculans can be found in sand and mud, burrow in dead coral or other rocks and within algal mass as well as seagrass bed. According to Maiorova and Adrianov (2013), some species can be found a few centimetres beneath the soft sediment while *Sipunculus* burrow down a meter in coarse and silty sand. Sipunculans cannot be found in the area that strongly affected by river as they are sensitive to salinity change (Ha et al., 2007).

2.5 The Burrow of Sipunculans

According to Kaladharan (2000), the burrows can be observed during low tide. The burrow usually present in high tide water marks. There were a shallow depression just a few inches away from burrow.

2.6 Feeding Habit of Sipunculans

Most of sipunculans are deposit feeder and a few of them are filter feeder (Maiorova and Adrianov, 2010). They feed on detritus and fecal material, bacteria, algae, protozoan and small invertebrates. On the other hand, they were eaten by fish, gastropods, cephalopods, carnivorous worms, crabs, starfishes, anemones and predator including man (Maiorova and Adrianov, 2010).

2.7 Ecological Groups of Sipuncula

Based on their mode of feeding and motility, the sipuncula consist of 4 ecological groups which are burrowers, generally swallowing the substratum, worms hiding in empty shelters and collecting detritus, waiting sestonophages and sessile worms, scraping off food from the substratum (Murina, 1984).

2.8 Burrowing Behaviour

Cutler (1984) reported that Sipunculans which are removed from their normal habitats then are place in a container with only seawater, they will ceases its probing or searching behaviour. They will frequently extend their introvert and test the surroundings then retracts back. They will quickly burrows in when they are place into a container fill with sand or gravel but if there are other worms with no sediment in a container, they will entangle themselves into a twisted mass. On the other hand, sipunculans will immediately buried inside the substrate after removed it from its burrow and place on the surfaced of wet sand. (Trueman and Smith, 1976)

2.9 Techniques to Collect Sipunculans

According to Cutler and Cutler (1984), there were varieties techniques to collect Sipunculans as they survive in different habitat. They can be collected by snorkelling or scuba diving if the pieces of rubble where sipunculans live were deeper. On the other hand, for sipunculans that live in died coral or soft rocks at intershallow subtidal zone, they can be collected by breaking the rocks with a hammer and chisel. *Siphonosoma,* a few *Golfingia, Thysanocardia* as well as *Sipunculus* can be collected by digging in the sand or muddy areas. Kaladharan (2000) reported that sipunculans were extracting from their burrow by inserting a spear-like spatula which were made out of midrib of coconut leaf into shallow depression. The

sipunculans were pulled out with two hands which one hand hold the spear while the other hand dig the sand. This is done after fixing like ‘spring action’ were felt.

2.10 Benefits of Sipunculans

Sipunculans provides benefits to man as well as to the environment. They play an important role in bioturbation of sediments (Acik, 2010), used as popular seafood, fish bait in some North Mediterranean countries (Toledo et al., 2005) and in India (Kaladharan, 2000), as a species to adsorb pollutants in sediments besides giving the pharmaceutical value (Ha et al., 2007) and bioerosion of coral reef (Salazar et al., 2013).

3.0 Materials and Methods

3.1 Sampling Sites

Few sampling sites were chosen in during this study located at northwest of Sarawak as shown in. Figure 2. The samples were collected from Kg. Pugu, Kg. Lebak, Kg. Belungei, Pantai Siar and Trombol. These study sites were chosen as a huge number of sipunculans that can be collected based on previous visit. Besides that, these area are less affected by human activities. Therefore, there are less disturbance for sipunculans.

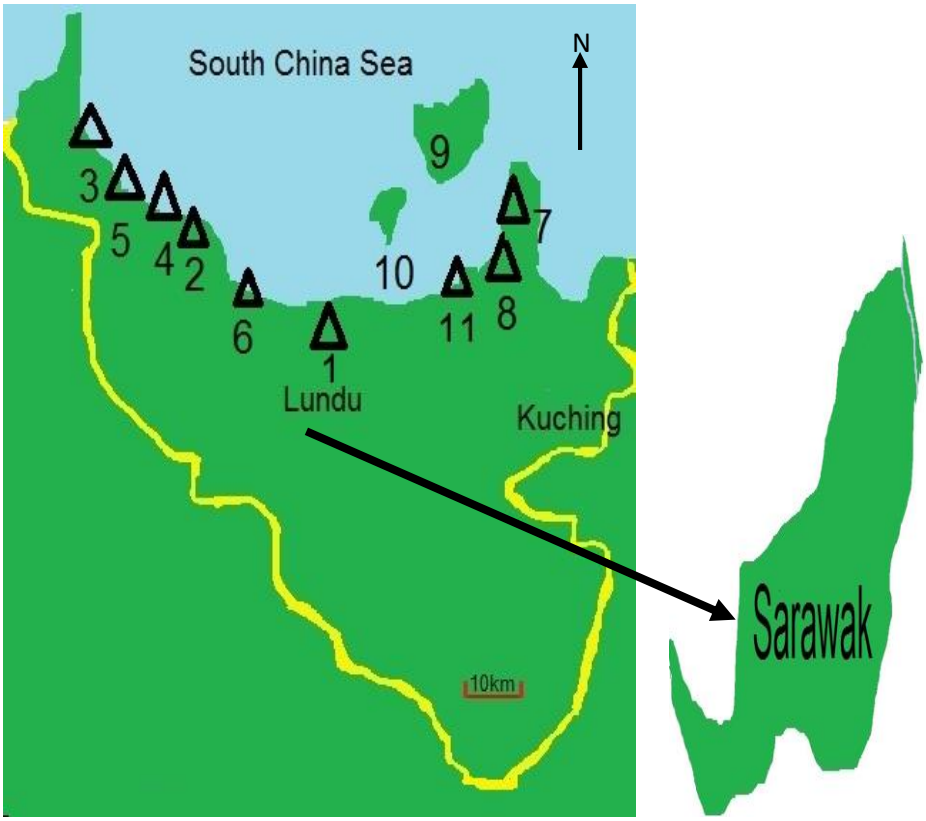

Figure 2: Sampling sites: (1) Pandan Beach (2) Kg. Lebak (3) Kg. Siru (4) Kg. Pugu (5) Pantai Siar (6) Kg. Belungei (7) Pantai Puteri (8) Pantai Pasir Panjang (9) Pulau Satang (10) Sampadi (11) Pantai Trombol.

3.1.1 Research Activities

This study was carry out based on two purposes which are studying the sipunculans distribution and observing their burrowing behaviour that involve laboratory work. Figure 3 summarise the research activities.

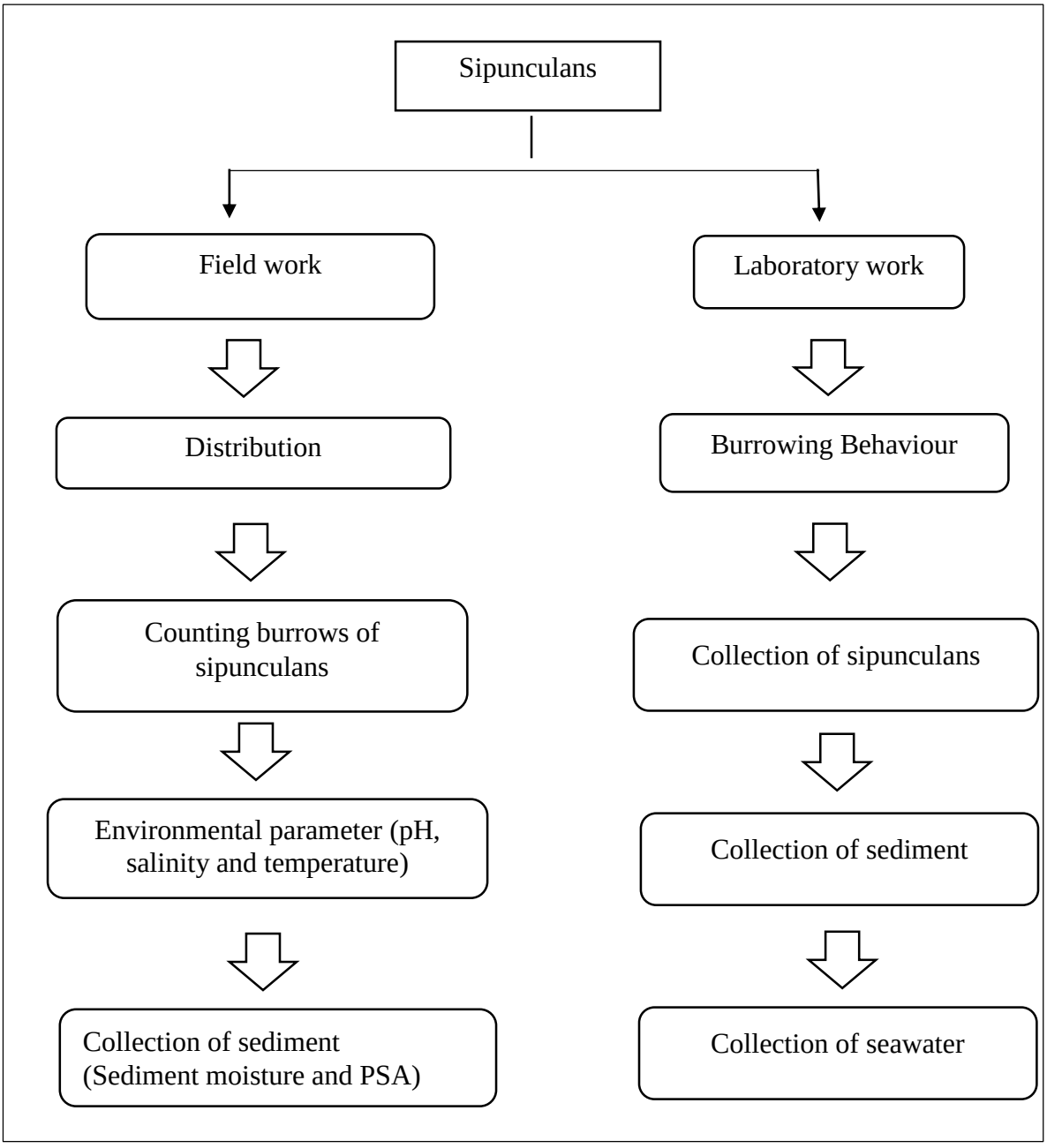

Figure 3: Summary of research activities

3.2 Field Work

3.2.1 Distribution of Sipunculans at Kg. Pugu, Kg. Lebak, Kg. Belungei, Pantai Siar and Trombol.

Population density were carried out at Kg. Pugu, Kg. Lebak, Kg. Belungei, Pantai Siar and Trombol. One plot (200 m x 100 m) was made during low tide. As there were burrow observed at both Kg. Pugu and Kg Lebak, the distribution of sipunculans' burrows were observed and counted before extracting the sipunculans from their burrow while at Kg. Belungei, Pantai Siar and Trombol where no burrow show can be observed, the sipunculans were collected by digging several small plots (1.0 m × 1.0 m) into the sand.

The sediments were collected in triplicate using an acrylic corer with diameter of 3.6 cm for sediment moisture and particle size analysis. The distance between the burrows and the distance of burrows from high tide and low tide water marks in Figure 4 were recorded using range finder (Bushnell, Model ELITE 1500).

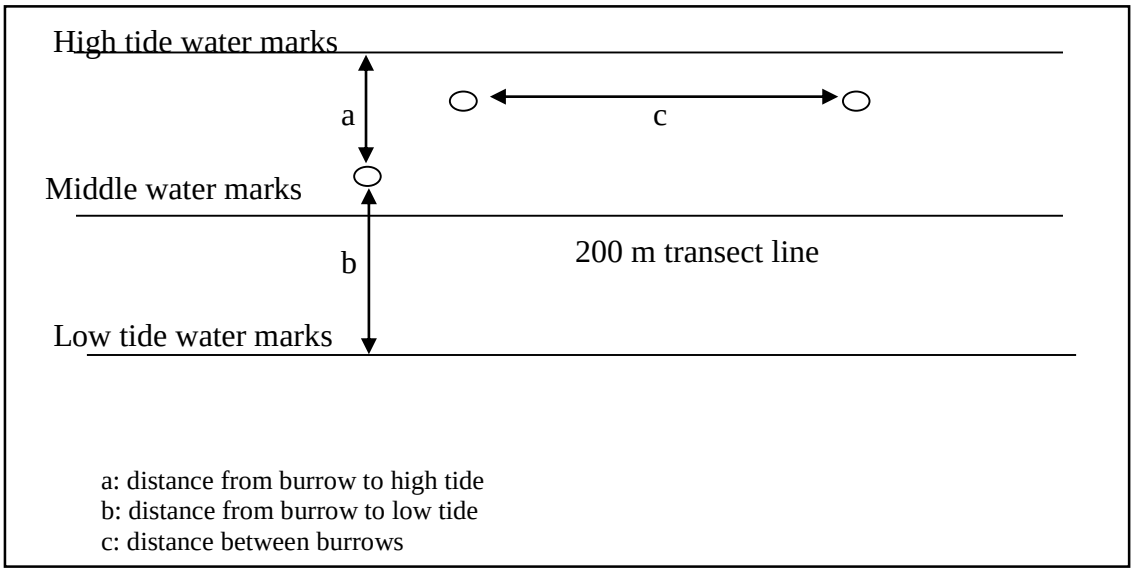

Figure 4: Sample collection at Kg. Pugu, Kg. Lebak, Kg. Belungei, Pantai Siar and Trombol.

3.2.2 Environmental Parameters (*In- situ* measurements)

Salinity of sediment was measured using hand refractometer (ATAGO MASTER-S/MIILα) while pH value of the sediment were measured using pH meter (LAQUAtwin HORIBA). Temperature of sediment were measured using Traceable thermometer.

3.2.3 Sampling of Live Sipunculans at Trombol for Burrowing Behaviour

Samples for study of the burrowing behaviour were collected by digging in the sandy area (1.0 m × 1.0 m) using a shovel as shown in Figure 5 and were kept alive by putting them in 250 ml plastic bottle with seawater, labelled and put inside cooler box with ice in order to make them calm. They were immediately being processed upon reaching UNIMAS laboratory. The sediment and seawater were also obtained for burrowing behaviour experiment.

Figure 5: The sediment that had been dig to collect alive juvenile *S. nudus* at Trombol.

3.3 Laboratory Work

3.3.1 Determination of the Sediment Moisture

The outer layer of plastic bags were cleaned first before being weight. Each sediment sample was weighed together with the plastic bag using portable balance (OHOUS ARA 520). The empty plastic bag were weighed after being dried. The weight of an aluminium tray were recorded first then the sediment were transferred into the tray. The weight of sediment were recorded until the stable reading were obtained.

Sediment moisture percentage were determined by calculating the amount of weight lost during the drying procedure (McDonald et al., n.d.)

$$\text{Dry weight \%} = \frac{(\text{Aluminium foil} + \text{Dry weight sample}) - (\text{Aluminium foil})}{(\text{Aluminium foil} + \text{Wet weight sample}) - (\text{Aluminium foil})} \times 10$$

3.3.2 Particle Size Analysis

The particle size analysis was carried out using the method of Buchanan (1984). The sample of sediment were dried in oven (Felisia HORNO) at 65°C overnight. The dried sediment were weighed using portable balance (OHOUS ARA 520). The sediment were agitated using automatic shaker sieve in 15 minutes to separate the sediment according to their size.

3.3.3 Burrowing Behaviour Observation

Figure 6 shows an aquarium (40 cm × 30 cm × 30 cm) filled with a container. Four sipunculans were tested in this experiments. The aquarium was filled with sediment and 5 L seawater to keep the sipunculans. Sipunculans were being feed with 10 ml of mixed algae culture (green colour). The seawater exchanged were done twice a week. To observe how they create their burrow, they were put on the surface of the sediment in the container as shown in Figure 7 and the reaction as well as their burrowing behaviour were observed using

video camera. Two different approach have been done to record the time taken for sipunculans to burrow (i) placed the sipunculans at the surface of the sediment only (ii) placed the sipunculans at surface of the sediment submerged with seawater. The range size of juvenile *S. nudus* were about 4.0 - 5.0 cm. This experiments were took place from 29th November until 30th March 2015.

Figure 6: An aquarium for studying burrowing behaviour

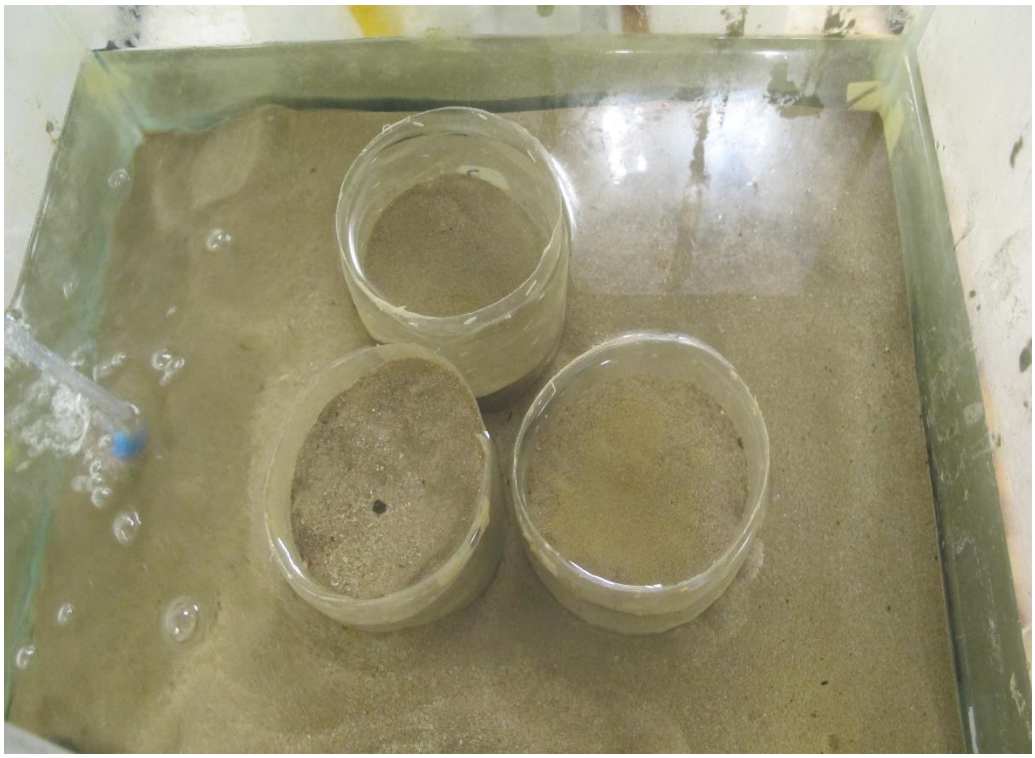

Figure 7: The container filled with sediment for placing peanut worm

4.0 Results

4.1 Distribution of Sipunculans at 12 Sampling Sites

The distribution of sipunculans at 12 sampling sites are shown in Table 1. These field work were done three times: (i) 10th August 2014 - 15th August 2014) (ii) 22th November 2014 - 26th November 2014 and (iii) 2nd February 2015 - 4th February 2015. The sipunculans were found at all the sampling sites except at rocky shore in Pantai Pasir Panjang, Pantai Pasir Pandak and Pulau Satang as well as mangrove area in Sampadi and Kg. Belungei. Three species were identified namely *Antillesoma antillarium, Aspidosiphorids elegans and S. nudus.*

Table 1: The distribution of sipunculans at all sampling sites. (+ present; - absent)

Sampling sites	**Sampling sites description**	**Sipunculans**		
		A. antillarium	*A. elegans*	*S. nudus*
Pantai Pandan	Rocky shore	-	-	-
	Sandy area	-	-	+
Kg. Lebak	Sandy area	-	-	+
Kg. Siru	Sandy area	-	-	+
Kg. Pugu	Sandy area	-	-	+
Pantai Siar	Sandy area	-	-	+
Pantai Puteri	Rocky shore	+	-	-
	Sandy area	-	-	+
Pantai Pasir Panjang	Rocky shore	-	-	-
Pantai Pasir Pandak	Sandy area	-	-	-
Pulau Satang	Coral rubble	-	-	-
	Rocky shore	+	-	-
Sampadi	Mangrove area	-	-	-
	Rocky shore	+	+	-
Trombol	Sandy area	-	-	+
Kg. Belungei	Sandy area	-	-	-