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

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Abstract

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 lipid metabolism 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 lipid estimation in bivalves from all four groups (including control) was measured on 3^{rd}, 6^{th}, and 9^{th} day.

The study revealed that, the lipid content was significantly decreased from all the tissues in progesterone injected group as well as from mantle and hepatopancreas in ganglionic extract injected group. It was also decreased significantly from mantle and gonad from estradiol injected group on 3^{rd} day. The content of lipid showed significant decrease from mantle and gonad in progesterone injected group and in ganglionic extract injected group, it was significantly decreased from mantle and foot, while in estradiol injected group it decreased significantly from mantle and gonad on 6^{th} day. Whereas on 9^{th} day the lipid content increased significantly from hepatopancreas, gonad and foot in progesterone and ganglionic extract injected groups and in estradiol injected group it increased significantly from mantle tissue only.

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

Introduction

A lipid is defined as a water-insoluble biomolecule which has a high solubility in nonpolar organic solvents such as chloroform. The simplest lipids are the fats, which are triesters made up of one glycerol and three fatty acids. The term fats is also used as a general synonym for lipids, so the more precise terms triacylglycerols or triglycerides are preferable for the simplest lipids. Triacylglycerols are used primarily for energy storage in animals. More complex lipids, the phospholipids, glycolipids, and cholesterol, are the major constituents of biological cell membranes. Seasonal changes in biochemical composition have been reported by many workers.

Lipids are an essential organic constituent of the tissues and play a key role in energy metabolism. Next to protein and carbohydrate, lipids are the best energy producers of the body. In the tissues, stored lipids are found in the form of cholesterol, lipoproteins, lipopolysaccharides, and so forth. When there is insufficient energy from the diet, stored lipids in the body may be catabolized to meet energy requirements (Vijayavel and Balasubramanian, 2006).

Lipid contents are directly related to the gametogenic cycle since they play two important roles in the reproductive process: i) as structural constituents in membranes of gonads, and ii) as energy reserves in gametes for embryonic development (Freites et al., 2003; Gabbot, 1983; Narvaez et al., 2008; Ojea et al., 2004; Pazos et al., 1997).

It is well known that neutral lipids are used as energy whereas polar lipids are mainly a component of cell structures (Gadner and Riley, 1972; Pazos et al., 1997; Swift, 1977).

There is general agreement that phytoplankton are the major source of essential fatty acids in the marine environment and that some of the fatty acids or their ratios can be used as biomarkers for different classes of phytoplankton (Pazos et al., 1997; Parrish et al., 1998; Budge et al., 2000). Various authors have discussed fatty acid nutrition and the requirement for ω3 and ω6 PUFA in diets of marine molluscs (Whyte et al., 1989; Robinson, 1992; Berntsson et al., 1997; Pazos et al., 1997; Leonardos and Lucas, 2000; Farias et al., 2003), particularly at the egg and larval stages. Leonardos and Lucas (2000) found that PUFA and ω3 fatty acids were positively correlated with growth of mussel larvae (*Mytilus edulis*) and Berntsson et al. (1997) observed a significant correlation between growth rate and 22:6ω3 content in *oyster* larvae (*Ostrea edulis*). Furthermore, lipid class distribution has been used as an index of growth and viability in larvae of three bivalve species (Gallager et al., 1989) and has been proposed as a condition index for fish and crustacean larvae (Fraser, 1989).

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 approximately 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 lipid 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 lipid. The lipid estimation has been done by sulpho-phospho-vanilline method given by Barnes and Blackstock (1973) and cholesterol used 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, the lipid content in hormone progesterone injected animals, on 3rd day, decreased significantly (2.2006 ± 0.1067, 51.89 %, $P < 0.001$) from mantle, (3.373 ± 0.212, 51.16 %, $P < 0.001$) from hepatopancreas, (4.1503 ± 0.4413, 67.74 %, $P < 0.001$) and (2.314 ± 0.212, 59.13 %, $P < 0.001$) from foot respectively. On 6th day the content decreased significantly (5.5636 ± 0.1238, 55.19 %, $P < 0.001$) from mantle as well as (7.8526 ± 0.2552, 44.35 %, $P < 0.001$) from gonad. On 9th day, the lipid content increased significantly (9.2373 ± 0.2413, 50.71 %, $P < 0.001$) from hepatopancreas as well as (13.2646 ± 0.2472, 115.42 %, $P < 0.001$) from gonad respectively as compare to control. In ganglionic extract injected group, on 3rd day, the lipid content decrease significantly (3.0903 ± 0.1223, 32.44 %, $P < 0.01$) from mantle, (5.8463 ± 0.2447, 15.34 %, $P < 0.05$) from hepatopancreas and significantly increased (14.6776 ± 0.2447, 14.05 %, $P < 0.05$) from gonad. On 6th

day, the content increased significantly (4.8146 ± 0.1243, 34.30 %, $P < 0.05$) from mantle as well as (6.7223 ± 0.1363, 42.55 %, $P < 0.001$) from foot respectively. The lipid content on 9th day, significantly increased (8.46 ± 0.1044, 38.03 %, $P < 0.01$) from hepatopancreas, (16.1616 ± 0.4413, 162.47 %, $P < 0.001$) from gonad and (6.4823 ± 0.0922, 74.61 %, $P < 0.001$) from foot as compared to respective controls. The lipid content in hormone estradiol injected animals, on 3rd day, decreased significantly (3.585 ± 0.0422, 21.62 %, $P < 0.05$) from mantle, as well as (9.2373 ± 0.1223, 28.22 %, $P < 0.01$) from gonad. Whereas on 6th day, the content decreased significantly (5.5353 ± 0.0329, 23.0 %, $P < 0.05$) from hepatopancreas and (7.6126 ± 0.2638, 46.05 %, $P < 0.001$) from gonad. On 9th day, significant increase found in the lipid content (5.2103 ± 0.0547, 40.35 %, $P < 0.01$) from foot

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

compare to respective control.

Table-1.

Fig.1

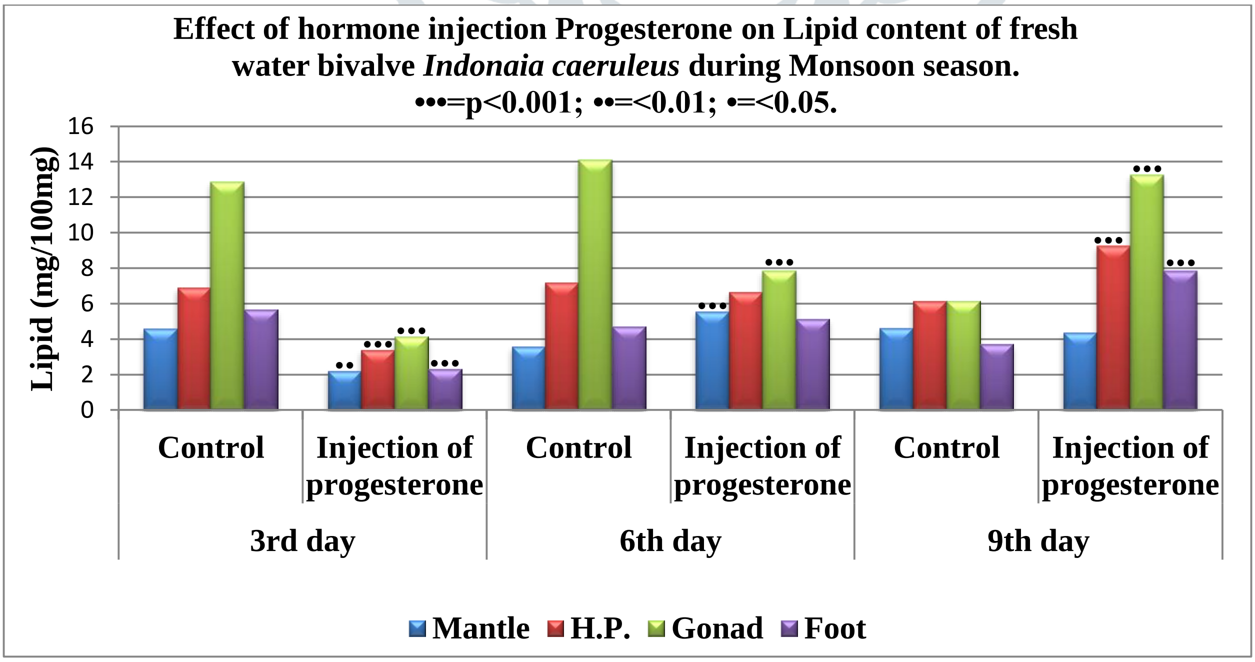


Fig.2

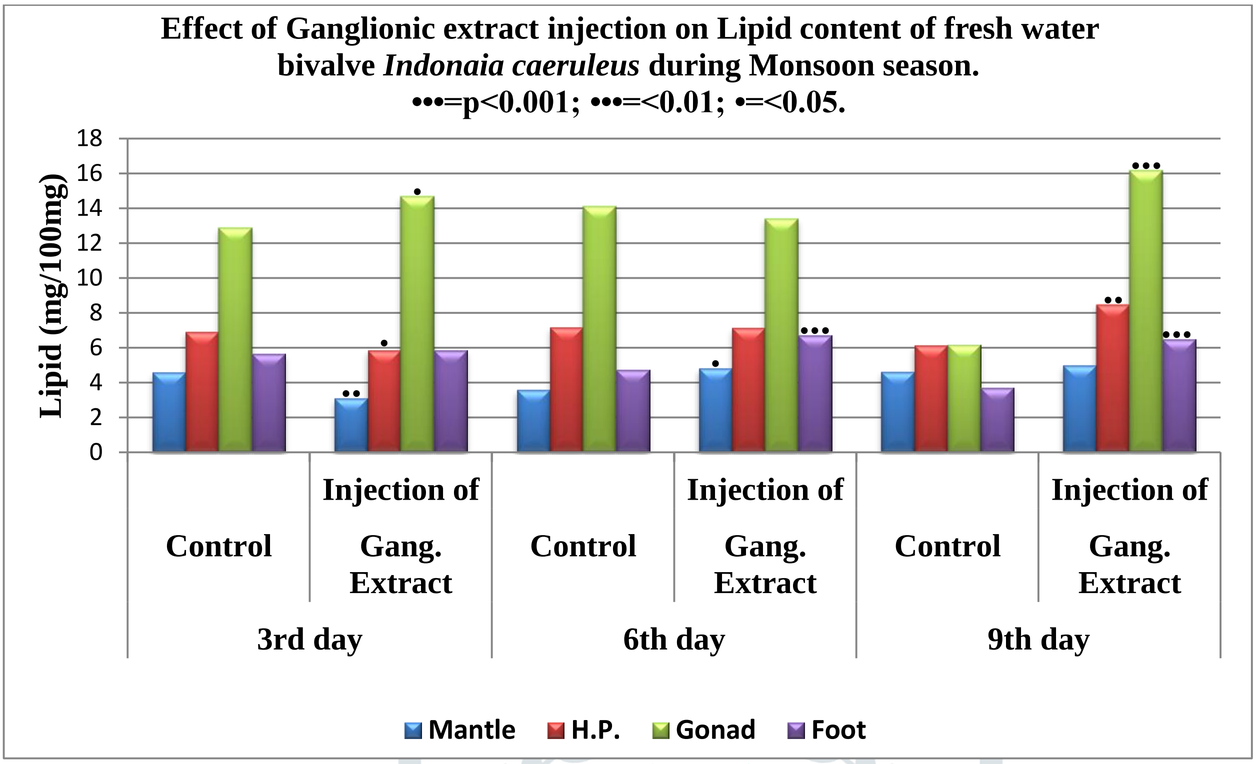

Effect of Ganglionic extract injection on Lipid content of fresh water bivalve *Indonaia caeruleus* during Monsoon season.
•••=p<0.001; •••=<0.01; •=<0.05.
Lipid (mg/100mg)
18
16
14
12
10
8
6
4
2
0
Control
Injection of Gang. Extract
Control
Injection of Gang. Extract
Control
Injection of Gang. Extract
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

Fig.3

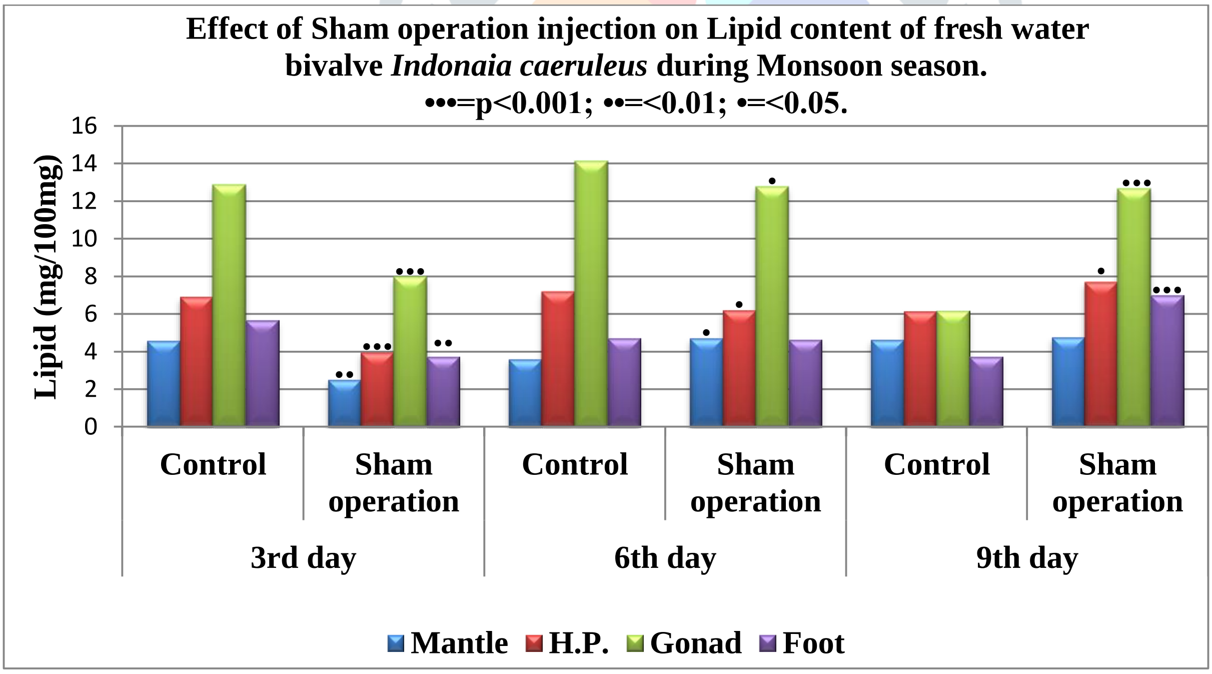
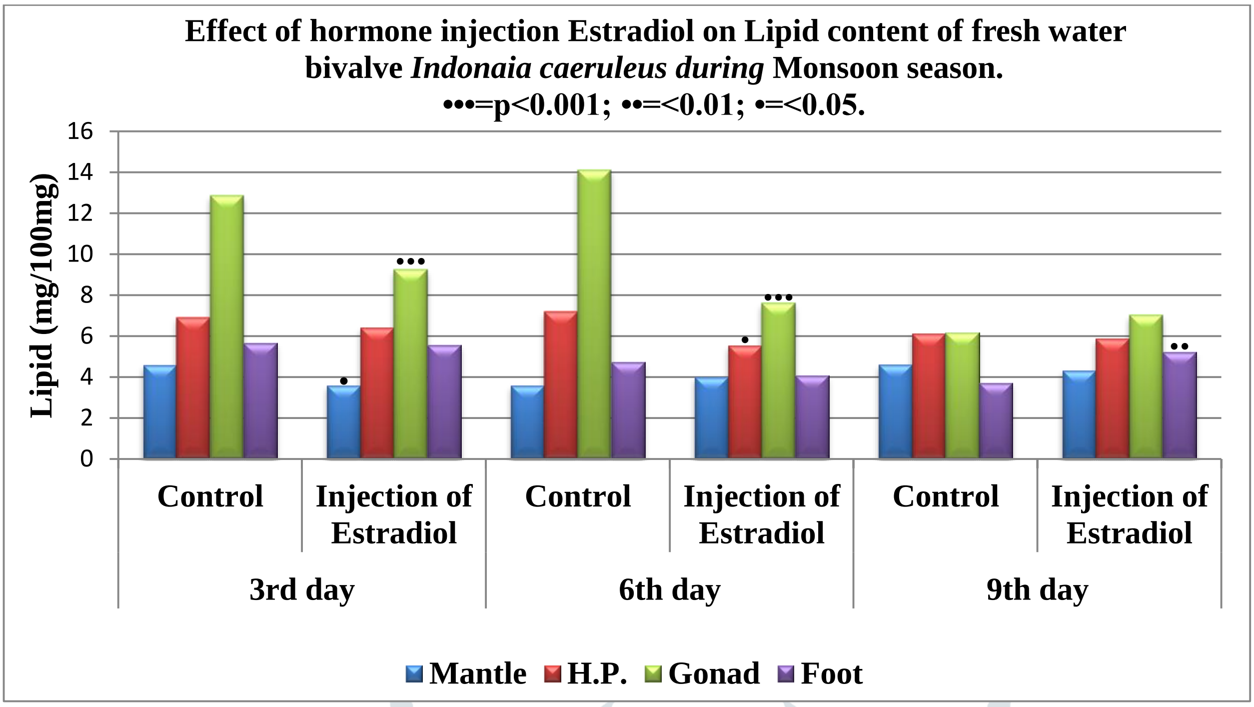

Effect of Sham operation injection on Lipid content of fresh water bivalve *Indonaia caeruleus* during Monsoon season.
•••=p<0.001; ••=<0.01; •=<0.05.
Lipid (mg/100mg)
16
14
12
10
8
6
4
2
0
Control
Sham operation
Control
Sham operation
Control
Sham operation
3rd day
6th day
9th day
Mantle
H.P.
Gonad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

Fig.4



Discussion

Lipids are an essential organic constituent of the tissues and play a key role in energy metabolism. Next to protein and carbohydrate, lipids are the best energy producers of the body. In the tissues, stored lipids are found in the form of cholesterol, lipoproteins, lipopolysaccharides, and so forth. When there is insufficient energy from the diet, stored lipids in the body may be catabolized to meet energy requirements (Vijayavel and Balasubramanian 2006).

In *Indonaia caeruleus* lipid reserve is build up in mantle, hepatopancreas, gonad and foot; the highest being in hepatopancreas and gonad. 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). In *Indonaia caeruleus,* 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.

Several studies on bivalves have indicated that lipids play an important role in the physiology of marine animals particularly during the reproductive process (Pollero et al. 1979; Besnard et al. 1989; Park et al. 2001; Ojea et al. 2004). H. Yan et al (2010) observed that, the lipid content of the female gonads of *S. constricta* showed higher levels before mass spawning occurred, and then markedly decreased. Seasonal variations in lipid content of the female gonads are inversely related to glycogen content, demonstrating that a part of glycogen may be converted to triglycerides during gametogenesis which is in agreement with previous data (Gabbott 1983; Napolitano et al. 1992). The total lipid content has been considered to be one of the principal energy sources used by the larvae during embryonic and early larval stages of the bivalves (Holland 1978; Gallager & Mann 1986; Fraser 1989; Couturier & Newkirk 1991), so it may be a good index of gonad maturity (Pazos et al. 1997).

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

Monsoon is the period of active gametogenesis due to abundant food availability and already biochemical reserves have been moved from depot tissues to gonad. It can be concluded that, progressive gametogenesis is occur because of sufficient amount of biochemical reserves converted to lipid, which is necessary for gamete formation and spawning purpose. As it is having more caloric or energy generating efficiency than protein and glycogen. Hence, commercial hormone and ganglionic extract play significant role by increasing metabolism with the help of neurosecretion to mobilize as well as interconvert significant biochemical reserves to reproductive tissues.

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