Effect of commercial hormones and ganglionic extract injections on glycogen metabolism in freshwater bivalve Indonaia caeruleus (Prashad, 1918) during summer season.

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ABSTRACT

Considering the importance of neuroendocrine control on the metabolic activities of freshwater bivalves, we report here the effect of injections of cerebral ganglionic extract and equivalent commercial hormones (Progesterone & Estradiol) on glycogen metabolism of freshwater bivalve mollusc *Indonaia caeruleus* (Prashad, 1918) from Godavari River. During summer 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 glycogen estimation in bivalves from all four groups (including control) was measured on 3rd, 6th, and 9th day. The study revealed that, the glycogen content was significantly decreased from mantle in progesterone and from mantle as well as gonad in ganglionic extract and estradiol injected group on the 3rd day. The content of glycogen also showed significant increase from mantle, hepatopancreas and gonad more in progesterone, ganglionic extract injected and slightly in estradiol injected animals on 6th day. Whereas the content of glycogen on 9th day increased significantly in mantle of ganglionic extract injected and mantle as well as gonad in estradiol injected group.

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

Introduction

In some marine and freshwater systems, bivalve molluscs are dominant filter-feeders that make up most of the biomass and exert control over ecosystem structure and function (Dame, 1996; Strayer et al., 1999). These bivalves also can be important filter feeders and in addition can directly impact benthic processes as they burrow through sediments. One reason to predict that freshwater bivalves influence ecosystem processes is that in marine and estuarine systems, both epifaunal and burrowing bivalves have been shown to have large ecosystem impacts.

These bivalves are filter feeder, these bivalves in temperate climate undergo seasonal cycles in their physiological condition in association with their reproductive cycle and the nutritional requirement of these animals may also vary seasonally with the changing reproductive condition (Kreeger, 1993). Studies on changes in biochemical constituents in relation to the reproductive cycle in bivalve molluscs have been carried out extensively and have reviewed by many workers (Bayne, 1976; Sastry, 1979). Though

considerable data is accumulating on several aspects of endogenous and exogenous regulation in reproduction and energy metabolism in bivalve molluscs, the data appears to be restricted exclusively and specially for marine dioecious species. Very little work on involvement of neurosecretion in reproduction and energy metabolism reported in case of freshwater species (Kulkarni, 1987).

During gonadal maturation marine bivalves showed changes in biochemical composition related to energy storage and utilization, for gamete production (Barber and Blake, 1981, 1985; Ojea et al., 2004; Ruiz et al., 1992). In general, energy is stored prior to gametogenesis in the form of glycogen, proteins and lipids in the mantle and the digestive gland. Glycogen and proteins are used in reproductive organ formation and gamete proliferation, while lipids are very important energy reserves in the oocytes, assuring the viability of larvae (Gallager et al., 1986; Leonardos and Lucas, 2000; Napolitano et al., 1992; Whyte et al., 1991). In mussels glucose is stored as glycogen during the sexual resting stage in special cells, named vesicular and adipogranular cells. These reserves are depleted during gametogenic development since it plays a central role in energetic and metabolic supply of gametogenesis in bivalves (Bacca et al., 2005; Berthelin et al., 2000; Gabbot and Peek, 1991; Lenoir et al., 1989; Ruiz et al., 1992; Zandee et al., 1980).

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 summer 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, according to availability of the animals during different seasons. Water has been changed twice in a day with regular interval of 12 hours 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 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 glycogen 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 glycogen. Glycogen has been estimated by De-zwaan and Zandee (1972) method by using glucose 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.

Result

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 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 changes in the biochemical constituent glycogen from different tissues in control, hormone progesterone injected, injection of ganglionic extract and hormone estradiol injected groups on 3rd, 6th and 9th day during summer season was given in fig. 1- 4. During summer season, on 3rd day, the glycogen content from mantle significantly decreased (4.06 ± 0.1057, 14.59 %, $P < 0.01$) as well as (1.961 ± 0.0727, 21.89 %, $P < 0.05$) from hepatopancreas and significantly increased (1.1823 ± 0.0711, 20.95 %, $P < 0.05$) from foot. On 6th day, the content showed significant increase (5.5313 ± 0.1057, 66.85 %, $P < 0.001$) from mantle, (4.1193 ± 0.1366, 135.43 %, $P < 0.001$) from Hepato -pancreas as well as (4.8473 ± 0.1728, 161.81 %, $P < 0.001$) from gonad and significantly decreased (1.4533 ± 0.0598, 37.71 %, $P < 0.01$) from foot, in hormone progesterone injected animals. In ganglionic extract injected groups, on 3rd day, the glycogen content significantly decreased (4.204 ± 0.0281, 11.57 %, $P < 0.05$) from mantle as well as (3.2303 ± 0.0479, 18.21 %, $P < 0.01$) from gonad. The content on 6th day, showed significant increase (5.1343 ± 0.1039, 54.88 %, $P < 0.001$) from mantle, (3.6367 ± 0.1070, 107.84 %, $P < 0.001$) from hepatopancreas, (4.2463 ± 0.1359, 129.40 %, $P < 0.001$) from gonad and (1.3263 ± 0.0439, 25.67 %, $P < 0.05$) from foot respectively. The glycogen content on 9th day, showed significant increase (3.3573 ± 0.1051, 43.37 %, $P < 0.01$) from mantle in ganglionic extract injected group. Whereas, in hormone estradiol injected animals, on 3rd day, the glycogen content showed significant decrease (4.3563 ± 0.1264, 8.36 %, $P < 0.05$) from mantle, (2.1303 ± 0.0388, 15.15 %, $P < 0.05$) from hepatopancreas, (2.7057 ± 0.1345, 31.50 %, $P < 0.01$) from gonad and (1.2587 ± 0.1464, 15.85 %, P <

0.05) from foot respectively. The content on 6th day significantly increased (3.2557 ± 0.2220, 86.07 %, P < 0.001) from hepatopancreas, (2.8493 ± 0.1360, 53.93 %, P < 0.001) from gonad as well as (1.4363 ± 0.0560, 36.10 %, P < 0.01) from foot respectively. On 9th day, the glycogen content increased significantly (3.1033 ± 0.0839, 32.52 %, P < 0.01) from mantle, (2.7647 ± 0.1108, 18.04 %, P < 0.05)

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

from hepatopancreas and (3.696 ± 0.1261, 45.72 %, P < 0.01) from gonad respectively, whereas significant decrease (0.924 ± 0.0433, 24.55 %, P < 0.01) found from foot in hormone estradiol injected animals.

Table-1

Fig-1

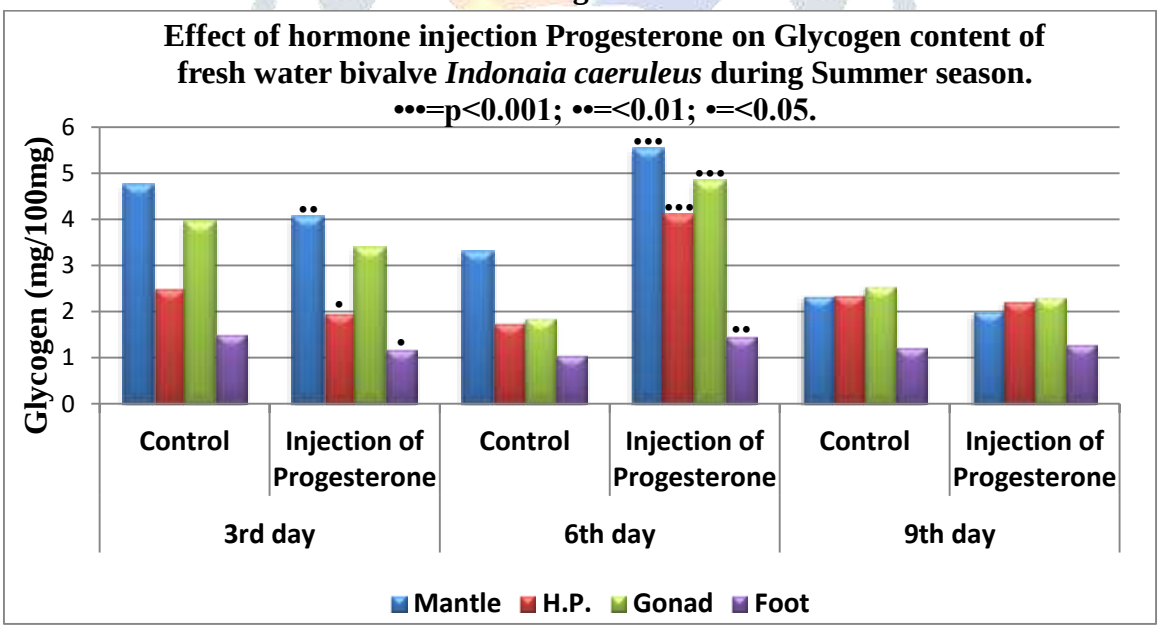


Fig-2

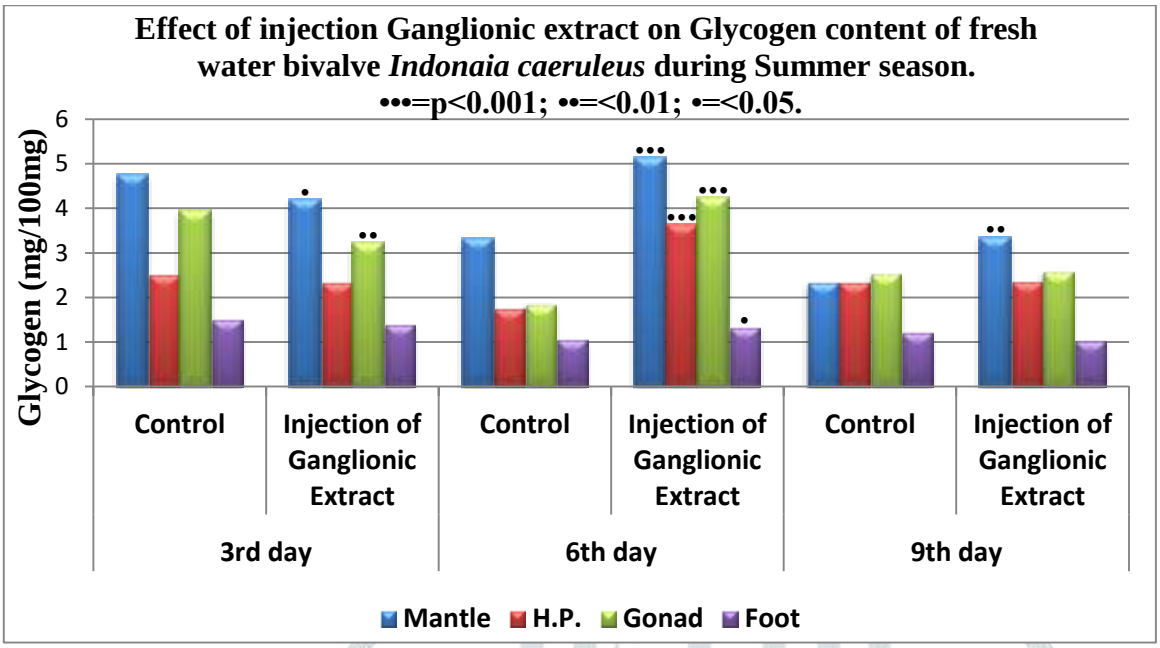


Fig-3

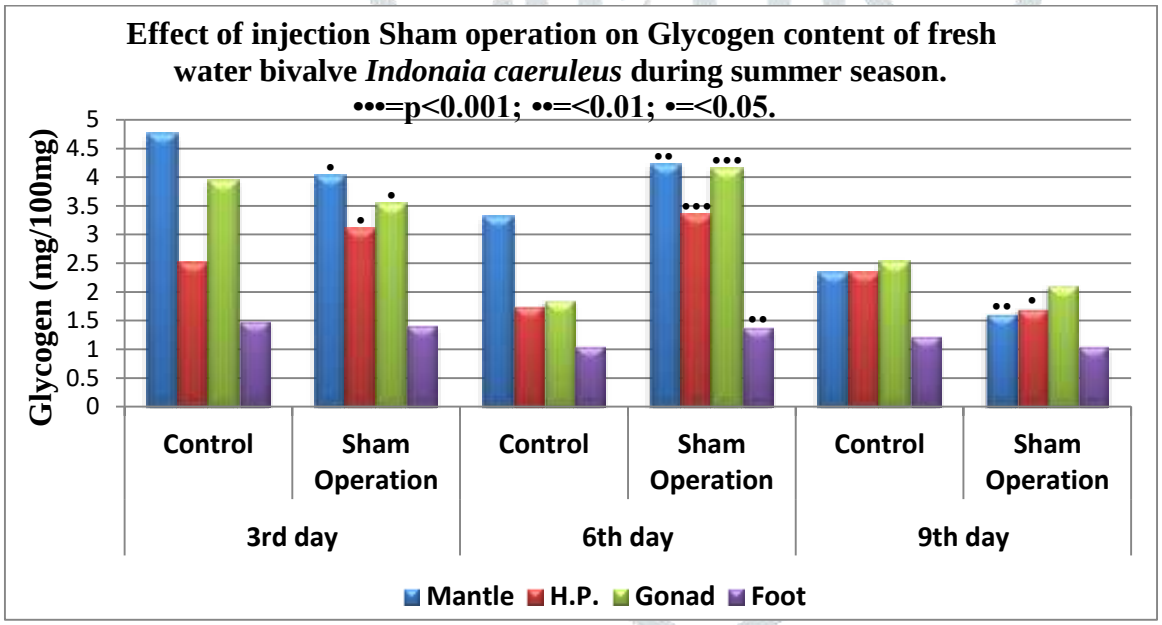
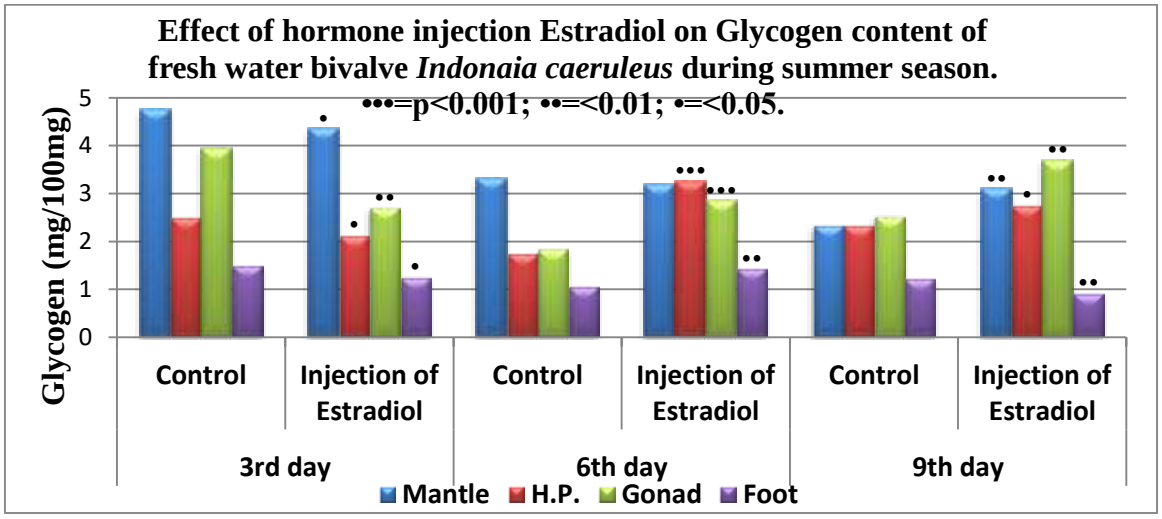


Fig- 4



Discussion

Studies on changes in the biochemical constituents in relation to reproductive cycle in bivalve molluscs have been carried out extensively and has been reviewed by many workers (Bayne, 1976; Sastry, 1979; De Zwaan, 1983; Bishop *et al.*, 1983; Voogt, 1983). A great deal of energy is to be channelized to gonad during reproduction. Assimilated food is distributed to the body tissues by the controlling mechanism of hepatopancreas. Studies on the energy metabolism are concerned with the ways in which the major carbohydrates, lipid and protein fuels are used for an energy production. Almost all the studies show that the seasonal cycles of storage and utilization of organic reserves reflect the complex interaction between food supply and temperature, and between growth and reproductive cycle.

Carbohydrates in the tissues exist as glycogen, free sugars and protein-bound sugars, which serve as energy reserves for various metabolic processes. Among the nutrients, carbohydrates are first degraded for energy production. Even though protein is the major source of energy in animals, rapid depletion of stored carbohydrates primarily in tissues are used for the metabolism. Polysaccharides occur both in free and in bound states along with proteins. The stored glycogen content in tissues is released by anaerobic glycolysis and is utilized to meet the energy requirement when needed (Vijayavel et al. 2006b).

Vitellogenesis is one specific activity that may be controlled by estrogens in the gonad. Li et al. (1998) showed that estradiol stimulated vitellogenesis in the gonad and both Matsumoto et al. (2003) and Osada et al. (2004) confirmed that the vitellogenin gene is primarily expressed in the gonad. Their results suggest that the liver may also be a target organ for estrogens. 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.

Carbohydrates remain at a high level until the beginning of proliferation of gonads. Carbohydrate is found to be at maximum during summer season, which shows the development of gonads to attain maturation. During the rapid proliferation of these gonads, the reserve supply is used, and by the end of the reproductive cycle the amount of carbohydrate is at a minimum. Spawning occurs during the monsoon season which is represented by a large depletion in the protein and lipid contents. Soon after spawning, after a short period of relative inactivity during which the un-spawned sex cells are reabsorbed, the mussels begin to accumulate and store carbohydrates in their tissues. The decrease in carbohydrates with increase in protein has been suggested to be due to conversion of carbohydrates into proteins during gametogenesis (Giese 1967). In *Indonaia caeruleus* similar conversion of glycogen into proteins in body parts like, mantle, gonad and foot is suggestive during summer at the time of gametogenesis.

S. B. Dongre and N. B. Dongre (2012) have observed effect of cerebralectomy on biochemical components from gonad of bivalve *L. corrianus*, and tentatively suggested that the cerebral ganglion in

perhaps elaborate some factors which trigger the metabolic demand and control reproduction. These neurohumors are capable of inducing changes in the neurosecretory cells in the cerebral and visceral ganglia of the bivalve mollusc (Kapoor, 1986). Administration of sex steroids in bivalves may also stimulate gonadal differentiation by accelerating the metabolic rate to provide more materials and energy (Croll and Wang, 2007). 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).

W. Liu et al., (2010) have found that in their study, the glycogen content considerably decreased in all the body components, especially in the gonads with an increase in oocyte diameter during gonad development, suggesting that gametogenesis of *C. gigas* depended largely on the glycogen stored in the tissues. It is generally accepted that glycogen reserves are the main source of energy in bivalves (Kang CK et al., 2000 and Li Q et al., 2000) and also may be utilized for the formation of gametes under conditions of nutrient stress (Barber BJ, Blake NJ 1981 and Beninger PG, Lucas A 1984). The glycogen content was lower in the starved oysters than in the fed oysters, demonstrating that glycogen reserves were quickly mobilized and depleted because of food deprivation. The preferential utilization of glycogen in proportion to other biochemical components is probably a means of protecting against the loss of protein and lipid (the structural components of the animal) (Lane JM 1986). Moreover, a fast decrease in the glycogen content at the start of starvation but a slow decrease thereafter may be a response of the enzymatic machinery for cautious utilization of glycogen reserves to preserve the valuable reserves in case fasting is prolonged (Sa´nchez-Paz A et al., 2007).

Conclusion

Based on the evidence for direct involvement of hormones in mobilization of glycogen reserves in different bivalve molluscs, it has been concluded that the variation in organic reserves due to injection of progesterone, estradiol and injection ganglionic extracts is attributed to the stimulate neurosecretion from cerebral ganglia and the acceleratory neurosecretory product from visceral ganglia is well in different seasons.

References

Bacca, H., Huvet, A., Fabioux, C., Daniel, J.-Y., Delaporte, M., Pouvreau, S., Van Wormhoudt, A., Moal, J., (2005): Molecular cloning and seasonal expression of oyster glycogen phosphorylase and glycogen synthase genes. *Comparative Biochemistry and Physiology* 140B, 635–646.

Barber BJ, Blake NJ. (1981). Energy storage and utilisation in relation to gametogenesis in Argopecten irradians concentricus (Say). *Journal of Experimental Marine Biology and Ecology* 52:121-34.

Barber BJ, Blake NJ. (1981). Energy storage and utilisation in relation to gametogenesis in Argopecten irradians concentricus (Say). *Journal of Experimental Marine Biology and Ecology* 52:121-34.

Barber, B.J. and Blake, N.J., (1985): Intra-organ biochemical transformations associated with

Bayne B.L., (1976): Marine mussels: their ecology and physiology, *Cambridge University press, Cambridge London, New York and Melborne, pp.* 1-495.

Bayne B.L., (1976): Marine mussels: their ecology and physiology, *Cambridge University press, Cambridge London, New York and Melborne, pp.* 1-495.

Beninger PG, Lucas A (1984): Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *J. Exp. Mar. Biol. Ecol.* 79:19–37.

Beninger, P.G., Le Pennec, G., Le Pennec, M., (2003): Demonstration of nutrient pathway from the digestive system to oocytes in the gonad intestinal loop of the scallop Pecten maximus L. *Biol. Bull.* 83–92.

Berthelin C, Kellner K, Mathieu M (2000): Storage metabolism in the Pacific oyster *(Crassostrea gigas)* in relation to summer mortalities and reproductive cycle (west coast of France). *Comparative Biochemistry and Physiology Part B* 125: 359-369.

Bishop, S.H., Ellis, I.L. and Burchan, J.M. (1983): Amino acid metabolism in molluscs. IN: The molluscs vol. I, (Ed. Wilbur, K.M.), Accademic press, New York pp. 244-328.

Croll, R.P., and Wang, C. (2007): Possible roles of sex steroids in the control of reproduction in bivalve molluscs. *Aquaculture*, **272**(1–4): 76–86.

Dame R.F. (1996): Ecology of Marine Bivalves: an Ecosystem Approach. CRC Press, New York.

De Zwaan A. (1983): Carbohydrate catabolism in bivalves. In: "The Mollusca", (Ed- Wilbur, K.M.), Academic Press New York, London, Vol. 1, PP. 137-175.

Gabbot, A.P., Peek, K., (1991): Cellular biochemistry of the mantle tissue of the mussel *Mytilus edulis* L. *Aquaculture* 94, 165–176.

Gallager, S.M., Mann, R., Sasaki, G.C., (1986): Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture* 56, 81–103.

Giese, A.C. (1967). Some methods for study of the biochemical constitutions of marine invertebrates. *Oceanogr. Mar. Biol.*, 5: 253288.

Kang CK, Park MS, Lee PY, Choi WJ, Lee WC (2000): Seasonal variations in condition, reproductive activity, and biochemical composition of the oyster, *Crassostrea gigas* (Thunberg), in suspended culture in two coastal bays of Korea. *J Shellfish Res* 19:771–778.

Kapoor, S.G. (1986): Neuroendocrine studies of freshwater Lamellibranch, Indonaia caeruleus, Ph.D. thesis Marathwada University.113-201.

Kreeger D.A. (1993): Seasonal patterns in utilization of dietary proteins by the mussel Mytilus *trossulus*, *Marine Ecology Progress Series, Vol.* 95: 215 – 232.

Kulkarni D.A., (1987): A study on the reproductive endocrinology of fresh water molluscs. Ph.D. Thesis, Marathwada University, pp. 1-192.

Lane, JM (1986): Allometric and biochemical studies on starved and unstarved clams, *Rangza Cuneata* (Sowerby, 1831). *J Exp Mar Biol Ecol* 95:131–143.

Lenoir, F., Robbins, I., Mathieu, M., Lubet, P., Gabbot, A.P., (1989): Isolation, characterization and glucose metabolism of glycogen cells (=vesicular connective-tissue cells) from the labial palps of the marine mussel *Mytilus edulis*. *Marine Biology* 101, 495–501.

Leonardos, N., Lucas, I.A.N., (2000): The use of larval fatty acids as an index of growth in *Mytilus edulis* L. larvae. *Aquaculture* 184, 155–166.

Li Q, Osada M, Mori K. (2000): Seasonal biochemical variations in Pacific oyster gonadal tissue during sexual maturation. Fisheries Science 66:502-8.

Li Q, Osada M, Suzuki T, Mori K (1998): Changes in vitellin during oogenesis and effect of estradiol 17 on vitellogenesis in the Pacific oyster, *Crassostrea gigas. Invert Reprod Dev*; 33: 87-93.

Matsumoto, T.,Nakamura, A.M.,Mori, K.,Kayano, T., (2003): Molecular characterization of a cDNA encoding putative vitellogenin from the Pacific oyster *Crassostrea gigas. Zoolog. Sci.* 20, 37–42.

Mori, K. (1969): Effect of steroid on oyster-IV: acceleration of sexual maturation in female *Crassostrea gigas* by estradiol-17_. Bull. Jpn. Soc. Sci. Fish. **35**: 1077–1079.

Mori, K., Muramatsu, T. & Nakamura, Y. (1972a): Effect of steroid on oyster-VI. Indoor experiment on the acceleration of glycogenolysis in female Crassostrea gigas by estradiol-17b. *Bulletin of the Japanese Society of Scientific Fisheries*, 38: 1191–1196.

Mori, K., Muramatsu, T. & Nakamura, Y. (1972b): Effects of steroids on oyster-V. Acceleration of glycogenolysis in female *Crassostrea gigas* by estradiol-17b injection under natural conditions. *Bulletin of the Japanese Society of Scientific Fisheries*, 38: 1185–1189.

Napolitano GE, MacDonald BA, Thompson RJ, Ackman RG. (1992): Lipid composition of eggs and adductor muscle in giant scallops (*Placopecten magellanicus*) from different habitats. Marine Biology 113:71-76.

Ojea J, Pazos AJ, Martinez D, Novoa S, Sanchez JL, Abad M. (2004): Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. *Aquaculture* 238:451-68.

Osada M, Tawarayama H, and Mori K (2004): Estrogens in relation to gonadal development of the Japanese scallop, *Patinopecten yessoensis*: gonadal profile and immunolocalisation of P450 aromatase and estrogen. *Comp Biochem Physiol* 139C: 123–128.

Ruiz C, Martı´nez D, Mosquera G, Abad M, Sa´nchez J. L.(1992): Seasonal variation in condition, reproductive activity and biochemical composition of the flat oyster, *Ostrea edulis*, from San Cibran (Galicia, Spain). Marine Biology 112:67-74.

S. B. Dongre AND N. B. Dongre (2012): Effect of cerebralectomy on biochemical content in the gonad of the freshwater bivalve mussel, *Lamellidens corrianus*, in different season. The Bioscan 7(1): 69-72, 2012.

Sa´nchez-Paz A, Garcı´a-Carren˜o F, Herna´ndez-Lo´pez J, Muhlia- Almazan A, Yepiz-Plascencia G (2007): Effect of short-term starvation on hepatopancreas and plasma energy reserves of the Pacific white shrimp (*Litopenaeus vannamei*). *J. Exp. Mar. Biol. Ecol.* 340:184–193.

Sastry A. N., (1979): Pelecypoda (excluding Ostreidea). In: "Reproduction of marine invertebrates", (Eds. Geese A.C. and Pearse J.S.). *Academic press, New York, Vol.*5.pp113-292.

Sowmyashree Shetty, N C Tharavathy, Reema Orison Lobo, Nannu Shafakatullah (2013): Seasonal changes in the biochemical composition of freshwater bivalves, *Parreysia spp*. From Tungabhadra river, Karnataka. *International Journal of Pharma Sciences and Research (IJPSR). Vol* 4 No 5.

Strayer D.L., Caraco N.F., Cole J.F., Findlay S. & Pace M.L. (1999): Transformation of freshwater ecosystems by bivalves. *Bioscience*, 49, 19-27.

Vijayavel K, Gopalakrishnan S, Balasubramanian MP. (2006b): Biochemical constituents and bioaccumulation as biomarkers in the green mussel *Perna viridis* with reference to silver and chromium toxicity. *Toxicol Environ Chem* 89(2):353-361.

Voogt, P.A. (1983): Lipids: Their distribution and metabolism. IN: "The Mollusca" (Ed. Wilbur, K.M.) Academic Press, New York, London, Vol. 1, pp. 329-370.

Whyte, J.N.C., Bourne, N., Ginther, and N.G., (1991): Depletion of nutrient reserves during embryogenesis in the scallop *Patinopecten yessoensis* (Jay). *Journal of Experimental Marine Biology and Ecology* 149, 67–79.

Zandee, D. I., Kluytmans, J. H. and Zurburg, W. (1980): Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. *Neth. J. Sea Res.* 14:1-29