REPRODUCTIVE PERFORMANCE OF *Anodontia philippiana*

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ABSTRACT

Objective of this study was to determine reproductive performance of *A. philippiana*. Samples were collected from the oil affected mangrove mudflat in Pototan, Guimaras, Philippine on September 2007. *A. philippiana* were induced to spawn using the serotonin method. Only *A. philippiana* with shell length of approximately 4.0-5.5 cm were induced to spawn. Three pairs of one ripe female and one ripe male were chosen and placed in aquaria with 3 replicates. A 0.3 ml of 4 mM serotonin solution (Gros *et al.*, 1997) was injected into 1-2 mm of the gonad of both male and female clams using 0.65 x 25-mm bore hypodermic needle attached to a 5 ml plastic syringe during mid until late afternoon. Number of spawned eggs was calculated, and fertilization was conducted. At 47 h, the percentage of normal (D-larvae) veliger relative to the initial number of eggs was calculated (Maosapina *et al.*, 1999). Larvae from each spawner were reared seperately in aquaria for several days without feeding in order to estimate survival rates. The number of larvae were estimated every 24-h intervals until total mortality. The decrease in the number of larvae per container were calculated as the proportion of live larvae from the initial number of larvae (extinction rate) (Narvarte and Pascual, 2003). Result of this study are total Total spawned eggs ($x10^6$)is 424.50±128.21, Egg diameter (µm) is 86.11±3.80, Fertilization rate (%) is 83.01±3.13, and hatching rate is 36.52±8.66, Length of newly hatched larvae (µm) is 135.73±1.96, Number of days to total mortality (after hatching) without feeding is 9-10 days.

Keywords: reproductive, *Anodontia philippiana*

INTRODUCTION

Anodontia philippiana a bivalve belonging to Family Lucinidae, is widely distributed in the Indo West Pacific Region (Poutiers, 1998). This bivalve is an important source of food and livelihood. Bivalves are molluscs typically have two part valves, with both parts being more or less symmetrical (Poutiers, 1998). *A. philippiana* is a member of family Lucinidae (Taylor and Glover, 2004). Generally shells of Lucinidae are equivalve, lenticular, and slightly inequilateral (Poutiers, 1998). Its ligament is more or less deeply sunken in the posterodorsal margin with two cardinal teeth and lateral teeth in either valve, sometimes reduced to absent. There are two adductor muscle scars, the anterior narrowly elongate with an oblique ventral lobe detached from the pallial line with no pallial sinus. Lucinids have a somewhat elongate adductor muscle, part of which is separated from the pallial line. In the adult, the hinge is essentially toothless hence its name (Kelvin *et al.*, 2001a). They are interesting animals, because they harbor sulphur-oxidising bacteria in their gills from which they derive most of their nutrition (Lebata, 2001). Many lucinids have therefore lost their siphons and their ability to filter feed, and make connection with the outside world with their piston-like feet (Kelvin *et al.*, 2001a).

However, *A. philippiana* is a toothless bivalve clam commonly found buried in mudflats of intertidal and subtidal zones (Kelvin *et al.*, 2001b). They inhabit the sandy muddy substrate at 20-60 cm below the surface (Primavera *et al.*, 2002).

Reproductive performance is factors influencing growth and developement of bivalve larvae (O'Connor and Heasman, 1995). Once successfull spawning has occurred, gamete fertilization is the next critical step in the hatchery process (Gruffyd and Beaumont, 1970). High sperm concentration during fertilization may cause lysis of the egg membrane making the eggs unviable, or atypical embryogenesis that results in high numbers of abnormal trocophores (Clavier, 1992; Encena et al., 1998). Sometimes, eggs may remain unfertilized due to a very low sperm egg ratio used during manipulation or due to a delay or failure in male spawning. In addition, food quality has been identified as one of the important factors to be controlled in order to maximize growth and survival of pectinid larvae (Winter, 1977; Griffiths and King, 1979; Bayne and Newell, 1983). This study was aimed to determine reproductive performance of *A. philippiana*.

MATERIAL AND METHODS

A. philippiana were collected from mangrove mudflat in Petotan, Guimaras, Philippine on September 2007. The collected clams were temporarily stocked in an aerated plastic bucket prior to transport to the Wet Laboratory of the Institute of Marine Fisheries and Ocean Sciences, University of the Philippines Visayas. During transport, the clams were placed in a net bag lined with moisturized layers of newspapers to avoid shell breakage and desiccation. The body weight, total tissue weight, and gonad weight of every sample were measured.Before induce spawning, all aquaria and beakers to be used were sterilized by scrubbing them inside and outside with detergent, rinsed with hot water, and dried under the sun. The seawater that was used was filtered using 1 µm filter and was used allowed to stay for 3 days with aeration before use for conditioning. Moreover, the seawater was maintained at room temperature with 28 ppt salinity. *A. philippiana* were scrubbed with a soft brush and rinsed in flowing seawater to remove any adhering debris (Doyola, 1999). Samples were acclimatized for 10 days to adapt to hatchery conditions. The clams were placed in aquarium with strong aeration and fed *Tetraselmis* sp. at an initial density of 200 million cells per day (Utting and Spencer, 1991).

A. philippiana were induced to spawn using the serotonin method. Only *A. philippiana* with shell length of approximately 4.0-5.5 cm were induced to spawn. Clams with ripe gonads were chosen by doing biopsy before spawning induction. Mature clams were characterized by the presence of stalked oocytes for female and motile spermatozoa for male. Three pairs of one ripe female and one ripe male were chosen and placed in aquaria with 3 replicates. A 0.3 ml of 4 mM serotonin solution (Gros *et al.*, 1997) was injected into 1-2 mm of the gonad of both male and female clams using 0.65 x 25-mm bore hypodermic needle attached to a 5 ml plastic syringe during mid until late afternoon. Moreover, spawning was induced during full or new moon phase since this is their natural spawning period (Ellis *et al.*, 1998). The syringe and needle were flushed with isopropyl alcohol between injections of each clam. Serotonin is injected into a ripe animal to induce sperm and egg release usually within 5-10 min.

Female clams were injected first before the male because female spawn 30-60 min much later than the male (Helm *et al.*, 2004). Injected clams were placed

individually in glass beakers filled with 500 ml seawater in a shaded area to avoid temperature shock. The spawners were observed closely after injection until spawning was complete.

After spawning was completed, the spawners were removed from the glass beakers to a separate aquarium with slight by aerated seawater. The number of spawned eggs was calculated, and fertilization was conducted by adding about 3-15 ml of the released sperms to 500 ml of eggs (Ellis *et al.*, 1998). The mixture was stirred slowly with an up and down motion for 2 min using a perforated plunger to ensure fertilization. Two hours after mixing the eggs and sperms, fertilization was marked by the appearance of a polar body. The Fertilized eggs were then placed in aquarium with light aeration at a density of 20-25 eggs per ml (Mingoa-Licuanan and Gomes, 2007) to lessen growth abnormalities caused by overcrowding. At 47 h, the percentage of normal (D-larvae) veliger relative to the initial number of eggs was calculated (Massapina *et al.*, 1999).

The following procedures were done in estimating of spawned eggs, fertilization rate, and hatching rate (Helm and Bourne, 2004):

a) Five subsamples were taken from the 100-ml beaker/30-l aquarium using a pipette/hose.
b) All of the five subsamples were placed in a 10-ml beaker/1-l pail.
c) The eggs and larvae were concentrated using a 40-µm sieve and then placed in a 10-ml beaker.
d) The eggs or larvae in the beaker were counted using a Sedgewick Rafter Counter and microscope.
e) The picture of 40 samples (eggs or larvae) was taken to measure sample size using Image Tool Software.

Larvae from each spawner were reared separately in aquaria for several days without feeding in order to estimate survival rates. The initial number of larvae ranged from 20,000 to 935,000. Rearing water was changed with fresh filtered seawater every two days. The number of larvae were estimated every 24-h intervals until total mortality. The decrease in the number of larvae per container were calculated as the proportion of live larvae from the initial number of larvae (extinction rate) (Narvarte and Pascual, 2003). The extinction rate was considered as the difference in larval numbers between two successive periods during the experiment and was estimated using the maximum likelihood method (Cerrato, 1990); which was applied to the fitted curve for each larval stock.

RESULTS AND DISCUSSION

Differences in the reproductive performance of *A. philippiana* in terms of total spawned eggs, egg diameter, fertilization rate, hatching rate, length of newly hatched larvae show in Table 1.

Table 1. Means of reproductive performance parameters of *Anodontia philippiana*

Parameters	Value
Total spawned eggs ($x10^3$)	424.50±128.21
Egg diameter (µm)	86.11±3.80
Fertilization rate (%)	83.01±3.13
Hatching rate (%)	36.52±8.64
Length of newly hatched larvae (µm)	135.73±1.96
Number of days to total mortality (after hatching) without feeding	9-10 days

Note : Values are mean±SEM

Total spawned eggs from 3 individuals (4-5.5-cm size) of *A. philippiana* is 413.70±363.39. The range of egg diameter *A. philippiana* is 80.42-99.25 µm, the fertilization rate of samples is 83.01±3.13%, The hatching rate of *A. philippiana* area ranged from 25.39 to 53.54%. Range of length of newly hatched larvae is 107.62-149.83 µm. The hatched larvae when reared without feeding showed that the number of days to total mortality in *A. philippiana* can survive in 9-10 days after hatching. But there was almost 0% survival of larvae at day 4 after hatching. Thereafter, the survival rate was already very low. Egg diameter of clam on the same genus *A. edentula* in this study is similar to the egg size reported by Doyola (1999) (80-90 µm). Larval length in this study were 135.73±1.96 µm also similar to Doyola (1999) (148.7±33.8 µm). This study observed high SEM in the total spawned eggs of *A. philippiana*. Hankoop and Van der Meer (1997) reported that even if the sample were more than in this study (150 *Macoma balthica* samples) similar result was observed. This is because spawned egg were significantly correlated to Body Mass Index (BMI) value. Total number of spawned egg was also dependent on age of *M. balthica* (Hankoop *et al.* 1998).

The higher survival rate was only achieved within three days after hatching and started to be the same after 4-5 days after hatching. Without feeding, larvae of *A. philippiana* could survive 9-10 days from the unaffected area which declined faster than the former. This study also observed that there is almost 0% survival of larvae from day 4 after hatching. Navarte and Pascual (2003) reported survival of *Aequipecten tehuelchus* larvae fed *Tetraselmis* sp. at the first week was low (approximately 25%), while *Placopecten magellanicus* larvae had a mortality of 50% within the first 2 days (Culliney, 1974), and 99% larvae of bivalves in general die before metamorphosis (Vance, 1973). Narvarte and Pascual (2003) reported that bacterial infestation severely affected larval cultures, suggesting that antibiotics should be used at this phase. Because *A. philippiana* larvae were not fed during the experiment, they mainly depended on their stored nutrient which was passed on by their parent stock. Robinson (1992) have shown that broodstock lipids increase before spawning and decrease immediately after spawning, probably as a result of losses of lipid which goes with the spawned oocytes, leading to a decrease in the condition index.

CONCLUSIONS

Reproductive performance of *A. philippiana* are: total Total spawned eggs ($x10^3$)is 424.50±128.21, Egg diameter (µm) is 86.11±3.80, Fertilization rate (%) is 83.01±3.13, and hatching rate is 36.52±8.64, Length of newly hatched larvae (µm) is 135.73±1.96, Number of days to total mortality (after hatching) without feeding is 9-10 days.

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