Effect of ganglionic extract and equivalent commercial hormones injections on protein metabolism in freshwater bivalve *Indonaia caeruleus* (Prashad, 1918) during monsoon season.

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Considering the metabolic shifts in freshwater bivalve molluscs, during changes in the environmental parameters, we report here the effect of ganglionic extract and commercial hormones injections induced changes in protein content from mantle, hepatopancreas, gonad and foot 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 the five respective experimental groups are as follows- 1) injection of commercial hormone progesterone 2) injection of ganglionic extract 3) injection of sham operation 4) injection of estradiol and 5) control (normal) for 10 days. The protein estimation in bivalves from all four groups (including control) was measured on 3rd, 6th, and 9th day.

The study revealed that, the protein content was significantly increased from gonad in ganglionic extract injected group and foot in estradiol injected group on 3rd day. The content of protein showed significant decrease from all the tissues in all experimental groups compared to control on 6th day. Whereas on 9th day the protein content increased significantly from gonad and foot in progesterone and ganglionic extract injected groups.

Keywords: - Cerebral ganglionic extract, Progesterone, Estradiol, Protein estimation, Freshwater bivalve.

Introduction

The freshwater mussels are good source for some important nutrients such as proteins, steroids, minerals and vitamins. They have got important roles in food chain since they are consumed by fish, water birds, mammals and reptiles in the river. Certain chemicals found in bivalves are used in the treatment of antithrombotic, extravasation agent, arthritis, ischemic heart disease and hyperlipidemia. Chondroitin is a low molecular compound, a medicine for arthritis, is naturally occurring substance in the body of mussels and is responsible for elasticity of cartilage. Along with glucosamine, Chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis. 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).

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). Any sort of cellular metabolism occurring in body involves one or many different proteins. The proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during metabolism of proteins, amino acids, enzymes and co-enzymes (Harper *et al*., 1978). Protein is a versatile, complex and fragile macromolecule with high molecular weight. It served as fuel to yield energy and also play a vital role in every aspect of the structural and functional characteristics of the organisms.

The reproductive cycle of marine bivalves is closely related to cycles of nutrient storage and use and to environmental factors such as water temperature and food availability (Bayne 1976; Gabbott 1983; Berthelin et al., 2000; Pazos et al. 2003; Ojea et al. 2004; Dridi et al. 2007). Gametogenesis is an energy-demanding process that requires mobilization of nutrients from ingested food or the reserves from the body tissues. In general, when food is abundant, reserves accumulate in the tissues prior to gametogenesis in the form of glycogen, lipid and protein substrates, and subsequently are utilized in the production of gametes when metabolic demand is high (Barber & Blake 1981; Mathieu & Lubet 1993; Brokordt & Guderley 2004).

Materials and Methods

Site selection have been done on the back water of Godavari river for collecting active, healthy and sexually mature bivalves, *Indonaia caeruleus* throughout the year in different seasons. The experimentation has been set up and carried out for 10 days during monsoon season. As soon as after collection of the animals from banks of Godavari river, animals brought to the laboratory and washed with tap water to remove access muddy coarse particles and brushed to remove the sticky mud, fouling fungal and algal biomass. After cleaning the animals of 50-55 mm in shell length were selected and separated in 2-3 small containers having well aerated water and kept them for 24 hours for laboratory acclimatization. No food was given to the animals during laboratory acclimatization and subsequent experimentation.

After laboratory acclimatization, the animals were separated in five (5) different aquaria with sufficient water quantity (11-12 liter) and aeration for providing oxygenated water to every animal. Each group was having 20-25 animals and water has been changed twice in a day with regular interval of 12 hours approximately and at the same time spawning, behavior and mortality if any observed on every day of experimentation. Injections were prepared before every experimentation i.e. commercial hormone injection progesterone and estradiol 0.1 mg/ml respectively; injection of cerebral ganglionic 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 supernatant collected for injecting purpose; sham operated injection was prepared by using 1:1 solvent (i.e. ice cold distilled water and ethanol) used for dilution of other experiment injections. The control (normal) group has been kept as it is for comparing with the other injected (experimental) groups. After separation of animals in five groups, the aquaria were labeled and the animals injected with commercial hormones progesterone, estradiol, sham operated control with 0.1µl quantity; except ganglionic extract injection group, it was injected by 0.2 µl quantity (0.2 µl extract/animal *i.e.* equivalent to 2 ganglia/animal).

The five respective experimental groups are as follows- 1) injection of commercial hormone progesterone 2) injection of ganglionic extract 3) injection of sham operation 4) injection of estradiol and 5) control (normal). After injecting each group on 1st day of experiment, the protein estimation has been done on 3rd, 6th, and 9th day respectively and every time individual 2-3 animals dissected carefully to remove anterior and posterior adductor muscles; animal taken out from shell valve and blotted on filter paper and weighed on weighing balance. Then different tissues – mantle, hepatopancreas, gonad and foot were separated from animal body and crushed well the same tissues for intermixing and facilitate weighing. 100 mg of each tissue have been taken for estimating protein. Protein has been estimated by Lowry's et al., (1951) method by using Bovine serum albumin (BSA) as a standard. All values were subjected to statistical analysis; significance as well as percentage differences were also calculated for experimental group with compare to the intact control.

Results

The results of the experiments were shown in (Fig. 1- 4 and table 1). The physico-chemical characteristics of the water used in experiments during monsoon season were – Temperature (24.0^{0}C- 29.0^{0}C); pH (8.0-8.35); hardness in terms of bicarbonate (125- 132 ppm) and dissolved oxygen content (5.25 – 7.20 mg/l/h).

During monsoon season, on 6th day, the protein content from mantle was significantly decreased (4.866 ± 0.29, 30.98 %, P < 0.05), (2.972 ± 0.1454, 72.13 %, P < 0.001) from gonad and (8.635 ± 0.29, 42.11 %, P < 0.001) from foot in hormone progesterone injected group. On 9th day, in same group, the content of protein increased significantly (6.6053 ± 0.2895, 35.74 %, P < 0.01) from hepatopancreas, (11.302 ± 0.3069, 152.31 %, P < 0.001) from gonad and (12.0173 ± 0.3728, 113.09 %, P < 0.001) from foot respectively. In ganglionic extract injected animals, the protein content on 3rd day, decreased significantly (3.0296 ± 0.1668, 25.97 %, P < 0.05) from mantle and significant increase found (9.408 ± 0.3342, 44.55 %, P < 0.001) from gonad. Whereas on 6th day, the content decreased significantly (3.165 ± 0.2339, 55.10 %, P < 0.001) from mantle, (3.5513 ± 0.1459, 57.67 %, P < 0.001) from hepatopancreas, (3.706 ± 0.29, 65.25 %, P < 0.001) in gonad and (5.156 ± 0.29, 65.43 %, P < 0.001) from foot respectively. The protein content on 9th day, increased significantly (6.3156 ± 0.2895, 60.38 %, P < 0.001) in mantle (7.127 ± 0.3069, 59.11 %, P < 0.001) from gonad and (8.4803 ± 0.1728, 50.38 %, P < 0.001) from foot respectively. The protein content during monsoon, in hormone estradiol injected animals, on 3rd day, decreased significantly (4.9046 ± 0.2344, 24.64 %, P < 0.01) in gonad and significantly increased (12.2773 ± 0.1029, 58.11 %, P < 0.001) from foot. On 6th day, the content decreased significantly (5.156 ± 0.19, 26.86 %, P < 0.01) from mantle, (4.7693 ± 0.2429, 43.16 %, $P < 0.01$) in hepatopancreas, (7.5716 ± 0.1474, 28.99 %, $P < 0.01$) in gonad and (11.534 ± 0.0795, 22.68 %, $P < 0.01$) from foot respectively. On 9th day, the protein content decrease significantly (2.547 ± 0.29, 43.14 %, $P < 0.01$) from gonad. **Table.1**

Fig.1

Sr. No.	Seasons	Months	Temperature (0C)	pH	Hardness (ppm)	Dissolved Oxygen content (mg/lit.)
1	Monsoon	August	27-29	8.0-8.15	125-132	5.25-6.19
		September	24-26	8.2-8.35	130-142	6.45-7.20

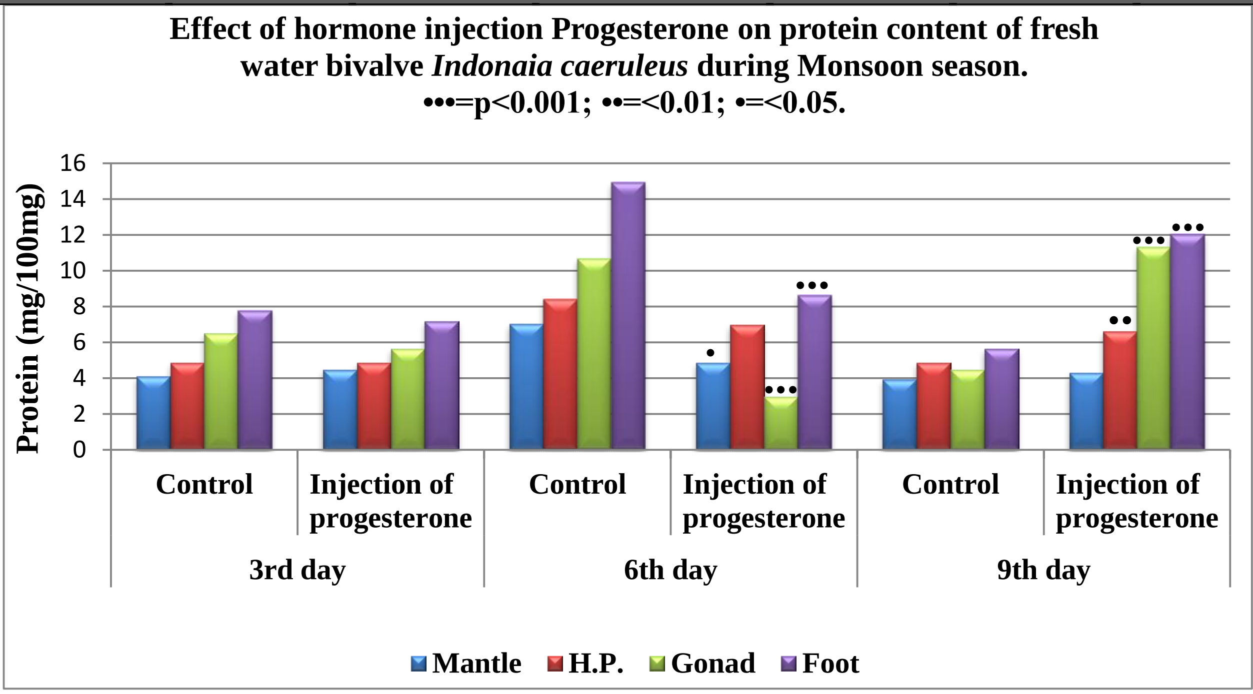
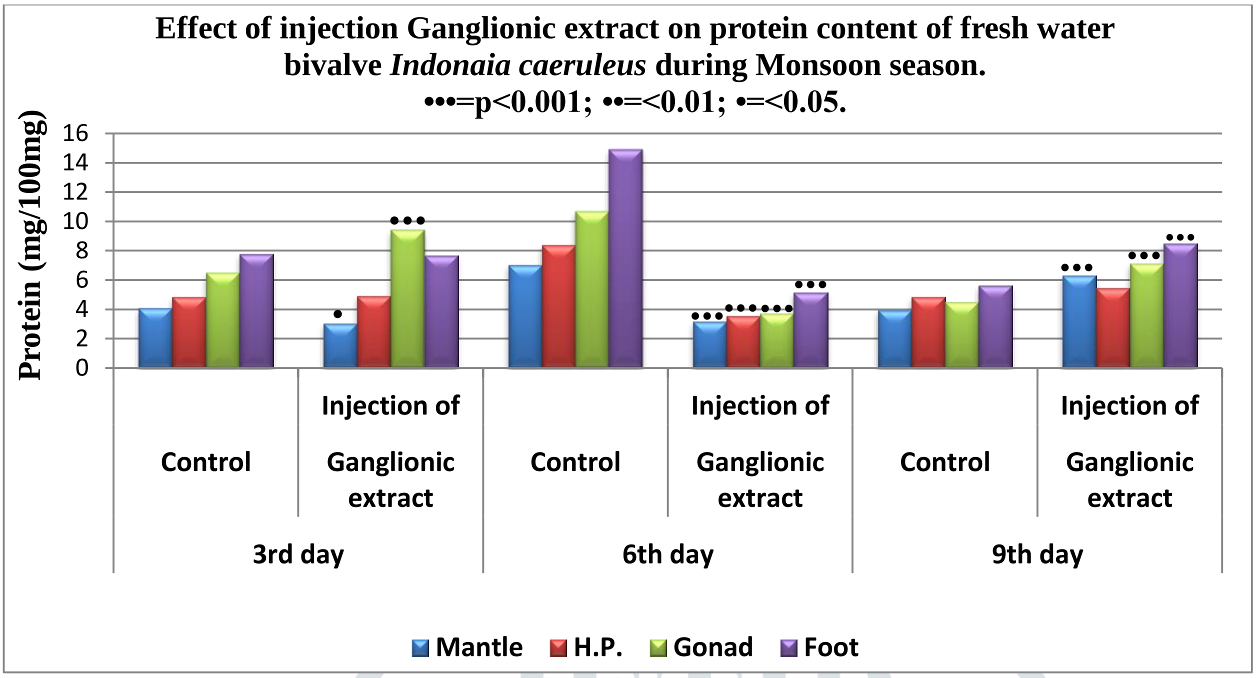
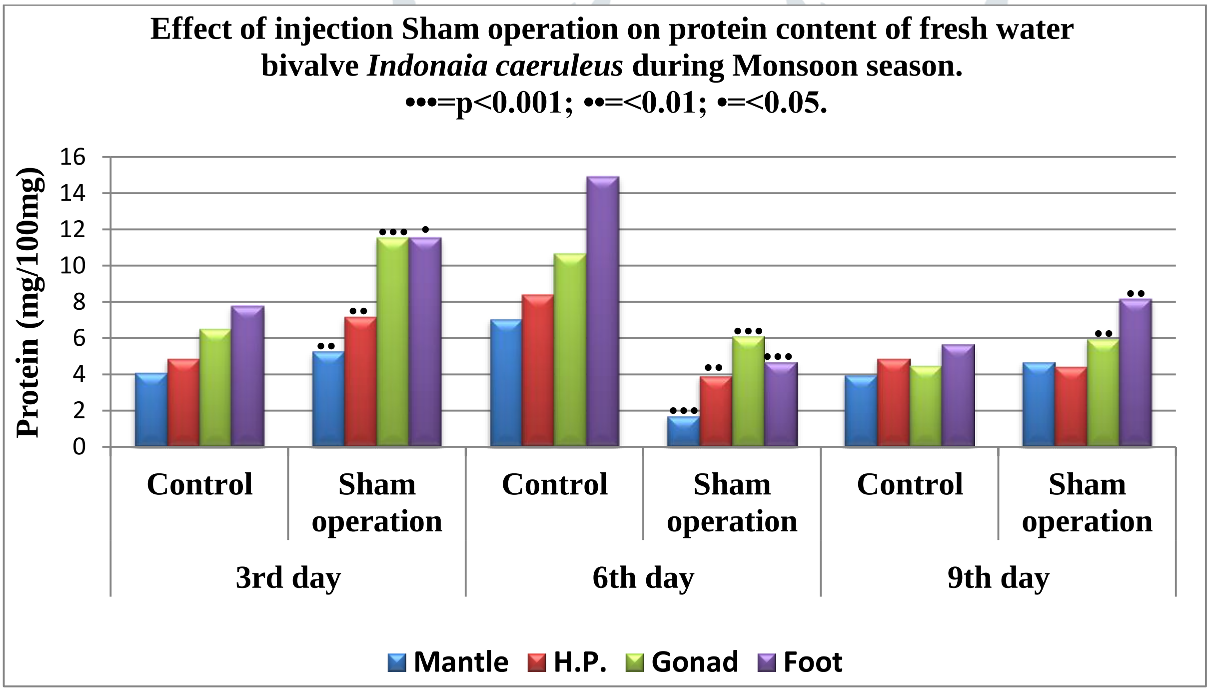


Fig.2



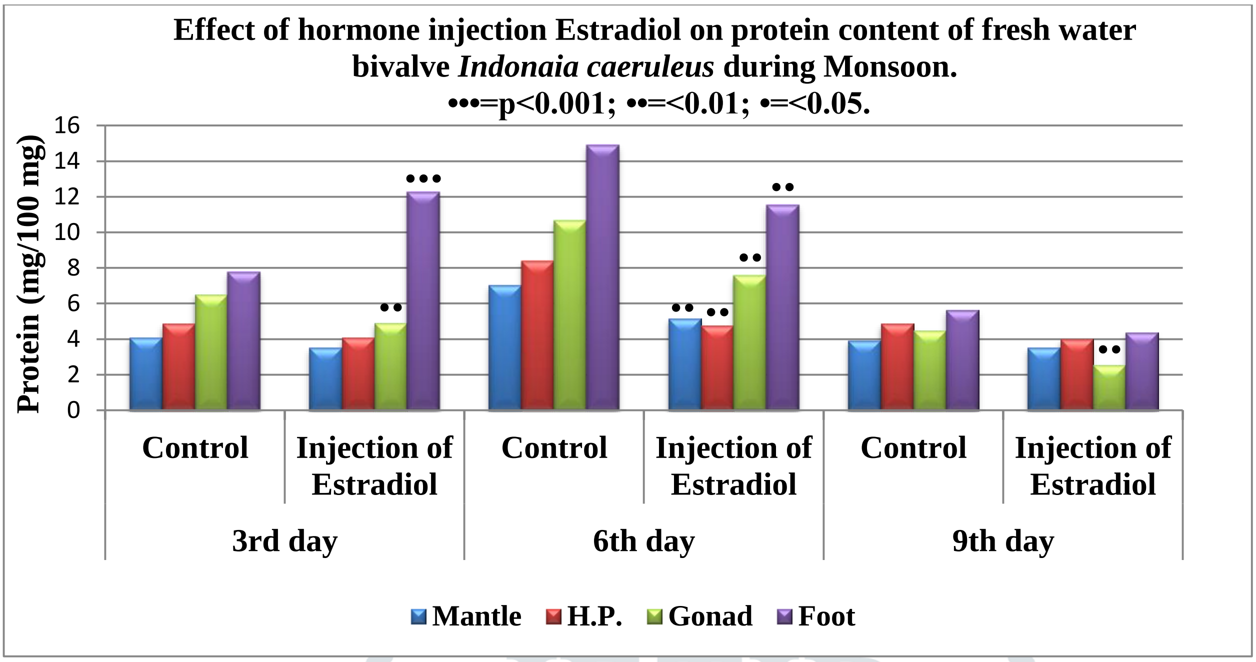
Effect of injection Ganglionic extract on protein content of fresh water bivalve *Indonaia caeruleus* during Monsoon season.
•••=p<0.001; ••=<0.01; •=<0.05.
Protein (mg/100mg)
16
14
12
10
8
6
4
2
0
Control
Injection of Ganglionic extract
Control
Injection of Ganglionic extract
Control
Injection of Ganglionic extract
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

Fig.3



Effect of injection Sham operation on protein content of fresh water bivalve *Indonaia caeruleus* during Monsoon season.
•••=p<0.001; ••=<0.01; •=<0.05.
Protein (mg/100mg)
16
14
12
10
8
6
4
2
0
Control
Sham operation
Control
Sham operation
Control
Sham operation
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

Fig.4



Discussion

Biochemical components such as protein, carbohydrates and lipids are very essential for body growth and maintenance. Protein in tissues occurs in the form of amino acids and other metabolites, which serves as building blocks. The energy gain or depletion from the body is due to the changes in the amount of protein content of tissues (Gonzalez et al. 2001). In *Indonaia caeruleus* protein content is also build up in mantle, hepatopancreas, gonad and foot. The highest accumulation is seen in foot. Thus, the depot tissue, hepatopancreas and germinal or reproductive tissue gonad stores more organic reserves in monsoon.

Metabolism of glycogen, lipids and proteins in the liver may be under the control of estradiol (Mori et al., 1972a, b). Moreover, Beninger et al. (2003) demonstrated a nutrient pathway from the digestive system to the gonads and such nutrient transfer may involve changes in the metabolic activity of the digestive gland. Sowmyashree Shetty et al., (2013) have demonstrated that, there is significant variation in the biochemical constituents in the bivalves according to seasonal changes. The nutritional composition of the bivalves can be affected by external (exogenous) factors, such as fluctuations in the environmental conditions (temperature and food availability), or by internal (endogenous) factors, such as metabolic and physiological activities (S. Brazao et al., 2003).The spawning cycle and food supply are the main factors responsible for this variation. It is well known that seasonal variations in nutritional contents of adult bivalves are closely linked to the reproductive cycle and climate changes and are affected by the availability and composition of the natural diet (M.J. F. Reiriz et al., 1996 and M. Caers et al., 2000). On the basis of their results, the authors stated that, the freshwater mussels are good source for some important nutrients such as proteins, steroids, minerals and vitamins. They have got important roles in food chain since they are consumed by fish, water birds, mammals and reptiles in the river. Certain chemicals found in bivalves are used in the treatment of antithrombotic, extravasation agent, arthritis, ischemic heart disease and hyperlipidemia. Chondroitin is a low molecular compound, a medicine for arthritis, is naturally occurring substance in the body of mussels and is responsible for elasticity of cartilage. Along with glucosamine, Chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis.

During spawning season, energy requirements are met by proteins and fats to a greater extent as compared to carbohydrates (Rodriguez-Astudillo *et al*., 2005). This is because; gonads consist mainly of protein and fat in bivalves (Pieters *et al*., 1980). Protein and fat content increases during gametogenesis whereas it decreases after spawning (Wolowicz *et al*., 2006).

Conclusion

Due to injection of commercial hormones and ganglionic extract, protein content from most of the tissues increased in monsoon. It can be concluded that, protein and glycogen contents in hepatopancreas and gonad decreased in monsoon due to injection of progesterone, estradiol and ganglionic extract suggesting that these reserves are utilized during maturation of gametes.

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.

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