Think India Journal
ISSN: 0971-1260 Vol-22, Special Issue-31
National Conference ETDAB-2019
Held on 23th & 24th December 2019
Organized by: Deptt. of Botany, Deogiri College, Aurangabad, M.S

Changes in the glycogen content of freshwater bivalve *Indonaia caeruleus* (Prashad, 1918) with injections of cerebral ganglionic extract and equivalent commercial hormones (progesterone and estradiol) during winter season.

[1]Thorat S. K*,[2]Vedpathak A.N.

[1]Department of Zoology, Smt. G. G. Khadse College, Muktainagar, Jalgaon. (M.S.).

[2,]Molluscan Endocrinology & Physiology Laboratory, Department of Zoology,

Dr.Babasaheb Ambedkar Marathwada University, Aurangabad-431004(M.S.).

Email: thoratsantoshk@gmail.com

Abstract

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, as we know the importance of neuroendocrine control on the metabolic physiology of freshwater bivalves. During winter season, the adult bivalve mollusc, *Indonaiacaeruleus* (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 and foot in all experimental groups, as well as the content increased significantly from gonadal tissue on 3rd day. During 6th day, the glycogen content increased significantly from mantle and hepatopancreas in all experimental groups. On 9th day, the glycogen content decreased significantly from hepatopancreas in progesterone and ganglionic extract injected group; whereas the content increased significantly from mantle in progesterone injected and from foot in ganglionic extract injected group.

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

The freshwater mussels (Order Unionoida) are distributed worldwide in lotic and lentic habitats. As filter feeders, freshwater mussels are ecologically important; they control seston, recycle nutrients, and provide a trophic link between primary producers and predators (Nalepa, Gardner & Malczyk, 1991). It has been known that the diet of suspension feeding bivalves consists mainly of phytoplankton (e.g. diatoms, flagellates) together with other sources of food such as bacteria and detritus debris (e.g. Parrish et al., 1998; Budge et al., 2000). However, the diet varies at different stages of the life history of the bivalve, owing in part to ontogenetic changes in feeding.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.

Glycogen is a major energy source under anaerobic conditions in many bivalves (Hochachika PW, 1976). Various advantages of anaerobic metabolism in bivalves as compared with normal glycolysis have been made clear (Hochachika PW, 1976; De Zwaan et al., 1976). However, because aerobic glycogen catabolism generally has great advantages over anaerobic glycogen catabolism with respect to energy yield, much less energy appears to be made available when glycogen in bivalves is converted anaerobically to various compounds than when it is oxidized aerobically to CO_2 and H_2O. Then, assuming the same amount of work, more glycogen needs to be consumed under anaerobic conditions. This speculation is consistent with the results reported by De Zwaan and Zandee for the mussel, *Mytilus edulis*, under laboratory conditions(De Zwaan and Zandee, 1972).

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 winter 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, theanimalswere separated in five (5) different aquaria with sufficientwater 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 ganglionicextract was prepared in 1:1 ice cold distilled waterand ethanol (*i.e.*20 ganglia in 2ml ice cold distilled water andethanol), 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.

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 winter season were – Temperature (18.0^0C- 24.0^0C); pH (8.13- 8.42); hardness in terms of bicarbonate (112-130 ppm) and dissolved oxygen content (6.10 – 7.45 mg/l/h).

The glycogen content during winter season, in hormone progesterone injected animals, on 3rd day, decreased significantly (2.0929 ± 0.0756, 35.77 %, $P < 0.01$) in hepatopancreas and (0.8923 ± 0.0256, 23.61 %, $P < 0.01$) in foot; whereas significant increase found (3.3196 ± 0.29.38, 39.88 %, $P < 0.05$) in gonad. On 6th day, the content increased significantly (4.3533 ± 0.1271, 102.95 %, $P < 0.001$) from mantle as well as (2.0316 ± 0.0503, 48.73%, $P < 0.01$) in hepatopancreas respectively. The glycogen content on 9th day, increased significantly (3.4506 ± 0.1017, 20.49 %, $P < 0.05$) in mantle, and significant decrease found (2.3553 ± 0.0651, 28.08 %, $P < 0.001$) in hepatopancreas as well as (0.796 ± 0.0359, 26.65 %, $P < 0.05$) in foot respectively as compared to control. In ganglionic extract injected group, the glycogen content on 3rd day, decreased significantly (2.11 ± 0.0659, 34.88 %, $P < 0.01$) in mantle as well as (2.1803 ± 0.0814, 33.08 %, $P < 0.01$) in hepatopancreas and increased significantly (3.9326 ± 0.1018, 53.18 %, $P < 0.001$) in gonad as well as (1.681 ± 0.0765, 31.53 %, $P < 0.01$) in foot respectively. Whereas on 6th day, the content increased significantly (3.407 ± 0.1038, 58.83 %, $P < 0.001$) in mantle, (1.935 ± 0.0408, 41.65 %, $P < 0.001$) in hepatopancreas, (3.232 ± 0.1137, 22.61 %, $P < 0.05$) in gonad and (1.2433 ± 0.0305, 35.34 %, $P < 0.01$) in foot respectively. The glycogen content on 9th day, decreased significantly (2.9953 ± 0.0396, 8.54 %, $P < 0.05$) from hepatopancreas and content found increase significantly (1.541 ± 0.0624, 41.98 %, $P < 0.01$) in foot compared to respective control. The glycogen content from estradiol injected animals, on 3rd day, decreased significantly

Sr. No.	Seasons	Months	Temperature (0C)	pH	Hardness (ppm)	Dissolved Oxygen content (mg/lit.)
1	Winter	December	19-24	8.13-8.27	112-118	6.10-6.80
		January	18-22	8.25-8.42	115-130	6.22-7.45

(2.5306 ± 0.1079, 21.90 %, $P < 0.05$) in mantle, (1.873 ± 0.0532, 42.51 %, $P < 0.001$) from hepatopancreas and (0.989 ± 0.0218, 22.61 %, $P < 0.05$) in foot, whereas significant increase found (4.476 ± 0.1152, 74.45 %, $P < 0.001$) in gonad. On 6th day, the content increased significantly (2.785 ± 0.1018, 29.83 %, $P < 0.05$) from mantle as well as (2.0223 ± 0.0948, 48.04 %, $P < 0.05$) in hepatopancreas. On 9th day, the glycogen content decreased significantly (1.8736 ± 0.0411, 34.57 %, $P < 0.01$) in mantle and (1.926 ± 0.0824, 41.19 %, $P < 0.001$) in hepatopancreas respectively as compared to their controls.

Table.1

Fig.1

Think India Journal
ISSN: 0971-1260 Vol-22, Special Issue-31
National Conference ETDAB-2019
Held on 23th & 24th December 2019
Organized by: Deptt. of Botany, Deogiri College, Aurangabad, M.S

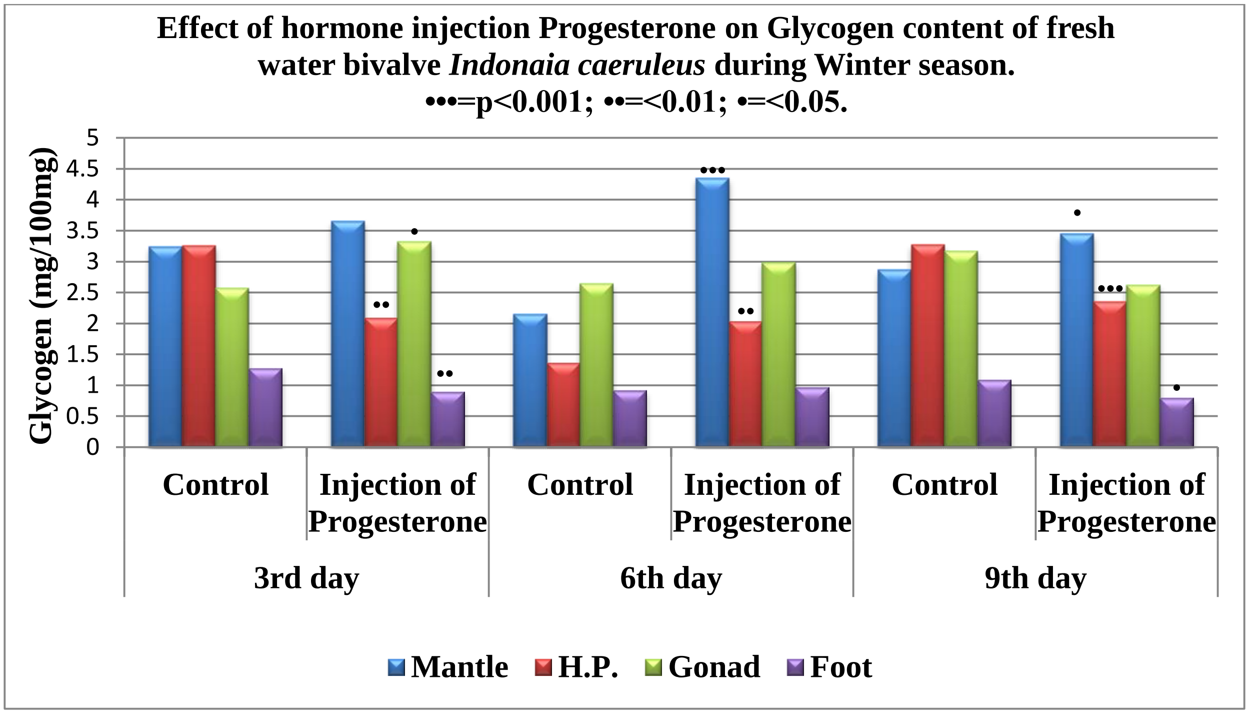


Fig.2

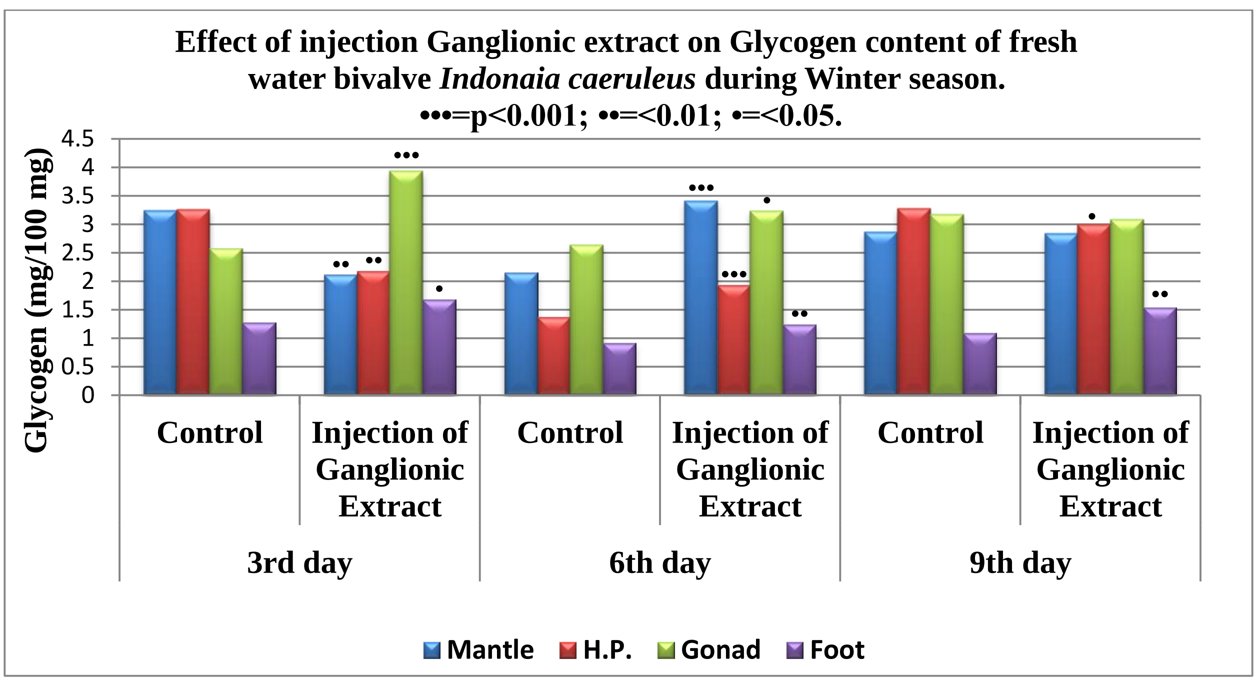


Fig.3

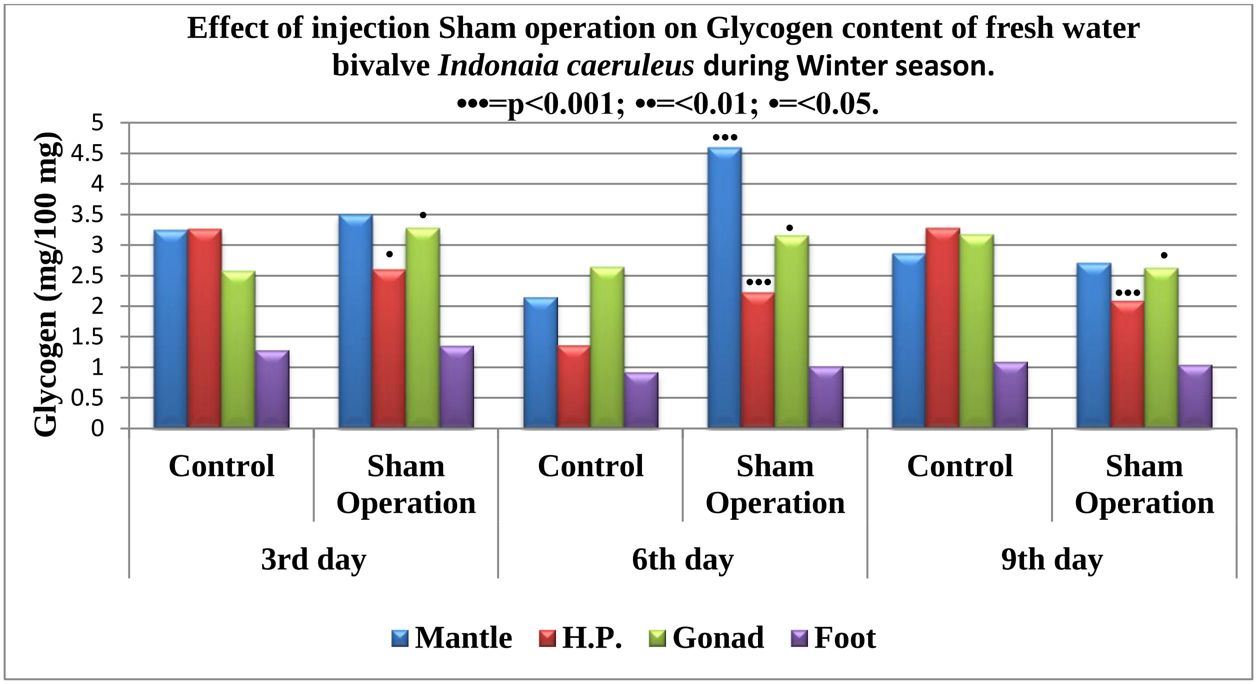
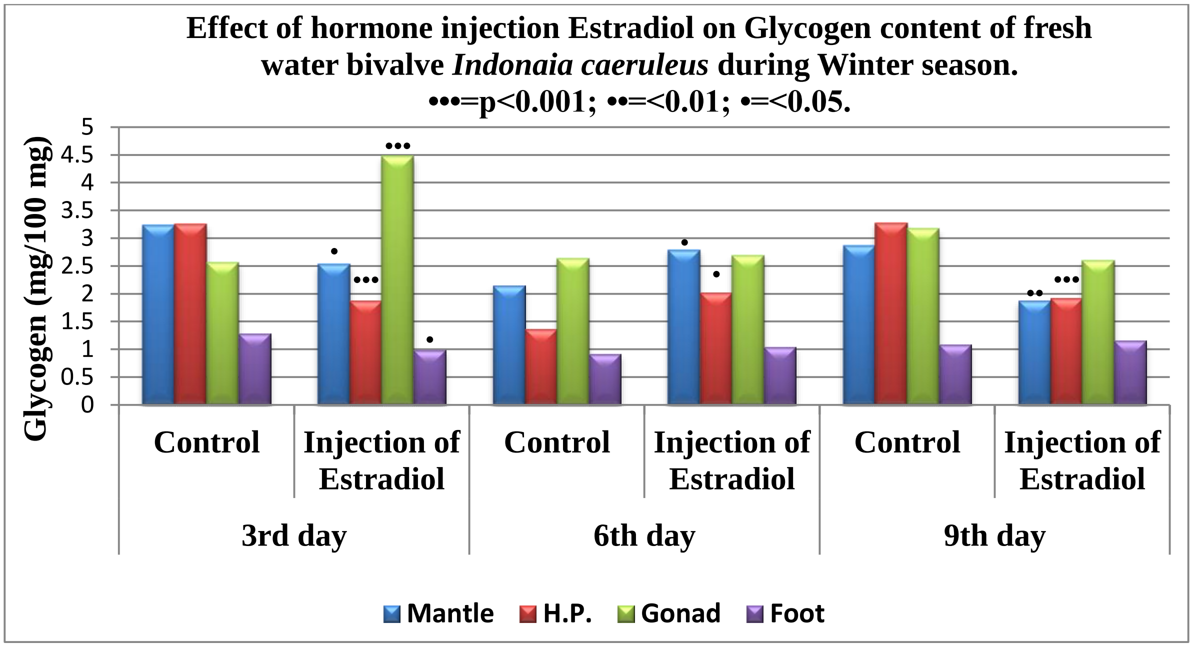

Effect of injection Sham operation on Glycogen content of fresh water bivalve Indonaia caeruleus during Winter season.
•••=p<0.001; ••=<0.01; •=<0.05.
Glycogen (mg/100 mg)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Control
Sham Operation
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

Fig.4



Discussion

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).

In *Indonaia caeruleus*,Seasonal cycle of storage and utilization of glycogen content is closely linked to food supply and gonad development. This content increases in mantle, hepatopancreas, gonad and foot in monsoon due to plenty of food available. The maximum accumulation occurs in hepatopancreas and mantle.Glycogen content from hepatopancreas and mantle as well as lipid content from all the tissues studied showed decrease in these contents during winter compared to monsoon.Metabolism ofglycogen, 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).

Conclusion

Endocrine control of carbohydrate metabolism as it occurs in an organism obviously cannot be considered separately from the metabolism of protein and fats, both of which are potential source of carbohydrates (by gluconeogenesis) and both of which can be formed from carbohydrate residues.Seasonal cycle of storage and utilization of organic constituents have been closely linked to the complex interactions between food supply, growth and reproductive cycle. It can be concluded that, the equivalent commercial hormones and ganglionic extract injections play significant endogenous role to mobilize primary organic constituent i.e. glycogen to the reproductive tissue from source tissues, which are necessary for active gametogenesis.

Acknowledgement

Authoris 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.

References

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.

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.

Budge, S.M., Parrish, C.C., Thompson, R.J., McKenzie, C.H., (2000).Fatty acids in phytoplankton in relation to bivalve dietaryrequirements. In: Shahidi, F. (Ed.), Seafood in health and nutrition.*ScienceTech Publishing Company, St. John's, Canada*. 495–520 pp.

De Zwaan A, Kluytmans JHFM, Zandee DI (1976): Facultative anaerobiosis in Molluscs. *Biochem. Soc. Symp.* 41: 133–168.

De zwaan, A. & Zandee, D.I. (1972): Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis. Comparative Biochemistry.*

Hochachika, PW (1976): Design of metabolic and enzymic machinery to fit lifestyle and environment. *Biochem. Soc. Symp.***41**: 3–31.

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.

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

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

Liu WG, Li Q, Yuan YD, Zhang SH. (2010): Seasonal variations in reproductive activity and biochemical composition of the cockle *Fulvia mutica* (Reeve) from eastern coast of China.*Journal of Shellfish Research* 27:405-11.

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.

Nalepa, T.F., Gardner, W.S. & Malczyk, J.M. (1991): Phosphorus cycling by mussels (Unionidae: Bivalvia) in Lake St. Clair. *Hydrobiologia*, **219:** 239–250. ORION RESEARCH INCORPORATED. 1990. Model 95–12 ammonia electrode instruction manual. 36 pp.

Parrish, C.C., Wells, J.S., Yang, Z., Dabinett P., (1998): Growth and lipid composition of scallop juveniles *Placopecten magellanicus* fed the flagellates *Isochrysis galbana*withvarying lipid composition and the diatom *Chaetoceros muelleri*. *Mar. Biol.* 133, 461–471.

S. Brazao, S Morais, D. Boaventura (2003): Spatial and temporal variation of the fatty acid composition ofpatella spp.(Gastropoda: Prosobranchia) soft bodies and gonads. *Comp. Biochem. Physiol*, 136B: 425-441.

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.

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.

Vokes, H.E. (1980): "Genera of the Bivalvia: A systematic and bibliographic catalogue" (revised and updated), Paleon tal. Res. Inst., Ithaca, New York.