# Effects of induced spawning on early development in snout otter clam, *Lutraria philippinarum* (Deshayes, 1854) (Bivalvia: Mactridae)

**Bantoto-Kinamot V.***

Received: December 2016 Accepted: May 2016

Department of Biology, Negros Oriental State University, Kagawasan Avenue, Dumaguete City 6200, Negros Oriental, Philippines.
*Corresponding author's Email: vdbbio@yahoo.com

**Keywords:** *Lutraria philippinarum*, Bais Bay, Spawning induction, Embryonic development

## Introduction

Throughout the world, bivalves play an important role in the national economy of many countries. In 2005, the contribution of bivalves to the total global trade of fish and fishery products was approximately US$ 78.9 billion (WHO, 2010). Though the contribution of the overall bivalve production to aquaculture increased, production from wild harvests exhibited a downward trend. Increased fishing efforts from rapidly growing population, habitat destruction, environmental changes, pollution, high seafood and shell trade global demand are the factors which lead to the decline of many mollusk resources. One of the commercially important bivalves which showed a declining catch is *Lutraria philippinarum* (Bantoto and Ilano, 2012). This species is served as a special dish in restaurants of the Philippines and Vietnam making it highly in demand.

A synergy between capture fisheries and aquaculture through hatchery technology is considered as a sustainable way to restore depleted stocks at the same time increase production to help meet the projected global demand for fishery resources. Release of cultured juveniles serves as a promising application to hatchery technology to augment the natural supply of juveniles, restore overexploited species and provide substantial yields (Bell *et al*., 2008). In the Philippine setting, this practice is still to be carried out for most commercially important bivalves like *L.*

*philippinarum* since most of its demands rely on wild harvesting.

Spawning induction in sexually matured broodstock is important in hatchery operations for the production of seeds (Alagarwami and Dharmaraj, 1983). Thus, the most effective method of spawning induction in species should be established. For instance, serotonin and sex steroids injection are mostly used for the giant clam (Beckvar, 1981; Crawford and Lucas, 1986; Neo *et al*., 2011). In the case of *Lutraria philippinarum*, a reliable method of spawning induction and description of its larval development are yet to be developed.

Thus, this study deals with the stages of early development in *Lutraria philippinarum* and the effects of induced spawning on its early development. Results of this study will add to the dearth of larval development and biology literatures on *L. philippinarum.* Description of *L. philippinarum* larvae reared under laboratory conditions will also help in the identification of the wild larvae since early larvae of bivalves are difficult to distinguish. A reliable method for spawning induction in *Lutraria philippinarum* for mass seed propagation, stock enhancement, restocking and coastal rehabilitation of this resource will also be established.

## Materials and methods

Mature clams with estimated length ranging from 6cm to 12cm were collected from North Bais Bay, Manjuyod, Negros Oriental (9º38' N; 123º08' E). The collected clams were placed in an ice bucket containing seawater and brought to the laboratory for spawning induction. In the laboratory, the clams were placed in a container with filtered seawater at room temperature for 24 hrs to acclimate the clam under laboratory conditions. After 24 hrs, each individual was cleaned individually to remove any encrusted epifauna and placed into separate spawning bottles.

During spawning induction, randomly selected clams were injected with 2ml of KCl, $NH_4OH$ and $H_2O_2$ at the foot. The concentrations of KCl used in this study were 0.5%, 2%, 4% and 6% while for $NH_4OH$ and $H_2O_2$, the concentrations used were 0.5%, 1%, 2% and 3%. After injection, each clam was placed in a single spawning bottle with filtered seawater at room temperature until they spawned.

As soon as the clam started to spawn, the gametes were immediately removed from the spawning bottle, transferred to a beaker and stirred carefully to allow fertilization to occur. The eggs or sperms released per clam sample after induction were counted using a Neubauer haemocytometer under a compound microscope. Fertilization success and early development were assessed under a compound microscope. Fertilized eggs were identified by active cell division into a blastula cell mass while undeveloped embryos were identified by no cell division. Each phase of larval

development was identified and documented.

**Results and discussion**

It was observed in this study that only high concentrations of the chemical stimulant used were able to induce spawning. Using $NH_4OH$, 17% clam samples spawned with 2% $NH_4OH$ and 42% with 3% $NH_4OH$. For KCl, 17% clam samples spawned with 6% KCl. On the other hand, 33% spawned with 3% $H_2O_2$. The timing of spawning among the clams induced with KCl, $NH_4OH$ and $H_2O_2$ did not differ. Release of gametes by the clams induced by KCl, $NH_4OH$ and $H_2O_2$ occurred 10-15 min after induction (Table 1).

*Lutraria philippinarum* spawned in response to relatively high concentrations of potassium chloride (KCl), hydrogen peroxide ($H_2O_2$) and ammonium hydroxide ($NH_4OH$) KCl, $H_2O_2$ and $NH_4OH$ which stimulated muscle contraction during spawning. In addition, hydrogen peroxide has been found to activate the endogenous enzymatic synthesis of prostaglandin related to spawning (Morse, D. 1984; Alagarwami and Dharmaraj, 1983). Prostaglandins produced by nerve cells are considered to play an important role in mollusk spawning. In the present study, though *L. philippinarum* spawned with $H_2O_2$, the percentage response was found to be relatively lower than that to $NH_4OH$ and KCl. According to Alagarwami and Dharmaraj (1983), peroxide induction has been found to be better in the alkaline medium of Tris. Alkalinity promotes both the peroxide activation and induction of spawning. In *Pinctada fucata*, alkaline medium increases the proportion of oyster to spawn in response to a given concentration of hydrogen peroxide.

Female clams released approximately 1.5 to 8.0 x $10^5$ eggs with a mean diameter of 20µm (Fig. 1B). Fertilization occurred 30 to 45 min after while gradual protrusion of polar body which marked the first cleavage was observed after 1 hr (Fig.1C-D). Most of the 2-celled stage was observed after 2 hrs (Fig.1E). As cleavage progressed, the micromeres became concentrated in the animal pole. Eight-celled stage during 3$^{rd}$ cleavage was observed after 2hrs and 30 min characterized by prominent micromeres and macromeres (Fig. 1H). The fifth cleavage occurred after 3hrs. The embryo during this stage was one-celled thick and cells adhered to the neighboring cells (Fig. 1I). After 3 hrs and 20min, the embryo underwent the 6$^{th}$ cleavage marked by the appearance of cilia and rotational movement (Fig 1K). Trochophores were observed after 24 hrs (Fig. 1L).

**Table 1: Timing of the early development of *Lutraria philippinarum* at 30 ºC and 35 ppt.**

| Stage | Time after spawning induction (hr) |
|---|---|
| Spawning | 10-15 min |
| Fertilization | 30-45 min |
| Perivitelline Membrane Formation | 1 |
| Polar Body Formation | 1 |
| 1st cleavage | 1-2 |
| 2nd cleavage | 2 |
| 3rd cleavage | 2 and 30 min |
| 5th cleavage | 3 hrs |
| 6th cleavage | 3 hrs and 20min |
| Trochophore | 24 |

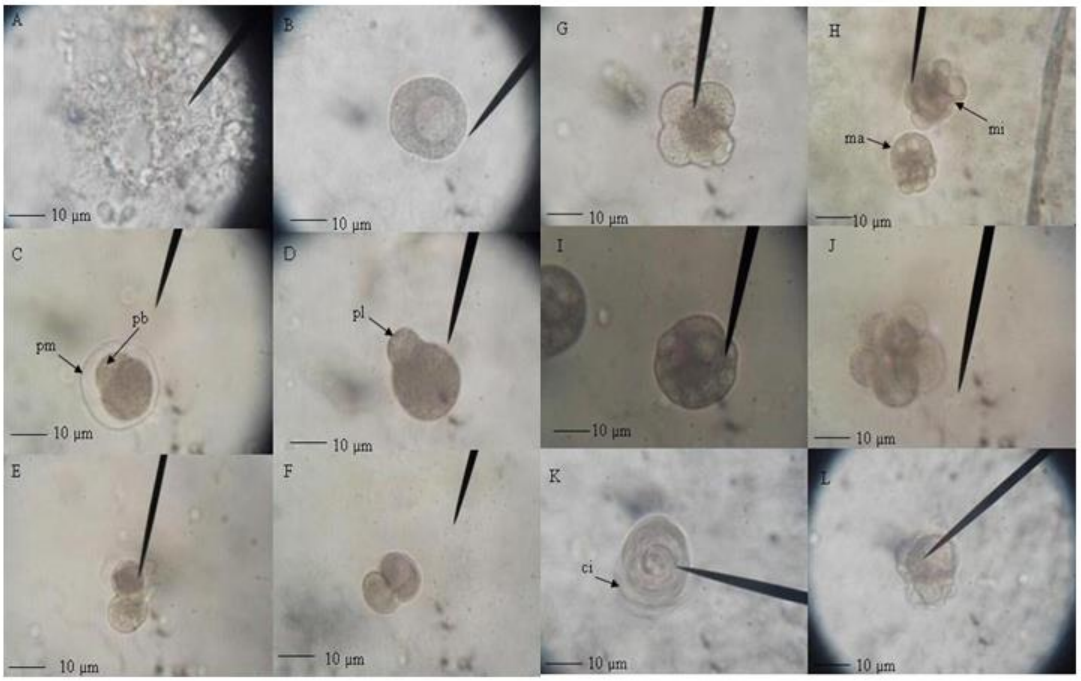


**Figure 1: Microscopic photograph of early development stages of *Lutraria philippinarum*, (A,B) sperms and egg, (C) zygote showing perivitelline membrane and polar body (D), zygote developing a polar lobe (E) 1st cleavage (F) 2nd cleavage; (G) 3rd cleavage, (H) prominent micromeres and macromeres, (I) 5th cleavage (J) morula (K) embryo with cilia that spin fast (L) trochophore. perivitelline membrane (pm); polar body (pb); polar lobe (pl) micromere (mi); macromere (ma); cilia (ci).**

The pattern of early embryonic development of *L. philippinarum* was comparable with that of *Pholas orientalis* in Iloilo, Philippines along Guimaras Straight (Ronquillo and McKinley, 2006). The embryos of *L. philippinarum* showed spiral cleavage. Cilia were observed in *L. philippinarum* as it reached the 6$^{th}$ cleavage stage. However, each stage of embryonic development in *L. philippinarum* during this study took longer than that in *P. orientalis*. The sixth cleavage occurred after 3 hrs and 20 min in *L. philippinarum* while it took only 2hrs and 55 min in *P. orientalis.*

Trochophore stage of *L. philippinarum* was observed after 24 hrs versus 10-11 hrs in *P. orientalis.* The eggs of *L. philippinarum* were smaller than that of *P. orientalis*. Temperature may be considered as the confounding factor for the difference in the timing of development as observed in *Potamocorbula amurensis* and *P. laevis* (Nicolini and Penry, 2000); *Bathymodiolus childressi* (Arellano and Young, 2009) and *Perna viridis* (Wong and Arshad, 2013).

Mortality of the larvae of *L. philippinarum* during this study could be due to water temperature, polyspermy and bacterial infections. Previous studies showed that water temperature is a major factor of larval development. In the larva of *B. childressi,* more individuals developed shells when temperature was increased to 12-14ºC than in cultures that remained at 7-8 ºC (Arellano and Young, 2009). The veliger stage of *Arctica islandica* is best reared at 10°C-15°C and a successful metamorphosis into shelled veliger requires above 10°C. At temperatures between 8.5°C-10°C, development and metamorphosis become prolonged and settlement may take 55days. In *Macoma balthica*, trochophore larvae developed at 4ºC within 2-3 days (Pekkarinen, 1986). According to Pekkarinen (1986), immature eggs released at high temperature will still develop into larvae but soon die. In this study, it was possible that immature eggs of *L. philippinarum* were also released due to high water temperature and fertilized but later died.

Polyspermy stopped cell division in fertilized eggs or produced malformed embryos. In the study of Neo *et al*. (2011) on Tridacna, it was found out that though giant clams release large amounts of gametes at each spawning, high larval mortality occurred before completing metamorphosis. Concentrated sperm suspensions and extracts can give rise to lytic activities against the egg membrane (Pekkarinen, 1986). In *Mytilus edulis*, embryonic development was reduced with high egg density beyond a certain concentration during the embryonic development (Wong and Arshad, 2013). Bacterial infection in the developing larvae also caused mortality as observed in five-day old *Tridacna squamosa* veligers (Neo *et al*., 2011). Thus, for future studies of *L. philippinarum* the effect of egg-sperm ratio on fertilization success should be determined.

The results of this study were encouraging because *L. philippinarum* was induced to spawn with chemical stimulants and early larval stages developed under laboratory conditions. Though more researches have to be conducted for hatchery operations and coastal rehabilitation of *L. philippinarum*, this study serves as benchmark to establish spawning induction and complete larval rearing protocols.

**Acknowledgements**

The author would like to thank Dr. Anthony Ilano and the anonymous reviewers for their comments and suggestions. The author also acknowledges the logistic support of the University of San Carlos and Negros Oriental State University.

**References**

**Alagarswami, K. and Dharmaraj, S., 1983**. On controlled spawning of Indian pearl oyster *Pinctada fucata* (Gould). Proceedings of the Symposium on Coastal Aquaculture. Vol. 2, 590-597.

**Arellano, S. and Young, C., 2009.** Spawning, development and the duration of larval life in a deep-sea cold-seep mussel. *Biology Bulletin*, 216,149-162.

**Bantoto, V. and Ilano, A., 2012**. The reproductive biology of *Lutraria philippinarum*(Veneroidea:Mactrida) and its fishery in the Philippines. *Revista de Biologia Tropica*, 60(**4**), 1807-1818.

**Beckvar, N., 1981.** Cultivation, spawning and growth of the giant clams *Tridacna gigas*, *T. derasa* and *T. squamosa* in Palau Caroline Islands. *Aquaculture*, 24, 21-30.

**Bell, J.K., Leber, H., Blankenship, L., Loneragan, N. and Masuda, R., 2008.** A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. *Reviews in Fisheries Science*, 16(**1-3**), 1-9.

**Crawford, C., Nash W. and Lucas, J., 1986**. Spawning induction and larval and juvenile rearing of the giant clam, *Tridacna gigas*. Aquaculture, 58 (**3-5**), 281-295.

**Morse, D., 1984**. Biochemical and genetic engineering for improved production of abalone and other valuable mollusk. *Aquaculture*. 39(**1-4**), 263-282.

**Neo, M., Todd, P., Chou, L. and Teo, S., 2011.** Spawning induction and larval development in the fluted giant clam, *Tridacna squamosa* (Bivalvia: Tridacnidae). *Nature in Singapore*, 4, 157-161.

**Nicolini, M. and Penry, D., 2000.** Spawning, fertilization and larval development of *Potamocorbula amurensis* (Mollusca: Bivalvia) from San Francisco Bay, California. *Pacific Science*, 54(**4**), 377-388.

**Pekkarinen, M., 1986**. Notes on the spawning, egg cleavage and early development of the bivalve *Macoma balthica*. *Annales Zoologici Fennici*, 23, 71-75.

**Ronquillo, J. and McKinley, R., 2006.** Developmental stages and potential mariculture for coastal rehabilitation of endangered Pacific angel wing clam, *Pholas orientalis*. *Aquaculture*, 256, 180-191.

**Wong, N. and Arshad, A., 2013**. Induced spawning and early development of *Modiolus philippinarum* (Hanley, 1843) (Bivalvia:Mytilidae). *Asian Journal of Animal and Veterinary Advances*, 8(**1**), 100-107.

**World Health Organization (WHO), 2010.** Safe management of shellfish and harvest waters. Edited by G. Rees, K. Pond, D. Kay, J. Bartram and J. Santo Domingo. ISBN:9781843392255. Published by IWA Publishing, London, K.http://www.who.int/water_sanitation_health/emerging/ bivalves.pdf. Retrieved date: 5 May 2014.