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ความดกของไข่และอัตราการฟักของไส้เดือนน้ำ *Limnodrilus hoffmeisteri* Claparede (Clitellata: Tubificidae) ในสภาวะการทดลองเลี้ยง

วฤชา ประจงศักดิ์

บทคัดย่อ

การทดลองครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการวางไข่และอัตราการฟักไข่ของไส้เดือนน้ำ Limnodrilus hoffmeisteri ภายใต้สภาวะการทดลองเลี้ยงในห้องปฏิบัติการ โดยนำไส้เดือนที่ไม่อยู่ในระยะเจริญพันธุ์ (immature specimens) ที่เก็บจากแหล่งน้ำธรรมชาติมาเลี้ยงในบีกเกอร์ ซึ่งมีทรายซิลิกาชั้นเป็นวัสดุรองรับ (substrates) เติมน้ำที่ผ่านการกรอง และเลี้ยงในสภาวะอุณหภูมิห้อง ทำการศึกษาและสังเกตทุก 2 วัน ผลการศึกษาพบโคคูน (cocoon) จำนวน 132 โคคูน และมีไข่จำนวน 264 ฟอง อยู่ภายใน จากการวิเคราะห์ข้อมูลพบค่าของจำนวนไข่ต่อโคคูน (eggs per cocoon) มีค่าอยู่ระหว่าง 1.55 และ 2.46 โดยมีค่าเฉลี่ยเท่ากับ 1.93±0.39 และมีค่าเฉลี่ยของจำนวนโคคูนต่อจำนวนตัวต่อวัน (cocoons per individual per day) เท่ากับ 0.09±0.02 (0.07-0.13) ไส้เดือนน้ำมีระยะเวลาระหว่างการวางไข่และการฟัก (time between cocoons laying and newborn hatching) ประมาณ 10 วัน และมีอัตราการฟักร้อยละ 39 ไส้เดือนน้ำสามารถให้กำเนิดตัวอ่อนได้ทั้งหมด 102 ตัว ซึ่งฟักในช่วงระยะเวลาระหว่าง 30 และ 36 วัน หลังจากเริ่มต้นการทดลอง โดยมีค่าเฉลี่ยของจำนวนตัวอ่อนต่อจำนวนตัวเต็มวัย (young worms per adult) เท่ากับ 2.04±0.74 การทดลองครั้งนี้ให้ข้อสังเกตได้ว่าไส้เดือนน้ำที่เลี้ยงในทรายซิลิกาชั้นและมีการเติมอาหารปลาเพื่อเป็นสารอินทรีย์ในขณะเลี้ยง ให้ผลผลิตไส้เดือนน้ำที่มีความดกของไข่ต่ำเมื่อเปรียบเทียบกับงานวิจัยที่มีการทดลองเลี้ยงโดยใช้ตะกอนธรรมชาติ

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Fecundity and Hatching Rates of Limnodrilus hoffmeisteri Claparede (Clitellata: Tubificidae) under Cultured Laboratory Conditions

Warucha Prajongsak

Abstract

The purpose of the present paper was to obtain information on the deposition and hatching rates of L. hoffmeisteri cocoons under laboratory condition. Immature specimens from natural site were placed in beakers containing moistened silica sand as the substrates filled with distilled waters and kept at room temperature. By every two days observation, it was shown that 132 cocoons contained of 264 eggs. The number of eggs per cocoon ranged from 1.55 to 2.46 with the average number of 1.93±0.39, with the mean number of cocoons per individual per day equal to 0.09±0.02 (0.07-0.13). The results indicated that the time between cocoons laying and newborn hatching was detected around 10 days with the mean hatching rate of 39%. The overall of 102 young worms hatched between 30 and 36 days from the beginning of the culture and the mean number of young worms per adult was 2.04±0.74. Laboratory studies suggested that individuals reared in moistened silica sand supplemented with dry fish food produced low fecundity compared to those reared in natural sediment.

Keywords: Cocoon, Hatching rate, Tubificid, Pligochaete

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Introduction

Aquatic oligochaetes are recognized as an important food sources for various fish. Yan and Liang (2004) reported that 90% of oligochaetes dry weight consists of protein and fat. Some species, such as *Limnodrilus hoffmeisteri,* gathered from the natural source of Thai water, is one of the major living diets for many of juvenile fish. Nutritional value of this worm species reported showed that the average values of protein and fat were 6.49±0.24 and 7.21±0.92%, respectively by Kanchana-Aksorn (2012).

L. hoffmeisteri Claparede, 1862 is one of the most widespread and abundant aquatic oligochaete in the world (Kennedy, 1965). It is widely recognized as an effective indicator of organically polluted aquatic environment. (Alves et al., 2006; Dorfeld et al., 2006, Martins et al., 2008). Additionally, this species has been used in ecotoxicological studies (Flores-Tena and Martinez-Tabche, 2001; Kanchana-Aksorn and Petpiroon, 2009; Prajongsak, et al., 2012.).

The biology of *L. hoffmeisteri* has received appreciable attention in many countries. From field studies, the life history of this species varied according to local conditions and worms could breed throughout the year (Kennedy, 1966; Ladle, 1971; Poddubnaya, 1980; Raburu et al., 2002; Yan and Liang, 2002; Zbikowski, 2007). In addition, the life cycle of L. hoffmeisteri had been facilitated by culturing the worms in laboratory conditions. Three groups of stage could be established in the life cycle of this worm, divided into cocoon, juvenile (immature) and adult (with mature eggs in the coelom) (Pasteris et al., 1999; Nascimento and Alves, 2009; Lobo and Alves, 2011b).

In Thailand, despite the previous data available on the population growth of *L. hoffmeisteri* in laboratory conditions (Kanchana-Aksorn and Petpiroon, 2008), there is a considerable lack of studies on its biology.

The aim of this study was to report on both fecundity and hatching rates of *L. hoffmeisteri* under laboratory conditions. The results from this observation could be available for applying in both ecotoxicological studies and further cultivation.

Materials and Methods

Preparation of worm specimens

L.hoffmeisteri were collected from the sediment of Chao Phraya river, Tok road waterside, Bangkok, between April and May 2012 by scrapping sediments with small shovel. The sediments were washed through 0.5 mm sieve with regional spring water. The specimens were transported to the laboratory and carefully sorted under stereoscopic microscope. Classification was followed by the original description (Brinkhurst and

Jamieson, 1971). The identification also was confirmed by Christer Erseus, Gothenburg University, Sweden, using the technique of mitochondrial 16s ribosomal DNA markers (Beauchamp et al., 2001).

Non-sexually mature individuals of approximately similar size (length ≅≅ 2.0-2.5 cm) were used in the culture. Firstly, worms were placed in a Petri dish filled with distilled water to evacuate their gut contents for 6 h (Lyes, 1979) prior to the culture started.

Preparation of substrate and culturing conditions

The substrate used in this study was silica sand (particle between 125 and 250 □m, total organic carbon (TOC) = 0%). Before the culture started, the sand was moistened with distilled water and 0.5 g of grinded fish food was added (Ducrot et al., 2007). It was kept to aerate for two weeks under static conditions in order to allow bacteria growth (Moore, 1979).

For the preparation of culturing conditions, dry fish food was added as a source of organic matter in the substrates. All beakers were exposed to the air. The culture was conducted at room temperature in a daily photoperiod of 12 h. Light was provided from cool-white fluorescent lamps. Every two days, the water was changes, the sands were washed, and the residual foods were removed and fresh foods were added.

Fecundity investigation

Five beakers were used for rearing to allow the specimen to reach sexual maturation. Each beaker (250 ml) contained 2-3 cm depth of substrate, 100 ml of distilled water, and ten immature individuals. All beakers were observed every two days for screening mature worms and cocoons deposition. The substrate of each beaker was washed in a 0.125 mm sieve and analyzed under a stereoscopic microscope. Specimens no contained cocoons were returned to the old beakers. The number of cocoons per individual per day and eggs per cocoon were determined.

Analysis of hatching time and rate

Both hatching time and rate were analyzed after the cocoons were observed. The cocoons were placed separately in another beaker containing 2-3 cm depth of substrate and 100 ml of distilled water. All cocoons collected on the same day were put in the same beaker by using a 3-ml Pateur pipette. These beakers were examined every two days for new hatchings. Young worms per adult were recorded by multiplying the number of newborn worms by the number of maturing specimens.

Results

Fecundity

One hundred thirty two cocoons contained 264 eggs were collected in the period of the culture. The overall number of eggs in cocoons ranged from 31 to 86.

The values of eggs per cocoon varied between 1.55 and 2.46 with the mean number of 1.93±0.39. Changes in the number of cocoons per individual per day were similar to the values of eggs per cocoon, with the mean number of 0.09±0.02 (Table 1).

Hatching rates

A total of 102 newborns hatched between 30 and 36 days from the start of the culture with mostly found in 34 days (Figure 1). The greatest number of young worms per adult was 3.00 and the average value was 2.04±0.74 (Table 1). The time between laying the cocoons and hatching was around 10 days. The mean hatching rate was 39%.

Table 1. Values of eggs per cocoon, cocoons per adult per day, young worms per adult and hatching rate of L. hoffmeisteri in the laboratory culture.

Beaker	Eggs per cocoon	Cocoons per adult per day	Young worms per adult	Hatching rate
1	1.68	0.09	1.9	0.45
2	2.23	0.11	2.6	0.39
3	2.46	0.13	3	0.35
4	1.55	0.07	1.3	0.42
5	1.73	0.08	1.4	0.37
Mean	1.93	0.09	2.04	0.39
SD	0.39	0.02	0.74	0.04

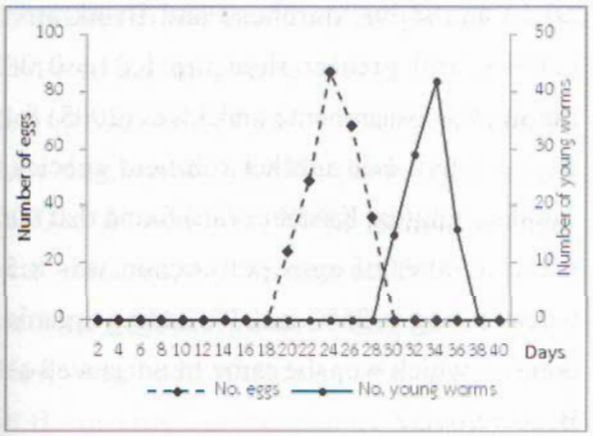

Figure 1. Period between egg laying and hatching of L. hoffmeisteri in the laboratory culture.

Since the wall of *L. hoffmeisteri* cocoons had been stuck to fine particles after laying, therefore cocoons individual were observed inside the mature specimens (Figure 2).

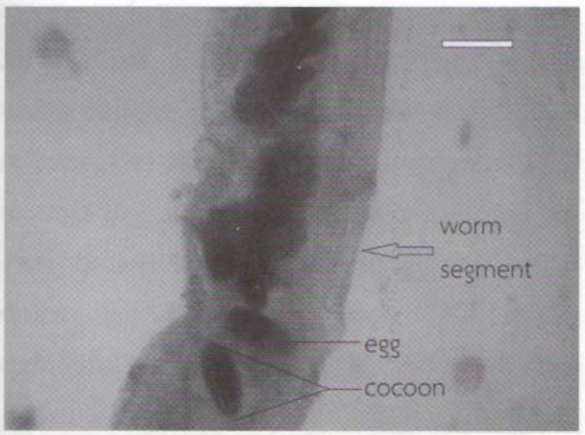

Figure 2. *L. hoffmeisteri* cocoons before hatching. White bar = 1 mm.

Discussion

The cocoons of *L. hoffmeisteri* from this study were covered with fine particles, which was the same evidence as shown in previous report (Aston, 1973; Lazim et al., 1989). This phenomenon decreases their detection in the substrate to provide more protection against organisms that can harm embryo development (Lobo and Alves, 2011b).

Fecundity

The number of cocoons obtained in this study was higher than the reports of Lobo and Alves (2011b), which was 115 cocoons reared at 25°C for 20 days in fine sand, and the observation by Nascimento and Alves (2009), which was 112 cocoons reared at 25°C for 21 days in fine sand. Also, the number of cocoons was higher than the report by Nascimento and Alves (2008) for another tubificid, Branchiura sowerbyi, which was 57 cocoons collected in 15 days at 25°C.

In accordance with the finding of Reynoldson et al. (1996), different populations of one given species may present difference in some biological features, such as number of cocoons per individual, as well as hatching rates. All organisms in the same population are in the reproductive stage at the same time. The first reproductive cycle is synchronized, while in the second cycle some organisms are differently reproduced (Ducrot et al., 2007). These evidences confirm the results from the present study that the specimens used in the culture might be in different generations. Consequently, different

intervals of the number of eggs in cocoons were detected.

An increase in temperature might lead to an increase in the number of cocoons of tubificid oligochaetes such as *L. hoffmeisteri*. Higher temperature accelerates the metabolism of organisms and causes to increase in the number of reproductive events (Howe, 1967). This reason supports the existence of condition appearing in this study which was conducted in tropical region and the room temperature was higher than 25°C.

The number of cocoon per individual per day was below the previous one investigated by Lobo and Alves (2011b) which was 0.37 ±0.22, for this species at 25°C. Likewise, it was lower than the averages observed by Marchese and Brinkhurst (1996), Nascimento and Alves (2008), and Lobo and Alves (2011a) which was 0.17, 0.13 and 0.12, respectively, for B. sowerbyi at 25°C.

The results from the present study have shown that the number of eggs per cocoon of L. hoffmeisteri found to be below those reports by Aston (1973), Nascimento and Alves (2009), and Lobo and Alves (2011b), which were 5, 3.25, and 3.12 eggs per cocoon, respectively.

The number of egg per cocoon was lower than that observed by Aston et al. (1982), which was 2.82, similar to the 1.94 ±0.13 found by Marchese and Brinkhurst (1996), and greater than the 1.21± 0.08 reported by Nascimento and Alves (2008) for B. sowerbyi. For another tubificid species, Tubifex. tubifex, Kaster (1980) found that the mean number of eggs per cocoon was 8.5 when rearing in 25°C and 3% adding organic content, which was the same trend as well as B. sowerbyi.

Hatching rates

The youngs had been observed between 30 and 36 days from this study. It showed quite similarly results to Pasteris et al. (1999) that has previously observed under laboratory condition, which was found that no worms hatched from cocoons older than 35 days

The presence of young individuals of *L. hoffmeisteri* from this study suggests that the species need about 10 days to develop the full embryonic period. This result was agreed with the period of time observed by Lobo and Alves (2011b), which was found between 8 and 12 day for this species. Further, T. tubifex required the same hatching period occurring around 15-18 days at room temperature and 10-12 days, as reported by Timm (1973) and Kosiorek (1974), respectively. For another one, B. sowerbyi, the hatching time was in the period, which was varied between 10 and 20 days (Nascimento and Alves, 2008).

L.hoffmeisteri cultivated under

laboratory conditions from the present study had low hatching rates (39%). Differently, the specimens reared in fine sand had the hatching rate of 84.83% (Lobo and Alves, 2011b). In addition, 97.3% of the hatching was observed in T. tubifex at 25°C with 3% adding organic content (Kaster, 1980). However, the low hatching rates were found in B. sowerbyi, as reported by Marchese and Brinkhurst (1996), which was between 30.2% and 39.4% at 20-30°C, together with Nascimento and Alves, (2008), which was 44.93% at 25°C. There by further studies are necessary to verify.

The results of many studies with respect to the number of cocoons per adult per day and the number of eggs per cocoon as mentioned above (Nascimento and Alves, 2009; Lobo and Alves, 2011b) were differ. These discrepancies come from the several external factors, including various pattern of methodology, for instance, differenced in population density, food quality or types of substrate, which makes it difficult to interpret and compare their results.

Silica sand with fine particle was adopted as the substrate in this study due to the lack of contamination of any organisms. However, when compared to the worms reared in natural sediments (Nascimento and Alves, 2009; Lobo and Alves, 2011b), it was mostly found that the specimens exhibited lower fecundity, because silica sand contained less nutritive matter than natural sediments (Ducrot et al., 2007). Although fish food was added at the substrate surface, the worms could not forage due to their feeding habit (Ewald et al., 1997; Othman et al., 2002). Therefore, natural sterilized sediment with fine fraction and enough for organic matter might be recommended for further culture.

Conclusion

In this study, it could be determined some substantial reproductive characteristics of L. hoffmeiteri, including the number of cocoons per individual per day, number of eggs per cocoon, the time between eggs laying and hatching, as well as hatching rate, which are assuredly essential aspects for the investigation on the reproduction. Although low fecundity was occurred when rearing in moistened silica and dry fish food was added on, eggs and cocoons could be produced in mature individual.

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References

Alves, R.G., Marchese, M.R. and Escarpinati, S.C. (2006). Oligochaeta (Annelida, Clitellata) in lotic environments in the State of Sao Paulo, Brazil. Iheringia Serie Zoologia. 96: 431-435.

Aston, R.J. (1973). Field and experimental studies on the effect of power station effluent on Tubifidae (Oligochaeta, Annelida). Hydrobiologia. 42: 225-242.

Aston, R.J., Sadler, K. and Milner, A.G.P. (1982). The effects of temperature and the culture of *Branchiura sowerbyi* (Oligochaeta, Tubificidae) on the activated sluge. Aquaculture. 29: 137-145.

Beauchamp, K.A., Kathman, R.D., McDowell, T.S. and Hedrick, R.P. (2001). Molecular phylogeny of tubificid oligochaetes with special emphasis on *Tubifex tubifex* (Tubificidae). Moecularl Phylogenetic Evolution. 19: 216-224.

Brinkhurst, R.O. and Jamieson, B.G.M. (1971). Aquatic Oligochaeta of the World. Edinburgh: University of Toronto Press.

Dorfeld, C.B., Alves, R.G., Leite, M.A. and Espindola, ELG. (2006). Oligochaeta in euthophic reservoir: the case of Salto Grande reservoir and their main affluent (Americana, Sao Paulo, Brazil). Acta Limnologica Brasiliensia. 18(2): 189-197.

Ducrot, V., Pery, A.R.R., Queau, H., Mons, R., Lafont, M. and Garric, J. (2007). Rearing and estimation of life cycle parameters of the tubificid worm *Branchiura sowerbyi*: application to ecotoxicity testing. Science of the Environment.384: 252-263.

Ewald, G., Berglund, O. and Svensson, J.M. (1997). Effect of oligochaete bioturbation on sediment accumulation of 2,2□4,4□-tetrachlorobiphenil. Ecotoxicology and Environmental Safety. 36: 66-71.

Flores-Tena, F.J. and Martinez-Tabche, L. (2001). The effect of chromium on the hemoglobin concentration of *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae). Ecotoxicology and. Environmental Safety. 50: 196-202.

Howe, R.W. (1967). Temperature effects on embryonic development in insect. Annual Review of Entomology. 12: 15-42.

Kanchana-Aksorn, W. (2012). Nutrition values of freshwater oligochaete *Limnodrilus hoffmeisteri* in different preservation conditions, pp. 829-837. In The Proceedings of 3rd Phranakhon Rajabhat University Annual Conference, Multidisciplinary Research for Sustainable Development: Thai Wisdom towards ASEAN, 25 July 2012. Phranakhon Rajabhat University. Bangkok (in Thai).

Kanchana-Aksorn, W. and Petpiroon, S. (2008). Study on Limnodrilus hoffmeisteri Population Response to Different Organic Enrichment in Laboratory Condition, 8 p. In The 1st PSU Phuket Research Conference. Prince of Songkla University, Phuket Campus, Phuket.

Kanchana-Aksorn, W. and Petpiroon, S. (2009). Toxic effect of naphthalene-spiked sediment to freshwater oligochaete *Limnodrilus hoffeisteri* from Chao Phraya estuary. Kasetsart Journal. (Natural Science). 43: 322-329.

Kaster, J.L. (1980). The reproductive biology of *Tubifex tubifex* Muller (Annelida: Tubificidae). American Midland Naturalist. 104(2): 364-366.

Kennedy, C.R. (1965). The distribution and habitat of *Limnodrilus hoffmeisteri* (Oligochaeta:Tubificidae). Oikos. 16: 26-38.

Kennedy, C.R. (1966). The life history of *Limnodrilus hoffmeisteri* Clap. (Oligochaeta: Tubificidae) and its adaptive significance. Oikos. 17: 158-168.

Kosiorek, D. (1974). Development cycle of Tubifex tubifex Mull. in experimental culture. Polskie Archiwum Hydrobiologii. 21: 411-422.

Ladle, M. (1971). The biology of Oligochaeta from Dorset Chalk streams. Freshwater Biology. 1: 83-97.

Lazim, M.N., Learner, M.A. and Cooper, S. (1989). The importance of worm identify and life history in determining the vertical distribution of tubificids (Oligochaeta) in a riverine mud. Hydrobiologia. 178: 81-92

Lobo, H. and Alves, R.G. (2011a). Reproductive cycle of *Branchiura sowerbyi* (Oligochaeta: Naididae: Tubificinae) cultivated under laboratory conditions. Zoologia. 28(4): 427-431.

Lobo, H. and Alves, R.G. (2011b). Influence of body weight and substrate granulometry on the reproduction of Limnodrilus hoffmeisteri (Oligochaeta: Naididae: Tubificinae). Zoologia. 28(5): 558-564.

Lyes, M.C. (1979). Bioavailability of a hydrocarbon from water and sediment to the marine worm Arenicolar marina. Marine Biology. 268: 518-519.

Marchese, M.R. and Brinkhurst, R.O. (1996). A comparison of two tubificid oligochaete species as candidates for sublethal bioassay tests relevant to subtropical and tropical regions. Hydrobiologia. 334: 163-168.

Martins, R.T., Stephan, N.N.C. and Alves, R.G. (2008). Tubificidae (Annelida: Oligochaeta) as an indicator of water quality in an urban stream in southeast Brazil. Acta Limnologica Brasiliensia. 20: 221-226.

Moore, J.W. (1979). Influence of food availability and other factors on the composition, structure and density on a subarctic population of benthic invertebrates. Hydrobiologia. 62: 215-223.

Nascimento Haroldo, L.S. and Alves, Roberto G. (2008). Cocoon production and hatching rates of *Branchiura sowerbyi* Beddard (Oligochaeta: Tubificidae). Evista Brasileira de Zoologia. 25(1): 16-19

Nascimento Haroldo, L.S. and Alves, Roberto G. (2009). The effect of temperature on the reproduction of *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae). Zoologia. 26(1): 191-193.

Othman, M.R., Samat, A. and Hoo, L.S. (2002). The effect of bed-sediment quality on distribution of macrobenthos in Labu river system and selected sites in Langat river, Malaysia. Journal of Biological Science. 2(1): 32-34.

Pasteris, A., Veccgi, M. and Bonomi, G. (1999). A comparison among different population models for *Limnodrilus hoffmeisteri* Clapared (Oligochaeta, Tubificidae). Hydrobiologia. 406: 183-189.

Poddubnaya, T.L. (1980). Life cycles of mass species of Tubificidae (Oligochaeta), pp. 253–317. In *Aquatic Oligochaeta Biology.* (Edited by Brinkhurst, R. O. & Cook, D. G.). New York:Plenum Press.

Prajongsak, W., Petpiroon, S. and Meksumpun, C. (2012). Concentration of naphthalene in oligochaete (*Limnodrilus hoffmeisteri*) and fingerling tilapia (*Oreochromis niloticus*) from a food chain experiment, pp. 367-376. In Full Paper Proceeding Book of Second International Symposium of Biopesticides and Ecotoxicological Network (ISBioPEN), 24-26 Sep. 2012, Maruay Garden Hotel. Department of Zoology, Faculty of Science, Kasetsart University, Bangkok.

Raburu, P., Mavuti, K.M. Harper, D.M. and Clark, F.L.(2002). Population structure and Secondary productivity of *Limnodrilus hoffmeisteri* Claparede and Branchiura sowerbyi Beddard in the profundal zone of Lake Naivasha, Kenya. Hydrobiologia. 488: 153-161.

Reynoldson, T.B., Rodriguez, P., and Madrid, M.M. (1996). A comparison of reproduction, growth and acute toxicity in two populations of *Tubifex tubifex* (Muller, 1774) from the North American Great Lakes and Northern Spain. Hydrobiologia. 334: 199–206.

Timm, T.E. (1973). Culture methods for aquatic Oligochaeta, pp. 166-184. In Aquatic Oligochaeta; systematic, ecology and studies of the Soviet Union. Fisheries Research

Board of Canada. Translation Series. 2721.

Yan, Y. and Liang, Y. (2002). Abundance and production of *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae) in algae-dominated Lake Houhu (Wuhan, China). Chinese Journal of Oceanology and Limnology. 20(1): 81-85.

Yan, Y. and Liang, Y. (2004). Energy flow in *Branchiura sowerbyi* (Oligochaeta: Tubificidae) in a shallow macrophyte-dominated lake, Biandantang Lake. Chinese Journal of Oceanograghy and Limnology. 22: 403-407.

Zbikowski, J. (2007). Various reproductive strategies of two species of Oligochaeta: *Limnodrilus hoffmeisteri* and *Tubifex tubifex*. Oceanological an Hydrobiological Studies. 36(4): 109-117.