PHOTOTHERMAL MANIPULATION OF GONAD RECRUDESCENCE IN *TRICHOGASTER LALIUS* (HAMILTON, 1822)

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of the requirements
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By

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DEDICATED

TO

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Dated : 30th June, 2017

CERTIFICATE

Certified that the dissertation entitled **"PHOTOTHERMAL MANIPULATION OF GONAD RECRUDESCENCE IN *TRICHOGASTER LALIUS* (HAMILTON, 1822)"** is a bonafide record of independent research work carried out by **Mr. Pranaysree Pravat Kumar** during the period of study from August, 2016 to June, 2017 under our supervision and guidance for the degree of **Master of Fisheries Science (Aquaculture)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

उपयुक्त फोटोपेरियोड और तापमान अग्रिमों या देरी की परिपक्वता के तहत मछली को बीज की मांग को पूरा करने के लिए और मछली पालन में वृद्धि के लिए आवश्यक होने पर किया जाता है। जननांग पुनरुत्पादन प्रक्रिया को आगे बढ़ने से, एक वर्ष में एक ही परिपक्व मछली से परिपक्व अंडे को बार बार प्राप्त करने का अवसर मिलता है, जो बालीअवधि बढ़ाता है और प्रति इकाई शरीर के वजन में, अंडे का उत्पादन बढ़ाता है। ड्वार्फ गौरामी, *त्रिचीगास्टर लालिअस* में, जननांग पुनरावृत्ति की प्रगति, को पहली बार स्पानिंग अवधि बढ़ाने और फोटो-थर्मल हेरफेर के माध्यम से, कैद में बीज उत्पादन बढ़ाने के लिए प्रयास किया गया है। इसके अतिरिक्त, विस्तारित परिपक्वता, मछलियों की सजावटी गुणवत्ता को समृद्ध करने के लिए पुरुष मछलियां में शानदार रंगों के लगातार लंबे समय तक सुनिश्चित करेगा। अध्ययन, फोटो-थर्मल हेरफेर के माध्यम से गोनैड रिक्रूससेंस को आगे बढ़ाने के उद्देश्य से किया गया था। पूरी तरह से यादृच्छिक डिजाइन (सीआरडी) के बाद, 90 दिनों के लिए परिवेश या इसके बाद के परिवेश के तापमान के साथ मछलियों को लंबे समय तक फोटोपेयोडिड (16 एल / 8 डी और 14 एल / 10 डी) या प्राकृतिक फोटोपेरियोड (एनपी) के संपर्क में रखा गया था। प्राकृतिक और लम्बी फोटोपेरोड के तहत, समय की प्रगति के साथ गोंनाडो-दैहिक सूचकांक (जीएसआई), विवेक और डिम्बाणु जनकोशिका के व्यास में अलग-अलग वृद्धि हुई। परिणाम बताते हैं कि 3 महीने लगातार लंबे दिनों (16 एल / 8 डी या 14 एल / 10 डी) और परिवेश के तापमान में दो महीनों के लिए डिम्बाणु जनकोशिका परिपक्वता में उन्नति हुई और मध्यम लंबे फोटोपेरियोड (14 एल / 10 डी) अकेले या उपरोक्त परिवेश तापमान के साथ संयोजन में सबसे अधिक था, कैद में, *त्रिचीगास्टर लालिअस*, प्राकृतिक मिलटिंग अवधि से कम से कम 2-3 महीने पहले वृषण परिपक्वता प्राप्त करने के लिए उपयुक्त हो जाता है। प्लाज्मा सेक्स स्टेरॉयड जैसे कि 11-केटो टेस्टोस्टेरोन (11-केटी), 17β-एस्ट्रैडियोल (ई-2) और 17α, 20β-डायहाइड्रॉक्सी प्रोजेस्टेरोन (डीएचपी), एकाग्रता में परिवर्तन के साथ सभी समूहों में डिम्बाणु जनकोशिका परिपक्वता की प्रगति के साथ भिन्न होता है। प्लाज्मा ई-2 के दृढ़ता से सभी समूहों में, वृद्धि के साथ डिम्बाणु से सम्बन्ध था और विभिन्न फोटो-थर्मल शासनों के साथ अलग-अलग चरम मूल्यों का प्रदर्शन किया गया और पाया गया कि 11-केटी शुक्राणु जनन की प्रगति में एक भूमिका निभाता है, जबकि प्लाज्मा डीएचपी स्तर को नर में स्पेर्मिएशन और मादा में डिम्बाणु जनकोशिका कि परिपकव्ता से सम्बंधित पाया गया। गोनाड और डिम्बाणु आवृत्ति वितरण के हिस्टोलॉजिकल स्टेजिंग, गोनैड विकास के अतुल्यकालिक प्रकार की पुष्टि करता है। वर्तमान अध्ययन ने यह निष्कर्ष निकाला कि विभिन्न शासनों के लंबे समय तक फोटोपेरियोडेड, नर और मादा ड्वार्फ गौरामी की उपयुक्तता पर निर्भर करता है।

ABSTRACT

Placing the fish under suitable photoperiod and temperature advances or delays maturity to cater the demand of seed as and when require for grow-out culture. Advancing gonadal recrudescence process provides an opportunity for harvesting ripe eggs repeatedly from same brood in a year which extends spawning period and enhances egg production per unit body weight. Advancement of gonad recrudescence in captivity in dwarf gourami, *Trichogaster lalius* has been attempted for the first time for extending spawning period and augmenting seed production in captivity through photo-thermal manipulation. In addition, extended maturity will ensure long persistent of brilliant color in males for enriching the ornamental quality of fish. The study was designed with an aim of advancing gonad recrudescence through photo-thermal manipulation. The fishes were exposed to either long photoperiods (16L/8D and 14L/10D) or natural photoperiod (NP) in combination with ambient or above ambient temperature in triplicates for 90 days following a completely randomized design (CRD). The gonado-somatic index (GSI), fecundity and oocyte diameter increased with the progress of time under natural and long photoperiod regimes differently. The results indicated that 3 months of constant long days (16L/8D or 14L/10D) and ambient temperature caused advancement in oocytes maturation for two months and the moderate long photoperiod (14L/10D) alone or in combination with above ambient temperature was most suitable to obtain testicular maturity at least 2-3 months earlier than the natural milting period in *Trichogaster lalius* in captivity. The changes in plasma sex steroids such as 11-keto testosterone (11-KT), 17β-estradiol (E-2) and 17α,20β-dihydroxy progesterone (DHP) concentration varied with the progress of gonad maturation in all the groups. Plasma E-2 was strongly related to oocytes growth in all the groups and exhibited peak values differently with various photo-thermal regimes and 11-KT plays a role in the induction and progression of spermatogenesis, whereas plasma DHP level was found associated with spermiation in male and oocytes maturation in female dwarf gourami. Histological staging of gonad and oocytes frequency distribution confirms the asynchronous type of gonad development. The present study concluded that long photoperiod of various regimes advances gonad recrudescence in dwarf gourami depending on the suitability for males and females.

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1. INTRODUCTION

Ornamental fish keeping has emerged as the most popular hobby with millions of enthusiasts worldwide. Ornamental fish is preferred due to their colour, shape and behaviour are often referred as living jewels. They are peaceful, generally tiny, attractively coloured and could be accommodated in confined spaces (Kasiri *et al*., 2012). In the past, it was mainly restricted to members of affluent families but now-a-days it has also become very popular activity among masses across different income groups (Pailan *et al*., 2012). The ever increasing demand for aquarium fishes has made a way towards global trade of ornamental fishes (Jayasankar, 1998) and in recent years, it has boomed as an important trade of multi-million dollar with more than one hundred countries involved (Mukherjee *et al*., 2000).

Since 1985 the value of international trade in exports of ornamentals has increased at an average growth rate of approximately 14 percent per year (FAO, 2013). A considerable growth has been seen from last 4-5 decades, not only in culture but also in diversification in the international trade in ornamental fishes (Pailan *et al*., 2012). The ever-increasing demand for aquarium fishes gradually paved the avenue towards global trade of ornamental fishes. The top exporting countries include Singapore followed by Hongkong, Malaysia, Thailand, Philippines, Srilanka, Taiwan, Indonesia and India. India's share in ornamental fish trade is estimated to be less than 1 percent of the global trade. The largest importer of ornamental fish is the USA importing fish worth over US$ 500 million every year followed by Europe and Japan. The emerging markets are China and South Africa (Biswas *et al*., 2014). Around 2000 species and millions of specimens are traded annually in the ornamental fish trade but only some 30-35 species of freshwater fish dominate the market (Livengood and Chapman, 2007).

India possesses rich resources viz., the lagoons and coral reefs of Lakshadweep and Minicoy Islands, Andaman and Nicobar Islands, Okha-pin tan, Gulf of Kutch complex, Coast of Kerala, Cape Comorin, Gulf of Mannar and Palk Bay are abound with highly attractive and varied species of ornamental fishes (Rani *et al*., 2014). India hosts 11 percent of the global ichthyic diversity of about 31,300

fish species, so far it possesses 930 described ornamental fish species and of these approximately 250 are now available to aquarium hobbyists (Biswas *et al*., 2014). India has recorded at least 150 commercially important indigenous freshwater ornamental fish species collected from rivers (Madhu *et al*., 2009). Prominent among the freshwater Indian ornamentals are Loaches, Eels, Barbs, Catfish and Goby (Ayyappan *et al*., 2006). About 90 percent of ornamental fish is traded from Kolkata port followed by Mumbai and Chennai (Ghosh *et al*., 2003).

India's share of global ornamental fish trade is less than 1 percent but still, she is projected as a "sleeping giant" because of yet untapped potential resources (Benjamin, 2012). Despite the huge potential offered by the rich diversity and environment, export of ornamental fish from India continues to remain negligible. Approximately 267 fish species belonging to 38 families have been identified as ornamental fishes from North East India comprising about 85 percent of the total aquarium fish trade of India (Biswas *et al*., 2014). However, India's marginal position is likely to change as trade is gradually increasing (Ghosh *et al*., 2003). At this moment it is appropriate to delineate India's position and competitiveness in emerging world market of ornamental fishes (Rani *et al*., 2014). Ornamental fish culture is one of the fastest growing segments of the fisheries sector. A considerable growth has been seen in last 4-5 decades, both in culture and in trade of ornamental fishes (Pailan *et al*., 2012). The value of annual trade of ornamental fishes is around US$ 15 million at an annual growth rate of 10 percent (Mohanta *et al*., 2013). Out of the total ornamental fish trade in the world, about 85 percent is contributed by freshwater ornamental fish (Mohanta, 2011).

The Anabantoids are one of these important group of freshwater ornamental fishes which contains about 16 generas and about 50 species and distributed throughout the southern Asia and central Africa (Degani, 2001). One of the major suborder of Anabantoidae is Gourami and *Trichogaster lalius* belonging to the family Osphronemidae is the smallest and popular fish in markets among the Gouramis (Welcomme, 1988). They are characterized by an accessory respiratory organ called labyrinth, which is located near the gills (Degani and Levy, 2013). It is indigenous to India, Pakistan and Bangladesh (en.wikipedia.org). They are found in varieties of colors such as powder blue to flame red. The popularity of the species is

due to its colour and peaceful behaviour in the aquarium. During breeding of this species, female lays the eggs in a bubble, which is built by the male nest using bits of plants, twigs or other debris. Once the nest is built, the male initiates courtship behaviour with the female. Temperature and light are the two main reproductive cues with little seasonal variation in tropical regions (Degani and Levy, 2013). Nest building occurs and eggs are laid in a temperature range of 23-29 ^{0}C or 73-84 ^{0}F (Degani, 1989). This is also the optimum temperature range for conditioning of the brood stock.

Though teleosts exhibit a great variety of reproductive strategies, a general characteristic of most of the teleosts is that the natural reproduction shows a long-term periodicity (Munro *et al*., 1990). Typically, gonad development from puberty leads to fully-grown gonads by the advent of the spawning season; if conditions are appropriate, then final gonad maturation leads to the production of viable gametes during one or more breeding bouts. Teleost fish growth development and reproduction are influenced by daily and seasonally variations of photoperiod and temperature (Falcon *et al*., 2003). Long day lengths stimulates body fattening and ovarian growth while short day lengths are inhibiting in stimulation of this response (Sarkar and Arora, 2001).

Many environmental and physiological factors e.g. temperature and photoperiod have their impact on fish gonadal maturation and spawning (Eyeson, 1983; Emit *et al*., 1989; El-Greisy, 1993 and El-Naggar *et al*., 2000). Ovarian recrudescence occurs at a time when day length begins to shorten and temperature reaches a seasonal minimum, while the ovarian recrudescence coincides with an increase in day length and temperature. Photoperiodism is the ability of an organism to assess and use the day length as an anticipatory cue to time seasonal events in their life histories (Bradshaw and Holzapfel, 2007). Many investigators have studied the influences of environmental factors on reproductive activity in fish, particularly the effects of photoperiod and water temperature on gonadal development (Lam, 1983). In some fishes, the developmental and maturation of gonads is to a great extent dependent on day length, while in others, temperature appears to be the important factor; in still others, both day length and temperature are operative (Poston, 1989; Baggerman, 1980 and Sundararaj, 1981). It has been

observed that photoperiod regulates the axis photoreceptors, central nervous system, hypothalamus, pituitary gonadotropin, ovarian estrogen secretion, oogonial proliferation and endogenous yolk formation (Yaron *et al*., 1980 and Hansen *et al*., 2001).

Body weight and testicular weight gain can be stimulated or inhibited depending on the time of the day when thermo-cycle commences. This interesting observation is similar to the presence of a daily rhythm in photo-responsiveness with sensitive phases occurring between 12-18 hours, after the onset of photoperiod in the case of *Gasterosteus aculeatus* (Baggerman, 1958) and between 16-17 hours and 20-21 hours after onset of photoperiod in *Heteropneustes fossilis* (Vassal and Sundararaj, 1975).

Fish exhibit both seasonal (day length induced) and daily (light / dark cycle influenced) endogenous rhythms indicating that they have an endogenous annual clock which is influenced by light, temperature, habitat conditions, social interaction and population density as well as pollutants. Though the endogenous clock cannot be suppressed by exposure to constant environmental factors it can be advanced or delayed by it. Photoperiod variation regulates the timing of sexual maturation, spawning, GnRH / GTH / sex steroid levels and thyroid hormone profiles in various species.

Gonadal maturation is stimulated or delayed by photoperiod alternations, which in turn is responsible for the change in spawning period or somatic growth (Lam, 1983). In cyprinids e.g. Carp, Catfish and other tropical and subtropical fish, high temperatures and long photoperiods appear to be important for the final maturation of the oocytes, ovulation and oviposition. There is no conclusive evidence to discriminate photoperiodic and temperature effects (Rocha, 2008). Photoperiod is the main proximal factor in the timing of maturation in the temperate fish, although water temperature most likely to play a modulating role. Though the absolute length of the photoperiod is not important for cueing the reproductive events, yet the relative change from short to long or vice versa seems to be critical for the entrainment of the true events (Carillo, 2007).

The knowledge on the physiological basis and ecological implications of the chronobiological approach are of prime importance to aquaculture and it provides the key to produce precociously gravid fish. Almost all fishes use the specific phases of the annually changing cycles of day length and temperature as cues for attaining maturity and spawning. By subjecting a fish stock to a constant photoperiod regimes maturity can be manoeuvred. This can be done by maintaining the fish stock permanently under light proof covers with controlled light and temperature. Now, by placing the fish under required photoperiod and temperature, maturity can be advanced or delayed as per requirement of the farm. By maintaining 3-4 separate brood stock under different yearly thermo-photo cycle's availability of gravid brood stock can be ensured throughout the year for conducting breeding. Thus by manoeuvring control over thermo-photo period multiple breeding and spawning can be undertaken to raise more crop from the same broodstock. Once standardized, this will add a new direction in the seed production industry (Chattopadhyay, 2016). Keeping in mind the importance of the above abiotic factors on the reproductive physiological process of the fish, the present study has been proposed with the following objectives:

1. To study effect of photoperiodic regimes on the gonadal recrudescence of *Trichogaster lalius*.
2. To study effect of temperature on photoperiod induced gonadal growth and maturation of *Trichogaster lalius.*

2. REVIEW OF LITERATURE

2.1. Aquarium keeping

Aquarium keeping is the most popular hobbies with millions of enthusiasts worldwide. In the past, ornamental fish keeping was mainly restricted to members of well to do families but now-a-days it has become ubiquitous and is very popular activity in town and in cities of the world (Pailan *et al*., 2012). Ornamental fish are often referred as living jewels due to their colour, shape and behaviour. They are peaceful, generally tiny, attractively coloured and could be accommodated in confined spaces (Kasiri *et al*., 2012 and Pailan *et al*., 2012) and colouration, which is one of the most important factors deciding the market value (Biswas *et al*., 2014).

2.2. Dwarf gourami

Among the ornamental species, *Trichogaster lalius*, previously classified as *Colisa lalia*, belonging to the Osphronemidae family, is the smallest and most popular among dwarf Gouramis and is widely marketed worldwide (Welcomme, 1988), both in the wild and as red and blue varieties. Being a species of peaceful behaviour and significant beauty, it is much appreciated by aquarists. The blue variety has received the most attention from producers of ornamental fish due to the high prices achieved for both males and females (Talwar and Jhingran, 2001 and Zuanon *et al*., 2013).

This species is one of the Labyrinth fish, members of the suborder Anabantoidei also called Anabantoids. These fish are characterized primarily by an accessory breathing organ called the labyrinth organ (Degani, 2001). However, it is unique in that it will use bits of vegetation in its nest. In nature, it adds such things as leaves, twigs, roots, peat fibers and other debris. Usually male build that bubble nest during breeding season. Once the female lays the eggs, the male will pick them up in his mouth and put them in his bubble nest and will continue to guard the eggs until they hatch (animal-world.com).

A male dwarf Gourami will be aggressive towards other dwarf Gouramis or similar looking fish and may bully a female. Males are usually peaceful

and non-aggressive, but when a female is introduced it will display territorial behaviour, developing a deep purple chest and flare up his dorsal fin. Yet even during the courtship and after building the nest the male sometimes considers the female as a rival and may attack her. Only in larger tanks that are well set up will territorial aggression be reduced (animal-world.com).

This species has been developed into several different types of dwarf Gouramis. Breeders selectively pair specimens with the most desirable characteristics, thereby strengthening those characteristics in their offspring. Today's varieties are the result of generation after generation of these improvements. Some of the most popular varieties include the powder blue dwarf Gourami, the flame dwarf Gourami, and the neon blue dwarf Gourami or rainbow dwarf Gourami. The dwarf Gourami has also been crossed with the thick-lip Gourami *Trichogaster labiosus*, however, in these hybrids, only sterile males have developed (animal-world.com).

2.2.1. Taxonomy

Kingdom : Animalia

Phylum : Chordata

Class : Actinopterygiii

Order : Perciformes

Family : Osphronemidae

Genus : *Trichogaster*

Species : *T. lalius*

India exported fish Gourami worth US$ 4,621 with the total quantity of 17,119. United States is the largest buyer of fish Gourami accounting for exports worth US$ 1,730 followed by Singapore and Japan which imported fish Gourami worth US$ 1,422 and US$ 460 respectively (animal-world.com).

2.2.2. Distribution

Anabantoids, which contains about 16 genera and about 50 species, are distributed throughout the southern Asia, India and Central Africa (Degani, 2001). The fish lives in habitats with various water sources and different soluble components (Degani, 1989 and Cole *et al*., 1999) and survives in foul waters (Rahman, 2005). These habitats are affected by the seasonal rain and might affect the standing water where the fish breeds (Degani and Levy, 2013). They are found together with other *Colisa* species in waterways and paddy fields of the tropical Far East inhabiting slow moving streams, rivulets, lakes, canals and other agricultural lands and occurring thickly vegetated areas such as wetlands of the Ganga, Jamuna, Brahmaputra and Baram rivers (animal-world.com).

2.2.3. Morphology of fish

Trichogaster lalius, previously classified *Colisa lalia*, is smallest and popular among the dwarf Gouramis (Welcomme, 1988) and is marketed worldwide as the wild and red and blue varieties (Zuanon *et al*., 2013). They generally grow to a size of 3.6-5.0 cm or to a maximum of 8.8 cm (Biswas *et al*., 2014). The males are bright coloured and have a wide range colour combination. The females are dull coloured and have a silvery light background only (animal-world.com). The body is strongly compressed. Mouth is small and upturned. Lips are normal. The dorsal fin is pointed in soft portion. Ventral fins are feeler like with extremely perceptive touch-sensitive cells used to touch things and to greet others. The caudal fin is usually rounded and sometimes notched. The lateral line is incomplete and interrupted. Width more in anal fin than in dorsal fin. Red spots on the dorsal fin and caudal fin. Scales number in lateral line series is 27-28 scales. Scales number above lateral line is 5-6 scales and below lateral line is 8-9 scales (Talwar and Jhingran, 2001 and Rahman, 2005).

2.2.4. Feeding habits

Trichogaster species is probably carnivorous though possibly omnivorous (Degani, 1989). In the wild, they feed mainly on invertebrates, zooplankton, detritus and occasionally on macrophytes (Conlu, 1986; Chung, 1994; Rainboth, 1996; Goodwin, 2003 and Talde *et al*., 2004). In the aquarium, they feed

on all kinds of live and flake foods (animal-world.com). Little has been published concerning about nutrition with artificial diets. The energy source of this species like other species is carbohydrates, proteins and lipids (Black and Love, 1986; Inui and Oshima, 1966 and Degani *et al.*, 1985, 1986).

Nutrition is known to have a profound effect on growth and maturation in various fishes (Wooton, 1982; Watanable *et al*., 1984 and James and Sampath, 2003) besides length, weight, sex and environmental factors (James and Sampath, 2003, 2006). Many authors have studied the effect of nutrition on gonad weight, egg weight, ova number and ova diameter in various fishes (Dahlgren, 1980; Watanable *et al*., 1984; Shim *et al*., 1989 and James and Sampath, 2006). *Trichogaster lalius* juveniles maintained with increased protein level and rearing period had increased mean body length and weight and females fed with high protein diets (35%) had a greater ovary weight and gonado-somatic index than females fed with low protein diets (5%) (Shim *et al.*, 1989).

2.2.5. Breeding biology

The Gouramis exhibit some sexual dimorphism either in the colour pattern or in the finnage pattern. The males generally grow to a size of 7.5 cm or to a maximum of 8.8 cm and the females reach a size of 6 cm. Males and females are easily distinguished by their colour. Males have total turquoise and orange-red iridescent vertical bands on the body and on the fins, mutants have a total orange-red body and turquoise dorsal fin or total turquoise body with redness at the fin edges. Females have total silvery with pale turquoise vertical stripes on the body (www.aqua-fish.net). Males have a longer dorsal fin, usually long enough to reach the caudal fin and females have a shorter dorsal fin, just long enough to touch the caudal peduncle when relaxed or laid down along the back of the fish. Mature males are somewhat compressed while mature females are much rounded in stomach region (Cole *et al*., 1999).

Fishes of genus *Trichogaster* are territorial (Forselius, 1957). *Trichogaster lalius* is a pair spawning species (Forcelius, 1957; Miller and Jearld, 1983 and Hayakawa and Kobayashi, 2010). In pair spawning species social status is divided into two types: the dominant males or territorial males which establish territories under floating aquatic plants to obtain females and to care eggs

(Forcelius, 1957; Miller and Jearld, 1983 and Hayakawa and Kobayashi, 2010) and the sneakers or streakers which releases sperm during pair spawning of dominant males (Gross, 1982, 1996; Taborsky, 1994, 1998 and Moyle and Cech, 2000). Forcing two males to compete for spawning with a female resulted in one male becoming dominant over the other and subordinate male did not attempt to sneak during spawning (Hayakawa and Kobayashi, 2010). The sneaker males possess relatively larger testis as compared to the dominant males for successful sperm competition (Parker, 1990 and Montgomerie and Fitzpatrick, 2009). It is well known that males exhibit clasping behaviour in which the male bends its body around the female under the floating bubble nest during mating (Hayakawa and Kobayashi, 2010). No occurrence of mating behaviour without floating nest has been reported, which suggests that establishment of the territory is quiet important to the reproductive success of males while no occurance of sperm emission without clasping behaviour has been reported, which suggests that clasping behaviour is strongly related to sperm emission and testis structure.

In mature *Trichogaster trichopterus* females, a closely related species of *Trichogaster lalius*, oogenesis has been described as 1. Chromatin nucleolar stage, 2. Peri-nuclear stage, 3. Chromatin nuclear stage, 4. Vitellogenesis (VTL), 5. Germinal vesicular breakdown (GVBD) and 6. Ripe egg stage (Degani and Boker, 1992). During the reproductive cycle, the first four stages are found in the ovary in varying proportions. Females with a low percentage of VTL in their oocytes are not ready to spawn. The male stimulates the female with high VTL by nest-building and other courtship behaviour and GVBD in the oocytes follows. The water in which the male builds nest contains steroid glucuronides which affect the female with high VTL, promoting maturation (Degani and Boker, 1992). Females spawn several times during a season in the wild (Pollak *et al*., 1978) but multiple spawning can take place (Pollak *et al*., 1981). They have a fecundity of about 300-800 eggs (Froese and Pauly, 2015). Egg production in this species is prodigious and it probably does not limit mating frequency. Both males and females are clearly capable of mating with as little as 24 hours successive spawning (Pollak *et al*., 1981). Commercial farms will condition and spawn the fish every 2-6 weeks depending upon the species. Females have been observed spawning twice in a period of 24-78 hour (Pollak *et al*., 1981) and also have been observed releasing

large numbers of unfertilized eggs. Rather than representing a conditioned of heightened spawning readiness, spontaneous egg release is more likely a method of rapidly eliminating over ripened eggs from the ovaries (Pollak *et al*., 1978). This is associated with large numbers of oil droplets on the water surface.

Similarly, in mature *Trichogaster trichopterus* males, all stages of spermatogenesis are found like the cellular organization of other fish testes and spermatogenesis and no differences in spermatogenesis is found among various stages of the reproductive cycle in testis (Degani and Jackson, unpublished data). Fishes are seasonal spawners, which are reflected in structural changes within the testis. The structural changes of fish testis and spermatogenesis have been described as 1. Spermatogonial proliferation, 2. Early recrudescence - spermatogonia and spermatocytes present, 3. Mid recrudescence - all stages of sperm development present, 4. Late recrudescence - tubules filled with sperm and number of developing sperm cysts is declining, 5. Functional maturity - tubules filled with sperm, very little if any spermatogenesis is occurring and 6. Post spawn (Grier, 1981). The testis undergoes several distinct stages of development through the annual reproductive cycle. Testes consist of lobules enveloped by membranes, very close to which are spermatogonia cells which have no specific forms (Degani and Jackson, unpublished data). The immature sperms are found very close to the tubule wall and the mature sperms are found in the middle of the tubule (Degani, 2001). Mature males defend an area in upper water coloumn, within which they build bubble-nest. If a ripe female is placed with a mature male in the suitably quiet tank, then spawning typically occurs between 36 and 60 hours later (Teo, 1984 and Munro, 1990), the resulting floating eggs are deposited in bubble-nest guarded by males. There is evidence that social signals from the male may be important in regulating processes leading up to ovulation and spawning in the female (Teo, 1984 and Munro, 1990).

Very scarce information is available on the breeding periodicity of *Trichogaster* species. For *Trichogaster fasciata* some have reported March-October as the breeding season in West Bengal (Mitra *et al*., 2007), while others have reported April-August as the breeding season in Assam (Das and Kalita, 2006). For *Trichogaster trichopterus* breeding occurs round the year but more profusely during heavy seasonal rains, when more areas are available for male territory in natural

habitats with plenty of vegetations (Degani, 2001). For *Trichogaster lalius* monsoon is a breeding season (Rahman, 2005).

2.3. Environment control of reproduction in fishes

A very wide and diverse range of processes is involved in reproduction in teleost fish. Fish species are diversified in terms of dioecious and hermaphroditic habits (including protandrous, protogynous and synchronous types) as well as oviparous and viviparous nature. Based on the mode of reproduction the basic anatomy of the gonadal structures and the hormonal profile during the reproductive cycle reflects the wide variety. There is a basic pathway with respect to the general endocrinology of gametogenesis and reproduction based on the activity of the hypothalamus-pituitary-gonad axis (Hoar, 1969; de Vlaming, 1974 and Schreibman *et al*., 1990). Production and emission of mature gametes are the final stage prior to reproduction. The development of the gonads can be divided into three phases: production of gametes (spermatogenesis and oogenesis), growth and maturation of the gametes (spermiogenesis and oocyte vitellogenesis, which are succeeded by oocyte maturation) and emission of the gametes (spermiation and ovulation). The seasonal events in an annual reproductive cycle are often synchronized with periodic changes in one or more environmental components in such a way that breeding occurs in the most favorable part of the year (Foster *et al*., 2006). Many environmental and physiological factors practically play an important role in fish gonadal maturation and spawning and so facilitate hatchery production (Bromage *et al*., 2001). The environmental factor such as photoperiod, dissolved oxygen, temperature, salinity and pH of water influence egg quality (Carrillo *et al.*, 1989) and in captivity they play a vital role in gonadal maturation (Mylonas *et al.*, 2007). Temperature and photoperiod are major environmental factors involved in the reproductive activity (Eyeson, 1983; Emit *et al*., 1989; El-Greisy, 1993 and El-Naggar *et al*., 2000). Seasonal cycles are mainly driven by photoperiod and temperature but other variables e.g. lunar phase and social interaction may also influence the sexual periodicity in most fish species living in mid and high latitudes (Bornestaf *et al.,* 2001; Garcia-Allegue *et al.,* 2001 and Pankhurst and Porter, 2003). However, this does not exclude the possible role of supplementary conditions (rainfall, an abundance of food, water pH and salinity, favourable growing conditions *etc*.). It is understood, for an environmental cue to be capable of phasing

or entraining reproductive development, it must be physiologically detectable, temporally predictable and generate endocrine changes that drive the reproductive process (Sumpter, 1990). Thus, without appropriate environmental cues, the endocrine control cannot continue which is stimulating reproduction (Fahmy, 2006).

Manipulations of environmental factors such as photoperiod and water temperature have been used to alter the timing of reproduction in a variety species (Bye, 1990) and biotic factors such as food availability, predators and pathogens (Bergman, 1980; Whittier and Crews, 1987; Hontela and Stacey, 1990; Munro, 1990 and Sumpter, 1990). However, it is well established among teleosts, in general, the importance of temperature and photoperiod on the stimulation of gametogenesis (Weil, 1992; Evans, 1995 and Moyle and Cech, 2000).

Processing of environmental information is a vital physiological process in the regulation of reproduction in animals. Studies in different teleosts found out that synchronization of physiological and environmental events is mediated by the system composed of sensors and circadian oscillators like the pineal organ, the lateral eyes and the suprachiasmatic nuclei of the hypothalamus (Falcon *et al*., 1992). Several experimental studies indicated that the pineal organ is able to translate environmental information (photoperiod and temperature) into rhythmic messages and the pineal hormone melatonin is the internal chemical messenger of environmental signal or zeitgeber (Begav *et al*., 1998 and Amano *et al*., 2000) and controls many functions, especially reproduction, in vertebrates (Sanchez-Vazquez *et al*., 2000).

Temperature and photoperiod are major environmental factors involved in cueing reproductive activity (Bromage *et al*., 2001). The most variable environmental parameter and also the most controllable in hatchery condition among them is temperature. It is the most thoroughly investigated environmental factor influencing fish reproduction. Though water temperature can affect reproductive development and spawning, yet photoperiod has been accepted as the most important factor synchronizing sexual maturation and reproduction in fish (Bromage *et al*., 2001).

2.3.1. Photoperiod and gonadal maturation

It is well established that both endogenous and exogenous factors regulate the onset of gonadal recrudescence and spawning in fish (Fostier *et al*., 1983 and Lam, 1983). Gonadal maturation in teleosts controls the endogenous rhythms (Bromage *et al*., 1993). Environmental cues of which photoperiod is considered as the most important influence endogenous rhythms resulting in synchronous spawning within each population at approximately the same time every year (Norberg *et al*., 2004). In many fish species, gonadal recrudescence and regression are to a large extent controlled by the length of photoperiod (Baggerman, 1972). Long day lengths stimulate body fattening and ovarian growth while short day lengths are inhibiting in stimulation of this response (Sarkar and Arora, 2001). Ovarian recrudescence occurs at a time when day length begins to shorten and temperature reaches a seasonal minimum, while the ovarian recrudescence coincides with an increase in day length and temperature (Sarkar and Upadhyay, 2011).

To stimulate or delay gonadal maturation, photoperiod alternations are used, which in turn is responsible for change spawning period or somatic growth (Lam, 1983). In cyprinids Carp, Catfish and other tropical and subtropical fish, high temperatures and long photoperiods are important for the final maturation of the oocytes, ovulation and oviposition (Rocha, 2008). In the temperate fish, photoperiod is an important proximal factor in the timing of maturation, although water temperature most likely also a modulating role to play, the absolute length of the photoperiod is not important for cueing the reproductive events. However, the relative change from short to long or vice versa seems to be critical for the entrainment of the true events (Carillo, 2007).

Photoperiod regulates the axis photoreceptors, central nervous system, hypothalamus-pituitary gonadotropin, ovarian estrogen secretion, oogonial proliferation and endogenous yolk formation. In some vertebrates, photoperiod plays an important role as a chief proximate factor in the regulation of seasonal reproduction. Photoperiod gets changed accurately and reliably around the year so that animals can use this for prediction of seasonal changes and accordingly programme their gonadal development (Fraile *et al.,* 1989).

Photoperiod manipulation can induce gonadal development in tropical species (Lam and Munro, 1987). However, the mechanisms that convey photoperiodic information to the reproductive and somatotropic axis are not clearly understood. Certainly, the clear effects of photoperiod on the timing of reproduction and growth and the corresponding diel and seasonal patterns of melatonin provide strong circumstantial evidence that melatonin may be an intermediary in the process (Randall *et al.,* 1991; Porter *et al.,* 1996 and Bromage *et al.,* 2001). In this respect, melatonin levels may act to provide a signal to entrain endogenous rhythms. Furthermore, the response of plasma melatonin to changes in light is affected by both light intensity and water temperature (Porter *et al.,* 2001). Modified photoperiod regimes can be used to advance or delay the spawning period and a year-round supply of eggs can be ensured by replacing, increasing and decreasing components of the seasonally changing day length with periods of constant long and short day length (Taylor and Migaud, 2008).

2.3.2. Temperature and gonadal maturation

Temperature has often not been considered in photoperiod studies, therefore, it is not clear whether the photoperiod effects reported are temperature dependent. Temperature dependency of photoperiodism has in fact been reported in a number of species. In tropics, seasonal variations in photoperiod and temperature are relatively small. Nevertheless, several species have responded to such changes (de Vlaming, 1974, 1975). Temperature is the main factor which is responsible for the ovarian development, spawning initiation (Egami, 1959) and also for embryonic development (Lagler *et al*., 1962; Ishii 1963 and Roberts, 1989).

Gonad maturation and other reproductive attributes can be influenced by temperature (Davies *et al*., 1986) and the secretion and action of reproductive hormones (Van Der Kraak and Pankhurst, 1997), frequency of reproduction (Hilder and Pankhurst, 2003 and Dhaneesh *et al*., 2012), clutch size and egg size (Brown *et al*., 2006), embryo development and duration (Taranger and Hansen, 1993; Rombough, 1997 and Hilder and Pankhurst, 2003), embryo hatching and their early ontogenesis (Kucharczyk *et al*., 1997 and Kujawa *et al*., 1999) and offspring size and quality (Brown *et al*., 2006 and Donelson *et al*., 2010). Controlled spawning through temperature manipulation in *Amphiprion sebae* showed increased

spawning frequency (Dhaneesh *et al*., 2012). A number of tropical species like Scianids, Perches, Gunthers, Rainbow fish show a well defined seasonal reproductive cycle typical of temperate species (Lam, 1974; Payne, 1975; Johannes, 1978; Beumer, 1979; Kuo and Nash, 1979 and Kumagai, 1981).

2.3.3. Photothermal and gonadal maturation

In temperate regions, photoperiod and temperature variations are the main cues controlling the reproductive cycle because they are both circannual oscillators. This external control allows acute adjustment of reproduction in the wild so that offspring have the greatest chance of survival (Bromage *et al*., 2001). In fish farms, photothermal programs (i.e. programs of variations in both photoperiod and temperature) are commonly used to control the reproductive cycle and obtain larvae all year round.

2.4. Endocrine control of reproduction in fishes

The neuroendocrine system initiates and controls the process of gametogenesis and steroidogenesis through the activation of the hypothalamus-pituitary-gonadal axis (Kah *et al*., 1993 and Lopez-Olmeda *et al*., 2006). The activation of this axis is controlled by environmental and endogenous cues. The brain receives the cues from the environment and activates the hypothalamus which in turn stimulates the release of gonadotropin releasing hormone (GnRH). Then GnRH stimulates the release of gonadotropins (GTH) from the pituitary (Okubo and Nagahama, 2008 and Kouril, 2009). Fish pituitaries, however, similar to those of other vertebrates, have been shown to secrete two kinds of GTH; GTH-I (similar to Follicle Stimulating Hormone; FSH) and GTH-II (similar to Luteinising Hormone; LH). GTH-I plays a role during initial gonadal growth and gametogenesis while GTH-II is prevalent during the final stages of maturation (Trudeau, 1997 and Kouril, 2009).

In ovaries, the gonadotropins stimulate the theca and granulosa cells of the ovarian follicle to secrete steroids including androgens (e.g. testosterone; T), estrogens (e.g. 17β-estradiol; E-2) and progestagens (e.g. 17α-20β-dihydroxy progesterone; 17α-20β-DHP) (Cavaco *et al*., 1997). During the early oocyte growth and development phases, FSH stimulates the follicular cells of the oocyte to

produce T which is then converted into E-2 in the granulosa cells via the activity of the aromatase enzyme (Redding and Patino, 1993). Increased E-2 levels in the blood stimulate the hepatic system to synthesize VTG. E-2 peaks generally, during the period of most active vitellogenesis and returns to basal levels before ovulation. T starts to increase in conjunction with E-2, but peak levels are not attained until 1-2 months after E-2 (Borg *et al*., 1983 and Frantzen *et al*., 1997). VTG is a large glycophospho-lipoprotein produced by the liver and transported via the circulatory system to the ovarian follicle, processed into yolk proteins then accumulated as yolk globules in the growing oocytes.

Once vitellogenesis is complete, plasma E-2 levels fall rapidly and a negative feedback on the hypothalamus and pituitary triggers the release of LH (Naor *et al*., 2003 and Kouril, 2009). LH stimulates the final oocyte maturation through the secretion of progestagens (17α-20β-dihydroxy progesterone; 17α-20β-DHP or 17,20β,21-trihydroxy-4-pregnen-3-one; 17,20β,21-P), acting as the maturation inducing hormone (MIH) (Okubo and Nagahama, 2008). This hormone then binds to the oocyte nucleus and forms the maturation promoting factors (MPF) which stimulate the germinal vesicle migration (GVM) towards the micropyle. The germinal vesicle then breaks down (GVBD) prior to the hydration stage and subsequent ovulation (Schulz *et al*., 1994 and Kouril, 2009).

In testes, the two GTH hormones play different functional roles. FSH levels are elevated during spermatogenesis while LH levels peak during spermiation (Redding and Patino, 1993). In fish, every single primary spermatogonium is enveloped by one or two Sertoli cells which support germ cells by providing the optimal microenvironment (Okubo and Nagahama, 2008). Leydig cells are steroidogenic, e.g. responsible for the production of steroids. FSH acts by regulating Sertoli cell functions to stimulate germ cells growth during spermatogenesis (Kouril, 2009). The LH receptor is then expressed by Leydig cells, which respond to LH and release androgens (testosterone; T and 11-keto testosterone; 11-KT) with T stimulating pituitary LH synthesis during puberty (Holzberg and Albrecht, 2003 and Okubo and Nagahama, 2008). Peak levels of 11-KT coincide with spermiation (Tvieten *et al*., 2005 and Okubo and Nagahama, 2008). In male fish, 11-KT is considered to be the main androgen hormone, stimulating the development of

secondary sexual characters and spermatogenesis. Seasonal cycles of gonadal activity have been described in many teleosts species.

2.4.1. Photoperiod and neuroendocrine regulation

Photoperiod acts via the hypothalamus-pituitary axis (Borg, 1994 and Nagahama, 2002). The photoperiod signals being transduced by melatonin (Reiter, 1993 and Lincoln, 2006), which act on the hypothalamic-pituitary-gonad axis (Amano *et al*., 2000; Bromage *et al*., 2001; Bayarri *et al*., 2004 and Falcon *et al*., 2007). This axis, in turn, controls reproduction timing and by means of neuroendocrine signals regulates the production of sexual steroids and growth factors in the gonads. The biosynthesis of melatonin and other indoles occurs in photoreceptor cells. The general mechanism of biosynthesis of melatonin in fish appears to be identical to that in mammals (Bromage *et al*., 1984).

2.4.2. Temperature and neuroendocrine regulation

Temperature change has the ability to affect the HPG axis at multiple sites by its reaction-rate-determining effects on hormone synthesis and action and its effects on hormone structure. This is reflected in a minimum temperature threshold for most endocrine events, increasing hormone synthesis, activity and metabolism across the physiological tolerance range and decreasing activity at the top end of that range (Pankhurst and King, 2010). Conformational changes in proteins (e.g. FSH, LH and their receptors, steroid-synthesising enzymes) and also the increasing tendency for steroid hormones to form water-soluble conjugates at high temperatures are inhibitory effects arisen out of higher temperature (Van Der Kraak and Pankhurst, 1997). Steroid conjugates (usually sulfates or glucuronides) being no longer able to pass through cell membranes to gain access to their intracellular receptors and of being more available for kidney filtration and excretion in the urine, significantly reduces their plasma residence times.

Thermal inhibition of reproduction is present across a widespread of taxas, habitats and temperature ranges irrespective of the mechanism involved. The main difference between species is the absolute temperature at which the suppressive effects occur. In cold temperate and sub-Arctic species, the inhibitory effects typically appear at 11-12 ^{0}C, among cold-temperate species at around 18

^{0}C, temperate species at 24 ^{0}C and tropical species at 30 ^{0}C and above. Though it supports the view that all species are likely to show similar responses to rising temperatures, yet the thresholds for these effects may vary in relation to specific thermal tolerance ranges. There is also evidence of the ranges over which normal function can be maintained may be broader in cool water and temperate species than among tropical species (Nilsson *et al*., 2009 and Donelson *et al*., 2010).

Exposure to elevated temperatures at the completion of vitellogenesis (late autumn) inhibits the synthesis of the maturational steroid 17,20β,21-P and subsequent progression of oocytes through final oocyte maturation (resumption of meiosis) and ovulation (Pankhurst and King, 2010).

There is also some evidence that high temperature may interfere with the transduction of photoperiod signals to the reproductive axis, although as noted earlier, the mechanism in fish is still not well understood. The amplitude of the nocturnal surge in melatonin in fish is greater at higher temperature and if as in some birds, melatonin exerts its role through stimulation of factors that inhibit GnRH synthesis (Migaud *et al*., 2010), then temperature-regulated increases in melatonin secretion could also result in an inhibitory effect at the pituitary level. With the caveat that the understanding of all of the possible effects of temperature on reproductive physiology is not complete, it is clear that temperature regulates reproduction at multiple sites in the reproductive pathway.

2.5. Role of sex-steroids in fish reproduction

Steroid hormones play a major role in growth regulation, development and reproduction in fishes. It is well investigated that the role of sex-steroids in controlling the maturation cycle in teleosts mainly during the spawning period is altered by environmental factors or hormonal manipulation (Flammarion, 2000). Among many hormones that influence the reproductive process of male, as well as female steroid hormones, is just one type which causes specific changes in serum sex-hormone levels during the different phases of gonadal development. Male hormones synthesized from Leydig cells and the major androgen synthesized by testicular tissue varies from species to species and also with the developmental stages.

2.5.1. Testosterone

Testosterone (T) is the prototypic and predominant circulating androgenic steroid, which plays a significant role in the growth and the function of many reproductive and non-reproductive tissues and also organs including muscle, liver and brain. It also directs the development of the male phenotype during embryogenesis and at puberty. By the enzymes 17,20-lyase and 17β-hydroxysteroid dehydrogenase, T is synthesized from 17α-hydroxy progesterone in the gonads of both males and females. It is reduced to 5α-dihydro testosterone in target cells, which mediates many of the biological actions of testosterone. It is then further metabolized to E-2 by aromatase (Vance, 1988).

2.5.2. 11-keto testosterone

11-keto testosterone (11-KT) is a second key androgenic steroid found in fish, which occurs in males together with T in amounts which vary from less than 1 ng/ml to as much as 50-100 ng/ml, depending on the species and the stage of the reproductive cycle. T concentrations are generally higher than 11-KT in Sea bass, with peak levels found after the spawning season, whereas 11-KT remains at levels less than 1 ng/ml but it rises abruptly to 4-6 ng/ml during spermiation at the height of the spawning season (Prat and Zanuy, 1990). 11-KT also shows individual variations in Arctic char, with dominant males having significantly higher 11-KT levels (Elofsson *et al*., 2000).

2.5.3. 17β-estradiol

17β-estradiol (E-2) is the major estrogen, which is secreted by the pre-menopausal ovary. Estrogens catalyze the development of the female genotype in embryogenesis and at puberty. E-2 is synthesized from testosterone primarily in the ovarian granulosa cells and placenta and from the adrenal gland in a small amount (Naka *et al*., 1992 and Miller and Tyrrell, 1995). Aromatase system accomplishes the conversion of T to E-2 and it consists of 3 enzyme activities localized to the endoplasmic reticulum of the cells in these tissues (Vance, 1988 and Erickson, 1995).

2.5.4. 17α,20β-dihydroxy progesterone

17α,20β-dihydroxy progesterone (DHP), which stimulates oocyte maturation in females of several teleost species, is an endogenous, maturation-inducing steroid. 1 µg/ml of DHP applied to Persian sturgeon oocytes has been shown to effectively induce GVBD, which is an essential step during oocyte maturation (Milla *et al*., 2008). GnRH and the GTH, FSH and LH (either in vivo or in vitro*)* have been shown to stimulate the production of DHP (Planas *et al*., 2008). DHP, when administered at 5 mg/kg to various male teleosts, has also been reported to influence spermiation by stimulating milt production (Milla *et al*., 2008).

2.6. Role of melatonin in fish reproduction

The impact of melatonin on the seasonal cycle of reproduction has been mostly found out using photoperiod manipulations, pinealectomy and melatonin administration (Ekstrzm and Meissl, 1997 and Mayer *et al*., 1997). In vitro study in the Atlantic croaker was the first concrete evidence of the effect of melatonin on reproduction (Khan and Thomas, 1996). In fish with fully developed gonads, low concentrations of melatonin stimulated in vitro LH release from pituitary cells in culture, in vivo melatonin elicited significant elevations in plasma LH levels late during the photophase of the day-night cycle, when administered in the basal diencephalon. This suggested that melatonin acted both at the hypothalamus and pituitary levels. This was recently confirmed in the Eel where melatonin implants induced a decrease in LH and FSH expressions as well as in plasma levels of some sexual steroids (Sébert *et al*., 2008). This was partly achieved through an action on the dopaminergic cells, which were the only brain catecholaminergic cells showing increased tyrosine hydroxylase (TH) expression. However, what applies to Eels might not be the rule for other fish. In cultured Carp hypothalamus, melatonin reduces dopamine levels, which would result in an increased LH secretion (Popek *et al*., 2000). Further evidence that melatonin plays a significant role in the regulation of annual testicular events was obtained from studies in *Catla catla* (Bhattacharya *et al*., 2007). Indeed, in this subtropical Carp, precocious testicular maturation was observed in both melatonin-treated fish and fish exposed to continuous darkness during the preparatory phase and inhibition of testicular function was observed during the pre-spawning and spawning phases. Also,

melatonin can accelerate the action of the (MIH) when added 4 hours prior to MIH in the incubation medium (Chattoraj *et al*., 2005) and conversely, serotonin inhibits the actions of MIH as well as those of melatonin on the MIH induced oocyte maturation in Carp (Chattoraj *et al*., 2008).

3. MATERIAL AND METHODS

3.1. Experimental site

The experiment was conducted over a period of 90 days from 6th December, 2016 to 6th March, 2017 at the Ornamental Fish Research Centre of I.C.A.R.-C.I.F.E., Kolkata Centre. The enzyme-linked immunosorbent assay (ELISA) for 11-keto testosterone (11-KT), 17β-estradiol (E-2) and 17α,20β-dihydroxy progesterone (DHP) was carried out in the Physiology and Biochemistry Laboratory and the Central Instrumentation Laboratory at I.C.A.R.-C.I.F.E., Kolkata Centre.

3.2. Experimental animal

The fishes with an average length of 42.6 cm and average weight of 1.55 g were procured from pet market, Gallif Street during the month of November, 2016. The fishes were packed in polyethene bags filled with 2:1 oxygen and water and transported to the Ornamental Fish Research Centre of I.C.A.R.-C.I.F.E., Kolkata Centre. The fishes were transferred to FRP tank of 500 liters capacity with sufficient aeration and left undisturbed the whole night. In order to ameliorate the handling stress the fishes were given a mild salt and potassium permanganate (KM_nO_4) treatment in the next day. The stock was acclimatized under aerated condition for a period of one month. All fishes were normal throughout the experimental duration.

3.3. Experimental design

The experimental groups consisted of a control (C) and 5 treatments (T1, T2, T3, T4 and T5) with 3 replications of each to study the effect of photothermal manipulation of gonad recrudescence with different combination of photoperiod and temperature, following a completely randomized design (CRD). The extended photoperiod and temperature combinations were created through artificial lightning with the help of led bulbs (3W) and temperature maintenance was made by thermostat heater (300W). To provide artificial day length, the experimental tanks were equipped with overhead lights and covered with black sheets. Water temperature during the experiment was measured 2 times a day at 6

AM for minimum and 2 PM for maximum. The combination of different photothermal regimes were conducted as follows:

1. Control: Natural Photoperiod (NP) and Ambient Temperature (AT)
2. Treatment 1: Long Photoperiod 16L:08D (LP-1) and Ambient Temperature (AT)
3. Treatment 2: Long Photoperiod 14L:10D (LP-2) and Ambient Temperature (AT)
4. Treatment 3: Natural Photoperiod (NP) and Above Ambient Temperature (AAT)
5. Treatment 4: Long Photoperiod 16L:08D (LP-1) and Above Ambient Temperature (AAT)
6. Treatment 5: Long Photoperiod 14L:10D (LP-2) and Above Ambient Temperature (AAT)

3.4. Experimental setup

The experimental tanks were housed indoor to maintain photoperiod and temperature. The setup consisted of 18 glass tanks of 80 litres capacity covered with hoods. The tanks were initially washed and filled with 2 ppm KM_nO_4 solution and left overnight. The tanks were flushed on the very next day and were thoroughly washed and filled with clean water. The total volume of the water in each tank was maintained at 70 litres. Round the clock aeration was provided. The aeration pipe in each tank was provided with an air stone and a plastic regulator to control the air pressure uniformly in all tanks. 360 healthy fishes were randomly distributed in 18 glass tanks, 10 males and 10 females in each tank.

3.5. Rearing

Uniform size fishes were kept in experimental tanks depending on experimental setup. Tanks were covered with hoods to prevent fishes from jumping out. Fishes were fed with a nutritionally balanced diet throughout experimental period. Environment conditions were maintained according to treatments and it was kept same throughout the experiment. The fishes were starved overnight before starting experiment.

3.6. Cleaning

Experimental tanks were cleaned manually at a regular interval of 3 days to remove the excess feed waste and remaining faecal matter. An equal volume of clean water replaced with the drained water. This was carried out throughout the experimental period.

3.7. Feeding

Feeding was done at 2% of the body weight throughout the experiment. The daily ration was divided into two equal parts and fed at 9 AM in the morning and 5 PM in the evening.

3.8. Water quality analysis

Water quality analysis of tank water was carried out by standard methods (APHA, 2005).

3.8.1. Temperature

Temperature was measured using a digital thermometer (Maxtech Technologies, India, Model No. ST 9269) by dipping the probe in water till the reading in the meter is stabilized and expressed as ^{0}C.

3.8.2. pH

pH was measured using a digital pH meter (Hanna Instruments, India, Model No. HI 98107) by dipping the probe in water till the reading in the meter is stabilized.

3.8.3. Dissolved oxygen

Dissolved oxygen was measured by Winkler method using N/80 sodium thiosulphate solution with manganous sulphate, alkaline potassium iodide, concentrated sulphuric acid and starch indicator and calculated by using the following formula:

Dissolved oxygen (mg/l) = Volume of titrant used

3.8.4. Total alkalinity

Total alkalinity was measured by titremetric method using 0.02 N sulphuric acid solution with phenolphthalein and methyl orange indicators and calculated by using the following formula:

Total alkalinity (mg/l) = Volume of titrant used X 20

3.8.5. Total hardness

Total hardness was measured by titremetric method using 0.01 M ethelenediaminetetraacetic acid (EDTA) solution with ammonium chloride-ammonium hydroxide buffers and erichrome black T (EBT) indicator and calculated by using the following formula:

Total hardness (mg/l) = Volume of titrant used X 20

3.8.6. Unionized ammonia

Unionizedd ammonia was measured spectrophotometrically at 640 nm wavelength by phenate method using phenol solution, sodium nitroprusside and oxidizing solution containing alkaline citrate and sodium hypochlorite and expressed as mg/l.

3.9. Proximate composition analysis

Proximate composition analysis of feed ingredients as well as experimental diets were carried out by standard methods (AOAC, 2006).

3.9.1. Moisture

The moisture content was determined by drying the pre-ground sample in a hot air oven at 105 ^{0}C for 12 hours and calculated by using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.9.2. Ash

The ash content was determined by burning the pre-dried sample in a muffle furnace at 550 ^{0}C for 12 hours and calculated by using the following formula:

$$\text{Ash (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.9.3. Crude protein

The crude protein content was determined by evaluating the total nitrogen content of the sample after digesting with acid and catalyst, followed by distillation and titration of the sample by kjeldahl method with digestion unit (Pelican Equipments, India, Model No. KES 6L), distillation unit (Pelican Equipments, India, Model No. DISTYL EM) and titration flask and calculated by using the following formula:

Crude protein (%) = Percentage of nitrogen in sample X 6.25

3.9.4. Crude lipid

The crude lipid content was determined by extracting the sample with petroleum ether by soxhlet method with extraction unit (Pelican Equipments, India, Model No. SCS 6R) and calculated by using the following formula:

$$\text{Crude lipid (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.9.5. Crude fibre

The crude fibre content was determined by digesting the sample with weak acid and weak alkali, followed by burning the sample by fibre cap method with digestion unit and muffle furnace and calculated by using the following formula:

$$\text{Crude fibre (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.9.6. Nitrogen free extract

The nitrogen free extract content was determined by subtracting the percentages of each nutrient from 100 and calculated by using the following formula:.

Nitrogen free extract (%) = 100 – [Moisture (%) + Ash (%) + Crude protein (%) + Crude lipid (%) + Crude fibre (%)]

3.10. Hormonal assay

The assay was performed according to the manufacturer instructions.

3.10.1. Sample preparation

Fishes from different experimental groups were collected using anaesthesia clove oil. Blood was collected using an anticoagulant EDTA and kept undisturbed at 24-25 °C for 2-3 hours. Thereafter, it was centrifuged at 3000 RPM for 10-15 minutes. The straw coloured plasma layer was pipetted carefully without disturbing the white coloured buffy layer by the help of micro pipette and stored at –20 °C till further analysis.

3.10.2. Steroid quantification

The quantification was done using commercially available ELISA kits.

3.10.2.1. 11-keto testosterone

11-KT was quantified in the plasma samples ELISA. The quantification was done using commercially available 11-KT ELISA kit (Cayman Chemicals, USA, Catalog No. 582751). The assay was performed according to the manufacturer instructions. The absorbance was read at a wavelength of 415 nm in the ELISA plate reader (Biorad, India, Model No. 1681135).The sample concentration was

determined from the standard graph plotted. The 11-KTlevel was expressed in ng/ml.

3.10.2.2. 17β-estradiol

E-2 was quantified in the in plasma samples through ELISA. The quantification was done using commercially available E-2 ELISA kit (Cayman Chemicals, USA, Catalog No. 582251). The assay was performed according to the manufacturer instructions. The absorbance was read at a wavelength of 415 nm in the ELISA plate reader (Biorad, India, Model No. 1681135).The sample concentration was determined from the standard graph plotted. The E-2 level was expressed in ng/ml.

3.10.2.3. 17α,20β-dihydroxy progesterone

DHP was quantified in the plasma samples through ELISA. The quantification was done using commercially available DHP ELISA kit (Cayman Chemicals, USA, Catalog No. 498504). The assay was performed according to the manufacturer instructions. The assay was performed according to the protocol provided along with the kit. The absorbance was read at a wavelength of 415 nm in the ELISA plate reader (Biorad, India, Model No. 1681135).The sample concentration was determined from the standard graph plotted. The DHP level was expressed in ng/ml.

3.11. Gonado-somatic index

The GSI was determined by correlating the gonad weight with the fish weight. The fish was brought and weight of the fish was taken after which the gonads were removed and weight of the gonads were taken. The GSI was calculated by using the following formula:

$$GSI = \frac{\text{Weight of the Gonad}}{\text{Weight of the fish - Weight of the Gonad}} \times 100$$

3.12. Fecundity

The fecundity was estimated by direct counting method. The total ovary of the fish was removed and weighed after which a small portion of the ovary was cut and weighed. It was then soaked in a petri dish containing water and the number of eggs were counted with the help of sterile needle and magnifying glass. The fecundity was calculated by using the following formula:

$$\text{Fecundity} = \frac{\text{No. of eggs in small portion of the ovary} \times \text{Total weight of ovary}}{\text{Weight of small portion of the ovary}}$$

3.13. Oocyte frequency distribution

The ova-diameter was measured by stage micrometer and occulometer. The total ovary of the fish was removed and weighed after which small pieces of ovary from the anterior, middle and posterior regions were cut and weighed. It was then teased on a glass slide and the ova-diameter was measured with the help of stage micrometer and occulometer. Oocyte frequency distribution was plotted for all stages of maturity.

3.14. Histological studies

For histological study, the gonads of the fish from different experimental groups were taken during sampling. The gonadal tissue was fixed in 10% neutral buffered formalin (NBF) and transferred to 70% alcohol within 24 hours for long term storage (approximately 9 months) before histological processing. It was then dehydrated in graded series of alcohol (70% ethanol for 15 minutes, 90% ethanol for 15 minutes, 100% ethanol for 15 minutes,100% ethanol for 15 minutes, 100% ethanol for 30 minutes and 100% ethanol for 45 minutes), cleared with xylene (xylene for 20 minutes, xylene for 20 minutes and xylene for 45 minutes), infiltrated in paraffin (paraffin for 30 minutes, paraffin for 30 minutes and paraffin for 45 minutes), blocked out in paraffin with a embedding centre (Leica Microsystems, Model No. EG1150) and sectioned at 5–7 µm with a microtome (Leica Microsystems, Model No. RM2125 RTS). The tissue sections were floated on a

water bath, picked on a microscope slide and placed on a hot plate. The microscope slides were stained with hematoxylin and eosin and covered with cover glass after which scanned using a light microscope (Leica Microsystems, Model No. DM3000 LED) and photographed using microscope camera (Leica Microsystems, Model No. DFC450 C) (www.leicabiosystems.com).

3.15. Statistical analysis

Statistical significance of different parameters was analyzed using one-way analysis of variance (ANOVA) using SPSS 16.0 for Windows. Duncan's multiple range test was used for post hoc comparison of means between different treatments. All data presented in the text, tables and figures are means ± standard error and statistical significance for statistical tests was set at $p<0.05$.

4. RESULTS

4.1. Water quality analysis

The water quality parameters during 90 days experimental period in 6 different experimental groups is shown in Table 1. Temperature varied in the range of 19.1 ± 0.058 ^{0}C to 26.4 ± 0.088 ^{0}C with an average of 23.6 ± 2.792 ^{0}C. pH varied in the range of 7.41 ± 0.052 to 7.78 ± 0.052 with an average of 7.58 ± 0.121. Dissolved oxygen varied in the range of 6.33 ± 0.220 mg/l to 7.67 ± 0.083 mg/l with an average of 7.11 ± 0.381 mg/l. Alkalinity varied in the range of 117 ± 4.055 mg/l to 150 ± 4.163 mg/l with an average of 138 ± 10.122 mg/l. Hardness varied in the range of 205 ± 3.528 mg/l to 225 ± 4.055 mg/l with an average of 215 ± 5.200 mg/l. Unionized ammonia varied in the range of 0.001 ± 0.000 mg/l to 0.007 ± 0.002 mg/l with an average of 0.004 ± 0.002 mg/l.

4.2. Proximate composition analysis

The proximate composition of feed ingredients as well as experimental diet is shown in Table 2. The experimental diet was prepared by mixing different feed ingredients like fish meal, wheat flour, soybean oil cake and groundnut oil cake. Crude protein and ether extract levels of fish meal was 32.43 ± 0.43 % and 2.76 ± 0.41 % respectively, wheat flour was 9.66 ± 0.35 % and 1.22 ± 0.36 % respectively, soybean oil cake was 52.94 ± 0.61 % and 1.92 ± 0.54 %, groundnut oil cake was 48.23 ± 0.31 % and 5.52 ± 0.54 % respectively and experimental diet was 30.65 ± 0.08 % and 9.53 ± 0.10 % respectively.

Table 1: Water quality parameters during 90 days experimental period.

Treatment	Days	Temperature (^{0}C)	pH	DO (ppm)	Alkalinity (ppm)	Hardness (ppm)	NH_3 (ppm)
C (NP & AT)	0 Day	21.2 ± 0.088	7.48 ± 0.052	7.50 ± 0.250	150 ± 4.163	225 ± 4.055	0.001 ± 0.000
	30 Days	19.1 ± 0.088	7.74 ± 0.079	7.50 ± 0.250	149 ± 3.528	223 ± 3.528	0.002 ± 0.000
	60 Days	20.5 ± 0.067	7.73 ± 0.079	6.83 ± 0.083	144 ± 3.464	221 ± 2.906	0.004 ± 0.001
	90 Days	24.1 ± 0.088	7.48 ± 0.079	6.83 ± 0.363	126 ± 3.464	215 ± 4.667	0.003 ± 0.001
T1 (16:08 & AT)	0 Day	21.2 ± 0.088	7.68 ± 0.079	7.33 ± 0.220	144 ± 2.603	219 ± 3.528	0.002 ± 0.000
	30 Days	19.1 ± 0.088	7.70 ± 0.079	7.33 ± 0.167	142 ± 3.055	215 ± 3.712	0.001 ± 0.000
	60 Days	20.3 ± 0.088	7.53 ± 0.079	7.00 ± 0.144	139 ± 3.712	214 ± 3.055	0.003 ± 0.000
	90 Days	23.9 ± 0.088	7.45 ± 0.079	7.00 ± 0.144	123 ± 3.528	211 ± 3.528	0.003 ± 0.001
T2 (14:10 & AT)	0 Day	21.4 ± 0.058	7.67 ± 0.104	7.67 ± 0.083	148 ± 3.055	223 ± 3.712	0.002 ± 0.001
	30 Days	19.1 ± 0.058	7.78 ± 0.052	7.67 ± 0.083	145 ± 3.528	219 ± 3.712	0.002 ± 0.000
	60 Days	20.5 ± 0.058	7.57 ± 0.104	6.87 ± 0.337	141 ± 3.712	217 ± 2.906	0.003 ± 0.001
	90 Days	24.0 ± 0.058	7.77 ± 0.052	7.33 ± 0.167	125 ± 3.712	212 ± 3.055	0.003 ± 0.000
T3 (NP & AAT)	0 Day	26.2 ± 0.115	7.43 ± 0.052	7.50 ± 0.144	147 ± 3.528	218 ± 4.163	0.006 ± 0.001
	30 Days	25.8 ± 0.115	7.54 ± 0.104	7.17 ± 0.220	147 ± 3.528	217 ± 4.055	0.007 ± 0.002
	60 Days	26.0 ± 0.115	7.53 ± 0.052	6.83 ± 0.220	133 ± 3.712	218 ± 2.000	0.006 ± 0.001
	90 Days	26.2 ± 0.115	7.52 ± 0.079	6.83 ± 0.083	123 ± 3.712	210 ± 3.055	0.005 ± 0.001
T4 (16:08 & AAT)	0 Day	26.4 ± 0.088	7.41 ± 0.052	7.33 ± 0.167	142 ± 3.464	211 ± 4.055	0.005 ± 0.000
	30 Days	25.7 ± 0.033	7.49 ± 0.079	7.00 ± 0.289	142 ± 3.464	211 ± 4.055	0.006 ± 0.001
	60 Days	26.0 ± 0.088	7.48 ± 0.079	7.08 ± 0.220	131 ± 4.055	209 ± 4.055	0.006 ± 0.001
	90 Days	26.1 ± 0.088	7.48 ± 0.052	6.33 ± 0.220	117 ± 4.055	205 ± 3.528	0.004 ± 0.000
T5 (14:10 & AAT)	0 Day	26.2 ± 0.088	7.48 ± 0.079	7.67 ± 0.083	145 ± 3.528	215 ± 4.055	0.006 ± 0.001
	30 Days	25.8 ± 0.088	7.56 ± 0.104	7.00 ± 0.250	144 ± 4.163	214 ± 4.619	0.007 ± 0.002
	60 Days	25.9 ± 0.088	7.55 ± 0.052	6.53 ± 0.117	138 ± 4.619	211 ± 4.667	0.006 ± 0.001
	90 Days	26.1 ± 0.088	7.78 ± 0.052	6.50 ± 0.144	121 ± 4.055	207 ± 3.528	0.006 ± 0.001

Data expressed as Mean ± SE (n = 3).

Table 2: Proximate composition of feed ingredients and experimental diet.

Ingredients or Diet	Moisture (%)	Crude Protein (%)	Ether Extract (%)	Crude Fibre (%)	Total Ash (%)	Nitrogen Free Extract (%)
Fish Meal	8.99 ± 0.27	32.43 ± 0.43	2.76 ± 0.41	3.34 ± 0.15	21.92 ± 0.26	30.56 ± 0.25
Wheat Flour	9.88 ± 0.29	9.66 ± 0.35	1.22 ± 0.36	8.77 ± 0.16	4.1 ± 0.13	66.37 ± 0.24
Soyabean Oil Cake	8.35 ± 0.21	52.94 ± 0.61	1.92 ± 0.54	8.12 ± 0.13	12.19 ± 0.16	16.48 ± 0.19
Groundnut Oil Cake	8.35 ± 0.23	48.23 ± 0.31	5.52 ± 0.54	9.08 ± 0.18	6.73 ± 0.15	22.09 ± 0.21
Experimental Diet	6.03 ± 0.13	30.65 ± 0.08	9.53 ± 0.10	7.89 ± 0.05	10.79 ± 0.13	35.11 ± 0.15

Data expressed as Mean ± SE (n = 3).

4.3. Gonado-somatic index (GSI)

The GSI (%) of males during 90 days experimental period in 6 different experimental groups is shown in Table 3 and presented in Figure 1, 2, 3 and 4. In Control (C), the highest GSI was 0.616 ± 0.042 in 90 days and the lowest GSI was 0.330 ± 0.033 in 60 days. In Treatment 1 (T1), the highest GSI was 0.510 ± 0.026 in 0 day and the lowest GSI was 0.400 ± 0.034 in 30 days. In Treatment 2 (T2), the highest GSI was 0.659 ± 0.067 in 60 days and the lowest GSI was 0.510 ± 0.026 in 0 day. In Treatment 3 (T3), the highest GSI was 0.542 ± 0.065 in 90 days and the lowest GSI was 0.487 ± 0.075 in 30 days. In Treatment 4 (T4), the highest GSI was 0.510 ± 0.026 in 0 day and the lowest GSI was 0.416 ± 0.044 in 60 days. In Treatment 5 (T5), the highest GSI was 0.710 ± 0.051 in 60 days and the lowest GSI was 0.494 ± 0.052 in 90 days.

The GSI (%) of females during 90 days experimental period in 6 different experimental groups is shown in Table 4 and presented in Figure 5, 6, 7 and 8. In C, the highest GSI was 4.690 ± 0.385 in 90 days and the lowest GSI was 0.921 ± 0.047 in 0 day. In T1, the highest GSI was 10.493 ± 2.167 in 90 days and the lowest GSI was 0.921 ± 0.047 in 0 day. In T2, the highest GSI was 7.048 ± 0.804 in 90 days and the lowest GSI was 0.921 ± 0.047 in 0 day. In T3, the highest GSI was 3.949 ± 0.453 in 60 days and the lowest GSI was 0.921 ± 0.047 in 0 day. In T4, the highest GSI was 3.279 ± 0.510 in 90 days and the lowest GSI was 0.921 ± 0.047 in 0 day. In T5, the highest GSI was 4.978 ± 1.169 in 90 days and the lowest GSI was 0.921 ± 0.047 in 0 day.

Table 3: Gonado-somatic index (% GSI) of males during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	$0.510^{a} \pm 0.026$	$0.365^{b} \pm 0.030$	$0.330^{d} \pm 0.033$	$0.616^{a} \pm 0.042$
T1 (16:08 & AT)	$0.510^{a} \pm 0.026$	$0.400^{b} \pm 0.034$	$0.430^{cd} \pm 0.026$	$0.402^{b} \pm 0.020$
T2 (14:10 & AT)	$0.510^{a} \pm 0.026$	$0.556^{a} \pm 0.031$	$0.659^{ab} \pm 0.067$	$0.552^{ab} \pm 0.053$
T3 (NP & AAT)	$0.510^{a} \pm 0.026$	$0.487^{ab} \pm 0.075$	$0.538^{bc} \pm 0.072$	$0.542^{ab} \pm 0.065$
T4 (16:08 & AAT)	$0.510^{a} \pm 0.026$	$0.478^{ab} \pm 0.037$	$0.416^{cd} \pm 0.044$	$0.500^{ab} \pm 0.057$
T5 (14:10 & AAT)	$0.510^{a} \pm 0.026$	$0.570^{a} \pm 0.066$	$0.710^{a} \pm 0.051$	$0.494^{ab} \pm 0.052$

Data expressed as Mean ± SE (n = 9).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

Table 4: Gonado-somatic index (% GSI) of females during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	0.921 ^{a} ± 0.047	1.363 ^{b} ± 0.143	1.584 ^{b} ± 0.241	4.690 ^{bc} ± 0.385
T1 (16:08 & AT)	0.921 ^{a} ± 0.047	3.009 ^{a} ± 0.806	2.643 ^{ab} ± 0.346	10.493 ^{a} ± 2.167
T2 (14:10 & AT)	0.921 ^{a} ± 0.047	1.528 ^{b} ± 0.452	3.358 ^{a} ± 0.702	7.048 ^{b} ± 0.804
T3 (NP & AAT)	0.921 ^{a} ± 0.047	1.346 ^{b} ± 0.226	3.949 ^{a} ± 0.453	3.519 ^{c} ± 0.276
T4 (16:08 & AAT)	0.921 ^{a} ± 0.047	1.198 ^{b} ± 0.220	2.509 ^{ab} ± 0.412	3.279 ^{c} ± 0.510
T5 (14:10 & AAT)	0.921 ^{a} ± 0.047	2.019 ^{ab} ± 0.353	4.149 ^{a} ± 0.952	4.978 ^{bc} ± 1.169

Data expressed as Mean ± SE (n = 9).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

4.4. Plasma sex-steroids concentration

The sex-steroids quantified in males are 11-keto testosterone and 17α,20β-dihydroxy progesterone and in females are 17β-estradiol and 17α,20β-dihydroxy progesterone.

4.4.1. 11-keto testosterone (11-KT)

The plasma 11-KT concentration of males during 90 days experimental period in 6 different experimental groups is shown in Table 5 and presented in Figure 1, 2, 3 and 4. In control and other treatment groups, except T1 and T5, the highest plasma 11-KT concentration was recorded in 90 days. The lowest plasma 11-KT values in control and other treatment groups were 0.157 ± 0.007 ng/ml in 0 day. The highest plasma 11-KT values in C, T2, T3 and T4 were recorded as 3.185 ± 0.084 ng/ml, 1.247 ± 0.074 ng/ml, 2.341 ± 0.067 ng/ml, and 2.072 ± 0.070 ng/ml respectively in 90 days. The plasma 11-KT concentration in T1 and T5 reached maximum at 60 days, which were 1.266 ± 0.034 ng/ml and 2.379 ± 0.031 ng/ml respectively.

4.4.2. 17β-estradiol (E-2)

The changes in plasma E-2 concentration of females during experimental period are shown in Table 6 and in Figure 5, 6, 7 and 8. In control and other treatment groups, except T4 and T5, the highest plasma E-2 concentration was recorded in 90 days. The lowest plasma E-2 values in control and other treatment groups were 0.216 ± 0.016 ng/ml in 0 day. The highest plasma E-2 values in C, T1, T2 and T3 were recorded as 0.631 ± 0.071 ng/ml, 1.957 ± 0.068 ng/ml, 0.541 ± 0.037 ng/ml and 1.200 ± 0.078 ng/ml respectively in 90 days. The plasma E-2 concentration in T4 and T5 reached maximum at 60 days, which were 0.605 ± 0.024 ng/ml and 1.113 ± 0.041 ng/ml respectively.

4.4.3. 17α,20β-dihydroxy progesterone (DHP)

The plasma DHP concentration of males during 90 days experimental period in 6 different experimental groups is shown in Table 7 and presented in Figure 1, 2, 3 and 4. In control and other treatment groups, except T2 and T5, the highest plasma DHP concentration was recorded in 30 days. The lowest plasma

DHP values in control and other treatment groups were 0.036 ± 0.004 ng/ml in 0 day, except T2, which was 0.014 ± 0.001 ng/ml. The highest plasma DHP values in C, T1, T3 and T4 were recorded as 0.648 ± 0.029 ng/ml, 0.259 ± 0.024 ng/ml, 0.720 ± 0.049 ng/ml and 0.792 ± 0.047 ng/ml respectively in 30 days. The plasma DHP concentration in T2 reached maximum at 90 days, which was 0.046 ± 0.004 ng/ml and in T5 reached maximum at 60 days, which was 0.050 ± 0.003 ng/ml.

The plasma DHP concentration of females during experimental period are shown in Table 6 and in Figure 5, 6, 7 and 8. In T2, T3 and T4, except C, T1 and T5 the highest plasma DHP concentration was recorded in 90 days. The lowest plasma DHP values in control was 0.065 ± 0.006 ng/ml in 0 day, in T1 was 0.064 ± 0.006 ng/ml in 60 days and in T2, T3, T4, T5 were 0.026 ± 0.002 ng/ml, 0.033 ± 0.002 ng/ml, 0.049 ± 0.004 ng/ml and 0.032 ± 0.003 ng/ml respectively in 30 days. The highest plasma DHP values in T2, T3 and T4 were recorded as 0.092 ± 0.006 ng/ml, 0.082 ± 0.006 ng/ml and 0.083 ± 0.006 ng/ml respectively in 90 days. The plasma DHP concentration in C and T1 reached maximum at 90 days, which were 0.413 ± 0.020 ng/ml and 0.226 ± 0.010 ng/ml and in T5 reached maximum at 60 days, which was 0.085 ± 0.006 ng/ml.

Table 5: Plasma 11-keto testosterone (11-KT) concentration of males during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	0.157 ^{a} ± 0.007	0.187 ^{d} ± 0.016	1.151 ^{d} ± 0.031	3.185 ^{a} ± 0.084
T1 (16:08 & AT)	0.157 ^{a} ± 0.007	0.136 ^{d} ± 0.008	1.266 ^{c} ± 0.034	0.547 ^{e} ± 0.021
T2 (14:10 & AT)	0.157 ^{a} ± 0.007	0.586 ^{a} ± 0.030	0.637 ^{e} ± 0.028	1.247 ^{d} ± 0.074
T3 (NP & AAT)	0.157 ^{a} ± 0.007	0.480 ^{b} ± 0.015	1.689 ^{b} ± 0.029	2.341 ^{b} ± 0.067
T4 (16:08 & AAT)	0.157 ^{a} ± 0.007	0.178 ^{d} ± 0.017	1.075 ^{d} ± 0.031	2.072 ^{c} ± 0.070
T5 (14:10 & AAT)	0.157 ^{a} ± 0.007	0.346 ^{c} ± 0.019	2.379 ^{a} ± 0.031	0.403 ^{e} ± 0.014

Data expressed as Mean ± SE (n = 3).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

Table 6: Plasma 17β-estradiol (E-2) concentration of females during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	0.216 ^{a} ± 0.016	0.158 ^{cd} ± 0.010	0.480 ^{d} ± 0.027	1.631 ^{b} ± 0.071
T1 (16:08 & AT)	0.216 ^{a} ± 0.016	0.198 ^{c} ± 0.015	0.528 ^{cd} ± 0.022	1.957 ^{a} ± 0.068
T2 (14:10 & AT)	0.216 ^{a} ± 0.016	0.442 ^{b} ± 0.016	0.284 ^{e} ± 0.016	0.541 ^{e} ± 0.037
T3 (NP & AAT)	0.216 ^{a} ± 0.016	0.499 ^{a} ± 0.021	0.979 ^{b} ± 0.041	1.200 ^{c} ± 0.078
T4 (16:08 & AAT)	0.216 ^{a} ± 0.016	0.131 ^{d} ± 0.009	0.605 ^{c} ± 0.024	0.130 ^{f} ± 0.010
T5 (14:10 & AAT)	0.216 ^{a} ± 0.016	0.202 ^{c} ± 0.016	1.113 ^{a} ± 0.041	0.959 ^{d} ± 0.069

Data expressed as Mean ± SE (n = 3).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

Table 7: Plasma 17α,20β-dihydroxy progesterone (DHP) concentration of males during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	0.036 ^{a} ± 0.004	0.648 ^{b} ± 0.029	0.055 ^{a} ± 0.002	0.045 ^{a} ± 0.004
T1 (16:08 & AT)	0.036 ^{a} ± 0.004	0.259 ^{c} ± 0.024	0.038 ^{c} ± 0.002	0.042 ^{a} ± 0.004
T2 (14:10 & AT)	0.036 ^{a} ± 0.004	0.014 ^{d} ± 0.001	0.039 ^{c} ± 0.002	0.046 ^{a} ± 0.004
T3 (NP & AAT)	0.036 ^{a} ± 0.004	0.720 ^{ab} ± 0.049	0.045 ^{bc} ± 0.004	0.053 ^{a} ± 0.005
T4 (16:08 & AAT)	0.036 ^{a} ± 0.004	0.792 ^{a} ± 0.047	0.044 ^{bc} ± 0.004	0.048 ^{a} ± 0.005
T5 (14:10 & AAT)	0.036 ^{a} ± 0.004	0.047 ^{d} ± 0.004	0.050 ^{ab} ± 0.003	0.046 ^{a} ± 0.005

Data expressed as Mean ± SE (n = 3).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

Table 8: Plasma 17α,20β-dihydroxy progesterone (DHP) concentration of females during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	0.065 ^{a} ± 0.006	0.413 ^{a} ± 0.020	0.058 ^{c} ± 0.004	0.072 ^{a} ± 0.006
T1 (16:08 & AT)	0.065 ^{a} ± 0.006	0.226 ^{b} ± 0.010	0.064 ^{bc} ± 0.006	0.083 ^{a} ± 0.006
T2 (14:10 & AT)	0.065 ^{a} ± 0.006	0.026 ^{c} ± 0.002	0.080 ^{ab} ± 0.008	0.092 ^{a} ± 0.006
T3 (NP & AAT)	0.065 ^{a} ± 0.006	0.033 ^{c} ± 0.002	0.065 ^{abc} ± 0.006	0.082 ^{a} ± 0.006
T4 (16:08 & AAT)	0.065 ^{a} ± 0.006	0.049 ^{c} ± 0.004	0.069 ^{abc} ± 0.006	0.083 ^{a} ± 0.006
T5 (14:10 & AAT)	0.065 ^{a} ± 0.006	0.032 ^{c} ± 0.003	0.085 ^{a} ± 0.006	0.082 ^{a} ± 0.006

Data expressed as Mean ± SE (n = 3).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

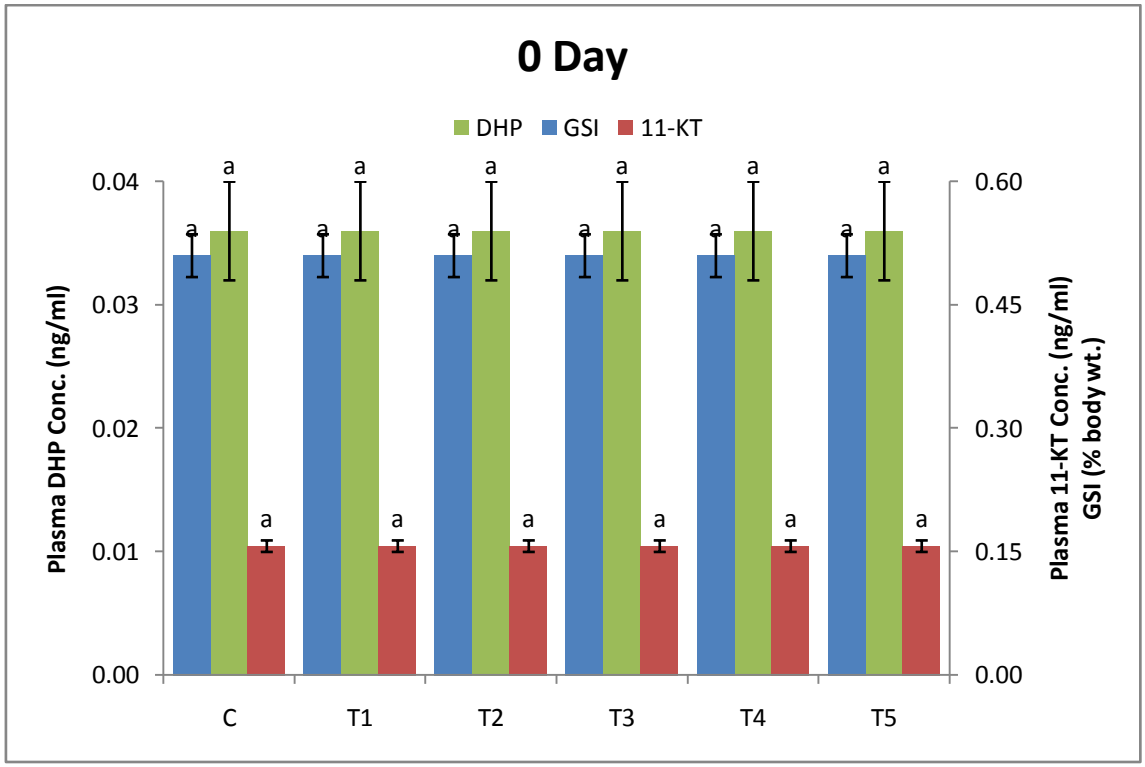


Fig. 1: GSI (%) and plasma sex-steroids concentration (ng/ml) of males under control and different treatments at 0 day.

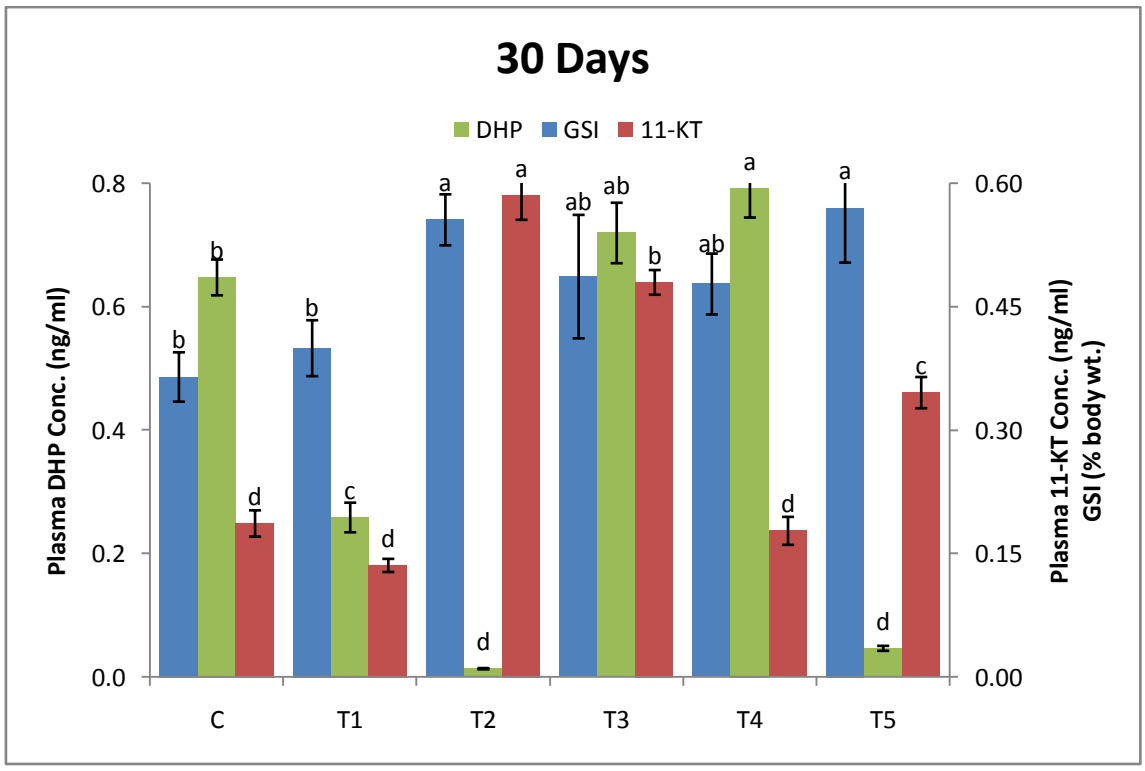


Fig. 2: GSI (%) and plasma sex-steroids concentration (ng/ml) of males under control and different treatments at 30 days.

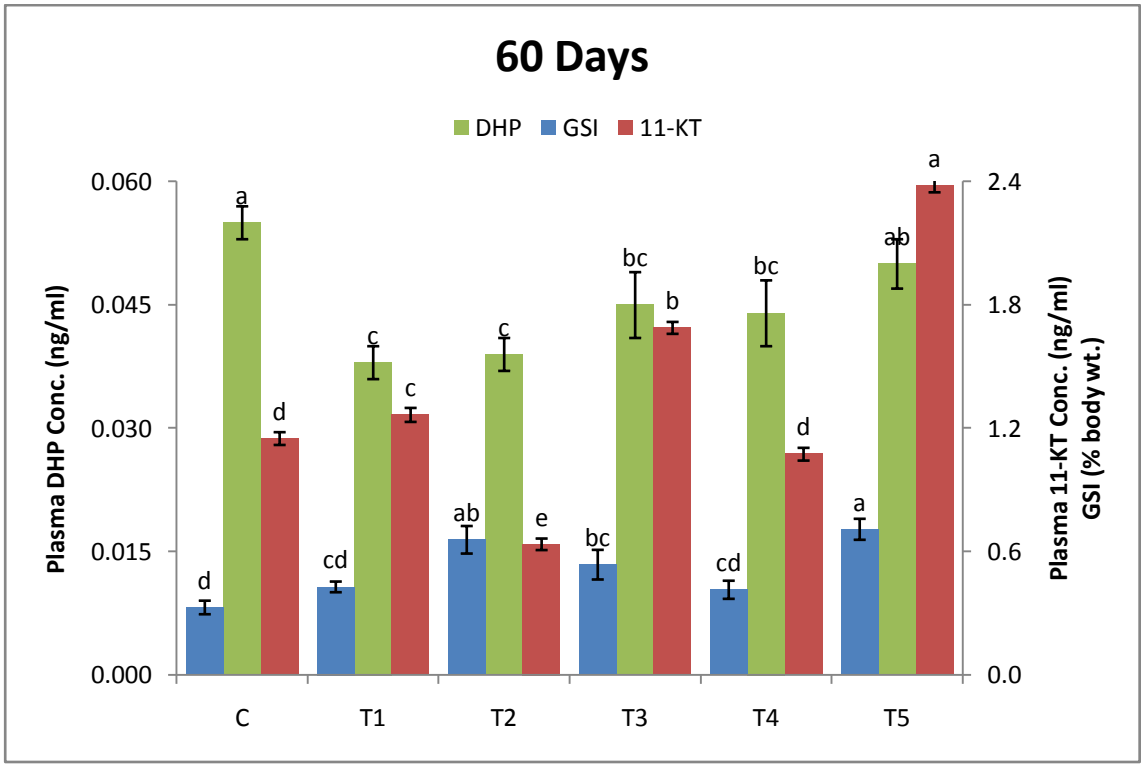


Fig. 3: GSI (%) and plasma sex-steroids concentration (ng/ml) of males under control and different treatments at 60 days.

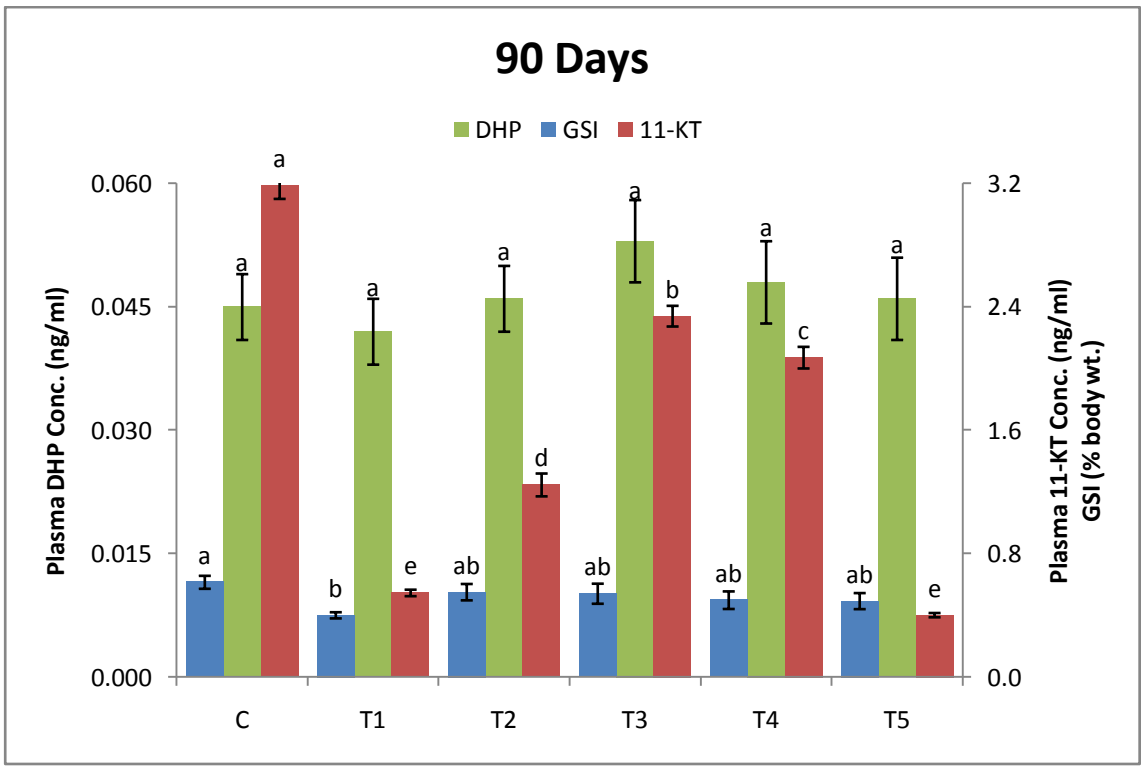


Fig. 4: GSI (%) and plasma sex-steroids concentration (ng/ml) of males under control and different treatments at 90 days.

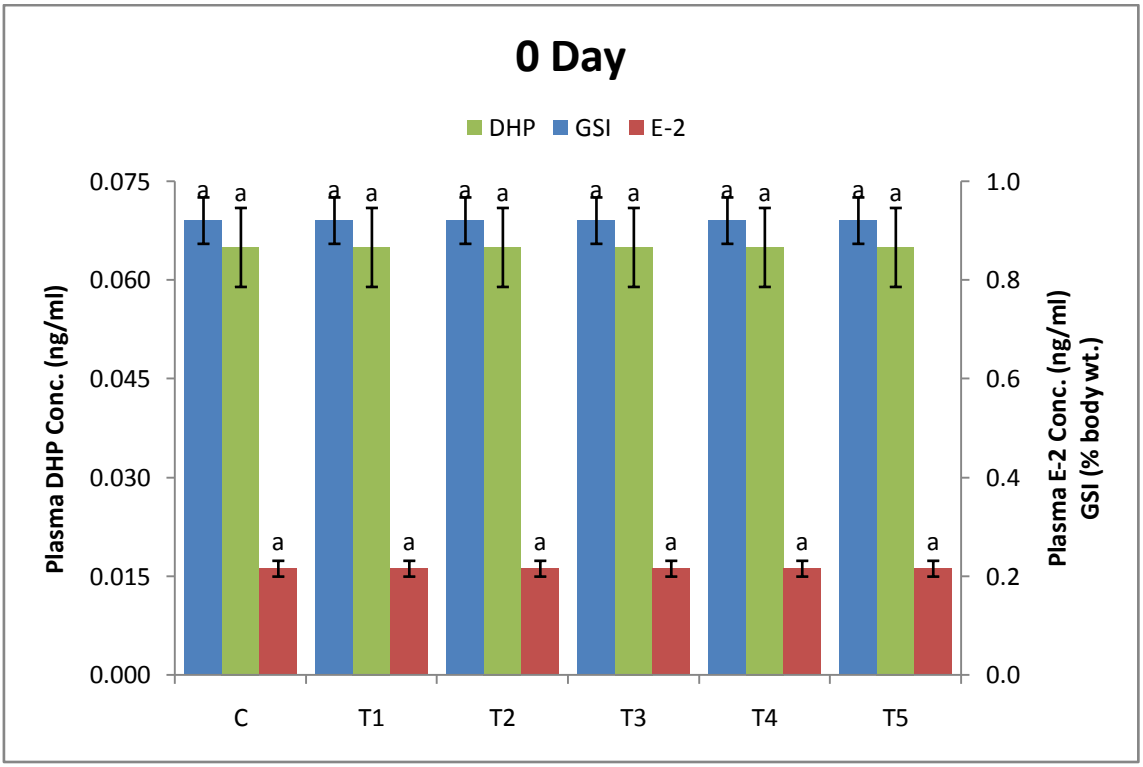


Fig. 5: GSI (%) and plasma sex-steroids concentration (ng/ml) of females under control and different treatments at 0 day.

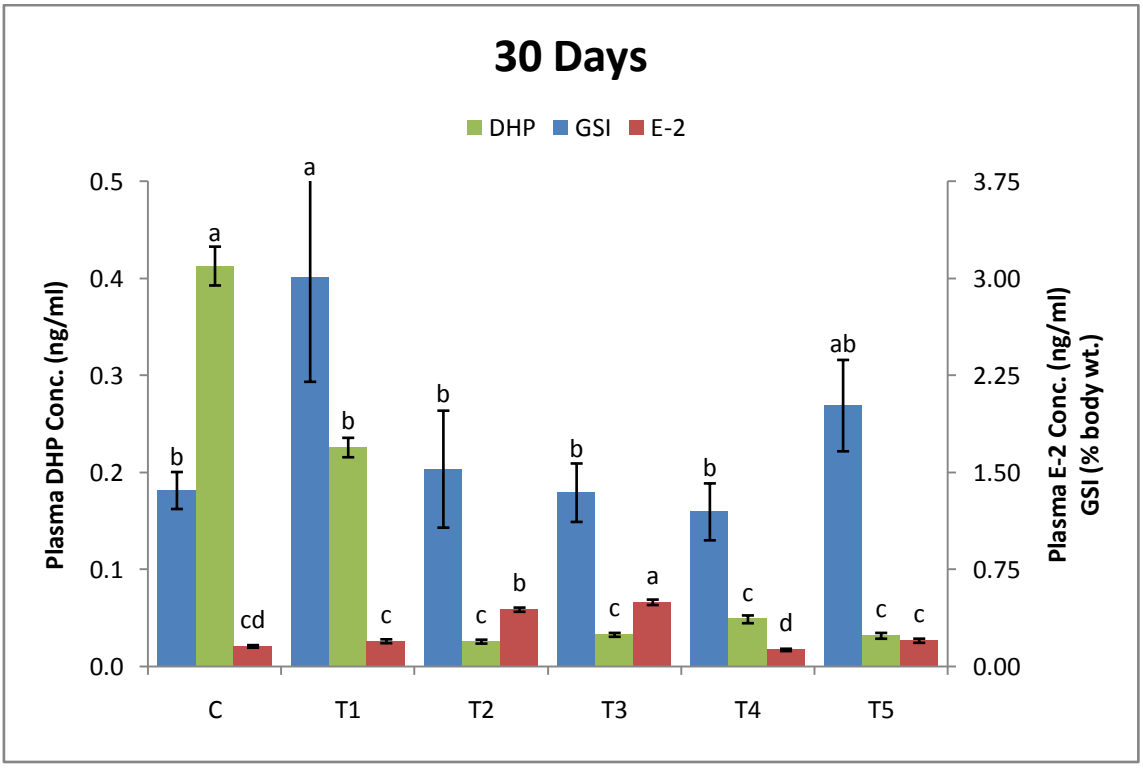


Fig. 6: GSI (%) and plasma sex-steroids concentration (ng/ml) of females under control and different treatments at 30 days.

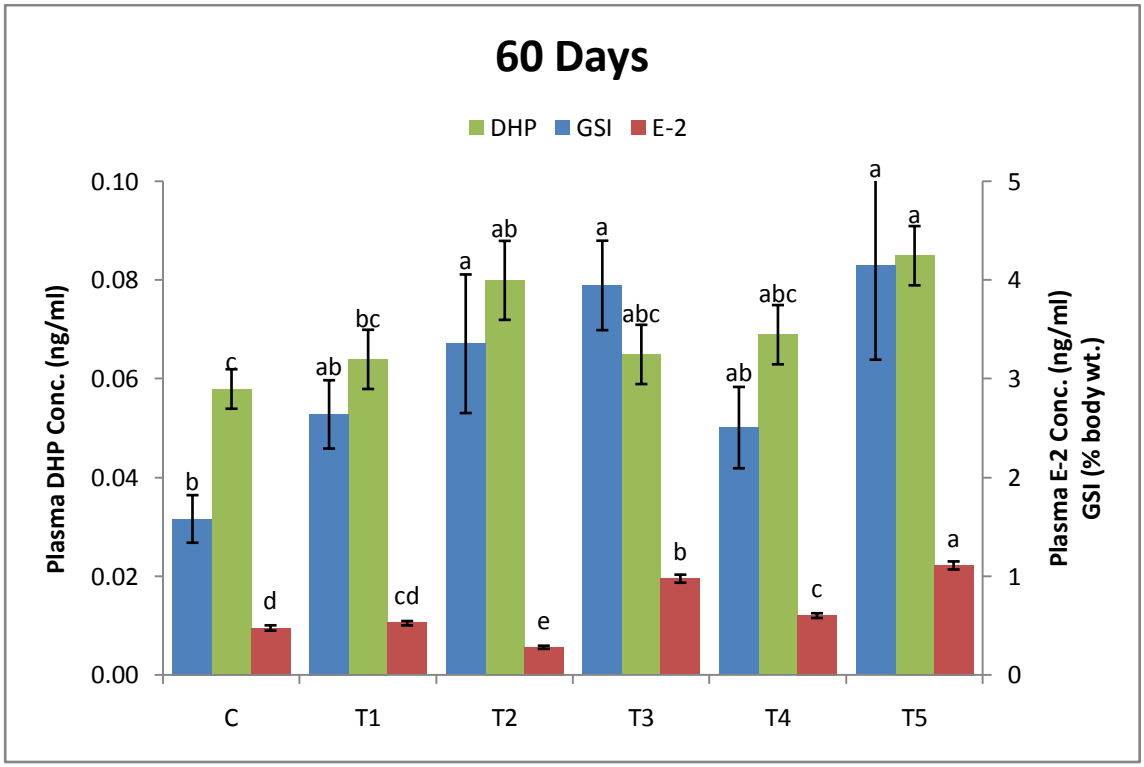


Fig. 7: GSI (%) and plasma sex-steroids concentration (ng/ml) of females under control and different treatments at 60 days.

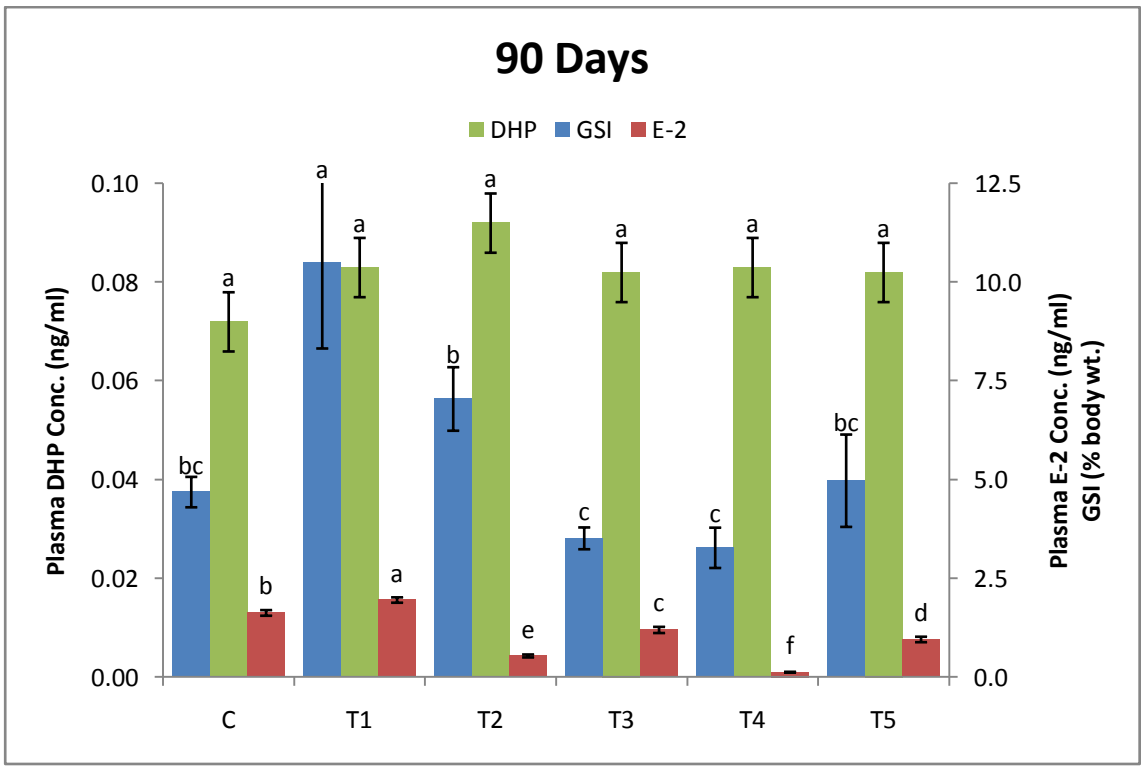


Fig. 8: GSI (%) and plasma sex-steroids concentration (ng/ml) of females under control and different treatments at 90 days.

4.5. Fecundity

The changes in fecundity during 90 days experimental period in 6 different experimental groups is shown in Table 9. The mean fecundity ranged between 352 ± 13.56 and 860 ± 70.02. At 0 day mean fecundity were 352 ± 13.56, which reached maximum values in the range between 608 ± 43.12 and 860 ± 70.02 at 90 days.

4.6. Oocyte frequency distribution

The oocyte frequency distribution in ovaries during 90 days experimental period in 6 different experimental groups is shown in Table 10 and presented in Figure 9, 10, 11 and 12. The oocyte diameter varied from 0.01 mm to 0.80 mm. The oocyte diameter increased with the progress of time in C and different treatments as the experimental duration increases. Maximum number of smaller eggs is found in between 0.01 mm and 0.50 mm at 0 day irrespective of groups. Larger size oocytes was appered in ovaries mostly at 90 days in C and all treatments. The percentages of larger oocytes were more in T1 and T2 compared to C and other treatments at 90 days.

Table 9: Changes in mean fecundity during 90 days experimental period.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	352 ^{a} ± 13.56	434 ^{b} ± 14.46	449 ^{b} ± 30.43	732 ^{ab} ± 27.53
T1 (16:08 & AT)	352 ^{a} ± 13.56	575 ^{a} ± 58.02	564 ^{ab} ± 34.36	860 ^{a} ± 70.02
T2 (14:10 & AT)	352 ^{a} ± 13.56	413 ^{b} ± 55.67	593 ^{a} ± 55.00	842 ^{a} ± 34.80
T3 (NP & AAT)	352 ^{a} ± 13.56	424 ^{b} ± 28.90	661 ^{a} ± 27.80	644 ^{b} ± 23.48
T4 (16:08 & AAT)	352 ^{a} ± 13.56	406 ^{b} ± 30.81	538 ^{ab} ± 30.30	608 ^{b} ± 43.12
T5 (14:10 & AAT)	352 ^{a} ± 13.56	500 ^{ab} ± 35.44	641 ^{a} ± 64.28	642 ^{b} ± 80.00

Data expressed as Mean ± SE (n = 9).

Mean values bearing different superscripts under each column vary significantly ($p<0.05$).

Table 10: Oocyte frequency distribution (%) during 90 days experimental period.

Treatment	Ova Diameter (mm)	Class	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	0.01 – 0.10	I	55	29	6	27
	0.11 – 0.20	II	26	34	15	26
	0.21 – 0.30	III	10	28	20	14
	0.31 – 0.40	IV	8	5	19	3
	0.41 – 0.50	V	1	2	18	6
	0.51 – 0.60	VI	0	2	19	10
	0.61 – 0.70	VII	0	0	2	9
	0.71 – 0.80	VIII	0	0	0	5
T1 (16:08 & AT)	0.01 – 0.10	I	55	59	2	13
	0.11 – 0.20	II	26	25	10	26
	0.21 – 0.30	III	10	10	24	13
	0.31 – 0.40	IV	8	6	25	1
	0.41 – 0.50	V	1	1	25	9
	0.51 – 0.60	VI	0	0	7	17
	0.61 – 0.70	VII	0	0	4	17
	0.71 – 0.80	VIII	0	0	3	4
T2 (14:10 & AT)	0.01 – 0.10	I	55	19	3	7
	0.11 – 0.20	II	26	32	22	27
	0.21 – 0.30	III	10	26	24	16
	0.31 – 0.40	IV	8	13	19	4
	0.41 – 0.50	V	1	9	23	11
	0.51 – 0.60	VI	0	2	4	23
	0.61 – 0.70	VII	0	0	4	11
	0.71 – 0.80	VIII	0	0	1	1
T3 (NP & AAT)	0.01 – 0.10	I	55	43	18	5
	0.11 – 0.20	II	26	27	18	23
	0.21 – 0.30	III	10	17	18	25
	0.31 – 0.40	IV	8	8	19	16
	0.41 – 0.50	V	1	3	12	22
	0.51 – 0.60	VI	0	1	14	7
	0.61 – 0.70	VII	0	0	2	3
	0.71 – 0.80	VIII	0	0	0	0
T4 (16:08 & AAT)	0.01 – 0.10	I	55	19	16	25
	0.11 – 0.20	II	26	35	20	22
	0.21 – 0.30	III	10	21	22	13
	0.31 – 0.40	IV	8	16	18	4
	0.41 – 0.50	V	1	7	14	7
	0.51 – 0.60	VI	0	2	5	14
	0.61 – 0.70	VII	0	0	3	13
	0.71 – 0.80	VIII	0	0	2	2
T5 (14:10 & AAT)	0.01 – 0.10	I	55	11	0	19
	0.11 – 0.20	II	26	24	2	13
	0.21 – 0.30	III	10	23	15	10
	0.31 – 0.40	IV	8	25	27	17
	0.41 – 0.50	V	1	9	32	13
	0.51 – 0.60	VI	0	6	14	23
	0.61 – 0.70	VII	0	1	8	5
	0.71 – 0.80	VIII	0	0	2	0

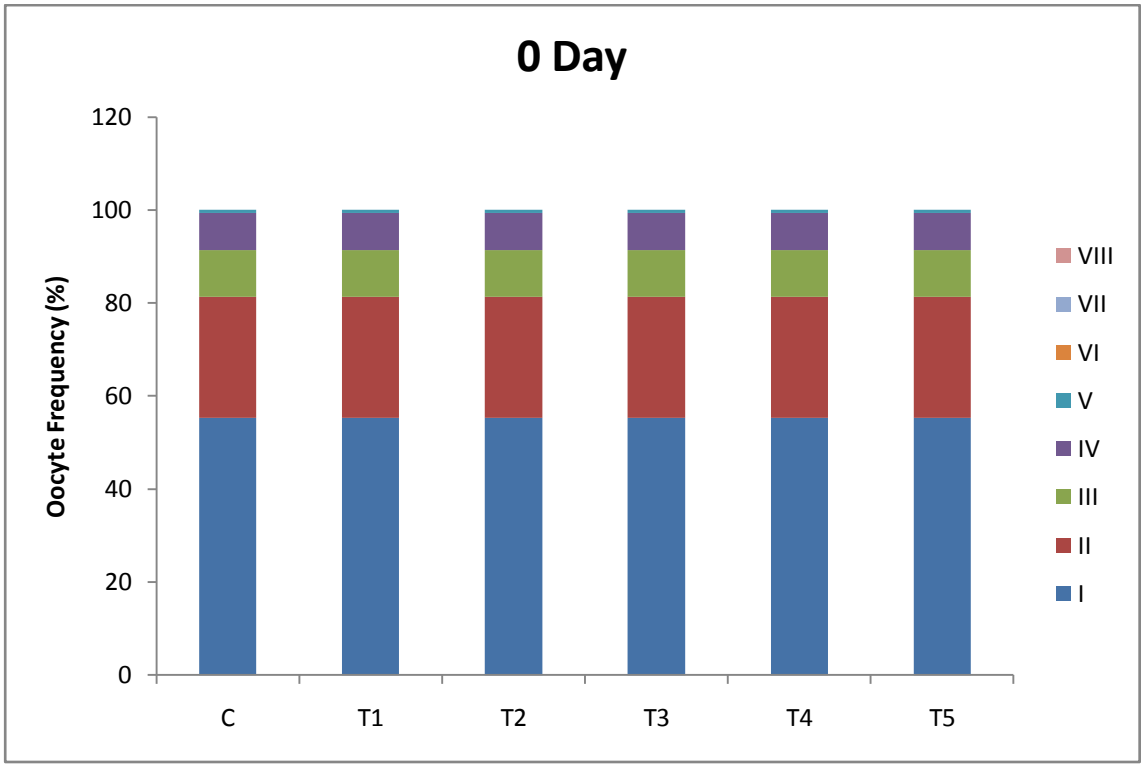


Fig. 9: Oocyte frequency distribution (%) under control and different treatments at 0 day.

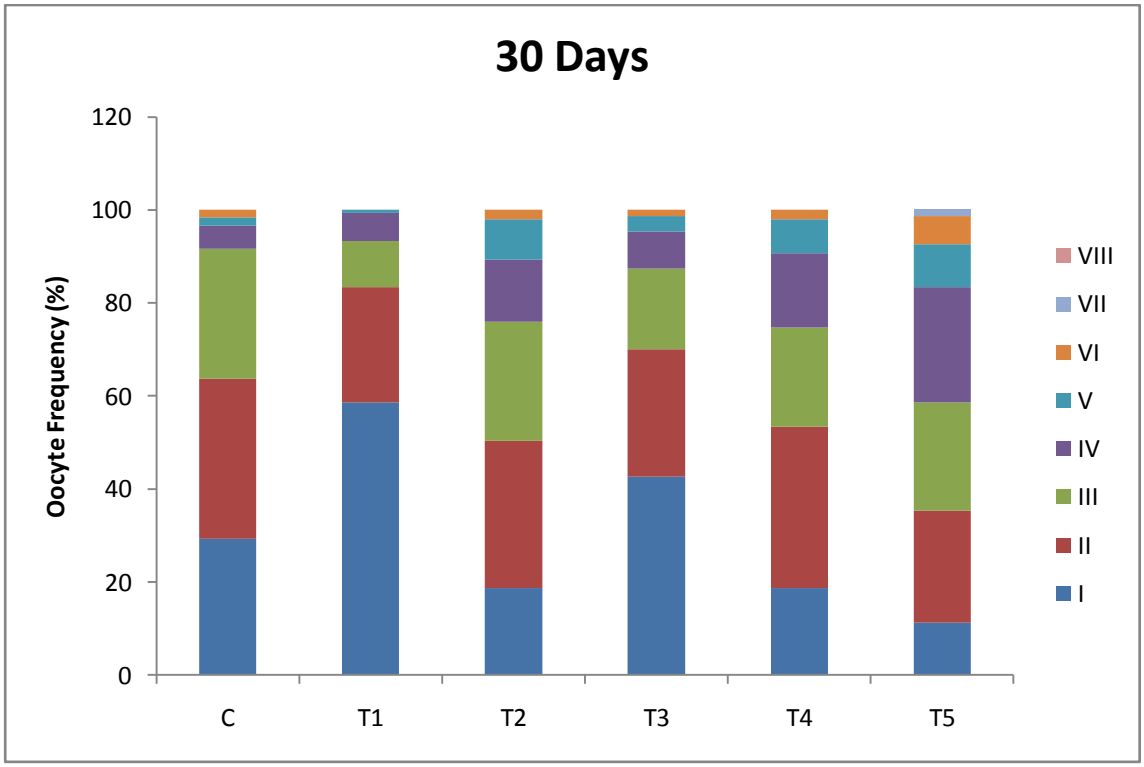


Fig. 10: Oocyte frequency distribution (%) under control and different treatments at 30 days.

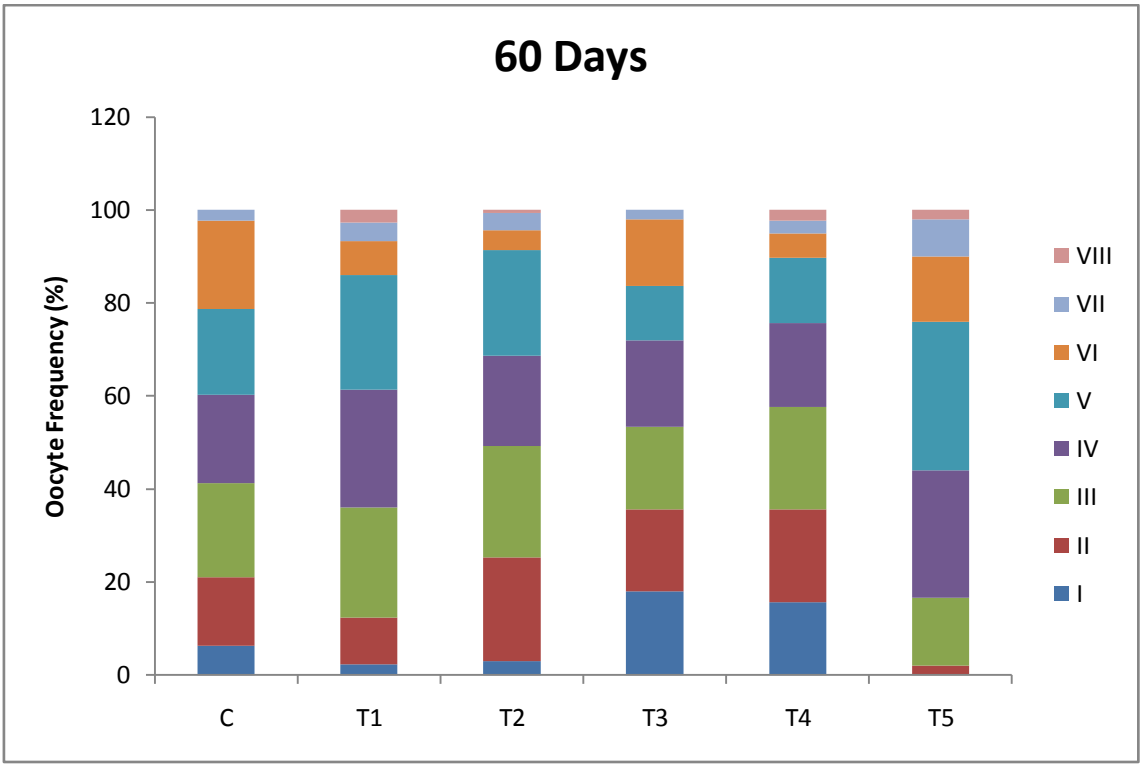


Fig. 11: Oocyte frequency distribution (%) under control and different treatments at 60 days.

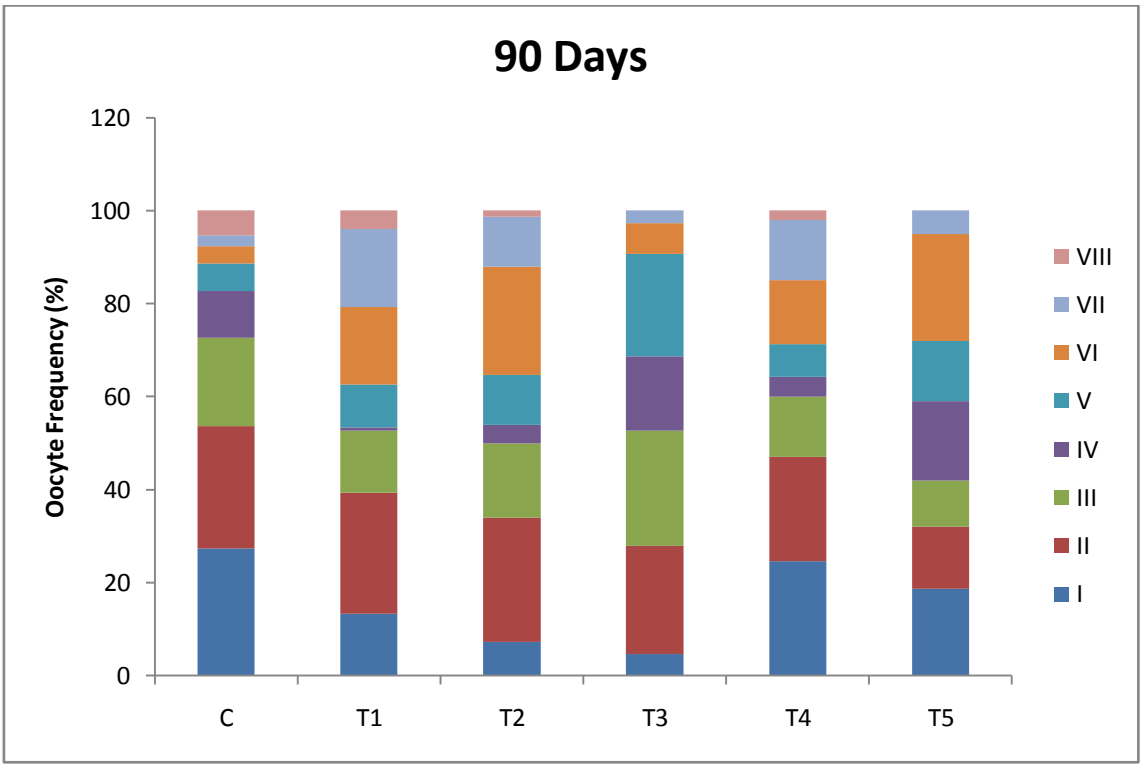


Fig. 12: Oocyte frequency distribution (%) under control and different treatments at 90 days.

4.7. Histology of gonads

The gonad development is staged on the basis of presence of different types of oocytes and spermatogenic cells in the ovary and testis respectively.

4.7.1. Oocyte development stages

The developmental stages of oocytes (Plate 1) in *Trichogaster lalius* can be classified as follows:

Stage I: Peri-nucleolar stage

The plasma area increased and the proportion between ooplasm and nuclear areas were changed. The nuclear membrane remained firm to an irregular outline, while several nucleoli penetrates into the granulations.

Stage II: Cortical alveolar stage

Cortical alveoli were seen on the periphery of the oocyte, containing yolk elements. Several small nucleoli can be observed, distributed around the periphery of the nucleus. The surrounding layer was further differentiated.

Stage III: Early vitellogenic stage

Densely stained globules appeared in the cytoplasm between lipid droplets and cortical vesicles.

Stage IV: Late vitellogenic stage

Lipid droplets and densely stained globules were dispersed homogenously in the whole cytoplasm.

Stage V: Germinal vesicle migration stage

Migrating nucleus (germinal vesicle; GV) migrated at the periphery of the oocytes. GV was surrounded by the ooplasm near the oocyte envelope and beginning of lipid droplets and dense globules condensation was taken place. The nuclear envelope was not well defined and the oocyte envelope invaginated slightly.

Stage VI: Germinal vesicle break down stage

Break down of the GV and disappearance of the nucleus occured. Deposition of yolk granules increased, supplanting the cortical alveoli and the ooplasm to the periphery of the oocyte. The nucleolemma disintegrated and strips of the nuclear envelope lay dispersed in the cytoplasm, which surrounded the nucleus.

Stage VIII: Post-ovulatory stage

Fully developed oocytes with large vacuoles were noticed after ovulation. Shrinkaged follicular wall along with a group of primary oocytes were present in the ovary. The yolk granules were completely disappeared.

4.7.2. Testicular development stages

The testicular developmental stages (Plate 2) in *Trichogaster lalius* is classified as follows:

Stage I: Spermatogonial proliferation stage

Mostly spermatocytes, few spermatogonia, spermatids and spermatozoa were present in testicular lobules.

Stage II: Early maturation stage

Continuous germinal epithelium appeared in lobules throughout testis. All stages of spermatogenesis were present, spermatozoa increased in lobules and ducts.

Stage III: Mid maturation stage

Continuous germinal epithelium in lobules in the periphery, discontinuous germinal epithelium in lobules near the ducts. Lobules enlarged in size and occupied with large number of spermatozoa. All stages of spermatogenesis were present.

Stage IV: Functional maturity stage

Discontinuous germinal epithelium were present in lobules throughout testis. Size of lobules largle extended and number of spermatozoa decreased and spermatogonial cells reappeared in more number along with other stages. Spermatocysts containing secondary spermatocytes, spermatids or spermatozoa were present, whereas, secondary spermatogonia or primary spermatocytes were absent. Primary spermatogonia were mostly located in the periphery, while spermatozoa appeared in lobules and ducts.

Stage V: Regression stage

Testis was in regression. All stages of developing germ cells were present. This stage was characterized by the presence of sertoli cells phagocytising spermatid residual bodies.

4.7.3. Progress of gonad development

Histological observations of the gonads of male and female specimens of *T. lalius* (Plate 3) revealed that the fish exhibits all stages of maturation, showing clearly the asynchronous type of ovarian and testicular development.

4.7.3.1. Female gonad development

Progress of ovarian development in control and different treatments during experimental period are depicted in Table 11. Initially all the ovaries were at stage I/II. At 90 days, III/IV stages observed in C, T3, T4 and T5 VI/V stages were recorded in T1, V/IV stages were recorded in T2.

4.7.3.2. Male gonad development

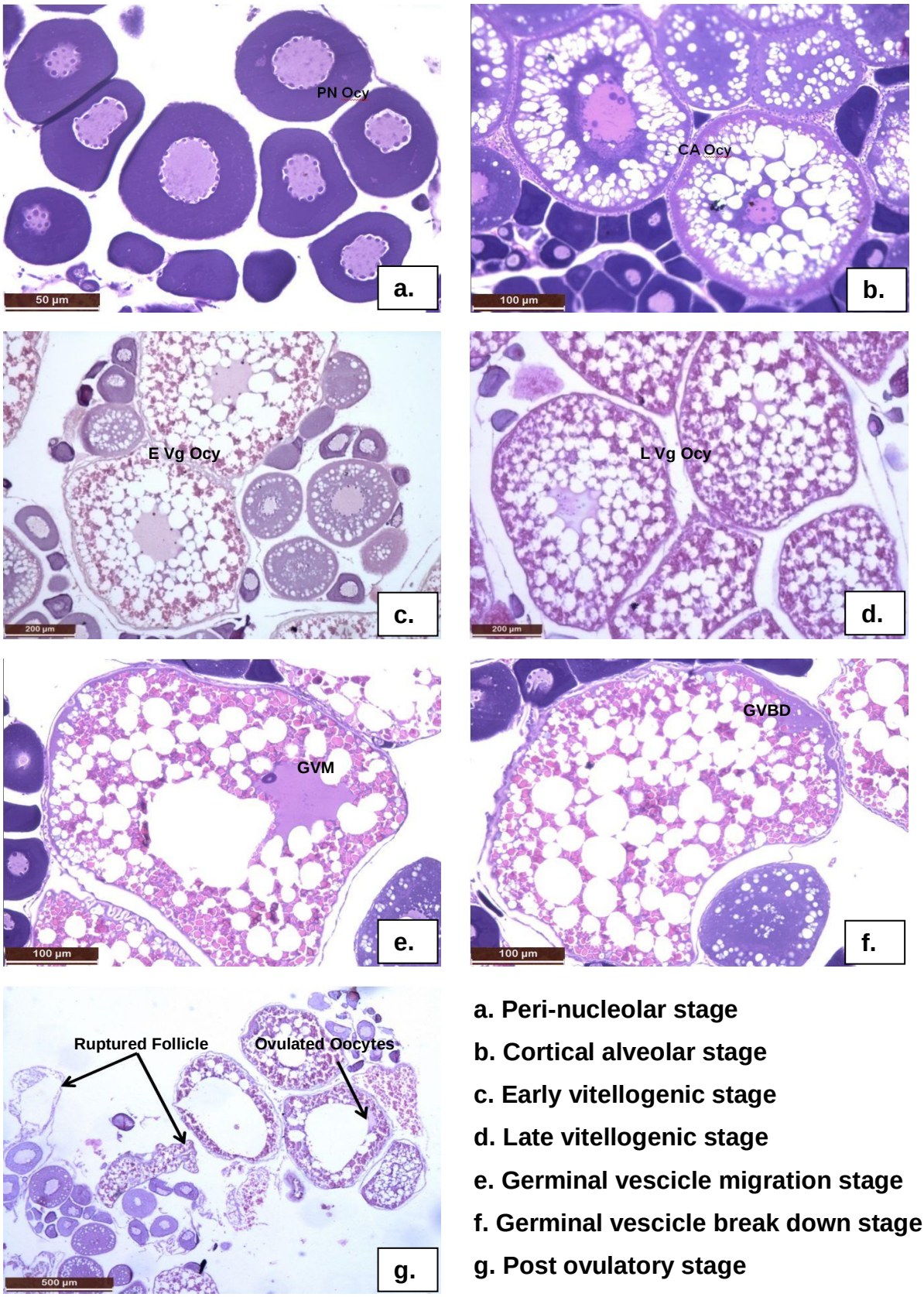
Progress of testicular development in control and different treatments during experimental period are depicted in Table 12. Initially all the testis were at stage III/IV, i.e. mid/late maturation stage. At 30 days V/I or V/I/II stages observed in C, T1, T3, T4 and I/II appeared in T2 and T5. At 60 days, mostly remained at III/IV stages in T2, T3 and T5, whereas, II/III stages were noticed in C, T1 and T4. At 90 days, testis remained at stage III in C, T3 and T4, whereas, stages III/IV and IV/V were noticed in T1and T5 and stages V/IV were noticed in T2.

Table 11: Progress of ovarian development in control and treatments.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	I/II	II	III	III/IV
T1 (16:08 & AT)	I/II	III	III/IV	VI/V
T2 (14:10 & AT)	I/II	II	III/IV	V/IV
T3 (NP & AAT)	I/II	II	III/IV	III/IV
T4 (16:08 & AAT)	I/II	II	III	III/IV
T5 (14:10 & AAT)	I/II	II/III	III/IV	III/IV

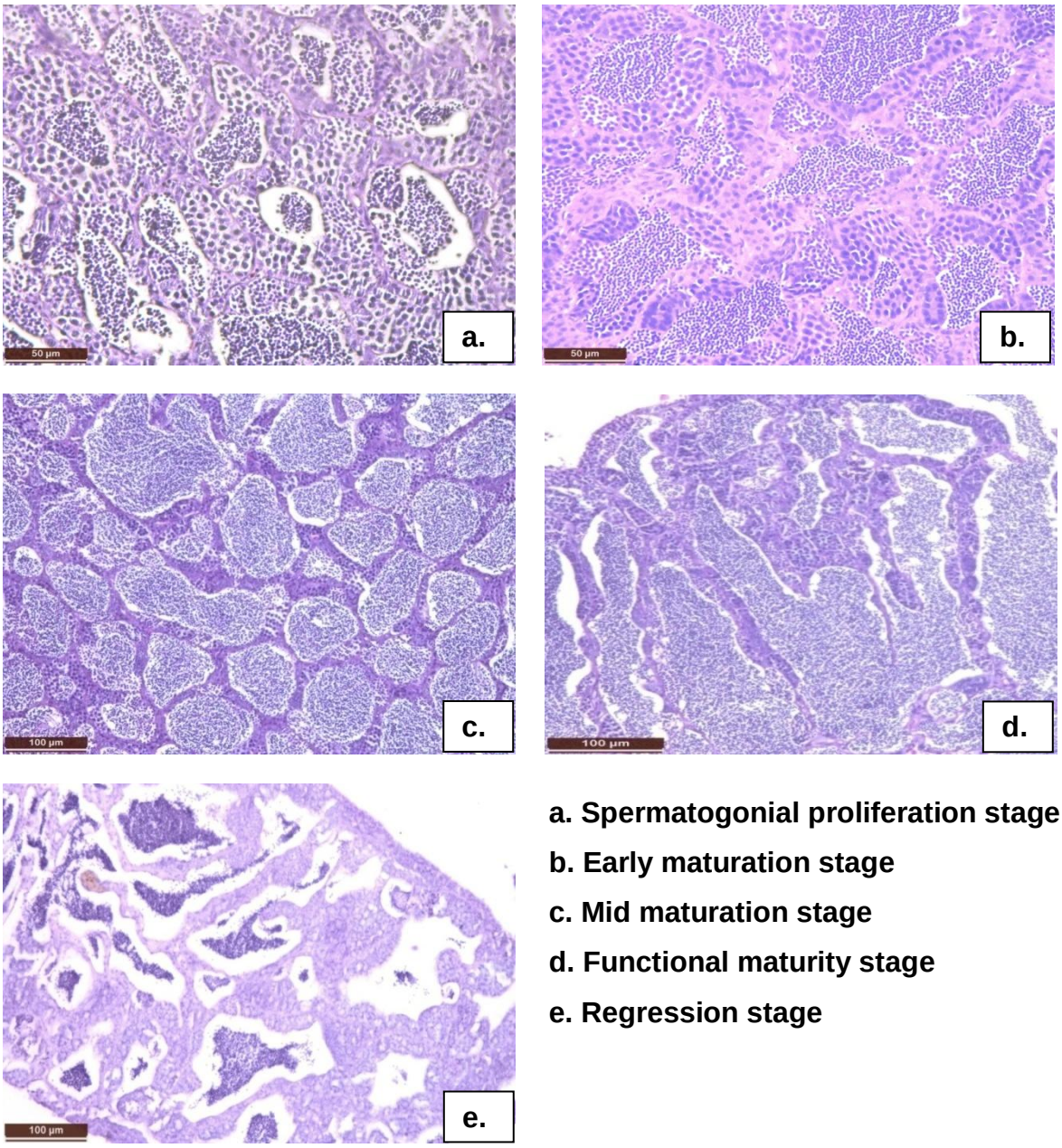
Table 12: Progress of testicular development in control and treatments.

Treatment	0 Day	30 Days	60 Days	90 Days
C (NP & AT)	III/IV	V/I	II/III	III
T1 (16:08 & AT)	III/IV	I/V	II/III	III/IV
T2 (14:10 & AT)	III/IV	II/I	III/IV	V/IV
T3 (NP & AAT)	III/IV	V/I/II	III/IV	III
T4 (16:08 & AAT)	III/IV	V/I	II/III	III
T5 (14:10 & AAT)	III/IV	I/II	III/IV	IV/V



a. Peri-nucleolar stage
b. Cortical alveolar stage
c. Early vitellogenic stage
d. Late vitellogenic stage
e. Germinal vescicle migration stage
f. Germinal vescicle break down stage
g. Post ovulatory stage

Plate 1: Oocyte developmental stages in *Trichogaster lalius*.



a. Spermatogonial proliferation stage

b. Early maturation stage

c. Mid maturation stage

d. Functional maturity stage

e. Regression stage

Plate 2: Testicular developmental stages in *Trichogaster lalius*.

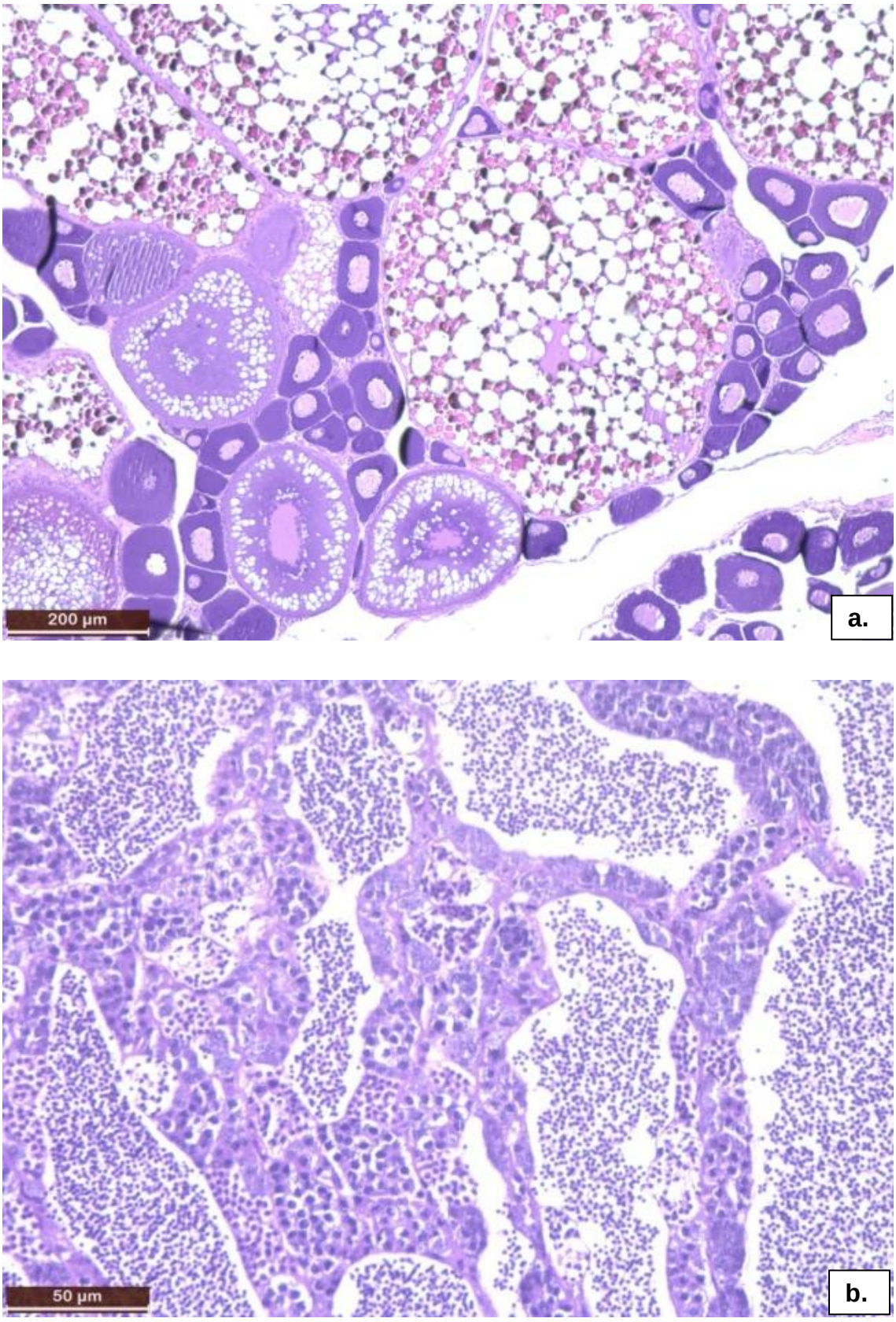


Plate 3: Histological observations of the gonads of (a.) male and (b.) female *Trichogaster lalius* showing various stages of maturation.

5. DISCUSSION

The Gouramis belongs to the Suborder – Anabantoidae which are popularly known as labyrinth fishes, characterized by an accessory respiratory organ called Labyrinth (Degani and Levy, 2013 and Welcomme, 1988). During breeding season, the male builds a bubble nest using bits of plants, twigs or other debris, in which the female lays the eggs. The reproductive performance is most often the deciding factor on the economic viability of fish production. Management techniques should be studied to improve reproductive performance and spawning frequency, number of eggs and hatching rate. However, with ornamental species, there are few studies directed to the development and optimization of these techniques, such as alternatives in induced spawning (Hill *et al*., 2005). Advancement of gonad recrudescence in captivity of this fish is not attempted earlier for extending spawning period through photo-thermal manipulation for enhancing period of active spawning phase of reproduction for augmenting seed production in captivity. As males exhibit brilliant color with the progress of testicular development, extended spawning phase provides a scope to maintain in brilliant color. Considering both issues the study was designed with an aim of advancing gonad recrudescence through photo-thermal manipulation.

5.1. Physico-chemical parameters of water

Maintaining optimum water quality suitable for a particular fish during the raising of brood stock is essential for obtaining suitable gonad maturity. The water quality parameters during the experimental period did not show much variation in between the control and treatment groups. All the parameters were in the optimum range in control and different treatments at different intervals. Average value of temperature, pH, dissolved oxygen, alkalinity, hardness and unionized ammonia were 23.6 ± 2.792 ^{0}C, 7.58 ± 0.121, 7.11 ± 0.381 mg/l, 138 ± 10.122 mg/l, 215 ± 5.200 mg/l and 0.004 ± 0.002 mg/l respectively and found suitable for gonad recrudescence in captivity.

5.2. Photo-thermal manipulation of reproduction

The timing of breeding to an optimal season is a requirement for a successful reproductive outcome and many organisms evolved to use changes in photoperiod as the primary signal that coordinates the breeding season. Environmental cues such as photoperiod and temperature can have important effects on the timing of gametogenesis, vitellogenesis and maturation in fish (Bromage *et al*., 2001). Manipulation of day length and water temperature, the primary environmental cues regulating gonadal development in temperate fish (Sumpter, 1990), which can advance or delay sexual maturation and spawning in commercially valuable fish. The present study provides information on the reproductive physiology of dwarf gourami, *Trichogaster lalius* reared in captivity. In addition, we expanded our knowledge by studying, for the first time in dwarf gourami, the plasma fluctuations of sex-steroids during gonad recrudescence. The plasma profiles of E-2, DHP and 11-KT were associated with the development of oocytes growth and maturation. On the other hand, investigation of the endocrine and gonadal changes in fish reared in natural conditions compared to fish exposed to experimental photoperiods alone or in combination with temperature, provide valuable information on the physiological effects caused by the photoperiodic manipulation of the captive maturation in both sexes.

5.3. Histological staging of gonads

Histological observations of the gonads of female *T. lalius* revealed that the successive cohorts of oocytes in different stages of development could be distinguished in ovaries of ripening and spawning females. Thus oocytes development was of asynchronous type (Plate 3). Histological analysis of the testicles reported the presence of individuals in all maturation stages, i.e. spermatogonia, spermatocytes and spermatids present in the lobular wall, whereas spermatozoa present in the lumen confirms the asynchronous pattern of development (Plate 3). The extreme is typified by species which have a prolonged spawning season, during which each individual may breed several times. There is a more-or-less continuous recruitment and subsequent development of gametes in individual fish over a prolonged period before and during the reproductive period. Thus, all stages of spermatogenesis are present in males (Billard, 1986), while

ovaries show the presence of more than one cohort of growing oocytes (on the basis of their diameters) during much of this time. This pattern will be identified as asynchronous here; it can be compared with the ecologist's category of multiple (batch) spawners (Marte *et al*., 1996). The histological observations are consistent with findings reported by studies with *Merluccius australis* (Alvarado *et al*., 2015) in males and *Merluccius hubbsi* (Cornejo, 1998 and Honj*et al.*, 2006) and *Merluccius merluccius* (Recasens *et al.,* 2008) in females.

Long photoperiod and high temperature appear to be important for the final oocyte maturation, ovulation and spermiation for cyprinids e.g. Carp, Catfish and other tropical and subtropical fish (Rocha, 2008). In the temperate country, photoperiod manipulation advances off-season breeding in Salmonids (Bromage *et al*., 1993), Scianids (Thomas and Arnold, 1993), Striped bass (Smith and Jenkins, 1984) and Atlantic cod (Hansen *et al*., 2001). In these species, photoperiod is considered as the main proximal factor deciding the timing of maturation (Carillo, 2007), although, the temperature is thought to be the secondary factor synchronizing the reproductive cycle (Van Der Kraak and Pankhurst, 1997).

Short daylength are found to be inhibitory to gonadal functions in *Cirrhina reba* (Verghese, 1975), *Heteropneustes fossilis* (Sundararaj, 1981) and *Channa punctatus* (Garg and Jain, 1985 and Srivastava and Singh, 1991), whereas, long daylength are found to be inhibitory to gonadal functions in *Liza ramada* (O'Donovan-Lockard *et al*., 1990), Salmonids (Bromage *et al*., 2001), *Gadus morhua* (Davie *et al*., 2007; Hansen *et al*., 2001 and Karlsen *et al*., 2006), *Sparus aurata* (Kissil *et al*., 2001) and *Melanogrammus aeglefinus* (Davie *et al*., 2007). Both long photoperiod and above ambient temperature are required for early gonadal maturation for off-season breeding in IMCs (Sarkar *et al*., 2010), *Cyprinus carpio* (Davies *et al*., 1986), *Carasius auratus* (Kawamura and Hanyu, 1950) and *Notemigonus crysoleuces* (De Vlaming, 1975). However, the effect of the photoperiod varies among the species.

Under natural conditions of photoperiod and temperature, captive dwarf gourami females attained maximum ovarian growth (stage III/IV) in 90 days. We showed that 3 months of constant long days (16L/8D or 14L/10D) caused advanced oocytes maturation for two months, as the natural spawning season

commences during May to June (Sutradhar *et al*., 2016). The GSI of females was 0.921 ± 0.047 % at 0 day, which reached in the range between 3.279 ± 0.510 % to 10.493 ± 2.167 % at 90 days. Highest GSI values of 10.493 ± 2.167 % and 7.048 ± 0.804 under 16L/8D and 14L/10D respectively were associated with advanced oocyte stages and larger oocyte sizes. GSI value was reported earlier as 6.218± 0.956 in female in June; the values increased from March to June with a peak value during May-June in captivity. The study confirms that the photoperiod manipulation caused an increase in ovarian growth revealed by significantly higher GSI values in the fish. The sudden drop of GSI value was noticed from July to October with a further subsequent increase from November onwards (Sutradhar *et al*., 2016).

The mean fecundity ranged between 352 ± 13.56 and 860 ± 70.02. It was 352 ± 13.56 at 0 day, which reached in the range between 608 ± 43.12 and 860 ± 70.02 at 90 days. The average pre-spawning fecundity of *T. lalius* was reported as 1024 to 1351 in number for the fish average length and weight of 43.60 mm to 46.33 mm and 1.244 g to1.808 g respectively (Sutradhar *et al*., 2016). The number of eggs increases with the increase in body weight, body length, gonad length and gonad weight of the fish. A similar observation was made by Dasgupta (1988), Musa and Bhuiyan (2007) and Chavan and Muley (2014).

The oocyte diameter varied from 0.01 mm to 0.80 mm. Maximum number of smaller eggs was found in between 0.01 mm to 0.50 mm at 0 day and maximum number of larger eggs was found in between 0.51 mm to 0.70 mm at 90 days. GSI (%), the progress of oocytes development and oocytes frequency distribution support the advancement of ovarian growth followed by maturation in the fish. The above ambient temperature did not show any additional effects on advancement, rather retarded the pace of ovarian development compared to natural photoperiod. The results of the present study is similar to that of previous work by Bromage *et al*. (1984), Duston and Bromage (1986, 1987) and Takashima and Yamada (1984), where spawning was advanced by constant long days, whereas, the present study contrasts earlier reports by Carrillo *et al*. (1989), Zanuy *et al*. (1995) and Mananos *et al*. (1997), in which spawning advancement achieved providing constant short photoperiod.

In majority of the Indian teleosts, spermatogenesis is controlled by increasing temperature and photoperiod, whereas, in some teleosts, the declining temperature and photoperiod seem to modulate spermatogenesis and some have winter spermatogenesis. The response of males to natural and long photoperiod regimes was different than exhibited by females in *T. lalius.* The maturation status in males was in little-advanced stages compared to females at 0 day. Most of the males were at III/IV stages of testicular development. At 30 days, most of the males under control and treatments showed a combination of stages V and I or II except in T2 and T5, which clearly indicates that the either late maturation or regression of testis occurred irrespective of control and treatments. Thereafter, all the groups showed the progress of testicular development at 60 days, which attained at most advance stages of maturity in T2 and T5, under long photoperiod of 14L/10D alone or in combination with above ambient temperature. Otherwise, there was no significant progress in testicular development in any groups, except in T1, in which slight improvement was noticed at 90 day. The study clearly showed that moderate long photoperiod was most suitable to obtain testicular maturity in *T. lalius* in two months period, which was almost 2-3 months prior to milting phase of the fish in captivity as reported earlier (Sutradhar *et al.*, 2016).

5.4. Changes in plasma sex-steroids concentration

5.4.1. Males

Spermatogenesis and spawning behavior are regulated by androgens (T and 11-KT). 11-KT is the main androgen in many teleost species (Billard *et al*., 1982 and Borg, 1994). In this species, 11-KT undergoes similar seasonal variations in most of the groups. The plasma 11-KT level in the dwarf gourami showed a sharp rise in all most all groups and was associated with spermatogenic growth of testicular cells. In C, T1 and T4 transformation of stage II to III was evident, whereas, in T2, T3 and T5 transformation from stage III to IV was noticed. In many species, 11-KT is considered to be the main androgen, regardless of the androgen plasma levels, which are generally high. However, a previous study reported that T and other androgens are dominant compared to 11-KT in several teleost species (Matsuyama *et al*., 1991 and Borg, 1994). In many cases, both androgens play a role in the initiation and maintenance of spermatogenesis, spermiation and

spawning behavior. The pattern of the timing increases and it is well known that 11-KT plays a dominant role in the control of spermatogenesis and reproductive behavior (Miura *et al*., 1991 and Borg 1994). The plasma concentrations of 11-KT increase and are maintained at high levels concomitant with reproductive phase III. This pattern is similar to that of other Cyprinids (Ching and Chen 1990 and Gerriero *et al*., 2005). These results suggest that 11-KT plays a role in the induction and progression of spermatogenesis in the dwarf gourami. DHP is known as a maturation-inducing steroid in many teleosts and plays an important role in spermiation and sperm maturation (Scott *et al*., 2010).

In many teleosts, high levels of DHP are observed during the spawning period when expressible milt and mature sperm are detected (Miura *et al*., 1992). However, in the dwarf gourami, relatively low level of DHP is observed throughout the experiment period, except at 30 days in all the groups, except T2 and T5. The rise in DHP was associated with sudden fall of GSI levels clearly indicated the progress of maturation and initiation of regressing of testes may be attributed to such decrease in testicular development. Moreover, Miura *et al*. (2006) and Scott *et al*. (2010) described that DHP induces the initiation of meiosis which initiates the process of spermiation. The observed levels of 11-KT are consistent with findings reported by studies with *Hucho perryi* (Amer *et al.*, 2001), *Clupea pallasii* (Koya *et al.*, 2002), *Verasper variegates* (Koya *et al.*, 2003) and *Solea senegalensis* (García-López *et al.*, 2006), where 11-KT was the most influential androgen for the spermatogenesis stage.

5.4.2. Females

The hormonal mechanisms controlling oogonium proliferation and oocyte recruitment are obscure in any vertebrate. In teleost, fragmentary information has been reported so far, gonadotropins (GTHs), steroids (E-2 and DHP) and growth factors may be involved in the process (Lubzens *et al*., 2010). The primary growth phase consists of two particular stages, chromatin nucleolus and peri-nucleolus stages. The chromatin nucleolus can be distinguished by a conspicuous nucleolus associated with chromatin thread. Multiple nucleoli become located around the periphery of the nucleus at peri-nucleolus stages in association with the increase in cell and nucleus sizes. Ovarian steroid hormones have been

implicated in the control of oocyte development. Measurement of steroid hormone levels in the plasma in relation to oocyte development provides some information about roles of steroid hormones on oocyte development.

Sex steroids concentrations in *T. Lalius* are at a very low level as compared to studies on *Catla catla* (Sehafii *et al*., 2014), *Dictyosoma burger* (Hwang *et al*., 2012), *Huso huso* (Bagheri *et al*., 2008), *Chalcalburnus tarichi* (Ünal *et al.*, 2005), *Perca fluviatilis* (Migaud *et al.*, 2003), *Coregonus clupeamorfis* (Rinchard *et al.*, 2001) and *Pleuronectes americanus* (Harmin *et al.*, 1995). However, studies on asynchronous spawners such as *Merluccius australis* (Alvarado *et al*., 2015), *Verasper variegatus* (Koya *et al.*, 2003) and *Gobio gudgeon* (Rinchard *et al.*, 1993) have also reported low-level sex steroid concentrations. The low-level sex steroid concentrations present in *T. lalius* is due to the fact that this fish is an asynchronous spawner and therefore the sex-steroids concentrations are diluted as consequence of prolonged spawning periods.

In teleosts, estrogen (E-2) is known to induce the synthesis and secretion of vitellogenins (VTGs) by the liver (Babin *et al*., 2007). High levels of plasma estrogen have been found in relation to vitellogenesis (Schreck, 1973 and Wingfield and Grimm (1977). In the present study, dwarf gourami showed an increase in plasma E-2 which was closely associated with increasing GSI and oocyte diameters. Long photoperiod caused a sharp rise in plasma E-2 at 60 days, whereas, natural photoperiod did not produce such rise in plasma E-2. The temperature effect was observed under natural photoperiod in causing a sudden rise in plasma E-2 level at 60 days. However, neither the level nor the gonad growth could attend such higher observed in long photoperiod groups. Long photoperiod in combination caused arise in E-2 level at 60 days, which could not sustain at 90 days, may be attributed to lower GSI in those groups. The observed levels of E-2 are consistent with findings reported by studies with *Engraulis ringens* (Cisneros, 2007), *Sardinops melanostictus* (Murayama *et al.*, 1994), *Salvelinus leucomaenis* (Kagawa *et al.*, 1981), *Mugil cephalus* (Tamaru *et al.*, 1991), *Acheilognathus rhombea* (Shimizu *et al.*, 1985) and *Oreochromis mossambicus* (Cornish, 1998), where E-2 was the most influential estrogen for the vitellogenesis stage.

Serum E-2 levels become low prior to oocyte maturation and spawning in many other single annually spawning species and it has been accepted that this change results from the shift of the steroid synthetic pathway from E-2 to DHP, which is considered to be a maturation inducing steroid (MIS) in many teleosts. In the present study, a sudden rise in plasma DHP level at 30 days in C and T1 was not clear, otherwise, increase in plasma DHP level was common in all the other treatment groups at 90 days, may be attributed for inducing oocyte maturational competence in the fish. The ovarian follicle acquires the ability to produce MIS and the oocyte acquires to respond to MIS during the first stage of maturation, whereas in the second stage the follicle produces MIS and consequently, the oocyte is released from meiotic arrest. Depending on the species, morphological indices such as the onset of lipid coalescence, germinal vesicle migration or hydration of oocytes also occur during the process of oocyte maturation and ovulation. (Nagahama and Yamashita, 2008 and Lubzens *et al*., 2010). In the present study, plasma E-2 concentrations declined to low levels whereas DHP surged at 30 days in C and T1, suggesting that the shift of the steroid synthetic pathway from E-2 to DHP also occurs in this species.

The profiles of 11-KT and T in males and E-2 and T in females exposed to constant short photoperiod were similar to those in control group, but fish exposed to constant long photoperiod have shown a bimodal pattern of these hormones (Prat *et al*., 1999). Variable changes in 11-KT and T in males of *Oncorhynchus mykiss* (Manning and Kime, 1985) and E-2 and T in females of *Carassiusn auratus* (Manning and Kime, 1984) were found in relation to temperature. Vikingstad *et al.* (2016) found that high temperature modulated the steroid profiles of the males with lower 11-KT level compared to ambient temperature and lowered DHP level compared to cold temperature, females with total inhibition of peri-ovulatory peak in DHP and prevented normal decline of T and E-2 levels associated with ovulation.

Short range variations of DHP levels may be due to lack of blood samples at the precise moment of increase in this hormone. Studies have shown that the increase in this hormone occurs for a short time period, just prior to ovulation when germinal vesicle breaks down (Tamaru *et al.*, 1991; Murayama *et*

al., 1994 and Mylonas *et al.*, 1997). Studies have also shown that DHP is detectable up to two hours after being synthesized (Migaud *et al.,* 2003). Furthermore, studies have also shown that 17,20β,21-trihydroxy-4-pregnen-3-one (17,20β,21P) act as MIS instead of DHP in *Micropogonias undulates* (Trant and Thomas, 1989), *Cynoscion nebulosus* (Thomas and Trant, 1989) and *Halobatrachus didactylus* (Modesto and Canário, 2002). Plasma DHP concentration did not show noticeable fluctuation during any maturation stages, may be due to the less number of individuals sampled or the continuous process of spermatogenesis and vitellogenesis or the short duration of DHP in the bloodstream.

In conclusion, the fish species *Trichogaster lalius* exhibits two distinct cohorts of oocytes at a time in ovary indicating the asynchronous type of ovarian development. Similarly, the presence of multiple types of spermatogenic cells in post-spawning males confirms the asynchronous type of testicular development. It is observed that the species is a prolonged breeder and spawns at least twice in a year. In the present study, males and females mature at different times, showing clearly the preferential difference in the environmental cues between sexes. This study provides the first description of the reproductive cycle and endocrine profile of the dwarf gourami. The data presented herein may be used as a baseline tool to develop techniques for seed production all-round the year manipulating photoperiod and temperature as cues.

6. SUMMARY

The Gouramis belongs to the Suborder – Anabantoidae which are popularly known as labyrinth fishes, characterized by an accessory respiratory organ called Labyrinth (Degani and Gal, 2013 and Welcomme, 1998). During breeding season, the male builds a bubble nest using bits of plants, twigs or other debris, in which the female lays the eggs. Advancement of gonad recrudescence in captivity in this fish are not attempted earlier for extending spawning period through photo-thermal manipulation for enhancing seed production in captivity. Extended spawning phase maintains fish particularly males in brilliant color. Thus, a study was designed with an aim of advancing gonad recrudescence through photo-thermal manipulation.

The experimental groups consisted of a control (C) and 5 treatments (T1, T2, T3, T4 and T5) with 3 replications of each to study the effect of photo-thermal manipulation of gonad recrudescence with different combination of photoperiod and temperature, following a completely randomized design (CRD). The combination of different photo-thermal regimes were conducted as three photoperiod regimes, natural (10-12 hours) and two long photoperiods (L16/D8 and L14/D10) in natural temperature (19-24 °C) or in combination with above ambient temperature (26°C).

The reproductive parameters during 90 days experimental period, including GSI, fecundity and oocyte frequency distribution shows significant difference ($p<0.05$) in between the C and treatment groups. The GSI, fecundity and oocyte diameter increased with the progress of time in C and different treatments as experimental duration increases. The GSI of males was 0.510 ± 0.026 % at 0 day, which reached in the range between 0.402 ± 0.020 % to 0.616 ± 0.042 % at 90 days. The GSI of females was 0.921 ± 0.047 % at 0 day, which reached in the range between 3.279 ± 0.510 % to 10.493 ± 2.167 % at 90 days. The mean fecundity ranged between 352 ± 13.56 and 842 ± 34.8. It was 352 ± 13.56 at 0 day, which reached in the range between 608 ± 43.12 and 860 ± 70.02 at 90 days. The oocyte diameter varied from 0.01 mm to 0.80 mm. Maximum number of smaller

eggs was found in between 0.01 mm to 0.50 mm at 0 day and maximum number of larger eggs was found in between 0.51 mm to 0.70 mm at 90 days.

The changes in plasma sex-steroids concentration during the experiment period in control and treatment groups showed significant fluctuations under different photo-thermal regimes. Long photoperiod caused a sharp rise in plasma E-2 in females at 60 days, whereas, natural photoperiod did not produce such rise in plasma E-2. The temperature effect was observed under natural photoperiod in causing a sudden rise in plasma E-2 level at 60 days. However, neither the level nor the gonad growth could attend such higher values in long photoperiod groups. Long photoperiod in combination caused a rise in E-2 level at 60 days, which could not sustain at 90 days, may be attributed to lower GSI in those groups. In the present, an increase in plasma DHP level was common in all the treatment groups except C and LP (16L/8D) in combination with above ambient temperature at 90 days may be attributed for inducing oocyte maturational competence in the fish. The 11-KT, E-2 and DHP varied with the progress of time in C and different treatments as experimental duration increases.

The plasma 11-KT level in the dwarf gourami showed a sharp rise in almost all groups and was associated with spermatogenic growth of testicular cells. Like many species, 11-KT is considered to be the main androgen in dwarf gourami regulating mitogenic proliferation of spermatogonial cells. In many teleosts, high levels of DHP are observed during the spawning period when expressible milt and mature sperm are detected (Miura *et al*., 1992). However, in the dwarf gourami, relatively low level of DHP is observed throughout the experiment period, except at 30 days in all the groups, except T2 and T5. The rise in DHP was associated with sudden fall of GSI levels clearly indicated the progress of maturation and initiation of regressing of testesmay be attributed for such decrease in testicular development.

In conclusion, the fish species *T. lalius* exhibits two distinct cohorts of oocytes at a time in ovary indicating the asynchronous type of ovarian development. Similarly, the presence of various types of spermatogenic cells in post-spawning males confirms the asynchronous type of testicular development. In the present study, males and females mature at different times, showing clearly the preferential difference in the environmental cues between sexes. This study provides the first

description of the reproductive cycle and endocrine profile of the dwarf gourami. The data presented herein may be used as a baseline tool to develop techniques for seed production all-round the year manipulating photoperiod and temperature as cues. Advancement of gonad recrudescence in captivity can be done through proper photo-thermal manipulation at the appropriate season for both males and females separately to extend spawning period and enhance seed production.

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8. ACRONYMS

%	Percentage
µ	Micron
µg	Microgram
µl	Microlitre
^{0}C	Degree centigrade
^{0}F	Degree Fahrenheit
11-KT	11-keto testosterone
17α,20β,21P	17α,20β,21-trihydroxy-4-pregnen-3-one
17α,20β-DHP	17α,20β-dihydroxy progesterone
AAT	Above ambient temperature
ANOVA	Analysis of variance
AT	Ambient temperature
BPG	Brain-pituitary-gonadal
C	Control
CA	Cortical alveoli
cm	Centimetre
CRD	Completely randomized design
D	Dark
DHP	Dihydroxy progesterone
e.g.	For example
E-2	17β-estradiol
EBT	Erichrome black – T
EDTA	Ethelenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FRP	Fibre-reinforced plastic

FSH	Follicle stimulating hormone
g	Gram
GnRH	Gonadotropin releasing hormone
GTH	Gonadotropin
GV	Germinal vescicle
GVBD	Germinal vescicle break down
GVM	Germinal vescicle migration
HPG	Hypothalamus-pituitary-gonadal
hrs	Hours
i.e.	That is
KM_nO_4	Potassium permanganate
L	Light
l	Litre
LED	Light-emitting diode
LH	Luteinizing hormone
LP	Long photoperiod
m	Metre
mg	Milligram
mg/l	Milligrams per litre
MIH	Maturation inducing hormone
min	Minute
ml	Millilitre
mm	Millimetre
MPF	Maturation promoting factor
N	Normal
NBF	Neutral buffered formalin
NFE	Nitrogen free extract

Ng	Nanogram
ng/ml	Nanograms per millilitre
Nm	Nanometre
NP	Natural photoperiod
OCY	Oocyte
pg/ml	Piccograms per mililitre
pH	Potential of hydrogen
PN	Peri-nucleolar
ppm	Parts per million
ppt	Parts per thousand
RPM	Revolutions per minute
SE	Standard Error
SPSS	Statistical package for the social sciences
T	Testosterone
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
T4	Treatment 4
T5	Treatment 5
US$	US dollar
VG	Vitellogenic
VTG	Vitellogenin
VTL	Vitellogenesis
W	Watts
µg/ml	Micrograms per mililitre