

## EFFECT OF RATE OF OXYGEN CONSUMPTION IN FRESHWATER BIVALVE MOLLUSC, *LAMELLIDENS CORRIANUS* FROM GODAVARI RIVER AT KAIGAON: IN THE EFFECT OF CEREBRALECTOMY AND INJECTION OF CEREBRAL EXTRACT DURING SUMMER.

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### ABSTRACT

The adult bivalve molluscs, *Lamellidens corrianus* of 80-85 mm shell length and 11.552-13.600 gm body weight were subjected to (a) control (normal) (b) removal of both cerebral ganglia (c) injection of their cerebral extract to intact control as well as (d) injection of their extract to ganglia removal bivalves and (e) injection of ice- cold distilled water to normal control for 12 days. The rate of oxygen consumption in bivalves from all four groups (including control) was measured on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day. The study revealed that, the rate of oxygen consumption was significantly increased in cerebral ganglia removed, as well as cerebral ganglionic extract injected to ablated group on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day compared to control. The rate also showed significant increase in injection of extract to normal control 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day. The rate of oxygen consumption showed more cerebral ganglia ablated group than extract injected one on 12<sup>th</sup> day.

**KEY WORDS:** Cerebralectomy, Cerebral ganglionic extract, freshwater bivalve molluscs, Oxygen consumption

### INTRODUCTION

In general, many exogenous environmental variables (Temperature, Salinity, pH, Light, Oxygen tension, Turbidity etc.) the rate of oxygen consumption in bivalve molluscs (Bayne, 1976; Samant and Agrawal, 1978). Most of the vital activities in bivalves are regulated by neuro-endocrine centers. The respiratory rate data of the animals reflect their general metabolic rate. The existence of neuro-endocrine modulations of metabolic rate will be the adaptive significance for the freshwater bivalves, which have to live in ever fluctuating environments. Comparatively, very work was done on the neuro-endocrine regulation in bivalve shell fishes and also comparatively, very less attention has been given on the role of neuro-endocrine centers in respiratory metabolism particularly from freshwater bivalves. In the field of neuroendocrinology, neuroendocrine regulation of oxygen consumption has been reported for crustaceans (Nagabhushnam and Kulkarni, 1979). Hanumante *et al.* (1980) has been shown that, neurohormones from pleurovisceral ganglia regulate the rate of oxygen consumption in gastropod mollusks. The role of cerebral and visceral ganglia in the respiratory metabolism has been reported by Mane *et al.* (1990) for estuarine clam, *Katylisia opima*, Shinde (2007) for freshwater bivalve, *Lamellidens corrianus* from Godavari River at Kaigaon. Jadhav (2011) studied on *Lamellidens marginalis* from Paithan some reports are available on respiratory physiology of freshwater bivalves mollusc from India and abroad (Salanki and Lukascsovice, 1967; Bayne, 1976; Zs- Nagy, 1974). In bivalve molluscs, two types of neuro-cycles like sudden changes in temperature, pH and salinity after cerebral neurosecretion and long cycle related to certain activities of reproduction and metabolism. Such neurosecretory cycles from neurosecretory cells was reported by Nagabhushnam and Mane (1973) for estuarine clam, *Katylisia opima* and by Kulkarni (1987) for freshwater bivalve, *Indonaiia caeruleus*. Shaikh (2010), reported the effect of mercuric and cadmium chloride on oxygen consumption of freshwater crab *Barytelphusa cunicularis*. Recently, Shaikh *et al.* (2012), observed Behavioral changes in *Lamellidens marginalis* due to acute toxicity of cadmium.

Review of the literature shows that very little information is known on the neuro-endocrine regulation in respiratory metabolism of freshwater bivalves since many features of aerobic metabolism can be studied directed by measurement of the rate of oxygen consumption by induced animals. Thus, considering the paucity of information on endogenous regulation in the respiratory metabolism (because the respiration is considered as one of the important aspect for understanding the physiological adaptation of a species). In bivalve shell fishes from the inland waters, hence the present study is taken on freshwater shell-fish, *Lamellidens corrianus* from Godavari River at Kaigaon

### MATERIALS AND METHODS

The adult freshwater bivalve molluscs, *Lamellidens corrianus* 80- 85 mm in shell length) were collected from banks of Godavari River at Kaigaon near Aurangabad, during summer season. After brought to the laboratory the shells of the bivalves were brushed and washed with water to remove the mud and fouling algal and fungal biomass. The bivalves were acclimatized for 24h. In laboratory conditions and subsequent experimentation without food. After 24 hr. acclimatization the animals were arranged in 5 groups, each group containing 15 animals in 10 lit. of aerated water. The first group of animals were served as normal control with intact ganglia and other four groups were experimental with (A) removal of both the cerebral ganglia; (B) injection aqueous ethanol ( water + ethanol) 1:1 to control animals, (Sham

operated) (C) injection of cerebral ganglionic extract to control and (D) injection of ganglionic extract to ablated bivalves. Total removal (ablation) of both the cerebral ganglia were done, with the help of fine sterilized forceps by inserting a rubber cork wedge of 3-4 mm thickness, inserted between 2 valves of the shell near anterior adductor muscles. The precaution was taken that the mantle should not get pinched in between the shell valves. For injection of cerebral ganglion extract, it was prepared in ice-cold distilled water and ethyl alcohol 1:1 (1 ganglia in 1 ml of solution), it was centrifuged with refrigerated centrifuge and injected (0.2 ml extract per animal) i.e. equivalent to 2 ganglia per animal, into the muscular tissue foot. For Sham operated control the animals were injected by 0.2 ml mixture of ice-cold distilled water and alcohol, (it was not run because it did not show significant change). The experiment was run for 12 days. The physicochemical characteristics of water used in experiments i.e. temperature, pH, hardness and dissolved oxygen contents were also measured. Temperature and pH were recorded daily, while hardness and dissolved oxygen contents of the water were determined on every two days throughout the experimental period.

The rate of oxygen consumption of individual animal from each group was determined by modified Winkler's method (Golterman *et al.*, 1978), in a specially prepared brown colored respiratory jar of 1.0 lit. capacity. Four closed respiratory jars, each with an inlet and outlet were kept in continuous circulation of water, in order to open the valves of animals. Once the animals were opened their valves, the flow of water was cutoff and sample of water from it, was drawn after 1hr., for determination of oxygen consumption. The flesh of the individual animal was taken out carefully from the shell and soaked on the blotting paper to remove the excess water. Blotted flesh was then weighed to obtain the wet-weight of the individual bivalve.

The oxygen consumed by each animal was then calculated and expressed as mg O<sub>2</sub>/l/h/gm wet-weight of the flesh. For confirmation of results all the values of four individual animals from each group were subjected to statistical analysis using 't' test (Dowd swell, 1975). Percentage differences were also calculated in experimental group.

## RESULTS

The results of the experiments were shown in table 1. The physio-chemical characteristics of the water used in experiments during monsoon season were- temperature (32.0° C – 33.0° C); pH (7.1 – 7.4); hardness in terms of bicarbonate (105 -110 ppm) and dissolved oxygen contents (4.59- 5.79 mg/l/h).

**Table 1. Effect of rate of oxygen consumption of freshwater bivalve, *Lamellidens corrianus* form Godavari River at Kaigaon during summer: as function of removal of cerebral ganglia and injection of their extract.**

Days	Normal control (with intact ganglia)- (a)	Ablation of both cerebral ganglia – (b)	Injection of cerebral ganglionic extract (to normal control)-(c)	Injection of cerebral ganglionic extract to ablated animals- (d)
2 <sup>nd</sup> Day	0.2506 ± 0.0130	0.3491 ●●● ±0.0166 (39.62%)	0.3200●● ±0.0287 (27.970%)	0.33085●●● ± 0.0171 (32.00%)
6 <sup>th</sup> Day	0.1814 ± 0.0118	0.2623 ●● + 0.0381 (16.825%)	0.1628 ± 0.005 (10.25%)	0.2482 ● ± 0.0267 (36.82%)
12 <sup>th</sup> Day	0.1419 ± 0.009	0.2313 ●● ± 0.0230 (22.601%)	0.1436 ± 0.0094 (1.19%)	0.1898 ● ±0.0187 (33.751%)

(Bracket values represent percentage differences)

●●●= P< 0.001    ●●= P < 0.01    ●= P< 0.05

As compared to control, the rate of oxygen consumption on was significantly increased (0.3491 ± 0.0166, 39.62%, P<0.001) in cerebral ganglia ablated, (0.33085 ± 0.0171, 32.0%, P< 0.001) in cerebral ganglionic extract injected to ganglia ablated group, as well as (0.3200 ± 0.087, P<0.01) in cerebral ganglionic extract injected to intact control group on 2<sup>nd</sup> day. On the 6<sup>th</sup> day, the rate of oxygen consumption showed significant increase (0.2623 ± 0.0381, 44.59%; P<0.05) in ganglia ablated group. On 6<sup>th</sup> the rate also showed similar significant increase (0.2482 ± 0.267, 36.82%, P< 0.01) in extract injected group. While on 12<sup>th</sup> day, the rate of oxygen consumption showed significant increase (0.2313 ± 0.0230, 63.00 % P<0.01) in cerebral ganglia ablated group, and (0.1898 ± 0.187, P< 0.05) in ganglionic extract injected to ablated animals compared to control. But the rated of oxygen consumption does not showed significant change injection of extract to normal control on 6<sup>th</sup> day and 12<sup>th</sup> day.

The rate of oxygen consumption in control group was (0.2506 ± 0.0130), (0.1814 ± 0.0118) and (0.1419 ± 0.0095) on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day, respectively, while the rate of oxygen consumption in ganglionic extract injected to intact animals

group was ( $0.3207 \pm 0.0287$ , 27.97%), ( $0.1628 \pm 0.005$ , 1.25%) and ( $14.36 \pm 0.0094$ , 1.198%) on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day respectively.

## DISCUSSION

The study revealed that, removal of the cerebral ganglia in bivalves causes significant increase in rate of oxygen consumption on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day. The rate of oxygen consumption in ganglionic extract injected to intact animals also caused significant increase on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day to control. But rate showed decrease in injected to cerebralectomised animals compared to cerebralectomised animals. The rate of oxygen consumption in ganglionic extract injected to intact animal (control) showed no significant decrease on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day compared to control. A significant increase in the rate of oxygen consumption in bivalves after cerebralectomised on 2<sup>nd</sup>, 6<sup>th</sup> and 12<sup>th</sup> day and decrease in the rate after injection of ganglionic extract to intact control on 6<sup>th</sup> day, suggest the possibility of feedback mechanism in regulation of oxygen consumption could be because of further stimulation of rate of oxygen consumption after injection of cerebral ganglionic extract to the ganglia removal animals, which is receiving the. The existence of cerebral ganglionic extracts and hence restore or recover the rate of oxygen consumption.

From the results of these experiments, it can be suggested that cerebral ganglia must possess the hormonal factors which is responsible for regulation of oxygen consumption. Injection of cerebral ganglionic extracts to the ganglia removal animals which did restore that the rate of oxygen consumption. An increase in the rate of oxygen consumption following injection of cerebral ganglionic extract to ablated animals which reached the normal intact control; this confirms that the regulating link is not through the nervous input but possibly by neurosecretory. This contention can further be supplemented by the fact that even in intact (normal) control animals, as after injection of extract to animals from control significantly decrease the rate of oxygen consumption than ablated bivalves. Hence, it is concluded that, cerebral ganglia must possess oxygen consumption controlling factor and which is neurosecretory. The integrity of these ganglia is essential in the normal functioning of physiological activities of the bivalve molluscs.

In the earthworm, *Perionyx excavatus*, the rate of oxygen consumption has been suggested to be under the influence of neurosecretory release of one or more hormonal agents from central nervous system (Nagabhushnam and Hanumante, 1977). The brain and subpharyngeal ganglia of the earthworm have shown to be the site of oxygen inhabiting and elevating hormones respectively. The concept of hormonal control of oxygen consumption has been evidenced in number of poikilotherm organism (Kale and Rao, 1973). In crab *Uca pugilator*, two independently activating hormones, regulates the rate of oxygen consumption (1) eyestalk factor regulating oxygen consumption and (2) the removal of most inhabiting hormone which enhances oxygen consumption (Silverthorn, 1975).

In gastropod mollusk, *Onchidium verruculatum*, removal of whole central nervous system or pleuropedal ganglia significantly inhibited oxygen uptake (Hanumante *et al.*, 1980). Replacement of pleurovisceral ganglia in pleurovisceralectomised gastropods recovers the rate of oxygen consumption up to the normal level. Similarly, in freshwater gastropod, *Limnaea stagnalis*, lateral neurohormones stimulates oxidative phosphorylation (Geraerts, 1976). In the present study, on freshwater bivalve, *Lamellidens corrianus*, it is possible that surgical bilateral decerebration and injection of their extracts to bilateral cerebralectomised animals could have resulted in initiation of the release of large quantities of serotonin and catecholamine as stated by Lubet (1970) in entry may be enhancing the role of non-specific stressors (Gold and Ganong, 1977) or neuroendocrine transducers (Wurtman, 1972), there by indicating the endogenous neurosecretory hormone/ hormones involved in regulation of oxygen consumption. This idea gives strength to the fact that the biogenic amines act as neurotransmitters to induce the release of neurohormones from hypothalamic nuclei of vertebrate (Maclead and Lehmeier, 1977) and probably also from those of invertebrate e.g. crustaceans (Fingerman *et al.*, 1974) and bivalve mollusk (Mane *et al.*, 1990) these neurohormones are capable of inducing changes in the neurosecretory materials from cells in the cerebral and visceral ganglia of the bivalve shell fishes (Kapoor, 1986).

Since the presence of these neurohormones in the ganglia of bivalve molluscs have already been established, regulation of oxygen consumption tentatively suggested as one of the physiological roles for these neurohormones in the metabolic economy in case of freshwater bivalve shellfishes.

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