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Probable role of ablation of cerebral ganglia and injection of its extracts on O:N ratio of *Lamellidens corrianis* during summer season

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Abstract
Amongst invertebrates, molluscs show great variability in their nervous system ranging from primitive arrangement in Chitons to the complex mass of fused ganglia forming the 'brain' of cephalopods. Most of the effector organs used for pharmacological or physiological experiments. The neurosecretory cells (NSCs) with their combination of neuronal and glandular capabilities are perfectly suited to translate a neuronal input into the hormonal output best suited to long-term process. In this capacity, the NSCs may produce hormones, which act directly upon the peripheral target or it may exert its effect indirectly by influencing the activity of other non-neural, endocrine organs. Neurosecretory cells have been detected in the cerebral, pedal and visceral ganglia of *Lamellidens corrianus*. The distribution and biology of freshwater bivalve like *Lamellidens corrianus* is influenced by local ecological factors (like temperature, pH, inorganic salts, type of soil etc.), water flow system and presence of micro-organisms, teleost fishes and seasonal variations in these parameters. Nitrogenous excretory changes which include ammonia in these molluscs is also influenced by these local ecological factors in which temperature is plays very crucial role. It is well known fact that the high stress conditions during drought or severe winter conditions have been conquered by several lineage of gastropods and sand bivalves and their ability to enter the resistant or dormant stages (low food) protein catabolism increased. Present investigation deals with the oxygen consumption and ammonia excretion ratio of these freshwater pelecypode *Lamellidens corrianus*. Study on *Lemellidens corrianus* directed in understanding the different behavioral and physiological aspects after cerebral ganglia removal and injection of their extracts revealed significant changes during summer (April-May).

Keywords: Cerebral ganglia ablation, Injectionic of cerebral ganglionic extract, *Lamellidens corrianus*, O:N ratio, summer season

Introduction
Mollusca are the second largest phylum of Animal Kingdom after the phylum Arthropoda (Verma and Prakash, 2020) [25]. In tropical regions like India, in general the conditions are hot-dry from March to May, these weather conditions can have considerable impact on the life of bivalve molluscs, especially in the areas lacking heavy rain fall. The bivalve molluscs living especially in lentic environment have to face drastic environmental changes than those in lotic conditions. Temperature rise above the optimum requirement can affect the physiology of bivalves (Bayne, 1976) [3]. The studies with polluting substances also revealed that the freshwater bivalves from Godavari River at Paithan are very sensitive to pesticide toxicity in summer than in monsoon and winter (Akarte, 1985; Muley, 1985) [1, 18]. Pesticide toxicity is also recorded in case of fishes (Prakash and Verma, 2014; Verma and Prakash, 2018; Kaur and Mishra, 2019) [19, 25, 11]. An oxygen concentration in natural waters is influenced by temperature, (Cairns *et al.*, 1975) [6], thus in summer *Lamellidens corrianus* from Jayakwadi back water faces severe environmental conditions. The physiological aspects or the endocrine aspects should be directed towards the studies through different seasons because the aquatic animal itself show cyclic changes in the aspects (Kulkarni, 1987) [15]. Stickel (1973) [22] stated that, nutrient deposition or depletion is dependent upon the balance between anabolism and catabolism and one measure of metabolic activity often used is the rate of oxygen consumption which is affected by factors that fluctuate seasonally such as food availability and water temperature.

Thus, in the present study this dual effect of changes in environmental parameters and starvation revealed that in summer the rate of oxygen consumption was decreased initially up to 12 days.

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) [4]. In mollusca, interactions with the environment are handled by the nervous system (including the sense organs) and the muscular system (the muscles that making the foot and those that attach the animal to its shell). Both systems, of course, have been of different forms in different group of animals. Basically, there are two extreme types of neural organization (with many intermediate forms).

The chord system, in which the neural tree tapers and branches in smooth lines; and the ganglia system, which is characterized by knots of nerve cells called ganglia, bound together by nervous tissue consisting of slender elongations of the cells.

The nervous and endocrine systems coordinate the activities of the various organs and the tissues in the body that the animal's function as individuals. Majority of the neurons with granular activity are known to be necessary for the transmission of transient impulses, with their highly localized production of chemicals such as neurohumors/neurohormones, which are rapidly destroyed. Neurons have similar properties throughout the animal kingdom, although their morphology and arrangement may vary.

The way in which nervous system operates may differ considerably between animal depending on the number of neurons involved and the individual shape, size and spatial arrangement of the component about fifty percent of the nervous system is composed of non-excitable satellite glial cells which are packed between and around the neurons. They are supposed to transport neurosecretory substance (Lubet, 1955a; Umiji,1969) [17, 23], provide physical support for the neurons and can modify action of nerve cells as well as a barrier or receiver for ions, metabolites and transmitters (Leak and Walker, 1980) [16].

The neurosecretory cells (NSCs) with their combination of neuronal and glandular capabilities are perfectly suited to translate a neuronal input into the hormonal output best suited to long-term process. In this capacity, the NSCs may produce hormones, which act directly upon the peripheral target or it may exert its effect indirectly by influencing the activity of other, non-neural, endocrine organs. In this later case, the NSCs organ may act via the production of blood-born hormone. Knowles and Bern (1966) [13] stated that the significance of NSCs as connecting link between nervous and endocrine systems and neurosecretory neurons participate either directly or indirectly in endocrine control and form all or part of endocrine organ. Yasmeen (2019) [26] also worked a lot on gonads of *Lamellidens.*

Hormones are consequently well suited to exert their effects over extended period of time and the endocrine system controlling-term process within the body such as the coordinated growth of organs or the maintains of appropriate metabolite concentration in the blood and tissues. Neurosecretory cells of cerebral, pedal and visceral ganglia secrete its secretion which help in regulation of body activity.

Removal of both cerebral ganglia (bilateral cerebralctomy) troubled in neuroecretion which might affect in metabolic activity and exert stress on animal which probably hamper the rate of respiration and ammonia excretion (O:N ratio).

Materials and Methods

The freshwater bivalve molluscs, *Lamellidens corrianus* were collected from Jayakwadi backwaters (Nathsagar) at Paithan, 45 km. away from Aurangabad. After brought to the laboratory, the shells of the bivalves were brushed and washed with fresh reservoir water so as to remove the fouling algal biomass and mud. The animals of 80-85 mm shell length were selected for experiment and they were acclimatized for 24 h. at laboratory condition in fresh aerated reservoir water (with renewal of water at the interval of 12-13 h.) and stocking capacity was given during this period and no food was given to the bivalves during laboratory acclimatization and subsequent experimentation. After 24 h., reservoir water was once again renewed and aeration was given. After a lapse of 1 h. the animals extended their organs (foot, mantle, siphons) to maximum and soon surgical operations and injection of the ganglionic extract were done. For removal of both the cerebral ganglia (bilateral cerebralectomy) active animal were chosen from the aquarium and a wedge (4-5 mm thick) was kept between the valves of the shell. Both the cerebral ganglia were removed by performing minimum injury to the animals within 30 seconds, with the help of fine, pointed sterilized forceps. For injection of ganglionic extracts, cerebral ganglionic extract was prepared in ice cold distilled water (10 ganglia in 1ml cold distilled water was centrifuged and the supernatant (0.2 ml/animal i.e., equivalent to 2 ganglia/animal) were injected into the foot (muscular region) of normal control and gangliatomized (both cerebral ganglia ablated) bivalves. In sham operated control animals were injected by 0.2ml cold distilled water. The result for control and sham operated groups were similar and hence a comparison was made between gangliactomized and control group and extract injected to normal control as well as ablated and control group of animals only. Soon after the operation and injection of ganglionic extracts to normal control, extirpated 20 animals of cerebralectomy, 20 animals of extract injected, and 20 animals of extract injected to ablated bivalves were transferred to separate aquaria. Each aquarium contained 15 liter well aerated reservoir water, and experiment was run for 12 days. The water from each aquarium was changed at an approximate interval of 12-13 h. throughout experimental period. The behavior and mortality of the bivalves were recorded before each change of water from all the aquaria. The experiments were conducted for 12 days on freshly collected animals during summer season (April - May). The adult bivalves were placed individually in respiratory jars with 1 liter water. The rate of oxygen consumption of the animals in each group during summer seasons was determined according to Winkler's modified technique (Golterman *et.al.,* 1978) [10], in a specially prepared brown colored respiratory glass jar of one liter capacity. The rate of ammonia excretion was measured on 2nd, 7th and 12th day according to phenol-hypochlorite method of Solorzano (1969) [19]. Every time four individual animals of each group were used and mean of triplicate of water samples were estimated for each group. The statistical analysis was done to express final data. The atomic equivalent values of ammonia excretion obtained for the same individual. All the values were subjected to statistical analysis for confirmation using student 't' test (Dowdeswell, 1957) [8]. Statistical and percentage differences were also calculated in experimental animals.

Results

Table 1: Effect of ablation of cerebral ganglia and injection of its extracts on O:N ratio of *Lamellidens carrianus* during summer seasons. (Bracket Values represents percentage difference)

	Summer season			
Days	Control	Ablation of cerebral ganglia	Injection of distilled water	Injection of cerebral gangilonic extract
On 2nd	47.55575±19.4535	26.0221±09.6193(45.28)	24.8675±05.3141	17.8820±02.7288(62.39)
On 7th	26.9100±05.3208(43.41)	15.6850±03.2732(41.37)	18.9100±03.0204	11.7844±01.9702(56.20)
On12th	10.0201±02.8612(78.93)	26.7002±11.8806(166.04)	07.2791±01.6874	15.6908±01.7687(56.58)

The changes in the O:N ratio from control, ablation of cerebral ganglia and injection of ganglionic extracts during different seasons, in freshwater bivalve mollusc *Lamellidens corrianus* were given in table-1. During summer season, in the control group, the O:N ratio was (47.5575 ± 19.4535) on 2nd day, (26.91 ± 5.3208) on 7th day, and (10.0201 ± 2.8612) on 12th day. The higher value of ratio was found on 2nd day. The ratio decreased (43.41%) on 7th and (78.93%) on 12th day, compared to 2nd day. Among the experimental groups O:N ratio in ganglia ablated animals was decreased (26.0221 ± 9.6193, 45.28%) on 2nd, (15.6850 ± 3.2732, 41.37%) on 7th and increased (26.7002 ± 11.8806, 116.4%) on 12th day. Whereas, the O:N ratio from ganglionic extract injected animals was decreased (17.882 ± 2.7288, 62.39%) on 2nd day, (11.7844 ± 1.9702, 56.20%) on 7th day, while it was increased (15.6908 ± 1.7687, 56.58%) on 12th day compared to respective controls.

Discussion

The O:N ratio is an index of protein utilization in energy metabolism. A few investigators also demonstrated the probable role of ammonia in the settlement of larvae of different bivalves. According to Coon *et. al*., (1990) [7] ammonia solution (pH 8.0) (2.5 mM concentration) induced stereotypical settlement behavior of larvae. The authors suggested that ammonia increased the intracellular pH. Fitt and coon (1992) [9] 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) [2] and thus changes in the rates 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) [12] in *Tapes decussalus* 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 depended 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) [12]. 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 (Krishnakumar and Damodharan, 1986) [14]. The author also stated that the mussel is dependent on protein for energy production, which is less than healthy *Mytilus edulis* from Cochin backwater. In addition, the author further stated that the ratio might vary according to gametogenic cycle, nutritive reserves and nature of food. It is well known fact that the high stress conditions during drought or severe winter conditions have been conquered by several lineage of gastropods and sand bivalves, and their ability to enter the resistant or dormant stages (low food) protein catabolism increased. Many workers have compared the excretion rates of bivalves related with their environment. Boucher and Boucher (1990) [5] have demonstrated the ammonia exchange processes of *Crassostrea gigas* and sediment. Authors compared the oyster biomass and lighting conditions (light and dark) with oxygen and ammonia exchange rates. The oxygen consumption increased with increasing biomass but the respiration rate was decreased, whereas, ammonia released never matched the rate predicted by adding sediment efflux to oyster excretion. The O:N ratio was influenced by oyster biomass. Further, the author suggested that both the organism and sediment were involved in regulation process (Boucher and Boucher, 1990) [5]. Food availability and the cells rapidly filled with material. With the close of monsoon, from October NM absorption phases extended till November NM. This was correlated with longer feeding period under short supply of food and further extended till February FM, showing tubules in either holding or absorption phases. The water temperature decreased with the onset of winter and no feeding occurred for a short period of November FM when the tubule showed re-organization phase (Saokar, 1994) [20]. The existing labor was undertaken to investigate the effect of endogenous parameters chiefly neuroendocrine on the aspects of O:N ratio. It reveals that cerebral ganglia ablation and cerebral ganglionic extract injection might exert some effect on O:N ratio along with the environmental parameters. As compare in cerebral ganglionic extract injection group, more profound effect of O:N ratio seen in the cerebral ganglia ablated group on 12th day of experimental period.

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