

# Effects of Culture Space on the Production of Tubificid Worms (*Oligochaeta*, *Naididae*)

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## Abstract

Tubificids are aquatic oligochaete worms distributed all over the world. The worms are very important, as they are used as live food for cultured fish and aquatic invertebrates. This study was carried out to determine the effect of culture space on the production of tubificid worms. The worms were cultured in three different treatments, namely treatment-I (40,000 cm<sup>3</sup>), treatment-II (96,000 cm<sup>3</sup>) and treatment-III (152,000 cm<sup>3</sup>) under flow-through conditions. The experiment was continued for 90 days using a culture medium containing a mixture of 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel. The observed highest yield ( $P < 0.05$ ) of 1,662.16 ± 28.32 mg cm<sup>-2</sup> was found after 70 days of culture in treatment-III. Only about 0.80 kg media ingredients valued at US\$ 0.24 were needed to produce 1 kg worms.

## Introduction

The tubificids (Subclass Oligochaeta, Family Naididae) are gatherers, feeding on decaying organic matter, detritus and vegetable matter commonly available in the sediment. They occur in a wide range of habitats and tolerate a spectrum of environmental conditions (Kaster 1980, Brinkhurst and Kennedy 1965). They are the only worms present in the deepest regions of lakes (Mackie 2001); however, the depth of a metre or so is the usual habitat for the great majority species belonging to the Naididae (Barnes 1966). All former tubificids are now regarded as members of the Naididae, the family Tubificidae now being considered a junior synonym of the family Naididae (Erseus et al. 2008). The family Naididae as presently conceived now includes the following subfamilies: Tubificinae, Naidinae, Telmatodrilinae, Phallogrilinae, Pristininae, Limnodriloidinae and Rhyacodrilinae.

Most tubificids have erythrocrurin, a red blood pigment, which can effectively extract oxygen dissolved in the water. They are segmented, bilaterally symmetrical worms with tapering ends (Brinkhurst and Kennedy 1965). Some freshwater forms burrow in the bottom mud and silt; others live among submerged vegetation. The worms lie with heads down and rear ends projecting from tubes and waving vigorously in order to increase aeration.

They are hermaphroditic, i.e., each individual contains both male (testes) and female (ovaries) reproductive organs. At maturity, the reproductive organs are clearly seen on the ventral side of the

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body (Jordan and Verma 1978). They have a fecundity of 92 to 340 eggs in a reproductive season and reproduce within a temperature range of 0.5-30 °C (Poddubnaya 1980).

The larvae of most of the commercially important cultivable fish species prefer tubificid worms among the live foods (Phillips and Buhler 1979, Alam and Mollah 1988, Mollah 1991, Mollah et al. 2009). Aquarium fish culturists, especially ornamental fish culturists have also become dependent on these worms to feed their fishes as well as to keep their aquaria clean. Tubificid worms are not commercially cultured in Bangladesh. They are usually collected from wild habitats to fulfill the demands of the aquarium trade. Worms are collected from habitats that are rich in organic matter, such as drains. Collection from these places is not only troublesome and hazardous but also unhealthy and sometimes causes outbreaks of disease in the larvae fed these worms. Ahamed and Mollah (1992) demonstrated the use of a suitable medium (20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% sand) for sustainable growth of the worms for the first time in Bangladesh. However, Mollah et al. (2012), working in the same laboratory, reported that a medium comprised of 35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% sand gave higher production compared to the medium used by Ahamed and Mollah (1992). They also established the best time interval for media inoculation as 10 days. Recently Mariom and Mollah (2012) identified a medium comprising 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel as the best for tubificid production. The above researchers carried out their experiments using a cemented tank system of size 160×25×10 cm. Research on the mass production of these worms based on information generated so far is of utmost value. Success in mass production of tubificid worms will ensure a steady supply for feeding the fish and fish larvae. In this context, this study was conducted using three different sizes of tank system to determine the effect of rearing space on the production of tubificid worms.

## Materials and Methods

A comparative study on the production of tubificid worms in tanks of three different size groups was conducted to observe the effects of culture space on the production of worms. In this regard, the worms were cultured in three treatments each having three replicates, namely: treatment-I (dimensions: 160×25×10 cm; volume: 40,000 cm<sup>3</sup>; surface area: 4,000 cm<sup>2</sup>), treatment-II (dimensions: 160×60×10 cm; volume: 96,000 cm<sup>3</sup>; surface area: 9,600 cm<sup>2</sup>) and treatment-III (dimensions: 160×95×10 cm; volume: 152,000 cm<sup>3</sup>; surface area: 15,200 cm<sup>2</sup>). Other parameters were kept constant so that a comparison could be made among their production capability. The best medium identified by Mariom and Mollah (2012) i.e., 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel was used in this experiment.

### ***Culture procedure***

The standard culture procedure of Mariom and Mollah (2012) was used. Culture tanks were first washed and cleaned thoroughly with fresh water and were then connected to a water reservoir tank by porous PVC pipe of 180 cm length and 1 cm<sup>2</sup> diameter. The required amounts of the ingredients (Fig. 1) were collected and measured on a proportional basis as previously mentioned to make up 1,000 g media·tank<sup>-1</sup> for treatment-I, 2,400 g media·tank<sup>-1</sup> for treatment-II and 3,800 g media·tank<sup>-1</sup> for treatment-III. The mixed ingredients were soaked with a sufficient amount of rice gruel in three separate fibre glass tanks. After 7 days of decomposition, a fixed quantity (250 mg·cm<sup>-2</sup>) of media was placed into each tank. The decomposition is to facilitate the availability of the nutrients. Then water flow was adjusted at the rate of 1.24±0.41 L·min<sup>-1</sup>, 2.48±0.13 L·min<sup>-1</sup> and 3.72±0.63 L·min<sup>-1</sup> in treatment-I, treatment-II and treatment-III, respectively in order to maintain the dissolved oxygen in each treatment above 5 ppm.

The colony of live tubificid worms (mixed species) was picked up from different drains of the Bangladesh Agricultural University Campus, Mymensingh, Bangladesh using a small plastic bowl. The collected worms were mixed together and cleaned by using continuous flow of water and held in a flow-through system for conditioning over 24 h before inoculating into the tanks. The worms were then inoculated at the rate of 1.25 mg·cm<sup>-2</sup> (wet weight) (i.e., 5 g·tank<sup>-1</sup>, 12 g·tank<sup>-1</sup> and 19 g·tank<sup>-1</sup> for treatment-I, treatment-II and treatment-III, respectively). The prepared media were introduced at the rate of 250 mg·cm<sup>-2</sup> after Mollah et al. (2012) in the respective tanks once in every 10 days to replenish the nutrients used by the worms. To determine the production, three samples of worms were taken (Fig. 2) from three randomly selected places of each tank together with water and media using a sampler. The samples were collected at 10-day intervals starting on the 40<sup>th</sup> day of culture. The sampler was a glass tube having a diameter of 2.2 cm. The collected worms were cleaned from media by water flow. Final separation of the unwanted particles was done by using forceps and dropper. Cleaned worms were then dried with tissue paper and weighed using a Mettler electric balance (METTLER TOLEDO, PG503- SDR, Switzerland). Before each sampling, water temperature, dissolved oxygen and pH were recorded. Harvesting was started on 40<sup>th</sup> day of culture. During each sampling, harvesting was done at the rate of 40 mg·cm<sup>-2</sup> (Mariom and Mollah, 2012) to maintain the sustainability of culture.

Data were analysed by using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc to identify significant difference between means. Statistical analysis was performed using the statistical software SPSS version 11.5 with the level of significance at  $P < 0.05$ .



a. Mustard oil cake.



b. Wheat bran.



c. Soybean meal.



d. Cow-dung.



e. Fine sand.

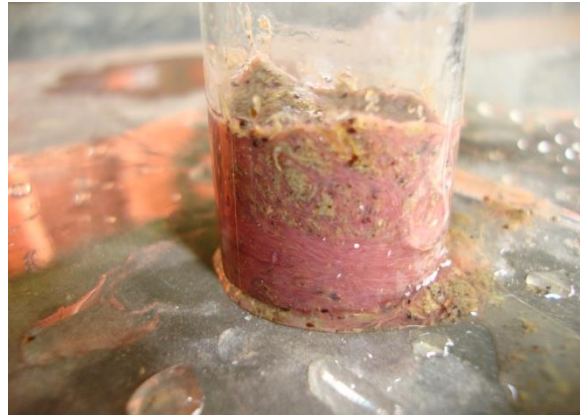


f. Rice gruel.

**Fig. 1(a-f).** Different ingredients for mass culture of tubificid worms.



a. Sample collection.



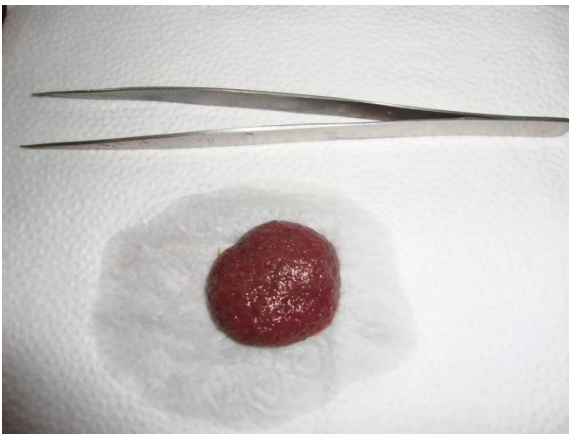
b. Samples pooled from one treatment.



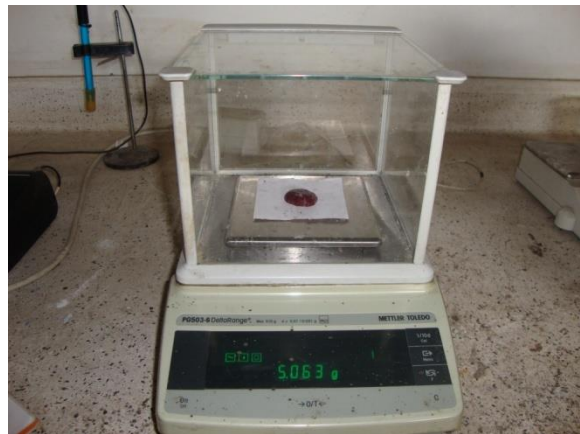
c. Collected sample in Petridish.



d. Cleaned sample in Petridish.



e. Cleaned sample on tissue paper.

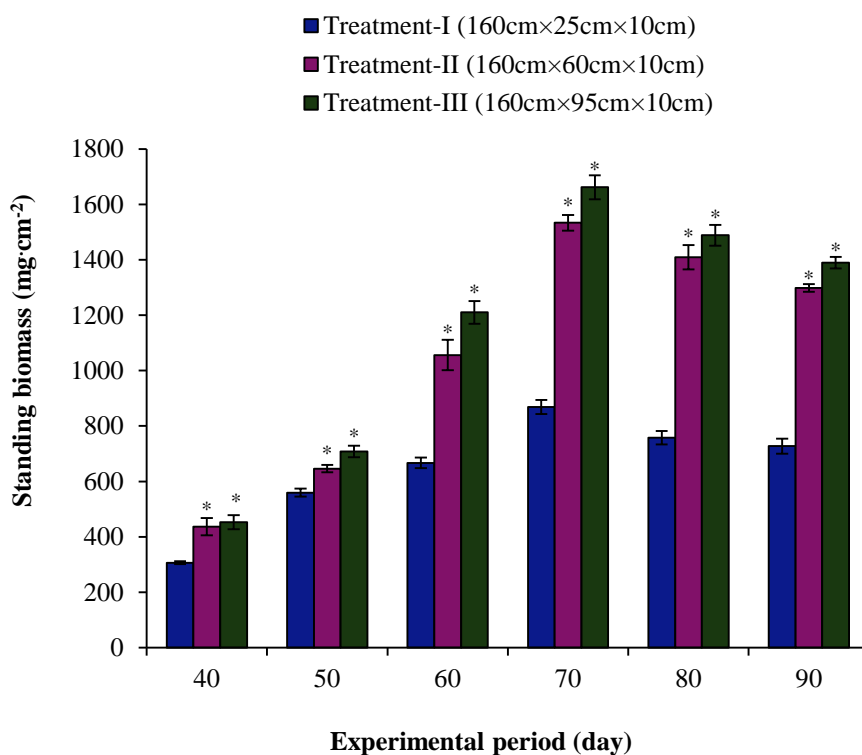


f. Weighing of sample.

**Fig. 2(a-f).** Different steps of sampling, cleaning and weighing of tubificid worms.

## Results

The standing biomass of tubificid worms in three different culture systems (Treatment-I, Treatment-II and Treatment-III) was calculated during the 90-day experimental period and is shown in Fig.3. The average standing biomass of tubificid worms was  $868.62 \pm 25.19 \text{ mg}\cdot\text{cm}^{-2}$ ,  $1,533.73 \pm 43.50 \text{ mg}\cdot\text{cm}^{-2}$  and  $1,662.16 \pm 28.32 \text{ mg}\cdot\text{cm}^{-2}$  in treatment-I, treatment-II and treatment-III, respectively at the 70<sup>th</sup> experimental day. The observed highest yield  $1,662.16 \pm 28.32 \text{ mg}\cdot\text{cm}^{-2}$  was found at the 70<sup>th</sup> day in treatment-III (Fig. 3). Test results (Table 1) showed significant differences ( $P < 0.05$ ) in mean total calculated production of treatment-II and treatment-III compared to treatment-I (Fig.3). However, statistical analysis showed no significant difference ( $P > 0.05$ ) between treatment-II and treatment-III throughout the experiment (Fig. 3). During the experiment, the standing biomass of tubificid worms in all the treatments gradually increased before peaking at day 70; biomass in all treatments then decreased up to the end of the experimental period (90<sup>th</sup> day) (Fig. 3).



**Fig.3.** Standing biomass ( $\text{mg}\cdot\text{cm}^{-2}$ ) of tubificid worms in three treatments (mean  $\pm$ SD) at different times. (ANOVA Test: \* $P < 0.05$ )

**Table 1.** ANOVA table for mean total calculated production ( $\text{mg}\cdot\text{cm}^{-2}$ ) of tubificid worms at 90 days experimental period.

Sources of variation	Degree of Freedom	Sum of Squares	Mean Square	F-value	Level of significance
Treatments between groups	2	2609412.699	1304706.349		
Errors within groups	51	6922006.301	135725.614		
Total	53	9531419.000		9.613	*

\*Significant at 5% level of probability.

Tubificid worms were harvested from all the replicates at the rate of  $40 \text{ mg}\cdot\text{cm}^{-2}$ , and treatment-III showed higher standing biomass ( $1,389.79\pm 14.21 \text{ mg}\cdot\text{cm}^{-2}$ ) than those of treatment-I ( $727.42\pm 27.30 \text{ mg}\cdot\text{cm}^{-2}$ ) and treatment-II ( $1,298.55\pm 21.06 \text{ mg}\cdot\text{cm}^{-2}$ ) at the 90<sup>th</sup> experimental day (Table 2). During the experiment, water temperature, dissolved oxygen and pH of treatment-I (temperature:  $29.2\pm 0.08 \text{ }^\circ\text{C}$ , DO:  $6.5\pm 0.1 \text{ ppm}$ , pH:  $7.1\pm 0.1$ ), treatment-II (temperature:  $28.7\pm 0.92 \text{ }^\circ\text{C}$ , DO:  $6.9\pm 0.2 \text{ ppm}$ , pH:  $7.3\pm 0.1$ ) and treatment-III (temperature  $28.3\pm 0.86 \text{ }^\circ\text{C}$ , DO:  $7.0\pm 0.2 \text{ ppm}$ , pH:  $7.4\pm 0.2$ ) were recorded and found suitable for culturing the worms.

**Table 2.** Total calculated production ( $\text{mg}\cdot\text{cm}^{-2}$ ) of tubificid worms over 90 days (mean $\pm$ SD)

Treatments	Standing biomass at 90 <sup>th</sup> day (S) $\text{mg}\cdot\text{cm}^{-2}$	Harvested biomass at 90 days (H) $\text{mg}\cdot\text{cm}^{-2}$	Total calculated production (S+H) $\text{mg}\cdot\text{cm}^{-2}$
I	$727.42\pm 27.30^b$	240	$967.07\pm 27.30^b$
II	$1,298.55\pm 21.06^a$	240	$1,538.18\pm 21.06^a$
III	$1,389.79\pm 14.21^a$	240	$1,629.25\pm 14.21^a$

Values with different superscripts in a vertical column are significantly different (one way ANOVA followed by Tukey's HSD Test,  $P<0.05$ ).

## Discussion

The experiment demonstrated the effects of culture space on the yield of tubificid worms. The highest yield of  $1,662.16\pm 28.32 \text{ mg}\cdot\text{cm}^{-2}$  was recorded at the 70<sup>th</sup> day in the largest culture unit (treatment-III). The results indicate that the better yield is produced in  $160\times 95\times 10 \text{ cm}$  rearing units. Also the larger culture units used in the present study are more suitable in terms of production of tubificid worms than the smaller ones used by previous workers. Marian and Pandian (1984) used a culture system of size  $150\times 15\times 15 \text{ cm}$  and reported a production of  $200 \text{ mg}\cdot\text{cm}^{-2}$  on a substrate containing 75% cow-dung and 25% sand. Ahamed and Mollah (1992) found better production

(419.4 mg·cm<sup>-2</sup>) on a medium containing 20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% sand in a culture system of size 160×25×10 cm.

From these experiments, it can be concluded that production differed due to differences in size of the culture unit under different media used.

This is an indicator that both the culture media and the size of culture unit might interact to play a significant role in the production of tubificid worms. More recently, Mariom and Mollah (2012) reported production of 999.16 mg·cm<sup>-2</sup> at the 70<sup>th</sup> day of culture using the same media ingredients used in the present study, i.e., 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel in a culture unit of size 160×25×10 cm. Hence, in the present study, the observed higher yields recorded in treatment-II (1,533.73±43.50 mg·cm<sup>-2</sup>) and treatment-III (1,662.16±28.32 mg·cm<sup>-2</sup>) at the 70<sup>th</sup> day of culture when compared with treatment-I (868.62±25.19 mg·cm<sup>-2</sup>), indicate the effects of culture space on the production of tubificid worms. Greater production in larger culture units might be due to larger surface area allowing more oxygen to diffuse into the tanks. Since the oxygen requirements of tubificid worms are high (Marian and Pandian 1984), this extra oxygen is beneficial to their growth and survival. The larger culture units may also help colonies to spread easily compared to smaller units.

The physico-chemical factors of the tanks i.e., water temperature, dissolved oxygen content and pH recorded during the period of study were suitable and within the productive range as reported by Poddubnaya (1980), Davis (1982), Marian and Pandian (1984) and Li (2001). Korotun (1959) stated that 11°C is the minimum temperature for the reproduction of *Tubifex tubifex* while 2.5 °C and 38 °C are regarded as lethal. The normal development of the embryo requires oxygen content between 2.5 and 7.0 ppm. Davis (1982) found that tubificids spontaneously grow in 2.4-8.8 ppm dissolved oxygen. The present study showed that it is economically feasible to mass culture tubificid worms, since only about 0.80 kg culture media costing US\$ 0.24 was needed to produce 1 kg worms. Ahamed and Mollah (1992) needed 2.85 kg raw materials for 1 kg worm production against 18 kg and 25 kg cow-dung reported by Marian and Pandian (1984) and Marian et al. (1989), respectively. Mariom and Mollah (2012) reported 1.01 kg culture media costing US\$ 0.29 to yield 1 kg worms. From a management point of view, the bigger culture unit is obviously easier and less troublesome to maintain.

## Conclusion

A number of studies have been conducted to develop a suitable culture technique for tubificid worms. Despite all these efforts it has not been possible to economically produce these worms commercially in Bangladesh. The findings of the present study can be of significant importance to the many fish hatcheries scattered over Bangladesh. The economical commercial mass production of



tubificid worms in the larger unit of 15,200 cm<sup>2</sup> as opposed to that of previously practised culture unit of 4,000 cm<sup>2</sup> will benefit the aquaculture industry.

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