Role of ganglionic extract and commercial hormones (progesterone and estradiol) injections on the rate of ammonia excretion in freshwater bivalve Indonaia caeruleus (Prashad, 1918) during monsoon season.

[1]Thorat S. K* and [2]Vedpathak A. N.
[1]Department of Zoology, Sharada Mahavidyalaya, Parbhani- 431401 (M.S.),
[2] Molluscan Endocrinology & Physiology Laboratory, Department of Zoology,
Dr.Babasaheb Ambedkar Marathwada University, Aurangabad-431004(M.S.),

ABSTRACT

Considering typically the importance of neuroendocrine control on the metabolic activities of freshwater bivalves, we report here the effect of injections of equivalent commercial hormones (Progesterone & Estradiol) and cerebral ganglionic extract on excretory metabolism of freshwater bivalve mollusc *Indonaia caeruleus* (Prashad, 1918) from Godavari River. During monsoon season, the adult bivalve mollusc, *Indonaia caeruleus* (50-55 mm shell length) were subjected to (a) control (normal) (b) injection of a cerebral ganglionic extract of same species to intact individuals (c) injection of equivalent commercial hormone progesterone to normal control and (d) injection of estradiol to normal control for 8 days. The rates of ammonia excretion in bivalves from all four groups (including control) were measured on 2^{nd}, 5^{th}, and 8^{th} day. The study revealed that, the rate of ammonia excretion was significantly decreased in all groups on 2^{nd} day compared to control. On 5^{th} day the rate of ammonia excretion increased significantly in Progesterone, ganglionic extract estradiol injected groups respectively as compared to the control. Where, on 8^{th} day, the ammonia excretion rate increased significantly in all experimental groups compared to the control group.

Keywords: - Cerebral ganglionic extract, Progesterone, Estradiol, Ammonia excretion, Freshwater bivalve.

Introduction

The freshwater mussels (Order Unionoida) are distributed worldwide in lotic and lentic habitats. As filter feeders, freshwater mussels are ecologically important; they control seston, recycle nutrients, and provide a trophic link between primary producers and predators (Nalepa, Gardner & Malczyk, 1991). It has been known that the diet of suspension feeding bivalves consists mainly of phytoplankton (e.g. diatoms, flagellates) together with other sources of food such as bacteria and detritus debris (e.g. Parrish et al., 1998; Budge et al., 2000). However, the diet varies at different stages of the life history of the bivalve, owing in

part to ontogenetic changes in feeding. In some marine and freshwater systems, bivalve molluscs are dominant filter-feeders that make up most of the biomass and exert control over ecosystem structure and function (Dame, 1996; Strayer et al., 1999). These bivalves also can be important filter feeders and in addition can directly impact benthic processes as they burrow through sediments. One reason to predict that freshwater bivalves influence ecosystem processes is that in marine and estuarine systems, both epifaunal and burrowing bivalves have been shown to have large ecosystem impacts.

In some marine and freshwater systems, bivalve molluscs are dominant filter-feeders that make up most of the biomass and exert control over ecosystem structure and function (Dame, 1996; Strayer et al., 1999). These bivalves also can be important filter feeders and in addition can directly impact benthic processes as they burrow through sediments. One reason to predict that freshwater bivalves influence ecosystem processes is that in marine and estuarine systems, both epifaunal and burrowing bivalves have been shown to have large ecosystem impacts.

The mussels are ecologically important because of their widespread distribution and biological filtration activity (K. Lewandowski, A. Stanczykowska, 1975 and K. Kasprzak, 1986) and also economically used as food and in the production of freshwater pearls (N. V. Subba Rao and A. Dey, 1989). Bivalves play an important role in the ecosystem equilibrium and constitute an important economic end point. The bivalves have not been the subject of intense studies despite the presence of rich diversity of edible and commercial species in India. Bivalve molluscs are potential sources of valuable proteins, carbohydrates and minerals and are abundantly available in India. The biochemical composition of mollusc is influenced by its size, growth and reproductive status. Vitellogenesis, which is an essential event for the development of female gametes, was also suggested to be controlled by estradiol (Li et al., 1998; Osada et al., 2003). In addition to these specific proteins, synthesis of total proteins in molluscs may also be under the regulation of steroids. Therefore, administration of sex steroids in the scallops may accelerate the metabolic rate in the gonad, providing more materials and thus more energy for the gonadal differentiation.

Materials and Methods

The adult freshwater bivalves, *Indonaia caeruleus* (50-55mm in shell length) were collected by hand picking method from Godavari River near Aurangabad, during monsoon season (August-September) 2014. After brought to the laboratory the shells of the bivalves were brushed and washed with water to remove the mud and fauling fungal and algal biomass and they were acclimatized for 24 hr. in laboratory conditions. No food was given to the animals during laboratory acclimatization and subsequent experimentation. The ammonia excretion estimated by phenol-hypochlorite method of Solórzano (1969) and always triplicate of a sample used in ammonia estimation for each experimental group. Considering the role of cerebral ganglia on the rate of oxygen uptake and ammonia excretion in freshwater bivalve, we designated experimental plan of 10 days i.e. the injection of cerebral ganglionic extracts and their equivalent commercial hormones

(progesterone and estradiol) to intact freshwater bivalves during monsoon season, the results are compared to respective controls of 2nd, 5th and 8th days. After 24hr. acclimatization the animals were arranged in four groups i.e. in individual aquarium, each group containing 20 animals in 10 liter of aerated water. The first group of animals was served as normal control and other three groups were experimental with (i) injection of cerebral ganglionic extract to intact control; (ii) injection of equivalent progesterone to normal intact control and (iii) injection of the equivalent commercial hormone estradiol to normal control bivalves. Injections were prepared before every experimentation i.e. commercial hormone injection progesterone and estradiol 0.1 mg/ml respectively and 0.1 ml quantity have been injected; for injection of cerebral ganglionic extract, extract was prepared in 1:1 ice cold distilled water and ethanol (*i.e.*20 ganglia in 2mL ice cold distilled water and ethanol), it was centrifuged and injected (0.2 mL extract/animal *i.e.* equivalent to 2 ganglia/animal), into the foot (muscular region). The experiment was run for 10 days. The physicochemical characteristics of water used in experiments i. e. temperature, pH, hardness and dissolved oxygen contents of the water were determined on every two days throughout the experimental period. The temperature determined with the help of thermometer, pH by ELICO pH meter, Hardness determined by EDTA method and dissolved oxygen of reservoir water determined by modified Winkler's technique.

The rate of oxygen consumption of individual animal from each group was determined by modified Winkler's technique, in a specially prepared brown colored respiratory jar of 1 liter volume. Five closed respiratory jars, each with an inlet and outlet. Every time five marked animals on their shells from each group were kept individually in the continuous circulation of water inside the jar by attaching inlet to the water reservoir with the help of plastic pipe, in order to open their shell valves. Once the animals were opened their valves, the flow of water was cutoff and animals were kept for 1 hour. Then sample of water from it was drawn after 1 hour in Erlyn Meyer's flask. For determination of ammonia excretion, the bivalves from each group dissected carefully and the flesh of the individual animal was taken out carefully from the shell and socked on the blotting paper to remove the excess water. Blotted flesh was then weighed to obtain the wet-weight of the individual bivalve, which required for calculating the rate of ammonia excretion of each individual animal.

The ammonia excreted by each animal was then calculated and expressed as mg NH_4/l/h/gm wet-weight of the flesh. The mean values of five individual animals from each group were used for statistical analysis. For confirmation of results all the values were subjected to statistical analysis using student't' test. Percentage differences were also calculated in the experimental group compared to their respective control.

Results

The results of the experiments were shown in (Fig. 1 and table 1-2). The physico-chemical characteristics of the water used in experiments during monsoon season (August- September) were – Temperature (24.0^0C-

29.0^{0}C); pH (8.0- 8.35); hardness in terms of bicarbonate (130- 142 ppm) and dissolved oxygen content (5.25 – 7.20 mg/l/h).

During monsoon season the rate of ammonia excretion in control group was (0.008116 ± 0.00023) on 2nd, (0.00312 ± 0.00015) on 5th and (0.005068 ±0.00017) on 8th day. The rate of ammonia excretion in control groups was decreased on 5th and 8th day compared to 2nd day. The rate of ammonia excretion in hormone progesterone injected animals was significantly decreased (0.005272 ± 0.00010, 35.04 %, P < 0.05) on 2nd and significant increase (0.00734 ± 0.00028, 135.32 %, P < 0.001) on 5th day as well as (0.007386 ± 0.0011, 45.74%, P < 0.001) on 8th day compared to respective controls. The rate of ammonia excretion in ganglionic extract injected group was decreased significantly (0.00252 ± 0.00018, 68.95%, P < 0.001) on 2nd, increased significantly (0.00474± 0.00017, 51.79%, P < 0.01) on 5th as well as (0.007386±0.00011, 45.74%, P < 0.01) on 8th day compared to respective controls. The rate of ammonia excretion in hormone estradiol injected animals was significantly decreased (0.00616±0.0009, 24.10%, P < 0.01) on 2nd day, while increased significantly (0.00431±0.00015, 38.08%, P < 0.01) on 5th day as well as (0.006732±0.00013, 32.83%, P < 0.01) on 8th day compared to their respective controls.

Table-1

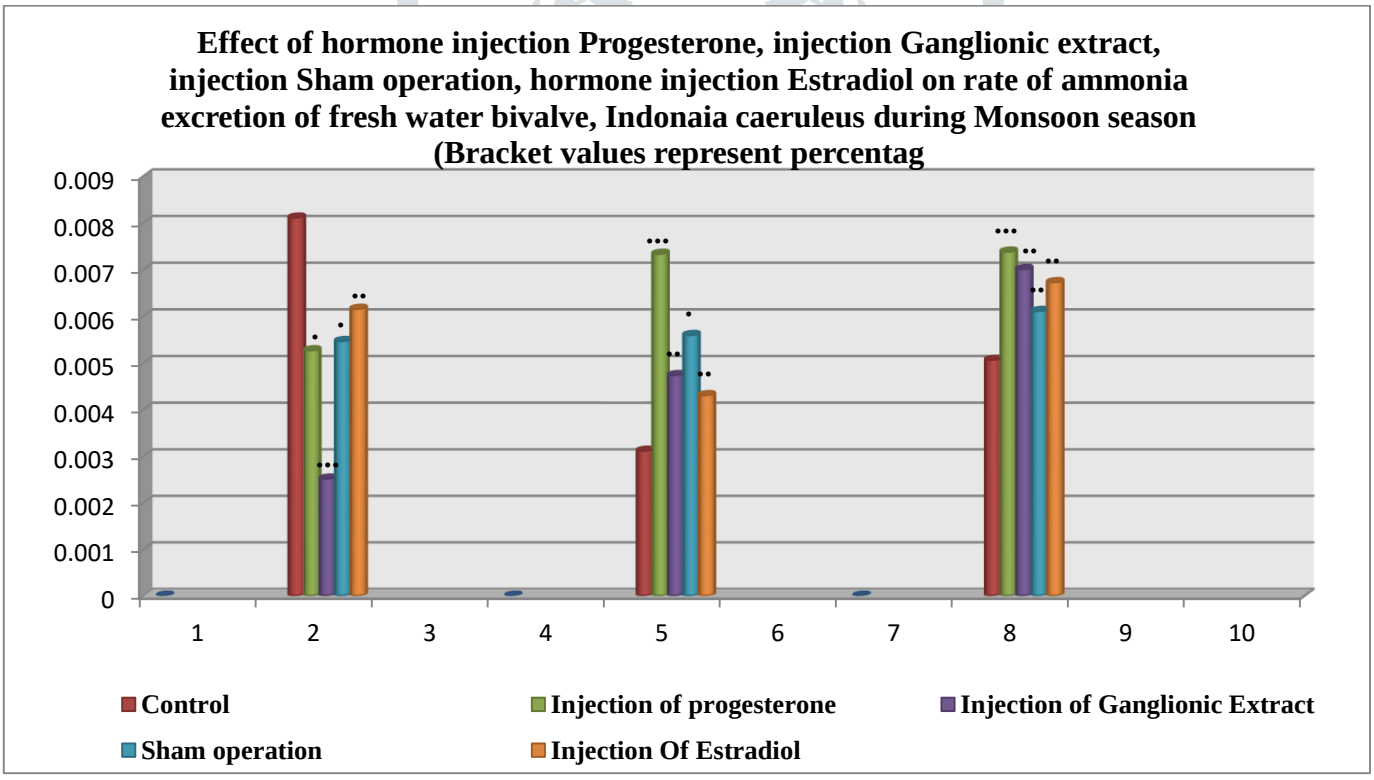
Table - 2.

Effect of hormone injection Progesterone, injection Ganglionic extract, injection Sham operation, hormone injection Estradiol on rate of ammonia excretion of fresh water bivalve, *Indonaia caeruleus* during Monsoon season (Bracket values represent percentage differences compared to control). •••=p<0.001; ••=<0.01; •=<0.05.

Ammonia Excretion					
Days	**Control**	**Injection of progesterone**	**Injection of Ganglionic Extract**	**Sham operation**	**Injection Of Estradiol**
2nd Day	0.008116 ±0.00023	0.005272 ±0.00010 (35.04%) •	0.00252 ±0.00018 (68.95%) •••	0.00547 ±0.00020 (32.60%) •	0.00616 ±0.00009 (24.10%) ••

5th Day	0.00312 ±0.00015	0.00734 ±0.00028 (135.32%) •••	0.00474 ±0.00017 (51.79%) ••	0.00560 ±0.0010 (79.62%) •	0.00431 ±0.00015 (38.08%) ••
8th Day	0.005068 ±0.00017	0.007386 ±0.00011 (45.74%) •••	0.007014 ±0.00015 (38.40%) ••	0.006114 ±0.00020 (20.64%) ••	0.006732 ±0.00013 (32.83%) ••

Fig.-1



Discussion:

Such effects on differentiation could be caused by the actions of sex steroids on the metabolism of the gonads. Evidence exists for possible actions of sex steroids in the regulation of the metabolism of glycogen, protein and lipids in bivalves. For example, estradiol may stimulate glycogenolysis and lipidogenesis by

regulating the activities of some important enzymes such as glucose- 6-phosphate dehydrogenase and malate dehydrogenase in molluscs (Mori, 1969; Mori et al., 1972a, b). Vitellogenesis, which is an essential event for the development of female gametes, was also suggested to be controlled by estradiol (Li et al., 1998; Osada et al., 2003). In addition to these specific proteins, synthesis of total proteins in molluscs may also be under the regulation of steroids. Therefore, administration of sex steroids in the scallops may accelerate the metabolic rate in the gonad, providing more materials and thus more energy for the gonadal differentiation.

Increased protein catabolism is indicated by high level of ammonia excretion and decline in oxygen: nitrogen ratio (Bayne, 1973) and thus changes in the rate of nitrogen excretion are best understood in the contest of physiological energetic and nitrogen balance, when related to overall metabolic rate by means of the oxygen : nitrogen (or O:N) ratio. Increased ammonia excretion indicated increased protein catabolism during starvation (Bhagde and Mane, 2005). Many authors have quoted that ammonia in general is a major nitrogenous excretory product of bivalves and there occurs a profound difference in loss of nitrogen between different sizes and seasons (Bishop *et al.*, 1983). This indicates shifts in physiological capacity with Change in temperature, season and reproductive cycle that affect the nitrogen economy and the metabolic rate in somewhat disparate fashions.

Conclusion- The monsoon is the season or period of active gametogenesis in bivalve molluscs, so that they require more amount of energy for active gametogenesis. It can be concluded that all essential biomolecules including proteins catabolized rapidly due to combine effect of seasonal physiological condition of gametogenesis and induction due to injections of synthetic hormones (progesterone and estradiol) as well as ganglionic extract injection which raised the ammonia excretion.

Acknowledgement

Author is thankful to UGC New Delhi, India for awarding Rajiv Gandhi National Research Fellowship and also thankful to Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS), India for providing the laboratory facilities.

References

Bayne, B. L. 1973. Physiological changes in *Mytilus edulis* (L.) induced by and nutritive stress. *J. Mar. Biol. Ass. UK*, 53: 39-58.

Bhagde, R.V. and Mane U.H. (2005): A study on the metabolism in green mussel, *Perna Viridis. J. Mar. Biol. Ass. India* 47(1): 106-110.

Bishop, S.H., Ellis, I.L. and Burchan, J.M. (1983): Amino acid metabolism in molluscs. IN: The molluscs vol. I, (Ed. Wilbur, K.M.), Accademic press, New York pp. 244-328.

Budge, S.M., Parrish, C.C., Thompson, R.J., McKenzie, C.H., (2000). Fatty acids in phytoplankton in relation to bivalve dietary requirements. In: Shahidi, F. (Ed.), Seafood in health and nutrition. *ScienceTech Publishing Company, St. John's, Canada*. 495–520 pp.

Dame R.F. (1996): Ecology of Marine Bivalves: an Ecosystem Approach. CRC Press, New York.

K. Kasprzak, (1986): Role of Unionidae and Sphaeriidae (Mollusca, Bivalvia) in the Eutrophic lake Zbechy and its outflow. *International Revue Gesamten Hydrobiologie.*, 71: 315-334.

K. Lewandowski, A. Stanczykowska, (1975): The occurrence and role of bivalves of the family Unionidae in Mikolajskie Lake. *Ecologia Polsca.*, 23: 317-334.

Li Q, Osada M, Suzuki T, Mori K (1998): Changes in vitellin during oogenesis and effect of estradiol 17 on vitellogenesis in the Pacific oyster, *Crassostrea gigas. Invert Reprod Dev*; 33: 87-93.

Mori, K. (1969): Effect of steroid on oyster-IV: acceleration of sexual maturation in female *Crassostrea gigas* by estradiol-17_. Bull. Jpn. Soc. Sci. Fish. **35**: 1077–1079.

Mori, K., Muramatsu, T. & Nakamura, Y. (1972a): Effect of steroid on oyster-VI. Indoor experiment on the acceleration of glycogenolysis in female Crassostrea gigas by estradiol-17b. *Bulletin of the Japanese Society of Scientific Fisheries*, 38: 1191–1196.

Mori, K., Muramatsu, T. & Nakamura, Y. (1972b): Effects of steroids on oyster-V. Acceleration of glycogenolysis in female *Crassostrea gigas* by estradiol-17b injection under natural conditions. *Bulletin of the Japanese Society of Scientific Fisheries*, 38: 1185–1189.

N. V. Subba Rao, A. Dey, (1989): Freshwater Molluscs in Aquaculture. *Zool. Surv. India, Calcutta.*, 225-232.

Nalepa, T.F., Gardner, W.S. & Malczyk, J.M. (1991): Phosphorus cycling by mussels (Unionidae: Bivalvia) in Lake St. Clair. *Hydrobiologia*, **219:** 239–250. ORION RESEARCH INCORPORATED. 1990. Model 95–12 ammonia electrode instruction manual. 36 pp.

Osada M, Takamura T, Sato H, and Mori K (2003): Vitellogenin synthesis in the ovary of scallop *Patinopecten yessoensis*: control by estradiol-17b and the central nervous system. *J Exp Zool* 299A: 172–179.

Parrish, C.C., Wells, J.S., Yang, Z., Dabinett, P., (1998): Growth and lipid composition of scallop juveniles *Placopecten magellanicus* fed the flagellates *Isochrysis galbanawith* varying lipid composition and the diatom *Chaetoceros muelleri*. *Mar. Biol.* 133, 461–471.

Solorzano, L. 1969. Determination of ammonia in natural waters by phenol-hypochlorite method. Limnology and oceanography. 14: 799-801.

Strayer D.L., Caraco N.F., Cole J.F., Findlay S. & Pace M.L. (1999): Transformation of freshwater ecosystems by bivalves. *Bioscience*, 49, 19-27.