Variation in the rate of ammonia excretion due to commercial hormones (progesterone and estradiol) and ganglionic extract injections in freshwater bivalve *Indonaia caeruleus* (Prashad, 1918) during summer season.

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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 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 shows that, the rate of ammonia excretion was significantly increased in ganglionic extract injected group and decreased significantly in estradiol injected group on 2nd day. While on 5th day, the rate of ammonia excretion increased significantly in progesterone injected as well as ganglionic extract injected group. The rate of ammonia excretion on 8th day of experimentation, decreased significantly in progesterone injected and ganglionic extract injected group, where it was increased significantly in estradiol injected group as compared to respective control group

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

Introduction

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.

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. For that reason nitrogen excretion, measured as ammonia excretion, is a good indicator of oxidation of amino acids. When shrimp are fed with different protein levels, the evaluation of nitrogen excretion may be used as an indicator of the capabilities of a species for using protein as energy source (Regnault, 1981; Da11 and Smith, 1986).

Masthanamma et al., (1985) revealed that the organic constituents viz. carbohydrate, glycogen, sugar, free amino acid, total protein and total lipids in whole body of *Lamellidens marginalis* subjected to 130 days starvation revealed elevator trend in amino acid and decrease in rest of the organic constituents. In comparison the protein level in gonad is high when gametes are present and decreases after their release with an accompanying increase in carbohydrates. The decrease in carbohydrates with increase in protein has been suggested to be due to conversion of carbohydrates into proteins during gametogenesis (Giese et al., 1967). Protein and lipid decrease during spawning period, coinciding with rapid increase in carbohydrates. Special connective tissue cells provide a means for removing and holding glycogen and protein for gametogenesis (Bayne et al., 1982).

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 summer season (April-May) 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 2^{nd}, 5^{th} and 8^{th} 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 summer season were – Temperature (28.0^0C- 35.0^0C); pH (7.2- 7.92); hardness in terms of bicarbonate (90- 117 ppm) and dissolved oxygen content (4.40 – 5.50 mg/l/h).

The rate of ammonia excretion in control was (0.00506 ± 0.00026) on 2^{nd}, (0.0043 ± 0.00013) on 5^{th} and (0.00498 ± 0.00021) on 8^{th} day. The rate of ammonia excretion was increased on 2^{th} day compared to 5^{th} and 8^{th} day. The rate of ammonia excretion was non significantly increased (0.00525 ± 0.00065, 3.71 %) on 2^{nd}; significantly increased (0.00579± 0.00012, 33.10 %, $P< 0.01$) on 5^{th} and (0.00321 ± 0.00014, 35.73%, $P< 0.01$) on 8^{th} day in hormone progesterone injected

animals compared to respective controls. The rate of ammonia excretion in cerebral ganglionic extract injected groups was significantly increased (0.00611 ± 0.00022, 20.73 %, P < 0.05) on 2nd, while significantly increased (0.00583 ± 0.00016, 34.02 %, P < 0.01) on 5th and significantly decreased (0.004246 ± 0.00023, 15.05%, P < 0.05) on 8th day compared to respective control. The rate of ammonia excretion in hormone estradiol injected group was significantly decreased (0.00420 ± 0.00018, 17.02%, P < 0.05) on 2nd day, while non significantly decreased (0.00420 ± 0.00078, 3.26%) on 5th day and significantly increased (0.005538 ± 0.00011, 10.80%, P < 0.05) on 8th day compared to respective control.

Table – 1

Sr. No.	Seasons	Months	Temperature (0C)	pH	Hardness (ppm)	Dissolved Oxygen content (mg/lit.)
1	Summer	April	28-30	7.2-7.5	90-102	4.40-5.35
		May	32-35	7.7-7.92	108-117	4.62-5.50

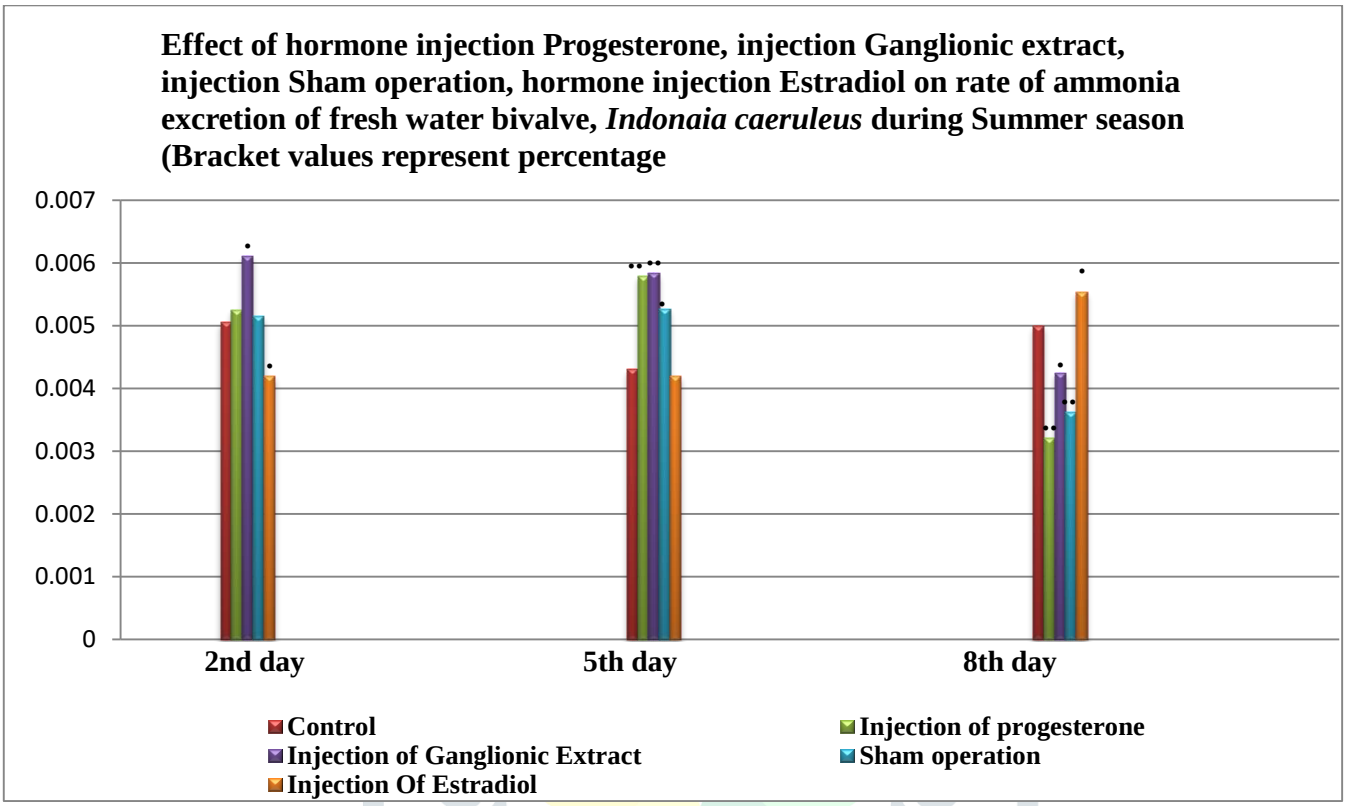
Table - 2

Ammonia Excretion					
Days	**Control**	**Injection of progesterone**	**Injection of Ganglionic Extract**	**Sham operation**	**Injection Of Estradiol**
2nd Day	0.00506 ±0.00026	0.00525 ±0.00065 (3.71%)	0.00611 ±0.00022 (20.73%) •	0.00515 ±0.00161 (1.70%)	0.00420 ±0.00018 (17.02%) •
5th Day	0.0043 ±0.00013	0.00579 ±0.00012 (33.10%) ••	0.00583 ±0.00016 (34.02%) ••	0.00527 ±0.00020 (21.49%) •	0.00420 ±0.00078 (3.26%)
8th Day	0.004998 ±0.00021	0.003212 ±0.00014 (35.73%) ••	0.004246 ±0.00023 (15.05%) •	0.003614 ±0.00016 (27.69%) ••	0.005538 ±0.00011 (10.80%) •

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

Fig. 1



Discussion

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. A few investigators also demonstrated the probable role of ammonia in the settlement of larvae of different bivalves. According to Coon *et al.*, (1990) ammonia solution (pH 8.0) (2.5 mM concentration) induced stereotypical settlement behaviour of larvae. The authors suggested that ammonia increased the intracellular pH. Fitt and Coon (1992) stated that the actual concentration of NH_3 was associated with the surface for the oysters *Crassostrea virginica* and *Crassostrea gigas.* 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. According to Khalil (1994) in *Tapes decussatus* ammonia excretion rate varied with body weight, temperature, and starvation. Ammonia excretion rates were steady during six days of starvation and higher excretion rate was dependant

on the temperature. The ammonia excretion rate was higher for starved clams than for fed clams of all sizes and at different temperatures, weight specific ammonia excretion rates were related to dry flesh weight of starved clams but were not related to fed ones in *T. decussates* (Khalil 1994). In the present study on *Indonaia caeruleus*, in control group, rate of ammonia excretion was increased (0.00506 ± 0.00026) on 2nd compared to 5th and 8th day during summer. 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).

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.

Conclusion- Exogenous factors play significant role in vital processes such as respiration, biochemical reserves and reproduction in bivalve molluscs, endogenous factors via neurosecretion modulating or regulating the above activities is also disputed (Bayne, 1976; Lubet and Mathieu, 1978; Mane, 1986).

It can be concluded that cerebral ganglia and commercial hormones possesses some factors which regulate metabolism of different tissues during different seasons. Therefore, integrated effects of season as well as injections of synthetic hormones (progesterone & estradiol) and ganglionic extract causes vital changes in excretion of ammonia.

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