

## Induced Breeding of *Pila globosa* (Swainson 1822) (Gastropoda: Prosobranchia) for Commercial Farming

Md. Sarwar Jahan, Md. Rafiqul Islam, Md. Redwanur Rahman and M. Monjurul Alam

Institute of Environmental Science, University of Rajshahi, Rajshahi – 6205, Bangladesh

e-mail: redwan\_rahman@lycos.com

**Abstract:** Studies were made on the number of egg clutch, copulating pairs, duration of mating, duration of egg laying, and weight of egg clutch of *Pila globosa* by inducing it with Pituitary Gland (PG) and Optic Tentacle cut (OT) under laboratory condition. The effect of inducing the animal with PG and OT cut showed that it resulted in an increase in the number of egg clutch in comparison with that of the control.

In control animals the number of egg clutch was found to be  $0.30 \pm 0.02$  which was 20.93% of total egg clutches laid. Whereas in animals with Pituitary Gland (PG) and Optic Tentacles (OT) cut the number of egg clutch were  $0.43 \pm 0.61$  and  $0.70 \pm 0.86$  which were 30.23% and 48.83% of total egg clutches laid respectively. The animals injected with pituitary gland extract and optic tentacle cut showed an increase in the number of egg clutch in comparison with that of the control animals.

In control animals, duration of mating, duration of egg laying, and weight of egg clutch were found as,  $3.23 \pm 0.29$  hrs.,  $5.37 \pm 1.51$  hrs., and  $8.38 \pm g$  respectively. The pituitary gland (PG) injected and optic tentacle (OT) cut, duration of mating, duration of egg laying and weight of egg clutch were found as,  $3.52 \pm 0.26$  hrs.,  $4.64 \pm 1.63$  hrs., and  $8.54 \pm 2.10$  g.;  $3.51 \pm 0.33$  hrs.,  $5.47 \pm 1.19$  hrs., and  $8.45 \pm 2.53$  g respectively.

**Key words :** *Pila globosa*, induced breeding, Commercial Farming.

### Introduction

A lot of evidences to indicate that the population of the species, *Pila globosa* is decreasing day by day. Consequently, a study of induced breeding of *P. globosa* was found to be essential in the sense that it may provide information and exploitation of this freshwater resource.

In spite of being densely populated Bangladesh possesses vast suitable habitats for freshwater edible molluscs which can supplement the animal protein for the people specially for the lower income group and various types of aquaculture such as prawn farming and cat fish farming. Female *P. globosa* starts the process of egg-laying generally about two weeks after the onset of rains. Though *P. globosa* is amphibious, water is essential for all of its biological activities except egg laying. Thus it passes its life mostly in water. The snails undergo aestivation by November or December when the field becomes dry, while those living in pond remain active as long as water is available (Raut, 1984).

The cytological evidence of neurosecretion in pulmonate molluscs has been shown by a number of workers. Lever (1957) demonstrated the neurosecretory phenomena for the first time in a freshwater pulmonate *Ferrissia* sp. Van (1962) described two types of neurosecretory cells in the land slug, *Arion*. Krause (1960) described cells in the cerebral and visceral ganglia of *Helix pomatia* and suggested that the cells of the visceral ganglion might produce a hormone which controls hibernation. The histological structure of the neurosecretory cells in the central nervous system of stylommatophoran pulmonates like

*Vaginulus* and *Ariophanta* were studied by Nagabhushanam & Swarnamayee (1963, 1964). Nolte & Kuhimann (1964) studied in detail the histology and the secretion of the cerebral ganglion in the stylommatophoran pulmonates. Quattrini (1964, 1965) identified elementary neurosecretory granules in the neurons of the slugs like *Milax* and *Vaginulus*.

Cells considered to be neurosecretory have been observed in the optic tentacles of certain stylommatophoran pulmonates (Rozsa and Nagy 1967). Lane (1962, 1964a,b) observed the fine structure of secretory cells in optic tentacle of the snail *Helix*. The histophysiological examination of the optic tentacle in pulmonates was made by Bierbauer & Torok (1967) and Bierbauer (1970).

Thus most of the observations so far made on the neurosecretion in pulmonates deal with the description of the cell types while the work on their functional aspects is very little. Pelluet and Lane (1961) and Pelluet (1964) showed the relationship between the neurosecretion and cell differentiation in the ovotestis of the slugs. Nisbet (1961) worked on some aspects of the structure and function of the nervous system of *Archatina marginata*, *Ayrion ater*, *A. subfuscus* and of *Milax*. Gabe (1965) observed a relationship between neurosecretory activity and certain function such as annual life cycle, control of water metabolism and reproduction. Rozsa & Nagy (1967) described physiological and histochemical evidence for neuroendocrine regulation of heart activity in *Lymnaea stagnalis*. Muthe & Naiksatam (1972) worked on the effect of extract of cerebral ganglia on the carbohydrate metabolism in a prosobranch, *P. globosa*.

In Bangladesh till today nobody has become interested in snail farming. Though snail capturing and its utilization as human food, poultry feed and in large scale as supplementary feed for prawn farms are lavishly going on. Specially over exploitation of *P. globosa* for prawn farming is in an alarming condition. The ecologically and economically important such a biological resource will become extinct within a very short time if no steps are taken to establish the commercial farming of this species immediately.

Considering the above facts the present research plan was chalked out to develop a juvenile hatching pond and induced breeding ponds for ensuring the sustainable natural population and stocking of this by commercial farming of *P. globosa*.

#### Materials and Methods

A set of experiment was conducted for the induced breeding of *P. globosa*. Glass made aquariums were set in the laboratory to determine the effect of pituitary gland treatment and ablation of optic tentacles on the induced spawning of *P. globosa*.

*Pituitary gland treatment:* Pituitary gland of *Labeo rohita* were collected from Natore Fish Hatchery. Then the pituitary gland solution was made by adding 5ml of distilled water. The solution was centrifuged for about 20 minutes. After centrifugation 0.1ml of the supernatant liquid was injected into the body cavity of each of the 50 snails in one of the aquarium by means of hypodermic syringe.

*Ablation of optic tentacles:* The tips of the optic tentacles were cut off in such a fashion that the eyes remained intact at their base. Thus the batches of 50 snails with cut off optic tentacles were kept in another aquarium.

*Control batch:* The rest batch of 50 snails was treated as control batch. Tap water was used for the experiment. Temperature of water and air in laboratory was recorded regularly by the help of the normal thermometer. pH of water was determined by digital pH meter and dissolved oxygen (DO) of water was determined by digital DO meter and recorded regularly.

Number of copulating snails, duration of mating, duration of egg laying, number of egg clutch and weight of egg clutches were recorded on constant observation during the experimental tenure.

#### Results

The results are shown in table 1. The studies on the animals injected with PG and OT cut showed that it resulted in an increase in the number of egg clutches in comparison with that of the control. In control animals the number of egg clutch was found to be  $0.30 \pm 0.02$  which is 20.93% of total egg clutches laid, whereas in animals with PG injected, and OT cut the number of

egg clutch were  $0.43 \pm 0.01$  and  $0.70 \pm 0.06$  which were 30.23% and 48.83% of total egg clutches laid respectively. In control animals, duration of mating, duration of egg laying, and weight of egg clutch were found as,  $3.234 \pm 0.293$  hrs.,  $5.375 \pm 1.505$  hrs., and  $8.380 \pm 3.933$  g. respectively. In the PG injected and OT cut the duration of mating, duration of egg laying and weight of egg clutch were found as  $3.517 \pm 0.256$  hrs.,  $4.64 \pm 1.633$  hrs., and  $8.544 \pm 2.100$  g;  $3.507 \pm 0.334$  hrs.,  $5.475 \pm 1.195$  hrs., and  $8.453 \pm 2.531$  g. respectively (Table 1).

The water temperature and laboratory temperature of control, PG injected, and ablation of optic tentacles treated aquarium were found as  $29.1 \pm 1.39^\circ\text{C}$ ,  $29.8 \pm 2.13^\circ\text{C}$ ;  $29.9 \pm 1.57^\circ\text{C}$   $29.8 \pm 2.13^\circ\text{C}$ .;  $29.1 \pm 1.43^\circ\text{C}$ ,  $29.8 \pm 2.13^\circ\text{C}$  respectively (Table 1).

The pH of water of control, PG injected and ablation of optic tentacles treated aquariums were found as  $7.151 \pm 0.319$ ,  $7.398 \pm 0.292$ ,  $7.234 \pm 0.285$  respectively (Table 1).

The Dissolved Oxygen of control, PG injected and ablation of OT treated aquariums were found as  $5.303 \pm 1.164$  mg/l,  $5.024 \pm 1.454$  mg/l,  $5.235 \pm 1.736$  mg/l respectively (Table 1).

Depending on the results of the experiments conducted in the glass aquariums in the laboratory an effective induced breeding ground for commercial farming of snail *P. globosa* could be designed. The structure of the conceived snail hatchery (induced breeding ground) was developed as presented below.

There were six small ponds viz. (i) two snail breeding ponds ( $15' \times 4' \times 4'$ ) separated by 4' of space in between the two for breeding ground, surrounded by 3' wide egg laying ground; (ii) snail food plant reserve pond ( $6' \times 4' \times 4'$ ); (iii) mature snail stocking pond ( $6' \times 4' \times 4'$ ); (iv) juvenile hatching and collecting pond ( $6' \times 4' \times 4'$ ) (v) yearling nursery pond ( $6' \times 4' \times 4'$ ).

Such small dimensions of the breeding pond are suggested for making the opportunity for spontaneous movement and close contact of *P. globosa* to copulate easily. Water depth (3'.0 - 3.6") should be ensured above 3" - 4" of bottom soil.

As *P. globosa* requires moist grassy soil for egg laying grass should be planted on the 3' wide egg laying ground surrounding the breeding ponds. Water line and head shower should be arranged in such a fashion that depending on the need the breeding pond could be filled and waste water drained out.

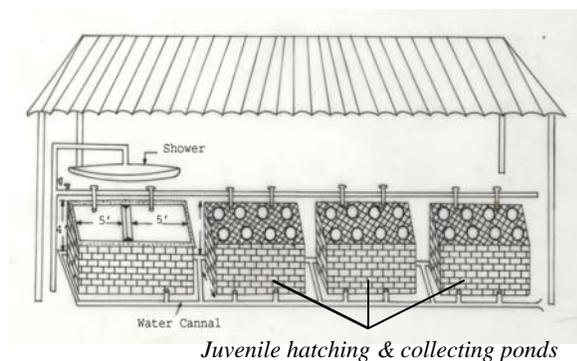
Juvenile hatching and collecting pond ( $6' \times 4' \times 4'$ ) covered by fine wire net having suitable mesh size for dropping down the juveniles after hatching out from the eggs kept on the petridishes. Arrangement for taking off the wire-

net and transferring the juveniles from the pond to nursery pond and nursery pond to stocking and aestivating pond should be ensured.

In all the cases aeration should be ascertained for maintenance of favourable temperature, Dissolved Oxygen (DO), pH etc. (Fig. 1a & 1b).

### Discussion

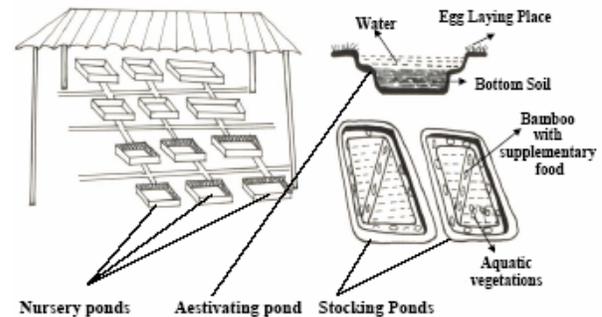
Actually no information is available on the induced breeding ground, induced breeding and stocking of *P. globosa*. But these are the most important components to play vital role for commercial farming of *P. globosa*. The snail, *P. globosa* is an important component of the biodiversity playing very vital role for the maintenance of the aquatic ecosystem in one hand and on the other hand recently the animal is being utilized as the supplementary food for prawn culture. This natural renewable resource is under threat due to over exploitation. Certainly, if not sustainable uses of the snail species can be achieved through commercial farming the biological resource will be extinct due to non-scientific management and unwise over exploitation. Development of artificial breeding ground, artificial breeding and stocking of *P. globosa* are the essential portion for the commercial farming.



**Fig. 1a.** Induced breeding and hatching ponds with shed for commercial farming of *P. globosa*.

Alam and Bhuiyan (1999) revealed that the broods of *Labeo rohita* did not respond at in April (first injection 0.5mg/kg body weight and second injection 2mg/kg body weight). This low dose was not able to induce fishes in early breeding season. The amount of doses depends with the advancement of maturity of fishes. Islam & Chowdhury (1976) reported similar report. The Dose 3.0mg/kg was effectively increased the rate of egg release, fertilization, hatching and survivability than other doses. So it was suggested that total 10mg of PG per kg body weight was the optimum dose for the month of April (first injection 3mg/kg body weight and second injection 7mg/kg body weight). Alikunhi &

Chaudhuri (1955) and Sinha (1971) stated similar observation.



**Fig. 1b.** Induced breeding ponds structure for commercial farming of *P. globosa*.

Chauhan & Sar (1993) observed that single injection of pituitary extract to Indian carp failed to induce spawning, but two injections at an interval of five to six hours was found successful.

Panday & Singh (1997) reported that when *Labeo rohita* was injected with ovaprim (female 0.4ml/kg body weight and male 0.ml/kg body weight), the rate of fertilization ranged from 65-98% with an average of 80%, and the survivability was 75%.

Kulkarni & Nagabhushanam (1973) revealed that the slug, *Laevicaulis alte* with tentacles ablation showed that it resulted in an increase in the number of eggs in comparison with that of the controls. In control animal the number of eggs was found to be 56 whereas in animals with tentacle ablation the number of eggs increased to 96.

Experiments were conducted in the laboratory simultaneously to examine the feasibility of induced breeding ground and effect of PG treatment and ablation of optic tentacles in glass made aquarium in laboratory. From the table 1 it is evident that in the animal in which the PG injected and OT cut the number of egg clutch exceeded than that of the control animal.

The experiments indicate that there is a possibility for culturing the snails *P. globosa* in induced breeding grounds as some sorts of treatments like PG injection and ablation of OT inspired the snails positively in spawning. It was found that number of copulating snails increased by 78.49% and 82.79% due to PG treatment and OT ablation to that of control snail. Similarly the number of egg clutch also increased by 30.23% and 48.83% for PG treated and OT ablation to that of control snail respectively. During the experiment the temperature, pH and dissolve oxygen (DO) of the water in different aquarium were slightly different from one another. These differences might be

Table 1. Results of control, Pituitary Gland (PG) injected and Optic Tentacle (OT) cut on the effective induced breeding.

Treatment	Number of snails used	Duration of mating (in hours) Mean $\pm$ SD	Duration of laying Egg. (in hours) Mean $\pm$ SD	No. of Clutch per day Mean $\pm$ SD	Percentages of total egg clutch laid	Weight of egg clutch (g.) Mean $\pm$ SD	Laboratory Temperature ( $^{\circ}$ C) Mean $\pm$ SD		pH of water Mean $\pm$ SD	DO of water mg/l Mean $\pm$ SD
							Chat Water	Air		
1. Control (Aquarium A)	50	3.234 $\pm$ 0.293	5.375 $\pm$ 1.505	0.30 $\pm$ 0.02	20.93	8.380 $\pm$ 3.933	29.1 $\pm$ 1.39	29.8 $\pm$ 2.13	7.151 $\pm$ 0.319	5.303 $\pm$ 1.164
2. Pituitary Gland (PG) injected (Aquarium B)	50	3.517 $\pm$ 0.265	4.641 $\pm$ 1.633	0.43 $\pm$ 0.01	30.23	8.544 $\pm$ 2.100	29.9 $\pm$ 1.57	29.8 $\pm$ 2.13	7.398 $\pm$ 0.292	5.024 $\pm$ 1.454
3. Optic Tentacle (OT) cut (Aquarium C)	50	3.507 $\pm$ 0.334	5.475 $\pm$ 1.195	0.70 $\pm$ 0.06	48.83	8.453 $\pm$ 2.531	29.1 $\pm$ 1.43	29.8 $\pm$ 2.13	7.234 $\pm$ 0.285	5.235 $\pm$ 1.736

due to differences in rate of degradation of food materials in deferent aquaria. However these variations were also negligible and did not exerts considerable effect on the concerned traits.

#### References:

- Alam, M.M. & Bhuiyan, A.S. 1999. Determination of optimum PG dose for induced spawning of *Labeo rohita* (Hamilton, 1822). *Univ.j.zool. Rajshahi Univ.* **18**: 103-108.
- Alikunhi, K.H. & Chaudhuri, H. 1955. Observation on the spawning in Indian carps by hormone injection, *Curr. Sci.* **26(12)**: 381-382.
- Bierbauer, J. & Torok, L.J. 1967. Histophysiological study of the optic tentacle in pulmonates I. Histological examination of the optic tentacle with special regard to the morphology of the collar and lateral cells. *Acta biol. Sci. lung.* **19**: 133-134.
- Bierbauer, J. 1970. Histophysiological examination of the optic tentacle in pulmonates. *Acta biol. Sci. lung.* **21(4)**: 3-1-387.
- Chauhan, R.C. & Sar, C.K. 1993. Induced breeding of Indian carps in Thar desert. *Uttar-Pradesh Journal of Zoology.* **13(12)**: 119-130.
- Gabe, P.M. 1965. La Neurosecretion chez les Mollasques et ses rapports avec la Reproduction. *Archives D. Anatomie Micro-scopique et de Morphoiigie Experimentale.* **54(1)**: 371-385.
- Islam, M.Z. & Chowdhury, A.Q. 1976. Induced spawning of major carps for commercial production of fry for fish seed in Bangladesh. *Bang. J. Zool.* **4(2)**: 51-61.
- Krause, E. 1960. Untersuchungen uber ne Neurosekretion in Schlundring von *Helix pamtia*. *L.Z. Zeilforsch.* **51**: 748-776.
- Kulkarni A.B. & Nagabhushanam, R. 1973. Endocrine control of reproduction in the slug, *Laevicaulis alte*. *Marathwada University Journal, India* pp.153-158.
- Lane, N.J. 1962. Neurosecretory cells in the optic tentacles of certain pulmonates. *Quarterly Journal of Microscopical Science.* **103(2)**: 221-226.
- Lane, N.J. 1964a. The fine structure of certain secretory cells in the optic tentacles of the snail *Helix aspersa*. *Quarterly Journal of Microscopical Science* **105**: 35-47.
- Lane, N.J. 1964b. Further observations on the secretory cells in the optic tentacles of with special reference to the results of the vital staining. *Helix aspersa Quarterly Journal of Microscopical Science* **105**: 61-72.
- Lever, J. 1957. Some remarks on neurosecretory phenomena in *Ferrissia* sp. (Gastropoda, Pulmonata). *Proc. Kon. Ned. Akad. Wet. Ser. C.* **70**: 510-522.
- Muthe, P.T. & Naiksatam, A.S. 1972. Effect of the extract of cerebral ganglia on carbohydrate metabolism in *Pila globosa*. *J. biol. Sci.* **15**: 80-84.
- Nagabhushanam. R. & Swarnamayee, T. 1963. Neurosecretory cells in the central nervous system of *Vaginulus* sp. (Gastropoda: Pulmonata). *J. Anim. Morph. Physiol.* **10**: 171-173.
- Nagabhushanam, R. & Swarnamayee, T. 1964. Neurosecretory cells in the central nervous system of *Ariopharta liquiata*. *Nucleus* **7**: 67-70.
- Nisbet, R.H. 1961. Some aspects of the structure and function of the nervous system of *Archachatna* (Calachatina) *marginata* (Swainson). *Proc. Roy. Soc.* **154**: 267-287.
- Nolte, A. & Kuhlmann, D. 1964. Histologie und Sekretion der Cerebraldruse adultery Stylommatophorea (Gastropoda). *Z. Zellforsch.* **63**: 550-567.
- Pandey, A.C. & Singh, R.N. 1997. Breeding of *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* by ovaprim injection for quality seed production. *Journal of Advanced Zoology* **18(1)**: 38-41.

- Pulluet, D. & Lane, N.J. 1961. The relation between acurosecretion and cell differentiation in the ovotestis of slugs (Gastropoda : Pulmonata). *Canad. J. Zool.* **39**: 789-805.
- Pulluet, D. 1964. On the hormonal control of cell differentiation in the ovotestis of slug (Gastropoda : Pulmonata). *Canad. J. Zool.* **42**: 195-199.
- Quattrini, D. 1964. Dati preliminari di microscopica elettronica dei neuroni centrali di *Vaginulus borellianus* (Colose) nel quadro del problema della neurosecrezione nei molluschi gastropodi. *Monn Zool. Ital.* **72**: 3-12.
- Quattrini, D. 1965. Osservazioni al microscopio elettronico su un gruppo di neuroni centrali appartenenti di *Milax gagates* Draparnaud Mollusca : Gastropoda: Pulmonata). *Basil. Sco. Ital. Biol.* **41**: 375-378.
- Raut, S.K. 1984. On the egg laying behaviour of the apple snail *Pila globosa* (Swainson) (Mollusca: Gastropoda). *Sci. Cult.* **50(6)**: 198-199.
- Rozsa, K. & Nagy, I. 1967. Physiological and histochemical evidence for neuroendocrine regulation of heart activity in the snail, *Lymnaea stagnalis*. L. *Comp. Biochem. Physiol.* **23**: 373-382.
- Sinha, V.R.P. 1971. Induced spawning in carp with fractional fish pituitary extract. *J. Fish. Biol. Lond.* **3**: 263-272.
- Van, M.J. 1962. Neurosecretory phenomena in the slug *Arion rufus* (Gastropoda, Pulmonata). *Gen. Comp. Endocrinol.* **2**: 616-617.

*Manuscript received on 26.07.07, accepted on 01.10.07*