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Biological characteristics and effects of salinity on reproductive activities of marine worm (*Tylorrhynchus heterochaetus*, Quatefages, 1865) in summer season in Hai Phong – Viet Nam

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Abstract

Tylorrhynchus heterochaetus (Quatrefages 1865) are not only an environmental indicator species, but also an economical aquatic animal. Production of this species mainly depends on natural exploitation, which currently decreases dramatically due to the changes of living environment, such as abuse of pesticide and salinization. The current study was conducted in order to understand the biological characteristics of *T. heterochaetus* in Hai Phong, Vietnam. Results showed that weight of ranged from 0.44 to 0.53 g. Length and number of node were different between tube worm and matured worm (10.60 and 4.81 cm, 61 and 166 nodes, respectively). The experiment was carried out with 11 of salt levels ranged from 2 – 20 ppt. to evaluate the effects of salinity on reproduction of *T. heterochaetus*. The results showed that 10 ppt. is optimum level for reproduction when it gave the best results of worm participate into reproduction (82.11%), the ratio of fertilization (85.33%), hatching (65.56%) as well as survival rate of larvae (69.56%). Artificial breeding of *T. heterochaetus* is feasible for large scale and having prospects.

Keywords: *Tylorrhynchus heterochaetus*; Reproduction; Salinity

1. Introduction

Tylorrhynchus heterochaetus (Quatrefages 1865) is a species of Polychaete family. They appear normally only once per year in their reproductive season when their body contains full of gametes and be ready for reproduction. Most of them die off immediately after spawning [1, 2]. *T. heterochaetus* inhabit in the mud of tidelands near the mouths of rivers in many countries, such as Russian, China, Japan, Indonesia, as well as Vietnam. In Vietnam, *T. heterochaetus* naturally distributed in Quang Ninh, Hai Phong, Hai Duong, Nam Dinh and some other provinces in the South [3, 4].

It was reported that *T. heterochaetus* plays an important role in bio-monitoring the marine environmental quality since they respond quickly to changes in environmental conditions. Canada, USA, UK and Germany used *T. heterochaetus* as indicators for toxic materials and pollution management [5]. *T. heterochaetus* are used in monitoring the marine environmental quality is due to their direct contact with the water and the sediments of their environment thus showing sensitivity to anthropogenic compounds which is expressed through changes in their reproduction, growth and mortality [6]. They also can reflex the condition and health of the benthic environment when they are the dominant microfauna within the fine sediments. Thus, marine worm *(T. heterochaetus)* is considered as an eco-friendly, innovative and sustainable technology in aquacultural waste degradation, especially nitrogen elimination due to filter feeding habit [5]. Moreover, during the degradation process low nitrogen organic waste is converted into nutrient-rich biomass, which has high potential as a protein source for human or animal feed.

Many reports pointed out that *T. heterochaetus* owned a much better nutritious profile than many other aquatic animals. Results of chemical composition showed that *T. heterochaetus* content proximately 82% of water, 12.40% of protein, 4.40% of lipid and 1.30% of ash [7]. In Vietnam, *T. heterochaetus* are well known as a special food, which are often available only in special restaurant with relatively high price. Farmers wanted to produce this animal for generating cash income but seed was still unavailable yet. Recently, due to change of environment quality including present of chemical residue, change of water flow, and salinization, production of *T. heterochaetus* is decreasing dramatically, even disappeared completely in some regions [8]. Bai and Chuong has investigated the reproduction activity of marine worm and concluded that marine worm mainly breeding on Autumn season (September and October according to lunar calendar) [4, 8]. High salinity of water (hypertonic) could be a factor that created contraction of marine worm's skin, broke it to release gametes [8]. However, it has not found any other publication about effect of salinity on reproduction of this animal, especially on summer season in Vietnam. Therefore, understand of their biological characteristics, especially the effect of environmental parameters to fertilization and larvae development of *T. heterochaetus* is required in order to develop this invaluable species on farm.

2. Materials and methods

Sample collection

Marine worm samples were collected monthly in Van Uc river mount, An Lao – Hai Phong where they inhabit naturally (20°76N, 106°55E). In highly reproductive season (from September to November), samples were collected every 3 days as method mentioned by Chuong [8]. Each time, 30 individuals were randomly selected and keep in a cool box to transport back to laboratory for analysis. The samples were either measured for length, height, weight, node, sex, and number of egg immediately or kept in alcohol (20%) and Bouin solution for later analysis. Besides, water parameters, such as salinity, dissolve oxygen, pH, water temperature and height of tidal were measured. Marine worm's movement and immigration were also monitored along the water flow at Van Uc river mount during reproductive season.

Reproductive experiment

Experiment design

Water was prepared for different salinity ranged from 2 to 22 ppt. with interval of 2 (determined by a spectrometer Jenco-3107). Different levels of salinity were prepared by diluting sea water (33 ppt. from Cat Ba Island) with water from sampling area. Both sources of water were filled through fine sand and stored in 50 m^3 tanks before used. Duplicates of, 100 pairs of marine worm (male:female = 1:1) were stocked in 200 litter tanks for preliminary screening test on reproductive activity of worm in different salinities. Number of worm participated to reproduction was recorded. Based on results of this experiment, the best salinity was selected to produce eggs to test for hatching rate, development of embryo and early stage larvae in different salinity levels. Fertilized eggs were kept in 8, 10, 12 and 14 ppt of salinity water with triplicates. Aeration was set up to ensure optimal dissolved oxygen (DO>4 mg L^{-1}) and pH (from 7.50 to 8.50). Number of egg and development of embryos and larvae were monitored every 30 minutes by counting under a microscope with a counter chamber until they reached spat stage according to the methods used by Josephine [9].

Observation parameters and formulas

Fertilization ratio = number of fertilized eggs/total number of eggs

Reproduction ratio = number of worm take part in reproduction/total number of worm

Hatching rate = number of hatched eggs/number of fertilized eggs

Survival ratio = number of survived/total number of larvae

Besides these parameters, all other abnormal activity as well as characteristic of egg, embryos and larvae were recorded. Data were analyzed by analysis of variance; significant differences between treatment means were determined by the Tukey test, using the SAS computer software [10]. Percent data will be normalized using an arcsine-transformation before analysis. Significance levels for all analysis were set at $p<0.05$.

3. Results and Discussion

Biological reproductive characteristics of marine worm

As the results of observation, marine worms were found popularly under the mud of the field during summer season. However, it was hardly found mature worm on May, whereas more available around middle of June. When they were matured, they swam up to water surface (floating worm) and followed the flow to migrate to the coastal sea. According to [8], environment could affect strongly to marine worm's maturation. Reproduction occurred during high tidal which probably can be helpful in distribution of gametes and larvae [8]. The results of water quality showed that all parameters did not change much during this season. Average DO was 5.80 ± 0.50 mg O_2 L^{-1}, temperature was 26.60 ± 2.40 °C and salinity was low (1.50 ± 1.80 ppt.). Thus, no reproduction activity was observed on the field. This result was in agreement with previous reports [8, 11].

Although results did not show much different in the weight, shape of the body and number of node of marine worm were changed during maturation and they could be divided into two types: unmature worm (tubed worm) and mature worm. Results of measurement showed that unmature marine worm had a hight of 2.31 ± 0.10 cm, much smaller than that of mature worm (5.65 ± 0.19 cm). In opposite, the length of unmature worm was 10.60 ± 0.25 cm, 2 times longer than that of mature worm (4.81 ± 0.25 cm). Similarily, number of node showed the same trend (166.0 ± 12.2 and 61.0 ± 3.9, repectively for unmature and mature worms; Table 1). The results also showed that length and number of node of marine worm in summer season (May to August) seemed to be higher than those of late autumn (September to November). According to Bai [4], worm body could be also divided into 2 parts: epitoque and atoque. In reproductive season, the first part was well developed in size. Other organs such as digestive tube and muscle become smaller to give internal space for gamete development. The second part was dissipated before worm became mature. [10] inferred that marine worm have both sexual and asexual reproductive types. However, in this study, it was not clear if the second part could develop to be a new body [8, 11], because asexual reproduction could not be monitored.

Table 1 Weight, length and number of node of marine worms in the study.

Type of worms	Height (cm)	Weight (g)	Length (cm)		Nodes	
	Summer	Summer	Autumn	Summer	Autumn	Summer
Tubed worm	2.31 ± 0.10	0.44 ± 0.12	8.07 ± 1.61	10.60 ± 0.25	110.74 ± 20.42	166 ± 12.20
Mature worm	5.65 ± 0.19	0.45 ± 0.10	4.15 ± 0.51	4.81 ± 0.25	54.25 ± 7.37	61 ± 3.90

During reproductive season, observations pointed out that sexual ratio (male $female^{-1}$) could fluctuate from 1.30 to 2.20 (1.70 ± 0.40 in average). Worms often swam on the water surface when they were ready for reproduction. In that period, males turned to white milky colour while females showed white-green colour. Reproduction of marine worms was strongly depended on tidal cycles.

They tended to swim down the river to the sea where water was saltier. Reproduction occurred when their thin body skin was easily broken down to release gametes.

Reproduction rate and fertilized rate in different salinities

Monitoring results showed that marine worm did not release gametes in low salinity water (at 2 and 4 ppt.) during reproductive experiment (Table 2). The highest number of worm took part into reproduction archived at salinity of 10 ppt. (82.11 ± 1.67%), followed by the worms in the groups 12 and 14 ppt. (75.33 ± 1.67 and 61.11 ± 0.96, respectively). These results were also in commitment with the data from Chuong [8] who reported that marine worm could produce gametes in above 5 ppt. salinity water. However, did not find any reproductive activity at salinity above 18 ppt. They were died after 1 – 2 hours being in salinity levels. Basically, high salinity can be a hypertonic environment that helps body membrane of *T. heterochaetus* broken to release gametes. However, it did not happen in this case although worms were matured both exteriorly and interiorly. This result suggested that salinity may not be the only factor that effects on worm's reproduction.

Results showed that above 16 ppt. of salinity, worms could still participate in reproduction. However, the fertilization rate was lower than those in less salty water. The highest fertilized rate was observed in group 10 ppt. with average of 85.33 ± 0.66%, followed by group 12 ppt., 8 ppt., and 14 ppt. (75.56 ± 0.66, 55.78 ± 0.66, and 30.22 ± 1.55%, respectively). These results suggested using salinity from 10 ppt to observe development of the embryo.

Table 2 Reproduction rate, fertilizer rate, hatching rate and survival of nectochaeta in different salinity levels.

Salinity (ppt)	Reproduction rate (%)	Fertilizer rate (%)	Hatching rate (%)	Survival rate (%)
2	0	-	-	-
4	0	-	-	-
6	12.89 ± 1.67^{e}	16.67 ± 0.66^{e}	9.33 ± 2.55^{d}	11.77 ± 0.44^{f}
8	59.28 ± 0.96^{c}	55.78 ± 0.66^{c}	35.78 ± 1.97^{b}	46.44 ± 1.11^{c}
10	82.11 ± 1.67^{a}	85.33 ± 0.66^{a}	65.56 ± 2.05^{a}	69.56 ± 0.89^{a}
12	75.33 ± 1.67^{b}	75.56 ± 0.66^{b}	55.56 ± 2.05^{a}	65.33 ± 0.67^{b}
14	61.11 ± 0.96^{c}	30.22 ± 1.55^{d}	15.78 ± 2.05^{c}	35.11 ± 1.11^{d}
16	29.78 ± 0.96^{d}	20.44 ± 0.89^{d}	7.33 ± 1.97^{e}	21.11 ± 1.78^{e}
18	11.56 ± 0.96^{e}	8.45 ± 0.45^{e}	2.22 ± 1.97^{f}	9.56 ± 1.11^{f}
20	0	-	-	-

Numbers in the same column have the different supercrisp are significultly different ($p<0.05$)

Effects of salinity level on development of marine worm embryos and larvae

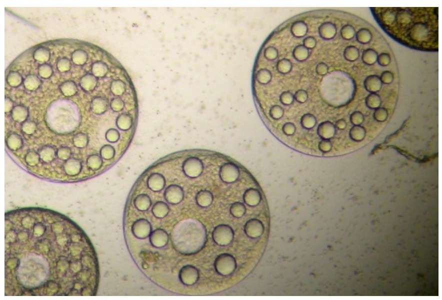
Environmental conditions

Observation on temperature in the second experiment showed that both air and water temperatures were in the suitable range for marine worm's reproduction (26.82 ± 3.61 to 24.13 ± 1.62 °C, respectively for air and water temperature). The pH values were stable with a small variation range (from 7.50 to 7.81). These results were also in commitment to the results from other reports. Chuong [8] stated that water temperature ranged from 24.40 to 29.70 and pH ranged from 7.21 to 7.82 were suitable for marine worm reproduction.

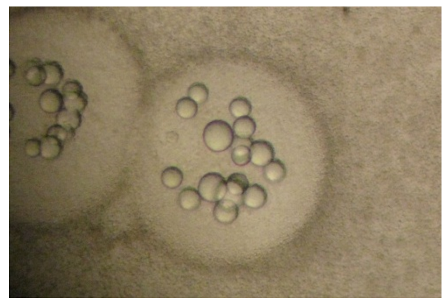
Life cycle and development of marine worm embryos and larvae (T. heterochaetus)

In general, development of *T. heterochaetus* is similar to that of many other aquatic animals when that also had 2, 4, 8 and 16 cells as well as morula and gastrula stage. Normal larvae reached the actively swimming trochophore stage after 24 hours post-fertilization. By that time, unfertilized eggs were decomposed. The trochophores appeared in spherical shape with peripheral band of cilia. By 48h, larvae reached the metatrochophore stage of development in which body segment, eyespots appeared. The normal larvae reached nectochaeta after 72h (Fig. 1). The life cycle of marine worm could be characterized as Fig. 2.

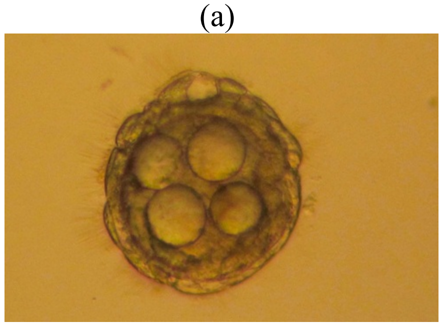
As the results, fertilized rate in the massive artificial reproduction was lower than that of the first experiment. It indicated that mature rate of marine was not uniform. Moreover, manipulation could affect to fertilization. The highest fertilized rate was recorded in the group 10 ppt. (37.68%), while the other groups gave 31.51, 24.10 and 22.92% respectively for 12, 8, and 14 ppt. salinity. Although the fertilized rate was not very high, the large number of egg still resulted in massive embryos.



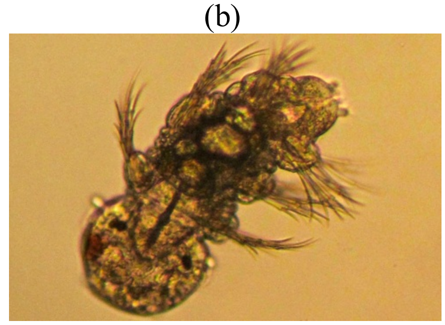
(a)



(b)



(c)



(d)

Fig. 1 The developmental stages of marine worm: (a) fertilized eggs; (b) un-fertilized eggs; (c) trochophore; and (d) nectochaeta.

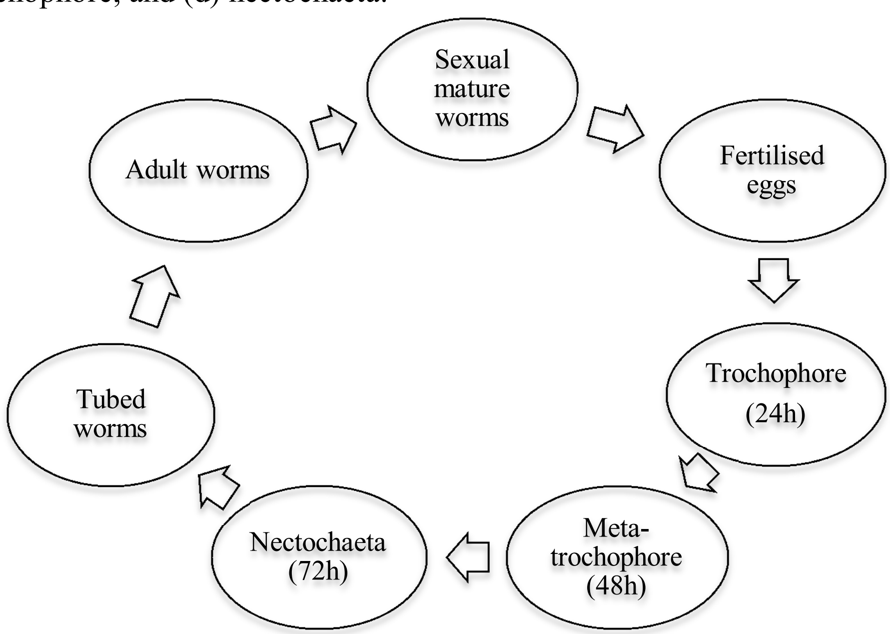


Fig. 2 Life cycle of marine worm *(T. heterochaetus)*

Similar to previous results, hatching rate was highest in the group 10 and 12 ppt. of salinity (67.42 ± 3.22 and 65.33 ± 2.72 ppt., respectively). They were significantly higher than that of the other groups. The lowest hatching rate was recorded in 14 ppt. salinity (51.11 ± 5.61 ppt.; Table 3). This group was also the lowest survival rate. However, it was not different from the other groups significantly. The survival rate in this experiment could be considered as high in comparison to that of many other aquatic species, especially this is one of the first investigations on reproduction of marine worm *(T. heterochaetus)*. If the knowledge about this animal is improved, the artificial reproduction will give better results.

Table 3 Fertilized ratio, hatching rate, and survival rate in the artificial reproductive experiment.

Salinity (ppt)	Fertilizer rate (%)	Hatching rate (%)	Survival rate (%)
8	24.10 ± 1.30^{b}	63.30 ± 4.10^{ab}	71.10 ± 2.90^{a}
10	37.68 ± 3.37^{a}	67.42 ± 3.22^{a}	65.83 ± 3.81^{ab}
12	31.51 ± 1.58^{ab}	65.33 ± 2.72^{a}	67.52 ± 4.64^{ab}
14	22.92 ± 3.71^{b}	51.11 ± 5.61^{b}	62.32 ± 3.48^{b}

Numbers in the same column have the different supercrisp are significultly different ($p<0.05$)

4. Conclusion

Based on the results of this study, it can be concluded that: beside the main reproductive season between November and December, marine worms could also reproduce in summer season. The suitable salinity for reproduction of this species is 10 – 12 ppt. In this salinity level marine worm gave the highest results of fertilization rate, hatching rate as well as survival rate. Artificial reproduction is feasible, especially when marine worms have high reproductive capacity (number of gametes). If reproduction of this species is completed, farmers can benefit more from this animal. It is suggested that other studies on biology of marine worm should be continue, especially for the effects of other environmental parameters on the development of *T. heterochaetus* on early stages.

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