Changes in the rate of ammonia excretion of freshwater bivalve *Indonaia caeruleus* (Prashad, 1918) with injections of cerebral ganglionic extract and equivalent commercial hormones (progesterone and estradiol) during winter season.

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ABSTRACT

Neuroendocrine control is important and which control the metabolic activities of freshwater bivalves, we report here the role of injections of equivalent commercial hormones (i.e. Progesterone & Estradiol) and cerebral ganglionic extract on excretion of freshwater bivalve mollusc *Indonaia caeruleus* (Prashad, 1918) from Godavari River. During winter 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 2nd, 5th, and 8th day.

The study demonstrates that, the rate of ammonia excretion was significantly decreased in ganglionic extract injected group and progesterone injected group on 2nd day. On 5th day, the rate of ammonia excretion increased significantly in progesterone injected group and significant decrease observed in ganglionic extract injected group. While on 8th day of experimentation, the rate of ammonia excretion decreased significantly in ganglionic extract injected group as compared to its respective control group.

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

Introduction

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. According to the classification of Vokes (1980), the living freshwater bivalve molluscan fauna is a primarily represented by three superfamilies Unionacae, Corbiculaecae and Dreissenacae. The freshwater mussels are falling under Unionaceae are documented by the members of families - Margaritiferidae and Unionidae. The family Unionidae is relatively large family in which *Indonaia caeruleus* belong. 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). Cultivation of filter-feeding bivalves is one of the potential and sustainable forms of mariculture which can be operated in large scale with no artificial food given, since the animals can get their nutrients from phytoplankton, microphytobenthos and other types of organic detritus (e.g., Grant, 1999; Hawkins et al., 2001). Bivalves play a key role in many coastal ecosystems due to their high filtration capacity and culture density (Smaal et al., 2001; Zhou et al., 2002). 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. 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.

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 winter season (December-January) 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 summer 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 NH4/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 winter season were – Temperature (18-22°C); pH (8.25-8.42); hardness in terms of bicarbonate (112-130 ppm) and dissolved oxygen content (6.10-6.80 mg/l/h).

During winter season, the rate of ammonia excretion in control group was (0.00541 ± 0.00015) on 2^{nd}, (0.0018 ± 0.00010) on 5^{th} and (0.0026 ± 0.00011) on 8^{th} day. The rate of ammonia excretion showed decrease on 5th and 8^{th} day compared to 2^{nd} day. The rate of ammonia excretion in hormone progesterone injected groups was significantly decreased (0.00272 ± 0.00067, 49.72%, $P < 0.05$) on 2^{nd} and significant increase (0.0031 ± 0.00021, 69.41 %, $P < 0.01$) on 5^{th} day, while it was non significantly decreased (0.00248 ± 0.00078, 7.38%) on 8^{th} day, compared to respective controls. The rate of ammonia excretion was significantly decreased (0.00225 ± 0.000403, 58.41 %, $P < 0.01$) on 2^{nd} day, (0.0011 ± 0.00054, 38.17%, $P<0.05$) on 5^{th} day and (0.0013 ± 0.00012, 48.44 %, $P < 0.01$) on 8^{th} day respectively in ganglionic extract injected group,

compared to respective controls. The rate of ammonia excretion in hormone estradiol injected groups was non significantly decreased (0.003942 ± 0.0017, 27.13%) on 2nd day, significantly decreased (0.0012 ± 0.00016, 30.37 %, $P < 0.05$) on 5th day and non significantly decreased (0.0023 ± 0.00056, 12.15%) on 8th day, compared to respective controls.

Table – 1

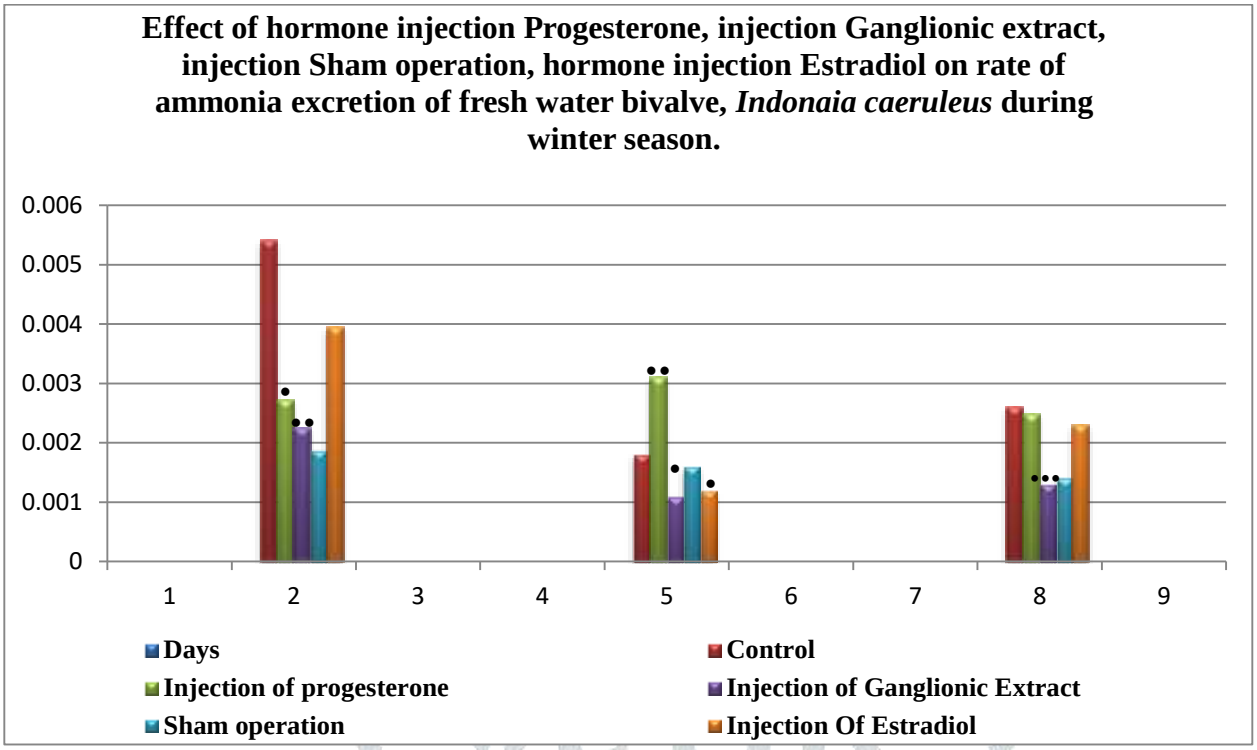
Sr. No.	Seasons	Months	Temperature (0C)	pH	Hardness (ppm)	Dissolved Oxygen content (mg/lit.)
1	Winter	December	19-24	8.13-8.27	112-118	6.10-6.80
		January	18-22	8.25-8.42	115-130	6.22-7.45

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 winter season. (Bracket values represent percentage differences compared to control). •••=p<0.001; ••=<0.01; •=<0.05.

Fig.1

	Ammonia Excretion				
Days	**Control**	**Injection of progesterone**	**Injection of Ganglionic Extract**	**Sham operation**	**Injection Of Estradiol**
2nd	0.00541 ±0.00015	0.00272 ±0.00067 (49.72%) •	0.00225 ±0.000403 (58.41%) ••	0.001882 ±0.00036 (65.21%) ••	0.003942 ±0.0017 (27.13%)
5th	0.0018 ±0.00010	0.0031 ±0.00021 (69.41%) ••	0.0011 ±0.00054 (38.17%) •	0.0016 ±0.00032 (9.98%)	0.0012 ±0.00016 (30.37%) •
8th	0.0026 ±0.00011	0.00248 ±0.00078 (7.38%)	0.0013 ±0.00012 (48.44%) •••	0.00141 0.00030 (47.39%) ••	0.0023 ±0.00056 (12.15%)



Discussion

Proteins are long chains of amino acids forming three dimensional structures. Proteins do play both structural and functional role of cellular level. Being an integral part of the cell membrane, intracellular and extra cellular passages are linked through it (Anilkumar and Meenakshi, 2012). It has been reported that proteins (Andrews *et al.*, 1972; Alava and Lim, 1983) provide the amino acids for growth or other metabolic functions (intracellular osmotic regulation, intermediate metabolism, etc.). Nitrogen metabolism that supports the oxidation of amino acids may follow different pathways depending on the feeding experimental conditions (Mayzaud and Conover, 1988). Ammonia is the primary excretory product of protein catabolism in crustaceans and accounts for over 70 % of the nitrogen excreted (Quetin *et al.*, 1980). For that reason nitrogen excretion, measured as ammonia excretion, is a good indicator of oxidation of amino acids. 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). 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.

In the present study on *Indonaia caeruleus*, in control group, rate of ammonia excretion was increased on 2^{nd} and 8^{th} day. Increase in the rate of ammonia excretion might be due to starvation, because during starvation there is more protein catabolism hence ammonia excretion rate increases. Increased ammonia excretion indicated increased protein catabolism during starvation (Bhagde and Mane, 2005). The

nutritional composition is an important factor governing the growth of living beings. According to Vijayavel and Balasubramanian (2006), growth represents a net outcome of a series of processes such as digestion, assimilation, metabolism, and excretion.

Conclusion- After the active gametogenesis in monsoon due to availability of more food required for more energy production. Bivalve gonads became mature and start spawning during late monsoon to winter. It can be concluded that, because of rapid catabolization of all essential biomolecules which already utilized for active gametogenesis and gonad maturation. In winter season as per rule of natural seasonal gonadal cycle only spawning remains but due to injection of ganglionic extract and synthetic hormone injections (progesterone and estradiol) may induces inter-conversion of biomolecules necessary for spawning and decrease the ammonia excretion.

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