The effects of different diets on survival of marine oligochaetes worm (Oligochaeta: Tubificidae)

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Abstract. This experiment is designed to observe the suitability of different diets on survival rate of marine oligochates worm in laboratory scale using flowing water system. The oligochaetes worms were cultured in five different diet treatments (commercial pellet, seaweed, goat dung, soybean meal and combination diet) each having triplicates. The oligochaetes were cultured for 90 days. The highest survival rate of oligochaetes culture were found significant ($p < 0.001$) in the combined diet containing goat dung (20%), seaweed (*Gracilaria* sp.: 20%) and soybean meal (20%). Results of the present study suggest that the combination diet is the best compared to single diet in order to obtain the maximum survival rate of marine oligochaetes worm. The present finding is important in providing the baseline data for marine oligochaetes culture. Further study might be extended in detail to investigate the reproductive cycle since marine oligochaetes is highly potential in mass culture production.
Key Words: marine oligochaetes, Tubificidae, diet, culture, survival rate.

Introduction. The oligochaetes worm belongs to the Phylum Annelida with 1700 species identified (Erseus 2002). However, 600 species which are reported from marine or estuarine species belong mostly from family Tubificidae, Enchytraeidae and Naididae (Brinkhurst 1982). Compared to terrestrial and freshwater species, marine oligochaetes are very small having a length ranging from 1 mm to 4 cm (Erseus 2002). Ecologically, oligochaetes worms serve as prey component in the marine food webs and are involved in sediment purification processes in marine ecosystem via bioturbation activities (Seys et al 1999; Mermillod-Blondin 2011; Pigneret et al 2016).

In recent years, ongoing researches are done for enhancing protein intake to be used in feed ingredients for finfish and shell fish aquaculture industries. One of the growing aspect of aqua-feed development is focusing on the use of aquatic oligochaetes worms as an approach to meet the nutrient requirements for cultured species (Marian & Pandian 1984; Bonacina et al 1987; Lietz 1987; Hossain et al 2011; Mollah et al 2012; Das et al 2012; Mariom & Mollah 2013; Vineetha & Maheswarudu 2013). This approach has been used extensively in terrestrial oligochaetes (Ghabbour 1966; Sogbesan et al 2007; Istiqomah et al 2009; Egbunu & Solomon 2012; Dedeke et al 2013; Monebi & Ugwumba 2013) and has been increasingly applied in freshwater and marine oligochaetes both as a complete live feed or supplement. The culture of aquatic oligochaetes was developed for several reasons including life cycle and ecology study (Moore 1978; Poddubnaya 1980; Adreani et al 1984; White et al 1987; Nascimento & Alves 2008, 2009; Lobo & Alves 2011; Mischke & Griffin 2011), live feed for aquaculture (Hilton 1983; Lietz 1987; Ahamed & Mollah 1992; Bouguenec 1992; Watanabe & Kiron 1994; Evangelista et al 2005), toxicological studies (Reynoldson et al 1991; Filipowicz et al 2007; Weinstein 2003; Ng & Wood 2008; Rodriguez & Reynoldson 2011) and for water treatment (Ratsak & Verkuijlen 2006; Wei & Liu 2006; Hendrickx et al 2009; Hendrickx et al 2010). Most widely used aquatic oligochaetes for aquaculture feed are contributed by freshwater oligochaetes, *Tubifex* sp. (Lietz 1987; Mollah et al 2012), *Branchiura sowerbyi* and *Enchytraeus* sp. (Watanabe & Kiron 1994; Memis et al 2009; Hossain et al

2012). These oligochaetes have been mass cultured as supplement or exclusive food for fish and crustacean rearing. However, only few species of marine oligochaetes have been introduced for cultured purposes such as intertidal oligochaetes, *Pontodrilus bermudensis* (Vineetha & Maheswarudu 2013). Aquatic oligochaetes worms play an important part in aquatic food chain as they are rich in protein and contain polyunsaturated fatty acid (PUFA) which is essential to animal feeding that are suitable for aquaculture feeds (Graney et al 1986; Hilton 1983; Vineetha 2001; Das et al 2012). This has been proven by several tests on culture species including cat fish - *Clarias microcephalus* (Evangelista et al 2005), sturgeon - *Acipenser gueldenstaedtii* (Memis et al 2009), tiger shrimp - *Penaeus semisulcatus* (Bouguenec 1992), snakehead - *Channa striatus* (Dayal et al 2012) and grass carp - *Ctenopharyngoden idella* (Cui et al 1992). In addition, the presence of prostaglandins and related compounds in oligochaetes worms are possible inducers of gonad maturation in several crustaceans and improving maturation. From previous study, Vineetha & Maheswarudu (2013) reported that intertidal oligochaetes, *P. bermudensis* was found to induce ovarian maturation and spawning in penaeid shrimp, *P. semisulcatus* and they pointed out the well balanced amino acid profile of *P. bermudensis*, make them as excellent alternative source of protein to replace fishmeal in commercial feeds.

The discovery of high nutritional properties of aquatic oligochaeteshas triggered a research for culturing oligochaetes. Dietary experiment is one of the important factors in establishing marine oligochaetes culture. The effect of diet on survival rate and growth has been well documented in freshwater species which are used in aquaculture industries as feed. Unfortunately, the information available on marine oligochaete is very scarce. Therefore, the objective of the present study was to determine the effect of diet on marine oligochaetes survival rate under laboratory condition.

Material and Method

Oligochaetes worm collection. All oligochaetes (*Monopylephorus* sp.) were collected from intertidal area of Buntal bay, Sarawak, Malaysia (N 1°41'52.03'', E 110°22'28.10''). This area receives a freshwater input mainly from Buntal River and Bako River (Figure 1). Buntal beach consists of a sandy substrate overlaid with mud closer to the river mouth. During low tides, almost one third of the beach turns into exposed sandflats and mudflats. Generally, pore water temperature and salinity ranged from 26.4 to 31.9°C and 22.4 to 32.9 PSU respectively during sample collection.

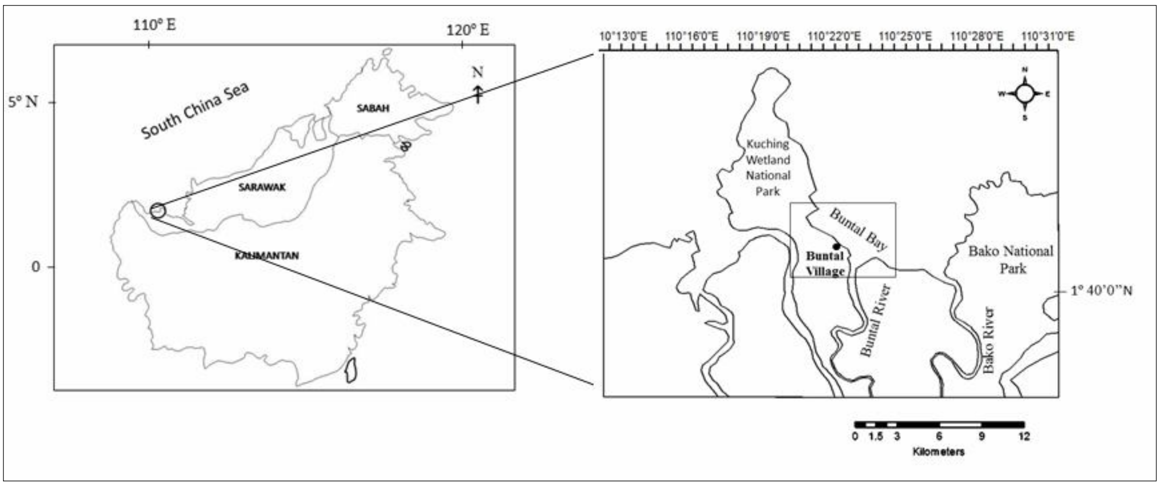

Figure 1. Map of Sarawak showing location of sample collection at Buntal bay.

The oligochaetes were randomly collected from sandy-muddy substrate by digging using a spade and sieved with 500 µm sieve. The sediment collected was sieved *in situ* in order to separate the sediment and organisms (macrobenthos). Sieving process was carried out with care so that the sediment does not rub against the sieve surface in order to avoid the worms being damaged. The retained material was placed in a plastic tray and carefully transported back to the laboratory. The sorting process was done immediately to isolate oligochaetes from other organisms in order to avoid predator.

A careful sorting was done by moving small quantity of the sieved sample into petri dish filled with field seawater, and observed under the stereomicroscope. Marine oligochaetes collected were placed in plastic storage container containing filtered seawater before subsequent process. The worms were acclimated to laboratory conditions for a week.

Experimental design. The experiment was carried out between January to November, 2014 in Aquatic Ecology Laboratory of Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). This experiment was designed to evaluate the suitability of diet for growing the marine oligochaetes worms under laboratory condition with five different types of diet treatment and sand substratum (250 µm – particle size mean). Based on literature, the population of marine and brackish tubificid appears to grow at the area of high sediment organic matter both in natural ecosystem (White et al 1987; Talley & Levin 1999) or introduced systems (Hossain et al 2012). Therefore, feed ingredients used in this study were chosen to provide organic material source in sediment. The components of organic matter used in this study derived from animal manures and decomposed plant. Animal manure used was dry goat dung which provides lots of organic matter but low nutrient value as reported by Irshad et al (2013). Therefore, decomposed plant-based media used in this study derived from seaweed (*Gracilaria* sp.) and soybean meal provides the nutrients in the sediment (Al-Asgah et al 2016; Tavoletti 2013).

Required amount of feed were weighed and mixed with sand in each container and left for three week to complete decomposition process as a source of organic matter (Table 1). The culture media were filled with seawater during this period for better decomposition. Basically, the cultures are kept in round plastic storage containers (13 cm x 7.5 cm, volume: 2112 cm^3) with autoclaved natural seawater. All containers were aerated with air pump (50-60Hz, 2W). Experiment was conducted by using flowing water system. The culture system runs for 72 hours in order to maintain the water flow at the rate of 2 mL min^{-1} before the worm was transferred.

Table 1

Summary of media treatment

Treatment	*Level of culture media ingredients*
1	Sand (40%) and commercial pellet (60%)
2	Sand (40%) and goat dung (60%)
3	Sand (40%) and seaweed (60%)
4	Sand (40%) and soybean meal (60%)
5	Sand (40%) and goat dung (20%), seaweed (20%), soybean meal (20%)

Ten oligochaetes worm were introduced per container and maintained by using five different types of feed with triplicate each. As the worms are very delicate, they were handled carefully during transfer to the culture systems. Salinity was maintained at 28 PSU and the dissolved oxygen at 4-6 mg L^{-1}. The container cultures were filled up with seawater for 24 hours (400 mL of sea water per container). Culture media and water exchange were periodically added once in ten days and the numbers of individuals were estimated. In order to observe the oligochaetes in culture system, the method of sieving the culture materials through series of sieves was employed (250 µm, 63 µm and 45 µm), the finest one having just a small enough to retain the small individual. After counting, oligochaetes worms were transferred back to their respective container for continuation of the experiment until day 90.

Data analysis. The main data collected in this study were the survival rate at the end of the experiment. The survival rates were expressed as percentage of the initial number of oligochaetes introduced. The statistical differences between diets in survival rate at the end were analysed by two-way analysis of variance (ANOVA) using GraphPad Prism 6.

Results and Discussion. Present study shows that there is a significant reduction in survival of oligochaetes in all diet treatments after 90 days. Analysis of variance of marine Tubificid culture implies that there are significant differences between the diet treatments (Table 2). The first diet media faced troublesome situation where drastic decrease of survival rate (Figure 2), where mortality reached 100% for Tubificid fed with shrimp commercial pellet after 10 days of experiment for all replicates. This showed that commercial pellet used was not suitable for culturing marine oligochaetes compared to other media. The survival rate of marine Tubificid fed with single diet goat dung (Figure 3), soybean meal (Figure 4) and seaweed (Figure 5) shows gradual reduction in survival rate after day 10 of experiment. In contrast, media containing mixture of seaweed, goat dung and soybean meal gives a better survival rate for all three replicates, which is the best survival rate at the end of the experiment reaching up to 60%-85% (Figure 6). Mollah et al (2012) in their study indicated that the media containing mixture of mustard oil cake, wheat bran, cow-dung and sand with ratio 35%, 20%, 25%, and 20% respectively shows the best media as it produced the highest yield of freshwater Tubificid on the 70th day. Hossain et al (2011) observed that the highest yield of freshwater Tubificid was detected after the 70th day over 80 days culture period by using mix culture media of soybean meal, mustard oil cake, sand, wheat bran and cow dung with ratio of 30%, 20%, 10%, 20%, 20% respectively.

Table 2

Two-way analysis of the variance of marine oligochaete culture from day 1st with different diet treatment; S, significant (p > 0.001)

Source of variation	*Degree of freedom (Df)*	*Sum-of-squares*	*% of total variance*	*F*	*Degree of significant*
Interaction	36	425.6	17.20	12.49	S
Media	4	1166	47.15	308.0	S
Time	9	787.2	31.82	92.39	S
Residual	100	94.67			
Total	149.0	2474			

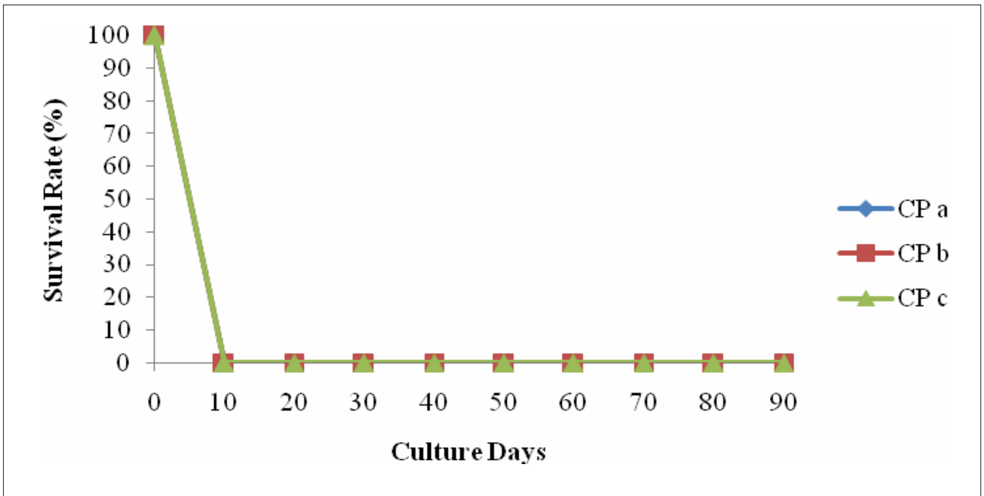

Figure 2. The survival rate of oligochaetes fed with commercial pellet with three replicates (CP a-c).

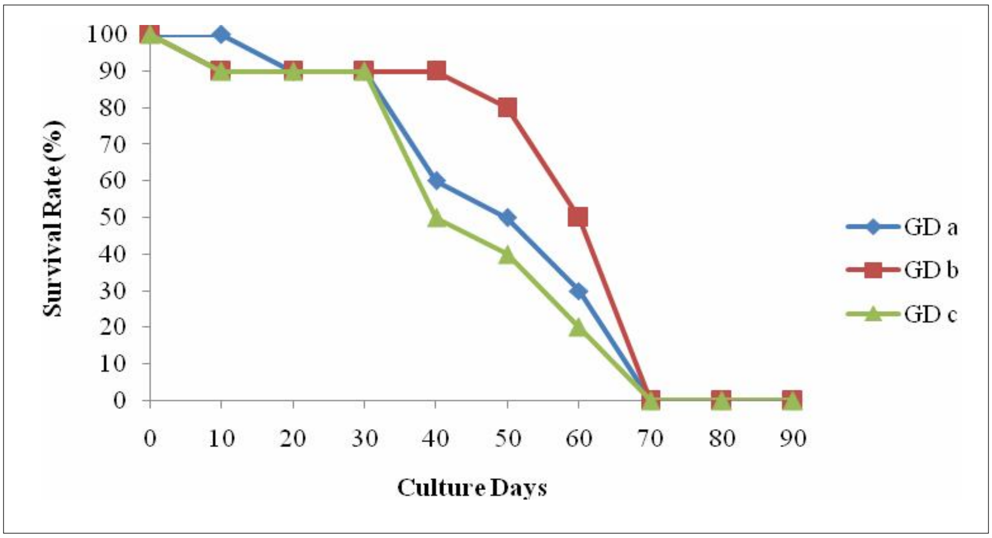

Figure 3. Survival rate of oligochaetes fed with goat dung with three replicates (GD a-c).

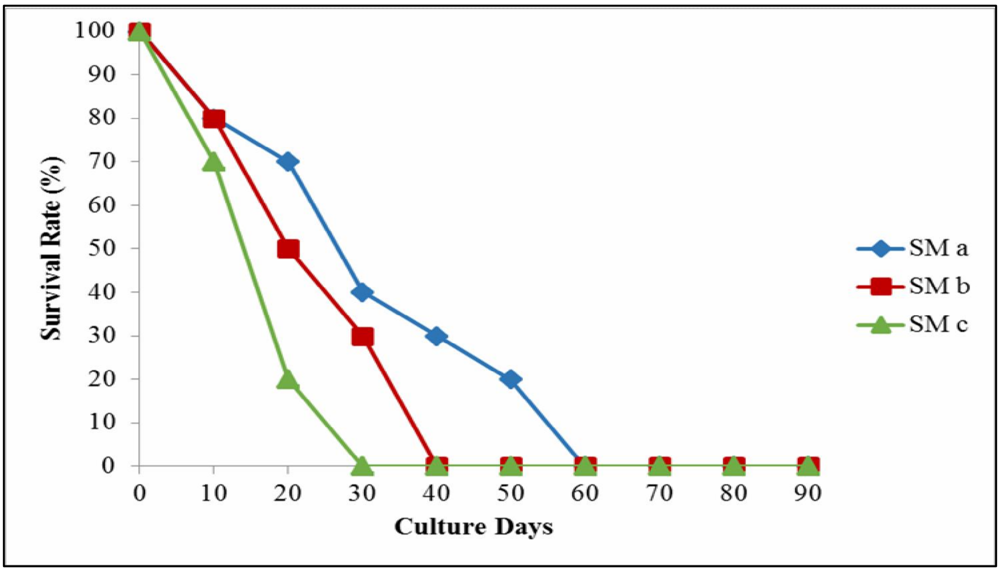

Figure 4. Survival rate of oligochaetes fed with soybean meal with three replicates (SM a-c).

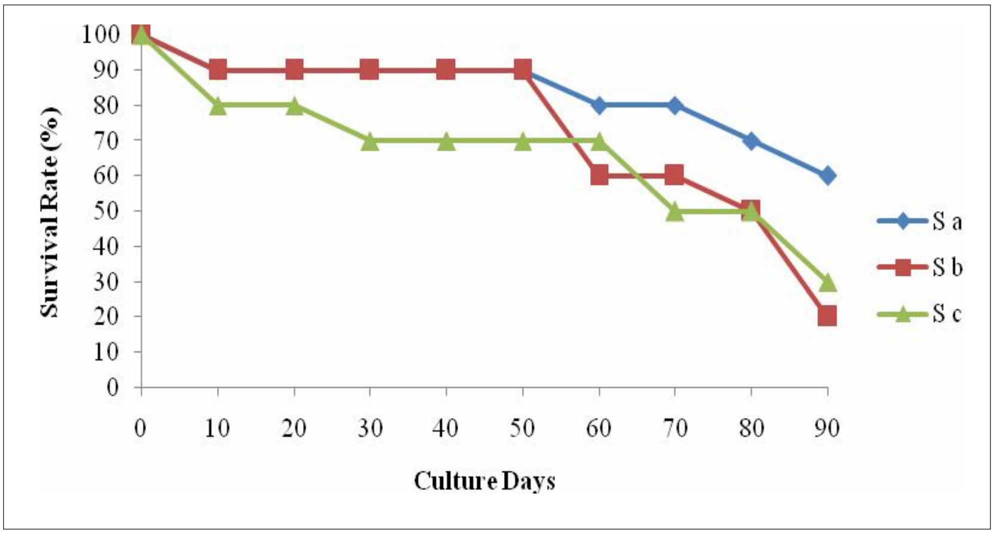

Figure 5. Survival rate of oligochaetes fed with seaweed with three replicates (S a-c).

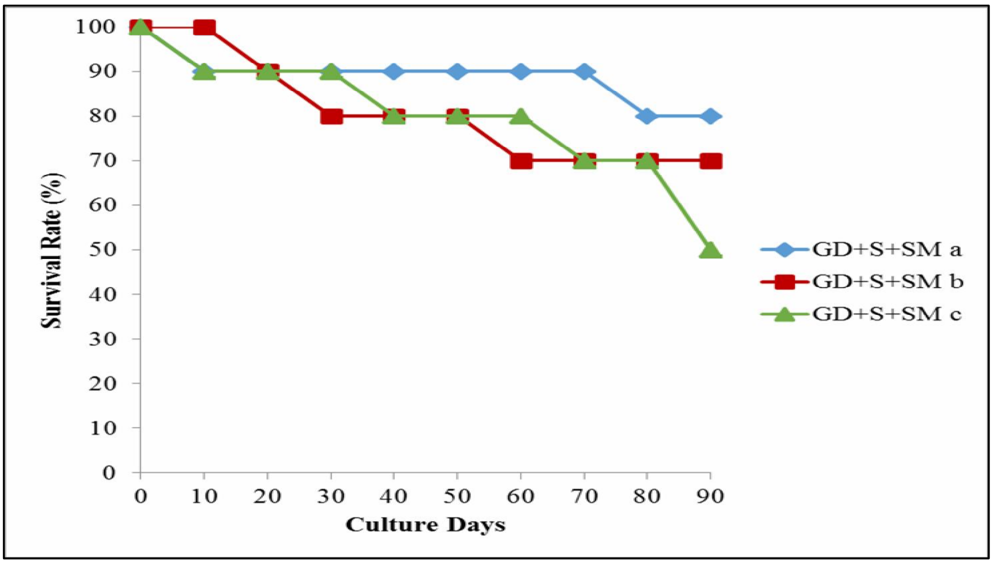

Figure 6. Survival rate of oligochaetes fed with combination diet (combination of goat dung, seaweed and soybean meal with three replicates (GD+S+SM a-c).

However, current study observes that, trend of survival rate decreased every week. This might probably due to stress during routine counting. These were in agreement with study by Brinkhurst et al (1972) showing culturing mix species of tubificid worms under laboratory condition was difficult to determine without careful handling and subject to individual response towards environmental laboratory conditions. Hossain et al (2012) reported that, the declination of culture after 80 days is due to decreasing carrying capacity of the culture medium. In addition, culturing the worms for more than 70 days is not suitable for commercial purpose in which earlier harvest will result in a smaller yield and longer culture period will increase the operation cost (Hossain et al 2012).

Despite the above matter, the ability of the culture system to operate for 90 days (3 month) shows that the culture water system is very stable and practical, works highly efficient and reliable for culturing marine oligochaetes worms in laboratory conditions for longer period of time. It is crucial to develop a standard culture system for mass production of feed organisms as a basis requirement for successful operation in live feed production in aquaculture (Marian et al 1989). Vineettha & Maheswarudu (2013) in their study on culturing the intertidal brackish oligochaetes *P. bermudensis* in plastic tray without water system with culture media containing mixture of cow dung (25%) and seaweed (75%) sprinkled with seawater resulted in increasing of population number after 30 days of culture period. However, the culture totally died after 60 days due to infestation by insect larvae.

The study by Moore (1978) reported that gut content of freshwater Tubificid composed of detritus and associated bacteria. Hence, large quantities of sediment are eaten in order to extract the bacteria associated with detritus. The current findings suggest that media used in this study was utilized by marine Tubificid worms to convert the waste nutrients into nutrient-rich protein source. The utilization of waste products into useful, highly palatable protein by oligochaetes worms can act as a fish feed and keep the culture system free of chemicals. It is important that aquaculture is perceived as environmentally friendly, especially when organic-based aquacultures are currently fostered and applied widely (FAO 2014).

To date, a comprehensive study of marine oligochaetes culture is scarce compared to freshwater and terrestrial oligochaetes. Undoubtedly, a culture of marine oligochaetes after this trial has added to our knowledge of the culturing marine oligochaetes. Therefore, with the increasing knowledge and development in aquaculture, it is undeniable that aquatic resources need to be properly managed considering their contribution to the nutritional and socio-economic needs. With the increasing costs of conventional protein sources such as fishmeal, it is believed that marine oligochaetes worms can be used as live feed and become an alternative source of protein in aquaculture industries.

Conclusions. The present study concluded that the mixture media of goat dung, seaweed and soybean meal is potentially used as diet in culturing marine oligochaetes worms compared to the use of any single diet. Based on this present finding, culturing the marine oligochaetes worms in the laboratory is important in order to achieve the best method for producing these worms. Hence, there is a need for research and development in Malaysia to develop and improvise new technology to culture live feed to further enhanced nutritional value and health of cultured of fish and shellfish species while at the same time reducing the operational cost. It is believed that the success in culturing marine oligochaetes worm is to understand the worms needs. Thus, there is a need to develop a suitable technique to culture marine oligochaetes worms. The finding of this study is expected to be useful as a basis for culture of oligochaetes worms particularly for aquaculture industry in the future.

Acknowledgements. This work was supported by Fundamental Research Grant Scheme (FRGS/STWN04 (01)/1062/2013(08)) from Ministry of Education, Malaysia as well as Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, for laboratory and technical support.

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Received: 19 September 2016. Accepted: 23 October 2016. Published online: 30 October 2016.
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How to cite this article:
Zakirah M. T., Shabdin M. L., Khairul-Adha A. R., Fatimah-A'tirah M., Ahmad-Nasir A. S., 2016 The effects of different diets on survival of marine oligochaetes worm (Oligochaeta: Tubificidae). AACL Bioflux 9(5):1144-1153.