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Survival rate and growth of Asian Clamworm *Perinereis aibuhitensis* Grube, 1878 cultured in the SPF cultured system

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Abstract. This study aims to observe the survival rate and growth of Asian Clamworm *Perinereis aibuhitensis* by raising them in aseptic culture systems from the two-day nectochaete stage to the two-month-old adolescence using vermiculite as a culture material. At the worm density of 3,000 individuals.m^{-2}, two distinct survival rates were found: 25.45 ± 86.49 and 55.26 ± 4.70 %. Those with a high survival rate had a specific weight rate of 0.006 g.d^{-1}, the specific length of 0.458 cm.d^{-1} and the specific segments of 1.058 segments.d^{-1}. On the other hand, those with the lower survival rate had the specific growth weight of 0.111 g.d^{-1}, the specific length of 0.089 cm.d^{-1} and the specific segments of 1.045 segments.d^{-1}. At the density of 1200 individuals.m^{-2}, the survival rate, specific weight rate, and specific length were 9.78 percent, -0.004 g.d^{-1}, and 0.075 cm.d^{-1}, respectively. At the density of 600 individuals.m^{-2}, the survival rate, specific weight rate, and specific length were 55.35 percent, 0.005 g.d^{-1}, and 0.099 cm.d^{-1}, respectively. At the density of 500 individuals.m^{-2}, the survival rate, specific weight rate, and specific length were 78.57 percent, 0.015 g.d^{-1}, and 0.125 cm.d^{-1}, respectively. These results revealed that sandworm *Perinereis aibuhitensis* could be cultured in an SPF farming system by using the vermiculite as a cultured substratum, producing specific pathogen-free sandworm with marketable size for marine shrimp broodstock within four months, which takes less time to produce than using natural sand. This study provides an effective method for sandworm farming in specific pathogen-free systems.

Keywords: sandworm, *perinereis aibuhitensis*, survival rate, sandworm farming

1. Introduction

Cultivation of sandworm as a maturation diet for marine shrimp broodstock is a solution for overcoming a disease-carrier problem. In order to balance the demand and supply, commercial-scale production of specific pathogen-free sandworms must be economically feasible and practical. Sandworm is a semelparous epigamy that breeds once and then die. In nature, sandworm breeds on a single night which is influenced by the lunar phase and tidal cycle, thus creating the problem of inter-mitten supply of the polychaete (Aranyakananda and Chunhabundit, 2007).

Sandworm *Perinereis aibuhitensis* is a marine polychaete found in sandy beaches in the subtropical regions of the world. This species is found across the Indo-Pacific region from Australia to Korea. It can consume carcasses of both plants and animals as food. The sandworm is a semelparous epigamy like a sandworm, which breeds once and dies. Successful spawning depends upon the synchronization of epitoky male and female, which displays nuptial dance before releasing the gametes. In nature, sandworm breeds on a single night which is influenced by the lunar phase and tidal cycle, thus creates the problem of inter-mitten supply of the polychaete. The reproductive activity of sandworms is based on the lunar phase with the peak during the second quarter moon. Water temperature in a rearing pond is a major environmental factor. Males and females, which are ready to breed perform saxophone like swimming with a little short shrinking body on the water surface during 04:00-09:00 am when the water temperatures in the rearing ponds range at 24-25ºC during the waxing moon (Suraphol and Thamasak, 2017). After two weeks of swimming, they developed into epitoky stage and released their gametes. Males and females show a very short shrinking body and swarm to release their gametes with nuptial dance (Porcham and Suraphol, 2007) in the early morning when the water temperature in the rearing ponds ranged at 25-26 ºC during the waning moon. Fertilized eggs float as planktonic larvae for 2-3 days and develop

into three segments nectochaete larvae and then settle into the substrate for further growing and living. Referred to the habitats from taxonomy and ecological research documents, this sandworm species distributes in the South region of China, South of Korea and North of Vietnam (WoRMS: www.marinespecies.org; Kristian Fauchald 1977). In Thailand, clamworm *Perinereis aibuhitensis* is found in sandy mud bottom of the rocky shore at Kung Krabaen Bay Royal Development Study Center, sandy beach of Por Bay, Chanthaburi; Hard Mae Ram-Pueng Beach, Rayong; Pra-Jaub Kirikhan, the provincials of the Upper Gulf and the Middle Gulf of Thailand.

Polychaetes were used to feed the marine culture worldwide (Xing, 2014; Deng et al. 2007; Cole et al. 2012; Leelatanawit et al. 2014; 2017; Liu et al. 2018; Meunpol 2007; 2010; Mouneyrac et al. 2003. Sandworm *Perinereis aibuhitensis* Grube, 1878 was a commercial polychaete species farmed for fishing baits in China (Lv et al., 2017). In Thailand, this species is also found in the sandy mud substrates at several locations in Thailand such as Trad, Chantaburi, and Prajuab-kirikhan, the Upper Gulf, and the Middle Gulf of Thailand (Suraphol and Malaiwan, 2008; Hylleberg, 1986). This species shows a highly nutritional composition, which is higher than other sandworms, e.g., *Perinereis nuntia* Grube, 1857; *Perinereis quatrefagesi* Grube, 1878 that are cultured in Thailand (Chunhabundit 1981; Chunhabundit and Aranyakanada, 2004; 2006). This study aims to observe the survival and growth rate of clamworm *Perinereis aibuhitensis*, 1878 cultivated in a specific pathogen-free system using vermiculite as the cultured substrate. The results of this study serve as important data for commercial aseptic sandworm farming for fresh food to marine shrimp broodstocks (Chunhabundit and Aranyakanada, 2005).

2. Materials and Methods

2.1 Location of study sites

The specific pathogen-free sandworm farming of OMG sandworm farming company is located at Had Chao Samran Petchaburi, Thailand, presented in Figure 1.

2.2 Data collections

Seawater storage ponds, nursing trays and polychaete rearing ponds

Seawater ponds are canvas ponds with a diameter of three meters. There were two seawater ponds with a capacity of 10 tons with a protein skimmer set to remove suspended proteins in the water. The seawater with the salinity of 30 ppt was prepared from the dilution of high salinity 90-110 ppt with fresh water, then precipitated with probiotics. Prior to discharging seawater into the rearing ponds and nursing trays, clean water was pumped through protein skimmer one more time.

Seven nectochaete nursing trays with the dimension of 1.0 x 1.0 x 0.15 meters were created with drainage pipes. Sixteen concrete rearing ponds with the size of 1.0 x 5.0 x 0.80 meters, each of which had a set of drainage standing pipes and airlift system installed. Vermiculite with a diameter of 1-2 mm was used as a nursing substrate of two-day nectochaete, whereas the vermiculite with a diameter of 2-3 mm was used as a rearing substrate of the two-month juvenile worm. Freshwater was ready to supply to all rearing ponds. Handheld salinometer and water quality test kits were used to monitor and maintain appropriate culture conditions. Chemical and probiotics used for this study included calcium chloride and magnesium chloride and Bactipose Plus red lid probiotics.

Two-day nectochaete of *Perinereis aibuhitensis* was obtained from Fisheries Research and Coastal Fisheries Development Center, Department of Fisheries Bangprakong, Chachoengsao Thailand, while two-month juvenile *Perinereis aibuhitensis* was from the nursing system of OMG sandworm farming.

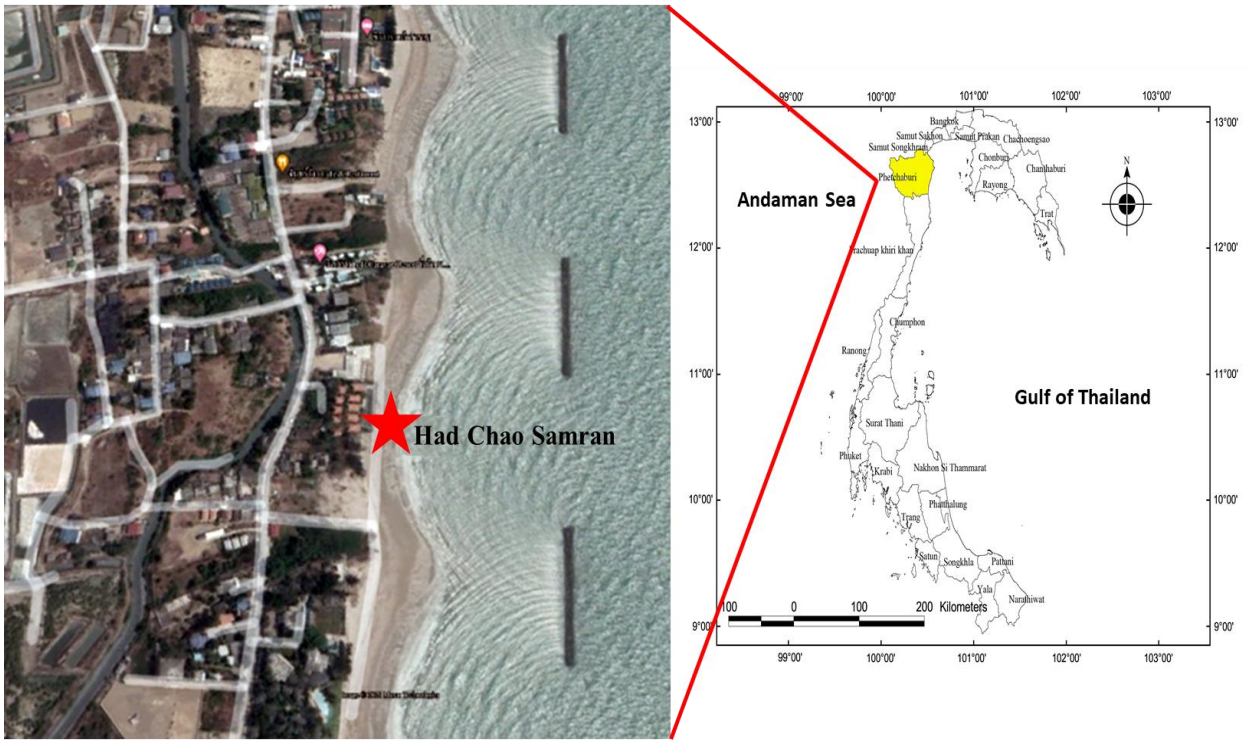

Figure 1. Location of research laboratory, OMG Sandworm Company Limited, Petchaburi, Thailand

Experimental procedures

The experiment was conducted from August 2019 to November 2019. The experiment started by putting 3,000 individuals of two-day nectochaete into each nursing tray with the three-centimeter height of vermiculite (1-2 millimeters diameter). In the first week, the nectochaetes were fed one time a day with 0.33 gram per square meter of feed (3-7 microns diameter). In the second week, fed them one time a day with 0.33 gram per square meter of feeds (7-10 microns diameter). In the third week, fed them one time a day with 1 gram per square meter of feeds (30-80 microns diameter). In the fourth week, fed them two times a day with 1.5 grams per square meter. During the fifth to seventh weeks, fed them two times a day with 1.5 grams of feeds (100-200 microns diameter).

During the eight-week nursing phase, the juvenile worm was fed two times a day with 1.5 grams per square meter of vannamai shrimp feed #1 (600-700 microns diameter). Twenty individuals of the two-month juvenile were randomly selected from each nursing tray for length and weighting measurements. Two-month juvenile sandworm was transferred from the nursing trays into five-square-meter concreate rearing ponds with different levels of juvenile density, including six ponds with 500 individuals per square meter, seven ponds with 600 individuals per square and three ponds with 1200 individuals per square meter. Each rearing pond was filled with the seven-centimeter height of vermiculites (2-3 mm diameter) with one airlift system. Cleaned seawater was filled into each pond until the water surface stayed over the rearing substrate surface for about three centimeters. The feeding regime for sandworm rearing was two times a day with 2-3 grams of vannamei shrimp diet pellets #1 per square meter. Water quality was monitored every week. Sandworms were sampled at the age of four months for individual length measurement and weighting using two decimal digital scales.

Specific Growth Rate and Survival Rate

Specific growth rate (SGR) and survival rates of two-month and four-month worms were calculated as the following equations:

Specific Growth Rate (SGR)

$$\frac{\text{Average Final weights (gm)} - \text{Average initial weight (gm)}}{\text{Rearing times (days)}}$$

Survival Rate

$$\frac{\text{Ended sandworm numbers of the experiment}}{} \text{X100}$$

Initial sandworm numbers of the experiment

Body weighting, length, growth rate and survival rate of experimented sandworms were compared at the end of the investigation

3. Results

Results from the investigation showed the growth of two-day nectochaete of *Perinereis aibuhitensis* raised at the density of 3,000 individuals per square meter from 7 nursing trays had two distinct levels of survival rates. At a high survival rate, the average highly survival rate was 55.265 ± 4.70 % or 1,661.75 ± 142.12 individuals were alive. The average body weight was 0.351 ± 0.20 gram. The average body length was 2.75 ± 0.433 centimeters and the average individual segment was 66.5 ± 14.292 segments. At a low survival rate, the average lower survival rate was 25.45 ± 6.49% or 572.75 ± 194.73 individuals were alive. The average body weight was 0.671 ± 0.01 gram. The average body length was 5.33 ± 0.94 centimeters and the average individual segment was 65.67 ± 6.65 segments (Table 1).

Nursing bran and nursing trays of 2-day juvenile worm of *Perinereis aibuhitensis*

Cleaned seawater pond

Brooder of *Perinereis aibuhitensis*

Artificial fertilization of *Perinereis aibuhitensis*

Two months of *Perinereis aibuhitensis*

Four months of *Perinereis aibuhitensis*

Figure 2. The experiment procedures

Table.1 Survival and growth rates of two-day nectochaete of sandworm *Perinereis aibuhitensis* raised to two-month worm and between high and low survival rates found in this experiment

Initial number per sq.m.	3000	3000
Avg survival number per sq.m.	1661.75 ± 142.12	572.75 ± 194.73
Avg survival rate %	55.26 ± 4.70	25.45 ± 6.49
Avg indv weight (g.)	0.351 ± 0.20	0.671 ± 0.01
Avg indv length (cm.)	2.75 ± 0.43	5.33 ± 0.94
Avg indv segments	66.5 ± 14.29	65.67 ± 6.65
SGR weight (g.)	0.006	0.011
SGR length (cm.)	0.458	0.089
SGR segments	1.058	1.045
N rearing trays	4	3
N of samples	20	20

The specific growth rate (SGR) of two-day nectochaete raised to two-month juvenile worm at 3,000 individuals per square meters showed that at the high survival rate, the specific weight rate was 0.006 gram per day, and the specific length was 0.458 centimeter per day, while the specific segments were 1.058 segments per day. At the lower survival rate, the specific growth weight was 0.111 gram per day, the specific length was 0.089 centimeter per day and the specific segments was 1.045 segment per day (Table 1.)

Results of survival rate in juveniles *Perinereis aibuhitensis* raised from two-month worm to 4 months worm at three different densities namely 500, 600 and 1,200 individual per square meter showed that: a) the survival rate of juveniles raised at 500 individuals per square meter was 78.57 percent. The average body weight was 1.43 grams and the average body length was 11.53 centimeters; b) the survival rate of juveniles raised at 600 individuals per square meter was 55.35 percent. The average body weight was 0.81 gram and the average body length was 9.99 centimeters; c) the survival rate of juveniles raised at 1,200 individuals per square meter was 9.78 percent. The average body weight was 0.282 gram and the average body length was 8.519 centimeters (Table 2).

The specific growth of four-month *Perinereis aibuhitensis* worm raised from two-month juvenile worm showed that: a) the specific weight rate of worm raised at 500 individuals per square meter was 0.015 gram per day and the specific length was 0.125 centimeter per day; b) the specific weight of worm raised at 600 individual per square meter was 0.005 gram per day and the specific length was 0.099 centimeter per day; c) the specific weight rate of worm raised at 1,200 individuals per square meter was -0.004 gram per day and the specific length was 0.075 centimeter per day (Table 2).The quality of raw and mixed seawater water used for sandworm aquaculture during three weeks (14 cultured ponds) was shown in Table 3

4. Discussion

This study conducted the experiments to observe survival and growth of sandworm *Perinereis aibuhitensis* raised from two-day nectochaete larvae to two-month juvenile worm in the SPF rearing system using vermiculite as the substratum. We found that when raised worm larvae at 3,000 individuals per square. There are two distinct survival rates, namely average highly survival raised worm was 55.26 ± 4.70 % and the average lower survival rate was 25.45 ± 86.49 %. This is because sandworm is an independent living creature, and it digs a burrow to stay inside the sand or sandy mud substrates. Sandworm has antagonistic behavior that fights each other in order to occupy living space. Raising conditions in a small area with a dense population may promote competition among them.

Table. 2 Survival, growth and specific growth rates of *Perinereis aibuhitensis* raised from two-month to four-month worm of this experiment

Initial number per sq.m.	500	600	1200
Avg survival number per sq.m.	405.87 ± 49.97	388.20 ± 83.74	117.33 ± 61.15
Survival rate %	78.57 ± 8.32	55.35 ± 14.03	9.78 ± 5.10
Avg indv weight (g.)	1.43 ± 0.38	0.81 ± 0.19	0.282 ± 0.11
Avg indv length (cm.)	11.53 ± 1.94	9.99 ± 1.32	8.519 ± 1.62
SGR weight (g.)	0.015	0.005	- 0.004
SGR length (cm.)	0.125	0.099	0.075
Number of rearing ponds	6	7	3
Number of samples	40	42	26

Table 3 Water quality of 16 cultured ponds for raising sandworm *Perineries aibuhitensis*

Weeks/ parameters	AVG RAW SW	AVG SW WK1	AVG SW WK2	AVG SW WK3
NH_3	0	0.01	0.03	0.066
Alkalinity	109.3	133.4	141.31	142.8
pH	8.3	8.25	8.22	8.15
Ca	640	287.5	311.25	324
Mg	800	858.75	933.75	972
Salinity	106.43	29.5	30.2	31.4
Sea water temp.	-	24.83	25.25	24.85
n	7	16	16	10

Therefore, the survival rate observed in each nursing tray was different as well as the average weight and average length of two-month juvenile individuals. At a high survival rate, the two-month worm had an average body weight of 0.351 ± 0.20 gram, an average length of 2.75 ± 0.43 centimeters. Whilst, at a low survival rate, the two-month worm had an average body weight of 0.671 ± 0.01 gram and an average body length of 5.33 ± 0.94 centimeters. The results of this study also show that the growth rates of the sandworm raised at the low density were higher than those raised at the higher density. The specific growth rates from raised two-day nectochaete to two-month worm were in the same direction. The high survival rate of sandworm had a lower specific growth rate in terms of body weight and average body length. Only the number of segments per individual was not different (table1).

Survival rate in juveniles *Perinereis aibuhitensis* raised from two-month worm to four-month worm in three different densities, namely, 500, 600 and 1,200 individual per square meter showed the difference in average body weight and length. High and low densities can affect the nutritional composition of raised sandworm, which is consistent with the study of Palmer et al. (2014). They found that the nutritional status of *Perinereis helleri* cultured in sand filters of mariculture wastewater when the stocking densities and graded size significantly affected their composition. They also stated that the higher total lipid contents were found in large and those grown at the lowest density than those who were small ones and those grown in high densities. When considering how the salinity of water affects the growth of raised sandworm, the salinity of seawater generally affects aquatic animals, especially the water regulatory system. In general, marine animals are able to adapt to changes in seawater concentrations over a narrow range. For polychaetes, Tangkrock-olan and Sinchareonsup (2013) studied the effects of salinity on survival rate and the ability to change in volume regulation of sandworm *Perinereis*

nuntia and found that the survival rate at 0 ppt of seawater was significantly lower than the survival rate of 10, 20, 35 and 40 ppt that were not significant. From the present investigation, the water salinity in the nursing trays was between 29.5-31.4 ppt. So, in this investigation, the seawater salinity did not affect the survival rate and growth and density of individuals (table 3). Fang et al. (2016) stated that the specific growth rate of *Perinereis aibuhitensis* was significantly affected by temperature and feed types and the worm achieved the high specific growth in AF group at 20 ºC. The slow growth in the group was attributed to the lower organic contents in sediment and the higher of C and N in metabolism.

Based on this study, it can be concluded that sandworm *Perinereis aibuhitensis* can used vermiculite as a cultured substrate. Specific pathogen-free sandworm can be cultured with an average body weight of 1 gram and an average body length of 12 centimeters, which is the marketable size for marine shrimp broodstock within four months. Using vermiculite as a cultured substrate takes less time to produce than using natural sand (Chunhabundit and Aranyakananda, 2006). However, the costs of vermiculite and the selection of sandworm species to be farmed appropriately with the vermiculite used as an aseptic cultured substrate should be considered as a critical parameter for the success of sandworm production.

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