BIOLOGY, CAPTIVE MATURATION AND BREEDING OF INDIGENOUS ORNAMENTAL FISH, DWARF GOURAMI, *COLISA LALIA* (HAMILTON, 1822)

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of the requirements
for the degree of

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by

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DEDICATED TO MY FAMILY

DECLARATION

I hereby declare that the thesis entitled **"BIOLOGY, CAPTIVE MATURATION AND BREEDING OF INDIGENOUS ORNAMENTAL FISH, DWARF GOURAMI, *COLISA LALIA* (HAMILTON, 1822)"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship fellowship or any other similar title.

Date:31st August, 2019
Place:Mumbai

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सारांश

एक्सपोर्ट मार्केट में स्वदेशी सजावटी मचलियोंकी बहुत ज्यादा मांग है, जो की नैसर्गिक स्त्रोतोंसे जमाकर के पूरी की जाती है और इस वजह से उन स्त्रोतोंमें भारी गिरावट देखि जा रही है। वामन गुरामी 'कोलिसा लालिया' इस पद्धति का एक उदाहरण है। प्रजनन और पालन के साथ बाजार की मांग लोगोको इस क्षेत्र में प्रवेश करने के लिए प्रेरणादायी होनेवाले कारक है। इसलिए कोलिसा लालिया के सामान्य जीव विज्ञान, खानपान तथा प्रजनन जीव विज्ञान का अभ्यास किया गया है। ग्रोथ, प्रजनन तथा लार्वा पालन पर खानपान के असर का अभ्यास करके, सबसे अच्छा फ़ीड का चयन करने हेतु तिन प्रयोग किए गए। आकृति विज्ञान के गणनीय लक्षनोंके अभ्यास से यह दिखता है की कोलिसा लालिया के शारीर का अकार छोटा, लांब गोल तथा दोनों बाजुओंसे दबा हुवा है और उसका फिन फोर्मुला D. XV/8; P.8; A. XVII/15; V. 2; C. 14 है। मादा तथा बच्चोंसे, नर ज्यादा रंगीन और आकर्षक होते है। सापेक्ष आंत की लंबाई, जो की 2.29±0.42 पाई गई, दर्शाती है की यह प्रजाति सबकुछ खानेवाली (सर्व-भक्षक) है। अधिकतम ग्यास्त्रोसोमाटिक इंडेक्स दिसम्बर माह में पाई गई, 3.00 ±0.01 (नर) और 4.75±0.13 (मादा) और न्यूनतम माह जून में, 1.88±0.03 (नर), 1.20±0.07 (मादा) में पायी गई। अधिकतम गोनाडोसोमाटिक इंडेक्स जून माह में पाई गई, 0.585±0.01 (नर) और 6.08±0.14 (मादा) और न्यूनतम माह नवम्बर में 0.305±0.01 (नर), 2.97±0.02 (मादा) में पायी गई। जननांग उतकीय अध्ययनमे गामेट्सकी एक से ज्यादा चरण देखा गया। ऊष्मायन अवधि 24 से 25 घंटा पाया गया। बंदी पालन स्थितिओ मे, 4.44±0.11 सेमी कुल लम्बाई तथा 1.53±0.07 ग्राम कुल वजन की मछली की अंडे देने के पूर्व की उपजाऊपन 439±49 पायी गई। अंडो का व्यास 0.265±0.003 मिमि पाया गया। प्रयोगंतार्गत, जिनमे 4% खाद्य पूरक मिश्रण (जिसमे 40% निर्मल लेसिथिन, 1.5% α टोकोफेरल एसीटेट, 2.5% सेलिन, 50% स्पिरुलिना और 6% एल-ट्रिप्टोफैन) था, उस उपचार का निष्कर्ष सजीव फ़ीड, टुबिफेक्स उपचार के निष्कर्ष से मेल खाता है, जो सर्व्वोत्तम निष्कर्षों है। सजीव फ़ीड, टुबिफेक्स के उपचार मे वजन में बढ़ोत्तरी 1.12 ± 0.02 ग्रा, वजन बढ़ोत्तरी प्रतिशतता 120.75 ± 1.63 % और विशिष्ट विकास दर 0.88 ± 0.01 % है। उपरोक्त फ़ीड और सजीव फ़ीड, टुबिफेक्स के संयोजन ने सबसे अच्छा प्रजनन प्रदर्शन दिया (गोनाडोसोमाटिक इंडेक्स 0.538 ± 0.006 (नर), गोनाडोसोमाटिक इंडेक्स 5.720 ± 0.013 (मादा), 100% स्पानिंग, निषेचन 70.67 ± 0.67% और हैचिंग 42.00 ± 1.15%), उसके बाद अच्छा प्रजनन प्रदर्शन टुबिफेक्स फ़ीड उपचार ने दिया। जहां उपचार केवल तैयार किए गए फ़ीड के साथ खिलाया जाता था, वहाँ कोई स्पॉनिंग नहीं देखी गई। लार्वा को जीवित फ़ीड, इन्फ्यूसोरिया के साथ खिलाए गए उपचार में, सर्वोत्तम परिणाम वृद्धि (10.54 ± 0.07 मिमी लंबाई) और जीवित रहने की दर (66.33 88 0.88%) देखा गया।

ABSTRACT

The indigenous ornamental fish has high export market. This market mainly depends upon wild collection, which has a depleting effect. Dwarf gourami, *Colisa lalia* is an example of this practice. Breeding and rearing coupled with market demand are the factors that encourage venturing into this sector. Therefore, general biology, feeding biology and reproductive biology of the *Colisa lalia* were studied. Three experiments were also conducted based upon nutritional interventions to decide the best feed for growth performance, reproductive performance and larval rearing. The Mophomeristics denoted that *Colisa lalia,* has small size oval body, which is strongly compressed with fin formula D. XV/8; P.8; A. XVII/15; V. 2; C. 14. Among both the sexes male is colourful and attractive in comparison to female and juveniles. The Relative Gut Length was found out to be 2.29 ± 0.42, denoting this species to be omnivorous. Highest Gastro-somatic index was observed in the month of December, 3.11 ± 0.1 (male) and 4.75 ± 0.13 (female) and lowest in June, 1.88 ±0.03 (male) and 1.20 ± 0.07 (female). Highest Gonadosomatic Index was observed in the month of June; 0.585 ± 0.01 (male), 6.08 ± 0.14 (female) and lowest in November; 0.305 ± 0.01 (male), 2.97 ± 0.02 (female). The histological study of gonads demonstrated the presence of multiple gamete stages. The incubation period was recorded 24-25 hours. Pre-spawning absolute fecundity in the captive conditions was 439 ± 49 for 4.44± 0.11 cm length and 1.53± 0.07 g weight of fish. Ova diameter observed was found to be 0.265± 0.003 mm. In the experiments conducted formulated feed with 4% supplementary diet mix (40% refined lecithin, 1.5% α Tocopheral acetate, 2.5% Celin, 50% Spirulina and 6% L-tryptophan) gave similar growth performance as live feed, *Tubifex,* which gave best results; weight gain (1.12 ± 0.02 g), weight gain percentage (120.75 ± 1.63%) and specific growth rate (0.88 ± 0.01). The combination of the above feed and live feed, *Tubifex* gave best reproductive performance (GSI male 0.538 ± 0.006, GSI female 5.720 ± 0.013, spawning 100%, fertilization 70.67 ± 0.67% and hatching 42.00 ± 1.15%), followed by the treatment fed with only *Tubifex*. There is no spawning observed where treatment is fed only with formulated feed. In case of larval rearing the treatment fed with live feed, Infusoria gave best results in growth (10.54 ± 0.07 mm length gain) and survival rate (66.33 ± 0.88 %).

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1.INTRODUCTION

Aquaculture practice in India is mainly based on the breeding and culture of carp and shrimp. Now-a-days ornamental fish culture is also a developing business of our country. These fishes are denoted as the 'living jewels' due to their lucrative colouration, unique shape of the body and there adaptive behavior. Ornamental fish form an important commercial component of fisheries, providing aesthetic requirements. Development of aquaria is picking up as hobby worldwide. However, the market of ornamental fishes in the world for public aquaria is less than 1% at present with 99% of the market for ornamental fish is still confined to hobbyist (Raja *et al*., 2016). The aquarium keeping has emerged as second most popular hobby in recent years only the next to photography. Ornamental fishes have brilliant and flamboyant colour and exotic appearance of appeal almost to one and all, children and aged alike. According to psychiatrists some mental aliments can be treated by keeping an aquarium with ornamental fishes in front of patient. Initially ornamental fishery practice was confined to the developed countries, but now due to their contribution to the export value, it is gaining popularity in the developing countries as well.

Globally this is a multibillion dollar industry, as according to FAO (2013), the total estimated worth of the ornamental industry is around US$15 billion. There is an average growth at the rate of 14 % per year in the international export trade, since 1985 (FAO, 2013). Asian countries have 60% share of ornamental fish export in the world market. India has a abundant potential to enhance the level of fish export to US$ 30 million every yea (Ziauddin *et al.*, 2007). It has also been noticed that Indian ornamental fishes are in greater demand in international market (Ayyappan and Jena, 2006). Approximately 2000 species are traded annually, out of which 30-35 fresh water species dominate the market (Livengood and Chapman, 2009). However exports from India have been just about a few crores of rupees only and 90% of the overall exports are wild caught fishes of fresh water origin (Livengood and Chapman, 2009). The industry continues to grow rapidly with active involvement of aquarium hobbyists but the main hindrance is that no precise statistical data are available to assess the ornamental fish trade dimensions.

Fish keeping in aquariums has developed as a very common practice and gradually people are getting attracted towards this leisure pursuit (Mahapatra *et al*., 2014). As per Ayyappan *et al*., (2006) Aquarium hobby in India dates back to pre-independence era. India is the main abode of the range of ornamental fishes available in the subcontinent. India possesses rich resources viz., Gulf of Mannar, Gulf of Kutch complex, the lagoons and coral reefs of Lakshadweep and Minicoy islands, Okha- pin tan, Andaman and Nicobar islands, Coast of Kerala, Cape Comorin, and Palk bay are abound with highly attractive and varied species of ornamental fishes. India, having a huge number of indigenous fishes of different ornamental characteristics has great potential to earn a huge foreign exchange (Mahapatra *et al*., 2014). As per Madhu *et al*., (2009) there are 150 commercially important ornamental fish species recorded in India and indigenous freshwater species which are collected from rivers are mainly traded. Prominent among the fresh water Indian ornamental fish are Eels, Loaches, Barbs, Catfish, and Goby (Ayyappan *et al.,* 2006). About 80% of ornamental fishes are from fresh waters and the rest from brackish and marine waters (Mahapatra *et al*., 2014). About 90 % of ornamental fish is imported from Kolkata followed by 8 percent from Mumbai and 2 percent from Chennai (Ghosh *et al*., 2003). Share of India's in global ornamental fish trade is less than 1 percent but still ornamental fishery is projected as a "sleeping giant" because of yet untapped potential resources (Benjamin, 2012). However, India's marginal position is likely to alter as trade is gradually increasing (Ghosh *et al*., 2003).

Due to ignorance about lucrativeness of the trade and lack of technical competence, India could so far manage to export ornamental fishes. Inability to take cognizance of our natural ornamental fish resource, unavailability of needed funding, inadequacy of local exporting agencies, lack of suitable low cost breeding technologies and transportation facilities are the major hurdles in the steeping up of our ornamental fish exports. Further, accessibility to oxygen packaging has gap to improper knowledge about conditioning of fish before transportation is seen be causing substantial mortality of these fishes especially in rural areas. Unlike other Asian countries, the native ornamental fishes are mostly collected from the wild, instead of adopting breeding techniques in confined facilities for their production.

Kolkata, Mumbai, Chennai, Cochin, Madurai are the most important breeding center for ornamental fishes with more than 150 full time and 1500 part time fish breeder in the country till the year 1996 (Prathvi Rani *et al.*, 2014)

There are increasing opportunities for augmenting the production of ornamental fish in India. The enormous ornamental fish resources are available in Indian waters and the climatic condition is suitable for culture, breeding and rearing. The farmers can undertake ornamental fish farming with comparatively lower investment, and get very good returns, as there are sustainable markets for ornamental fish both inside and outside the country (Prathvi Rani *et al.*, 2014)

The demand of ornamental fish is being satisfied by capturing them from wild resources. Due to over-exploitation and indiscriminate destruction of breeding and feeding grounds by applying pesticides and insecticides in agricultural field, leads to threatened condition of ornamental fish. Some species are considered as a vulnerable fish species in West Bengal (Menon, 1994; Das and De, 2002). To save fish population from getting vulnerable, the juvenile of these fish should be recruited to the natural ecosystem as precautionary measures. This is possible through captivebreeding and larval rearing.

Therefore, breeding and rearing coupled with market demand are the factors that encourage venturing into this sector. So the knowledge of general biology, feeding biology, reproductive biology, captive maturation, breeding and larval rearing is more essential for the propagation of any fish including ornamental fish.

There is a huge market for gourami in the ornamental fish industry. These are a group of freshwater anabantiform fishes that belong to the family Osphronemidae. There is a huge variety of gourami available in nature and some species can grow quite huge, and are unsuitable for the general hobbyist. In India presently 8 indigenous gourami species are very common and high in demand (Bhattacharya *et. al*., 2016). One among them is the dwarf gouramy, often kept in home aquaria. Life span of most dwarf gouramisis about four - five years and with proper care, they can live longer. Dwarf gouramis can be reared in public and community aquariums and are compatible with most fish, such as Platies, Mollies, Plecostomus, Tetras, and other docile fish. As per the F.A.O. Fish Base, the latest information of the nomenclature of dwarf gourami, *Colisa lalia* has been changed. The valid name is *Trichogaster lalius*. It belongs to the family- Belontiidae and Sub-family- Trichogasterinae. It is usually liked by the people for its good taste. It has high foreign demand as aquarium fish. It is collected from wild and exported to the foreign countries e.g. Singapore, USA, Japan, Republic of Korea, Sri Lanka, Germany, UK, Hong Kong, Taiwan, Bangladesh, Thailand, Netherlands, Malaysia and China (Biswas and Lepcha, 2004).

Originating from West Bengal, Assam, and Bangladesh, the dwarf gourami is an indigenous ornamental fish which is native to thickly vegetated waters and found with other species of Colisa. In northern India, they are one of the most common food fish, and are sold as dried fish or as fish meal in many of the markets. The common name fits this fish well, as it grows to a size of only two inches at best. Males are somewhat larger than the females and have a bright orange-red body with turquoise blue vertical stripes that outspread into the fins. The female have rounded dorsal fin in contrast to male. Females remain a duller silvery blue-gray colour, never achieving the brilliant colours of the male. Several colour hybrids exist, including Blue/Powder Blue), Neon, Rainbow, and Red/Blushing. Neons exhibit a brighter blue pattern than the standard variety. Reds are nearly solid red all over the body, with solid blue dorsal fins.

This species is not aggressive, so can be kept in community aquariums. Brightly coloured fish can sometimes cause males to become aggressive, as they are perceived as rivals. Peaceful, small schooling fish are suitable, as well as most bottom dwelling fish. Dwarf gourami is well suited to smaller aquariums, as well as community aquariums due to their peaceful nature. They should not be reared with very large or aggressive fish. It needs plenty of vegetation, including floating plants that cover part of the surface of the water. The optimum range of pH required is in the neutral range, and water hardness should be 40-100ppm. The ideal water temperature is 77^{o} F (25^{o} C). Dwarf gourami can be skittish when subjected to noise, and should be kept in a quiet location.

With a view to above, the present research work entitled "**Biology, captive maturation and breeding of indigenous ornamental fish, dwarf gourami, *Colisa lalia* (Hamilton, 1822)**" was undertaken in the laboratory conditions. It included studies on the general biology, feeding biology, reproductive biology, captive maturation, breeding and larval rearing of *Colisa lalia*, which would help in further standardization of induced breeding and larval rearing operation.

OBJECTIVES OF THE STUDY

- **To analyse the feeding and reproductive biology of dwarf gourami, *Colisa lalia.***
- **To evaluate the effect of nutritional intervention on growth and reproductive performance of dwarf gourami, *Colisa lalia*.**
- **To analyse the larval rearing of dwarf gourami, *Colisa lalia.***

2. REVIEW OF LITERATURE

Reproduction is the natural phenomenon of all the organisms to exist for next generations and fish is also one of them. Every fish species has immense power of prodigality and if the conditions are favourable then the population may increase to the explosive level. But it seldom happens because, from the beginning of gametogenesis to the attainment of maturity, there are a number of factors responsible for untimely death of embryos, juveniles and adults even before they start reproducing. Many commercial and productive fish species are adversely affected in modem times, due to severe fast and undesirable changes detrimental to their surroundings, survival and viability. Hence it needs conservation for the future generation. Therefore, the species are to be managed, conserved and exploited accordingly. For the proper management of the species, the fundamental knowledge on various aspects of biology, reproduction and breeding behaviour are very much necessary. In studies of fisheries biology, it is important to determine the cycle of maturation and depletion of gonads. Determination of maturity stages finds primary application in providing basic knowledge on the reproductive biology of a stock (Venkataramanujan and Ramanathan, 1996). According to Arocha (1997) knowledge on gonad development in individual fish is required to establish the duration of spawning season, the size and age at maturity and spawning pattern.

Maintaining aquariums is one of the widespread leisure pursuits in the world today. This growing interest has resulted in increase in global aquarium fish trade. As per Das, (2003), aquarium fish trade has a turnover of US$ 5 billion and it has annual growth rate of 8 percent. This offers a lot of scope for its development. There is moderate demand of *Colisa lalia* among the aquarium fish hobbyist but it is popular as beautiful small indigenous ornamental fish. This fish has been considered eatable fish due to its good taste. In Indian market, trade of *Colisa lalia* still relies upon wild collection, which is having depleting effect on natural stock. This situation points out the need to give attention on captive maturation and captive breeding of this species, which also demands the study of the biology of it. This work not only will help ornamental fish industry but also will help to conserve this indigenous variety.

2.1. General Biology

Morphometric characters are important for identify and classify fish species (Bhattacharya *et al*., 2016). Studies on external morphology are needed for identification of sex externally. Body shape, colour, belly structure, structure and colour of vent, fins and end of the caudal peduncle *etc.* are the external morphological features of fish. Some scientists such as Dehadrai *et al*. (1973) and Banerji and Prasad (1974) have studied external morphology of some air breathing fishes. Dehadrai *et al.* (1973) and Khan (2004) have been studied the external morphology, taxonomy and morphology of related fish *Colisa fasciatus*. The external morphology and taxonomy of dwarf gourami *Colisa lalia* have been studied by Hamilton (1822); Cuvier (1831); Jhingran and Talwar (1991).

General biology of any species mainly includes the study of morphometric and meristic characters. The body shape can be well described by the morphometric aspects. Meristic counts are the countable structures like rays and fins. Morphomeristics is commonly used for taxonomical identification and differentiation of individual species and species from different stocks (Lourie *et al*., 1999; Doherty and McCarthy, 2004; Jayasankar *et al*., 2004). The fin formula of *Colisa lalia* given by different workers are as follows; D. Xv- Xvii/7-10, P1. 10, P2.1, A. Xvii-Xviii/13-17 (Shafi and Quddus, 1982); D. Xv- Xvii/7-10, P1. 10, A. Xvii-Xviii/13-17 (Talwar and Jhingran, 2001); D. Xv- Xvii/7-9, P1. 8-9, P2. 1, A. Xvii-Xx/14-16. (Rahman, 2005). Talwar and Jhingran (2001) reported the lateral line series to be 27-28 scales in *Trichogaster lalius*. Similarly, length-weight relationship of fish are important in fisheries biology because they allow the estimation of the average weight of the fish of a given length group by establishing a mathematical relation between them (Beyer, 1987).

The body of *Colisa lalia* is oval shaped, strongly compressed. Mouth is minor, directed upwards. Slightly concavity at the dorsal side over the opercula region, dorsal and anal fins are spiny. Base areas of soft dorsal and anal fins are covered with scales.

Lateral line is not complete. Body is crossed by oblique bands of sparkling pale blue, almost translucent blue colouring highlighted with fine stripes of red or dark orange, caudal fin rounded, sometimes slightly, fins with scarlet barbs (Mahalder and Mustafa, 2011). *Colisa lalia* have accessory respiratory organ called labyrinth organ situated next to gill cavities. This delicate tissue has a great concentration of blood vessel and does the job like terrestrial lungs.

2.2 Feeding Biology

Feeding biology is obligatory for efficacious management practices in fisheries and aquaculture. Food is essential for growth, development; reproduction and survival of all organisms. Food and feeding habits of any species are intimately associated with the ecological niche that they inhabit in the natural environment. Studies on the food and feeding habit of different fishes have been carried out by many workers like Moffet and Hunt (1943), Khan (1947), Hynes (1950), Karim and Hossain (1972), Doha (1974), Dewan and Saha (1979), Jhingran (1983), Bhuiyan and Haque (1984), Bhuiyan and Islam (1990, 1991), Hossain *et al.* (1991), Bhuiyan *et al.* (1997, 1998, 1999) and others. Jhingran (1983) stated that the natural foods of fishes are classified under three groups, *viz.,* main food, occasional food and emergency food. The inducements to food are of two kinds, (a) factors affecting the appetite including season, light intensity, time of day, nature of last feeding, temperature and any internal rhythm that may exists; (b) food stimuli observed by the sense like smell, taste, sight and the lateral line system that release and regulate the momentary feeding act. Feeding with appropriate high energy diets rich in protein, vitamins and minerals is required for adequate breeding of ornamental fishes in confined waters (Encina *et al.,* 1997). The modern studies exhibited that the various effects on growth and reproductive performance of fish is triggered by live feeds (Salas *et al*., 2009). Incomplete information is available on the effects of food on broodstock, fecundity and larval survival of ornamental fishes.

2.2.1 Relative length of the gut

The ratio between the gut length and total length (RLG) has been estimated by dividing the gut-length by total length of the body (Al-Hussaini, 1949). Das and Nath (1965) calculated the relative length of the gut in *L. dero* as 8.9. They found RLG value in *Puntius conchonius* and *Barbus hexastichus* as 3.3 and 2.3, respectively, which indicated that the fish is omnivorous.

The length of the digestive tract is closely related to the type of food. Hugueny and Pouilly (1999) gave an opinion on relative gut length and assumed to be higher in magnitude in detritivores and herbivores. Piet (1998) found that relative gut length is linked to herbivory. Bowen (1988) gave a view that a functional explanation for the long intestine in herbivorous species is that one or more essential components of their diet are slow to be digested and both a long residence and extensive exposure to absorptive surfaces are required. Das and Nath (1965) recorded an average RLG value for *Tor tor* as 2.14. Bhattacharjee and Dasgupta (1998) reported that the RLG value for *A. morar* (Ham.) as 1.02 and Dasgupta (2001) reported the RLG values for *L. gonius, L. rohita, L. bata* and *L. calbasu* as 9.420, 8.08, 7.75 and 4.51 respectively.

2.2.2 Gastro-somatic Index

Feeding intensity denotes to the degree of feeding as specified by the relative fullness of the stomach. Singh *et al.* (2013) conveyed that there is difference in the feeding intensity with respect to maturity stages, seasonal variation, spawning season, and accessibility of desired food items. Gastro-somatic index determines the feeding intensity of the fish. The gastro-somatic index of mature fish is little during the spawning period, as compared to the non-spawning duration. Study of food and feeding habits of fishes have diverse significance in fishery biology (Singh *et al.,* 2013). A correlation of feeding intensity of *Trichogaster fasciata* with breeding periodicity has been reported by Mitra *et al.* (2007). He says that low feeding activity has been documented during the spawning months while intense feeding has been observed in post-spawning season of *Trichogaster fasciata*.

2.2.3 Feeding habits

As per Degani (1990), *Trichogaster trichopterus* are considered carnivorous and the natural diet constitutes of different species of invertebrates. In an intensive culture situation, where natural foods are inadequate, a complete diet must be used in order to accomplish optimum growth. As per Krystal Austin (2015) the three-spot gourami feeds on mosquito eggs, algae and other minor organisms. The larger gourami, like giant gourami is able to consume shrimp and other small fishes. Other worker like Conlu (1986); Chung *et al.* (1994); Rainboth (1996); Talde *et al.* (2004) states that the species is omnivorous but feeds mainly on zooplankton, (eg. copepods, cladocerans, ostracods), macroinvertebrates (insect larvae), and occasionally terrestrial macrophytes, and occasionally on detritus (Talde *et al.* 2004). Until now limited studies have been made on feeding biology of *Trichogaster fasciata* (Mitra *et al.* 2007; Das and Kalita, 2006). Some of the researchers (Mitra *et al.* 2007; Dasgupta, 2004) have reported it as herbivorous fish while others (Das and Moitra, 1963; Goodwin, 2003; Das and Kalita, 2006) have documented it as omnivorous in feeding habit. Pearl gourami nourishes *Daphnia*, *Tubifex*, small insects and commercial dried feeds (Akhter, 1995). The Pearl Gourami is omnivorous in nature, in the wild they feed on crustaceans, insect larvae, and zooplankton. In the aquarium they generally eat all varieties of live, fresh, and flake foods. Supplementation should contain white worms, blood worms, brine shrimp, or any other appropriate substitute. Fresh vegetables and vegetable tablets can also be given as feed (Akhter, 1995).

According to Bhatti (1943) and Rao (2014) *Trichogaster lalius* is a carnivorous fish which feeds on mosquito larvae. According to Sahu (2017), it is omnivorous. Other worker says the dwarf gourami is omnivorous, as in the wild they feed on small invertebrates, algae, and other aufwuchs (Hans-Joachim Richter, 1988).

2.3 Reproductive Biology

For an enhanced understanding of the biology of any fish population, information on reproductive cycles is needed (Lazarus, 1990). In case of major carps, natural spawning commonly overlaps with the South-west monsoon in North-Eastern

India and Bangladesh and continues from May to August. It continues from June to September in North India and Pakistan. The spawning season seems to be variable in the southern portions of India (Jhingran, 1968; Khan and Jhingran, 1975; and Jhingran and Khan, 1979). According to Natarajan and Jhingran (1963), under natural conditions Indian major carps breeds only once in a year. Recently, however in a season/year under controlled conditions Indian major carps have been bred more than four times (Gupta *et al.*, 1995).

According to Gratzek (1992) reproduction of any species involves successful accomplishment of a number of key steps, *viz.,* brood stock selection, brood stock conditioning, spawning, hatching and survival - growth of larvae and fry. By mismanagement of one or more of these steps, most problems can be traced. Brood stock must be in good health, colour, strong and vigorous with no deformities. Decreased productivity may be seen in older breeders because of age or acquisition of age related diseases. Often less fertility is seen in younger breeders and certain species might show more cannibalism and less parental care (Gratzek, 1992). The major factors that affect quality and productivity of fish eggs are age and nutritional status of brood stock (Izquierdo *et al*., 2001). Through maintenance of optimal water quality, nutrition, habitat and reduction of pathogenic load, brood stock conditioning is achieved. Omnivores and carnivores brood stock have need of high quality protein foods that contain essential fatty acids, carbohydrates, vitamins and minerals. In culture of ornamental fishes, reproductive capacity is also reduced due to external and internal parasites. To avoid unwanted spawning activities, there should be segregation of sexes as soon as possible (Gratzek, 1992).

2.3.1 Sexual dimorphism

Differentiating sex is the primary step in developing a captive breeding program for a fish species. Sexual dimorphism depicts the morphological features with which we can successfully segregate them sex-wise. Differences in the selective pressures experienced by the sexes can ultimately result in the evolution of sexual dimorphism of morphological traits (Andersson, 1994). Fish exhibits wide variations in the possession of sexual dimorphic characters. In some species, there exists a highly remarkable difference between male and female as they should have been mistaken to be a different species, while some others are found to be extremely identical in morphological features.

Sexual dimorphic characters of *Trichogaster fasciata* has been documented by Das and Kalita (2006), Swarup *et al.* (1972) and Dehadrai *et al.* (1973) to identify male and female of it. According to Swarup *et al.* (1972) upper lip is more pronounced, dorsal and pelvic fins are more pointed at the posterior end in male and upper lip is not much pronounced, dorsal and pelvic fins are not pointed at the posterior end in female. According to Das and Kalita (2006) body size is smaller in size, body colour is much brighter in male and body size is comparatively larger and comparatively less colourful in female. According to Dehadrai *et al.* (1973) and Das and Kalita (2006) the obliquely disposed lateral bands on the body are peacock blue in male and are dull steel-gray in female. According to Dehadrai *et al.* (1973) and Das and Kalita (2006) during breeding season, pelvic fin which is a single ray becomes orange-red; dorsal and anal fins become peacock blue with an orange border and belly is slender in male. According to Dehadrai *et al.* (1973) and Das and Kalita (2006) during breeding season, pelvic fin is yellowish-gray, anal fins become yellowish gray and abdomen is swollen in females.

2.3.2 Gonadosomatic index

Gonadosomatic index is an index of gonad size relative to fish size. It is a good indicator of gonadal development in fishes (Dadzie and Wangila, 1980). The percentage body weight of a fish is used to define the production of eggs by the gonadosomatic index. Gonad progress can encourage physiological or behavioural responses in the fish (Moyle and Cech, 1988). The Gonadosomatic index is one of the most important measures in the estimation of the reproductive period and maturity condition of a fish. The gradually increasing values of Gonadosomatic index gives the picture about the dynamics of gradual gonadal maturation and the maximum Gonadosomatic index means the point of maximum reproductive maturity and beyond that a steep fall because of spawning, the shedding of eggs. Sexually mature fish had high GSI values and in females these were as much as six times greater than in males (Chellappa *et al.*, 2003).

2.3.3 General morphology of gonads

The knowledge about morphology of gonads plays a vital role in the success of artificial propagation of any species. The gonads have cyclic variations in the development controlled by endocrine system, nervous system as well as ecological conditions. In this regard, a considerable literature is available with respect to morphology of teleost gonads. Afterwards, the reproductive structures and the reproduction in fishes have studied by Hoar (1955). He opined that the gonads have cyclic variation in development controlled by endocrine and nervous systems as well as by external ecological conditions. General morphology of gonads and GSI of *Colisa fasciatus* were studied by Banu and Bhakta (1985). Khan (2004) also studied on sexual dimorphism and gonadal development of an indigeneous ornamental fish, *Colisa faciatus* (Bloch and Schneider).

Gonadal maturity can be determined through direct eye observation of gonadal morphology and by microscopic observation of the histological survey. Morphological staging is generally done on the basis of its appearance, colour, size, weight, volume and intensity of blood vascularization, and the space occupied in the coelomic cavity (Venkataramanujan and Ramanathan, 1996).

The different stages of ovary exhibit their cytoplasamic and nuclear changes either synchronously or as in most cases asynchronously. This is reflected in parallel fluctuations of gonadosomatic indices. Different authors described various types of morphological staging of ovary. Wood (1930), Sen (1972) and Guraya *et al.* (1977) recognized 7 stages of ovarian maturity. Azad (1990) had classified the maturity of gonad into 7 stages based on macroscopic and microscopic observation. Literature about ovarian maturity of *Colisa fasciatus* is meager. Arockiaraj *et al.* (2004) have opted for 5 maturity stages and Koc *et al.* (2008) 4 maturity stages observed in case of Zebra fish. Banu and Bhakta (1985) described 3 maturity stages of ovary in *Colisa* *fasciatus* from Bangladesh. Agarwal (1996) divided the ovarian cyclicity of *Schizothorax plagiostomus* into 8 stages.

Testis is composed of a great number of seminiferous tubules or lobules bound together by a thin layer of connective tissues blood capillaries and interstitial cells (Diwan and Dhakad, 1996). Seminiferous tubules are filled up with sperm during breeding season, whereas after breeding season, empty and collapsing seminiferous tubules are seen, some of which contain residual or unexpelled sperm (Khanna and Pant, 1996). Das (2002) studied the testicular maturity of *Anabas testudineus* and reported that high spermatogenetic activity during spawning months (June and July) followed by an activity of the testicular maturity and identified three phases of testis i.e. spawning phase, post-spawning phase and preparatory phase. Agarwal (1996) classified the testicular cyclicity of *Schizothrax plagiostomus* into 5 stages based on histological observations.

2.3.4 Histological study of developing gonads

In a broad sense, histology means the study of cellular level of living things. Andrew Rogers (1983) has spelled out a truth about histology in the opening sentence of his book “Cell and Tissues”, as “Histology is the meeting place of biochemistry physiology and anatomy”. Agarwal (1996) in his book "Fish Reproduction" has stated "Monthly histological examinations of gonads are taken into consideration to determine the spawning season. Regular histological and histochemical examination of reproduction system could categorically define the size and age of a fish at first maturity, its reproductive rhythm and the changes in the reproduction cycle in the nature, controlled or in culture system. It is also an effective tool to study the ontogeny.

During the past years there has been vast expansion in the knowledge of histology of gonads. Comparatively more emphasis has been laid on the ovarian aspects than testes. Interestingly, differentiation of oocytes even from the components of the ovarian wall has been seen *Mystus seenghala* and *Wallago attu* (Dixit, 1956). A cursory glance at the reviews by Raven *et al.* (1961) leads one to infer that studies on one of the ovaries have been largely centered on one of the most important and dynamic aspects *viz.*, vitellogenesis, particularly because every organoid of the oocyte, either individually or in collaboration with one or two other organoids is considered to play a role in the production of yolk.

On the other hand, Dixit (1956) studied the atretic oocytes in the ovaries of two fishes, *Mystus seenghala* and *Wallago attu*, which exhibits atresia mostly to be observed in mature oocytes. They also observed that atretic oocytes in these oviparous fishes are fewer then in viviparous fishes.

Guraya *et al.* (1975, 1977), Guraya (1982), Lehri (1967) and Junhro and Boron (2006) studied the histomorphological changes in gonads, the percentage and duration of different developmental stages of oocytes, the average diameter and the gonosomatic index and distinguished different reproductive phases during reproductive cycle namely, resting, preparatory, maturing, pre-spawning, spawning and spent of the teleostean ovary. Growing oocytes have been observed with minor variation accommodating a few histochemical changes in almost all the cases (Merwe *et al.,* 1988; Verma, 1997; Khanna, 1996; Borah, 2002; Verma *et al*., 2002). Banu and Bhakta (1985) observed two distinct batches of eggs during spawning period of *Colisa fasciatus* and stated that the fish spawned more than once in a single year.

2.3.5 Sex ratio

According to Joshi and Pathani (2009), the operational sex ratio *i.e.,* ratio of fertilizable females to sexually active males at a given time, is a major factor prompting the intensity of sexual selection and hence maximizing seed production in fish. The female fish were larger in size than the male of the same age during the spawning season (Joshi and Pathani, 2009). Mitra *et al.* (2007) have informed male dominance in their studied population and 5.7 cm as length of first maturity for *Trichogaster fasciata*. Das and Kalita (2006) have stated that in captivity this fish species is used to mature at around 10-12 months age and at length of 6.5 cm and 5.2 cm for male and female respectively.

2.3.6 Breeding behaviour

According to Moyle and Cech (2004) some species are nest spawners and lay eggs in the nest and others called substrate spawners, which lay eggs in the spawning substratum. According to Mohanta *et al.*, 2008 spawning often take place all through the early sunrise hours. During the commencement of breeding season, males chase females, stimulating them to discharge their eggs by bumping and pushing them. Occasionally the courtship lingers for numerous hours or even days and then the female discharge the eggs, which are inseminated by the milt released simultaneously by male. Within 12 to 48 hours, the eggs may perhaps be hatched out and the yolk sac fry developed. The freshly hatched early larvae carry on to nurse off their yolk sacs for around 4 to 7 days till they become free swimming. Proper feeding should be done at this time and water quality must be maintained (Gratzek, 1992).

Significant research has been led on the reproductive behaviour of *T. trichopterus*. According to Tooker and Miller (1980), Hollis *et al.* (1984,1989,1995, 1997,1999) particularly the male fish, display innate complex behaviours related with establishing and protecting reproductive territories. According to Lee and Ingersoll (1979); Pollak *et al.* (1978,1981); Becker *et al.* (1992); Degani and Boker (1992), Degani (1993); Degani and Schreibman (1993), Jackson *et al.* (1994) maturation of female can be provoked either by pheromones, released by territorial males or in reaction to male nest building and courtship behaviors.

According to Cole *et al.* (1999) male *T. trichopterus* constructs “bubble nests” by gulping air at the surface and expelling mucus-lined bubbles which adheres to each other at the water surface, usually between the floating or emergent vegetation. According to Picciolo (1964) males are territorial and exhibit violent behavior to intruders that approach the nest, except receptive female for spawning activity. Degani (1989) concluded that females lay their eggs according to the size of the nest by finding out a correlation between parental body size, nest size and number of larvae. Unlike others in the genus, *T. trichopterus* breeds only with a limited scattering of bubbles that barely resemble a nest (Pinter, 1986).

According to Pinter (1986) male initiates spawning with stroking the ventral side of the female with his dorsal fin. Then he wraps his body around the female to exercise pressure on her to eject her eggs, which he inseminates later. Through each "nuptial embrace", the female expels 40 to 80 eggs. The eggs float upwards into the nest, or the male retrieves sinking eggs, if any, in his mouth, and expel them into the bubble nest with numerous bubbles. This is repeated until all eggs have been ejected by the female. The male then guards the young ones for several days (Hodges and Behre, 1953; Miller, 1964; Picciolo, 1964). After spawning, the male becomes highly violent towards conspecifics, as well as the just spawned female. After the larvae leave the nest, the male stops caring, but usually maintains the nest and spawns with other receptive females (Hodges and Behre, 1953; Miller, 1964; Pollak *et al*., 1981).

Rossi (1969) revealed that female pheromone could provoke male nest building activity in *Tricogaster lalius*. Up to 2000 eggs may be laid within a bubble-nest that is built on the surface between submerged plants. The male guards the nest, eggs and the young, until they are able to swim freely. The incubation period is about 24 hours.

Froese and Pauly (2014) reported that *T. trichopterus* increases offspring survival and recruitment with the help of specialized nesting and parental care which, when pooled with the species elongated breeding period, allows fast population growth.

Mitra K. *et al*. (2006) reported that under controlled environment Honey Gourami, (*Colisa sota*) was bred in captivity and it lay about 200-400 eggs in bubble nest built by the male. After 28-30 hours of laying eggs, hatching started. By 3 - 4 hours of hatching, the hatchlings became free swimming. Territoriality and parental care was shown by the male by guarding the eggs and hatchlings. There was 30-35% of larval survival.

2.3.7 Sexual maturity

According to McKinnon and Liley (1987) *T. trichopterus* can reach sexual maturity at 7 cm of total length and 12 - 14 weeks of age. The sexual maturity of giant gourami, *Osphronemus goramy* female,was considered by determining gonadosomatic index (GSI) and fecundity. It was found that the size at sexual maturity of female giant gourami was with 33.88±7.42 cm in total length (mean ± SD) and 968.75± 68.78 g in body weight (Amornsakun T. *et al.,* 2014).

According to Rahman (1989) and Froese and Pauly (2014) length of dwarf gourami reaches up to 3.5 inches (8.8 cm) in length but most males grow up to about 3 inches (7.5 cm) of length with the females growing a bit smaller. According to Shim *et al.* (1987) in temperate areas like northern India, dwarf gourami takes 8 to 12 months to mature whereas in Singapore it can breed all year round with newly hatched fry reaching sexual maturity within four months.

2.3.8 Breeding periodicity

Based on oocyte size distribution, Wallace ad Selman (1981) and Dietrich and Krieger (2008) classified ovaries into three basic types:

(i) **Synchronous ovaries** - All oocytes develop and ovulate in unison and there is no replenishment from the earlier stages. Such ovaries are found in species that spawn once and then die. The oocyte size distribution consists of a single mode (semelparous fishes.)

(ii) **Group synchronous ovaries**- At least two size groups of oocytes are present at the same time; the larger group or clutch usually being more homogenous than the smaller.

(iii) **Asynchronous ovaries**- Oocytes at all stages of development are present at the same time. The oocyte size frequency distribution is continuous except in the ripe stage where, there may be a clear separation of the yolked oocytes.

Based on spawning frequency (Prabhu, 1956; Karekar and Bal, 1960), the fishes are categorized into four groups. This classification is based upon the works of Hickling and Rutenbnerg (1936).

(i) **Category A**: Spawning takes place once in a season during a short duration, the individual spawning once. Ovary contains a ripe stock distinctly and clearly separated from immature stock.

(ii) **Category B**: Spawning takes place once in a season but with longer duration. Range in size of the ripe ova nearly one half of the total range of intra-ovarian eggs.

(iii) **Category C**: Spawning more than once during a protracted spawning season. Ovary with a batch of ripe stock, an immature stock and an intermediate ripening stock in between the ripe and immature ones.

(iv) **Category D**: Spawning extended over a very long period or almost round the year but intermittently, the individual spawns many times in the spawning season. Batches of eggs in the ovary are not well differentiated from one another, usually shown by fishes in tropical structured communities.

Some species are multi-spawners and others are not. A perusal of literature on spawning season of Indian freshwater fishes revealed that most species not only have relatively short spawning season, but most of them spawn after the onset of the south-west monsoon (Verma, 1997). The species spawns more than once throughout the year (Hyndes *et al*., 1992). Arocho (1997) described the sword fish (*Xiphias gladius*) as multispawner. The GSI of *A. mola* varies from 1.88 to 20.42, lowest value recorded in January and maximum in June and November i.e., it breeds twice in a year (Pal and Mahapatra, 2016). The spawning season of *Anabas testudineus* mentioned by different authors is about April and May (De, 1910); mid-April to mid-June Mookerjee and Mazumder (1946); pre to post monsoon (Alikunhi, 1957); May to October (Hora and Pillay, 1962). According to Banu and Bhakta (1985) the spawning season of *Colisa fasciatus* is from April to June in Bangladesh. Very limited information is available on breeding periodicity of *Trichogaster fasciata*. Mitra *et al.* (2007) have conveyed March-October to be its breeding season in West Bengal, while Das and Kalita (2006) have documented April-August to be its breeding season in Assam.

2.3.9 Fecundity

Fecundity study is very important with respect to weight, age and length of the fish and it is also important to estimate the reproductive potential (Mahapatra *et al.,* 2004). Fecundity may be defined as the number of mature eggs in the ovary of female fish prior to spawning (Bagenal and Braum, 1978). Many fishery researchers have worked on the fecundity of diverse fishes (Naeem *et al*., 2005; Jakobsen *et al*., 2009; Mekkawy and Hassan, 2012; Shinkafi *et al*., 2011). The awareness of fecundity is one of the significant parts of the reproductive biology (Nikolsky, 1963). According to Khallaf and Authman (1991) fecundity is not a persistent feature but it is altered with differences in ecological conditions and species specific reasons. Fecundity is useful to apprehend if fish has attained maturity and is able to spawn the number of eggs in the spawning period. The difference in fecundity may occur due to the dissimilarities in environmental conditions and food consumption by the fish . Doha and Hye (1970) reported that the disparity of fecundity is very common witnessed in fishes. The number of eggs spawned by female is at the mercy of several factors like age, size, and ecological conditions.

Zukal (1983), Richter (1988) and Pethiyagoda (1991) conveyed that in *T. trichopterus* fecundity is dependent on size and usually ranges from 300 for smaller females, up to maximum of 2000 to 4000 eggs for larger females. Under experimental conditions Reyes-Bustamante and Ortega-Salas (2002) recorded a higher mean absolute fecundity of 8021 eggs, and a maximum value of 9104 eggs.

According to Amornsakun *et al. (*2014) fecundity of *Osphronemus goramy* was 5,508±1,547 per fish and gonadosomatic index (GSI) was 2.32±0.5%. By semi-controlled natural method, newly hatched larvae of giant gourami were produced.

In case of *Trichogaster fasciata*, Behra *et al.* (2005) documented absolute fecundity range of 599-5,522 and later Mitra *et al.* (2007) have reported the range of 1,095-19,291 for the same. Das and Kalita (2006) have stated 4,100-4,700 as the relative fecundity range for this fish species.

2.4 Larval rearing

In order to increase the survival chance, the fish larvae need to complete their morphofunctional systems so that they can escape predation and find their food (Osse *et al.,* 1997). In freshwater fish culture, the transition phase from endogenous to exogenous feeding is one of the most perilous stages and the accessibility of suitable food is necessary for a high rearing success on marketable measure of aquaculture (Gulbrandsen 1993; Jahnichen and Kohlmann, 1999). According to Giri *et al.* (2002) success of larval rearing mainly depends on the accessibility of suitable diets that can be readily consumed, well digested and provides the essential nutrients for good growth and health. Larval survival is affected by number of factors, *viz.,* duration from embryo stage to the first feeding stage, the preferred food and its availability, the interactive relation between the larva and its prey, the accomplishment of feeding responses, the swimming capability of larvae in hunt of food and the essential food ration required for growth and metabolic expenditure.

The complications in larval rearing of fishes are largely related to nutrition. The first few days after hatching makes feeding over crucial because of the minor mouth gap of the larvae (Dhert *et al.,* 1998). The small mouth size of larvae should be considered while selecting the size of the diet particles. According to Kim *et al.* (1996) very small feed particle can lead to nutrient leaching problems because of very high ratio of surface area to volume. According to Mohanta *et al*., 2008 due to their small mouth gap and changes in feeding habit, the larval stages of many fishes are critical in fish breeding.

Appropriate knowledge regarding food preference, particularly the first food of fish larvae is vital for accomplishing good survival rates through fish culture (Ghosh *et al.*, 2003). According to Gupta and Banerjee (2009) there is deficient information regarding the age specific food preference of fish and there is less survivability which needs research thrust for enhancing larval survival and it also profoundly depends on the supply of appropriate live feed (Madhu *et al*., 2012). Though a small food organism is required for fish larvae, the usage of larger organisms is more advantageous as long as the size of the food organism does not affect with the ingestion mechanism of the larvae (Merchie, 1996). According to Dou *et al*. (2002) the first feeding of a fish larva is very important for its consequent growth and survival. Most fish larvae display deformed growth and failure to swim and prey the feed if they do not start successful first feeding immediately after the mouth opening (Dou *et al*., 2002).

According to Blaxter and Ehrlich (1974) fish larvae must successfully establish the transition of exogenous nutrition, when yolk sac is exhausted else it will suffer progressive starvation. During this critical phase, food availability and the larva's capability to ingest it are the significant factors for establishing successful exogenous feeding for larval survival, growth and recruitment of the next generation (Sanderson and Kupferberg, 1999).

According to Houde (1974) the nutrients stored in the yolk sac and also the environmental factors decides the length of time from hatching to first feeding. Due to little quantity of yolk material, many of the larvae starts feeding within 2-3 days after hatching and initially the feeding strikes is low but rapidly rises later during early development. The selective feeding is determined by the size of the larva and its mouth in relation to live food size (Hempel, 1965; Sherman *et al*., 1981). The initial amount of endogenous yolk reserves and the developmental rate prior to external feeding is controlled by maternal inheritance and temperature control (Theilacker and Dorsey, 1980).

In the cycle of fish seed production in hatcheries, the rearing of the larvae to the fry stage is most critical and development of specific culture techniques is required for its rearing under controlled hatchery condition (Olaniyi and Akinbda, 2013). According to Duray *et al*. (1997) and Toledo *et al.* (1999) survival of early stage larvae is still low in aquaculture production. But according to Tlusty (2002) 90% of presently marketed fresh water ornamental fish are cultured due to the research developments in larviculture and early rearing technology. In current years, many studies have been conducted to observe their first and succeeding feeding behaviour and their survival mechanism (Kailasam *et al*., 2007and Wan *et al*., 2014).

For determining the effects of both live and formulated diets on first-feeding fish larvae, the powerful tools are growth and survival data. Proper availability of lipid, protein, carbohydrate, vitamins and minerals through the diet results in successful rearing of fish larvae (Watanabe and Kiron, 1994 and Kanazawa, 2003). High mortality, low feed efficiency, anemia and poor growth results due to deficiencies of these nutrients (Sargent *et al*., 1999 and Olivotto *et al.,* 2003).

2.5 Feed Formulation

Fish feed formulation in aquaculture with balanced nutrition was established by Hardy (1980). Singh and Srivastava (1985) developed another approach to formulate low cost diet with suitable feeding management strategies. De Silva (1985) assessed the general problem of high feed cost in fish culture by minimizing it with cheaper ingredients as protein sources. Mollah and Hossain (1990) observed the effect of artificial diets with different protein levels on growth and feed efficiency of *Clarias batrachus*. Similar work on trouts was done and reported by Tacon (1990). Efficacy of formulated feeds for juvenile green-back grey mullet, *Liza subviridis* was examined by Das *et al*. (1993). Studies regarding the raw materials used to produce fish feed and its quality control in aquaculture were done by Sitasit (1993). Using blue gourami (*T. trichopterus*) as a model for labyrinth fish, Degani (2002) compared the digestibility of protein from plant (Soybean meal) and animal (fish meal). For spawning of common carp, Mishra and Singh (2002) determined the requirement of protein, fat and carbohydrate. Ahilan and Kumaran (2003) suggested that nutritionally well balanced feeds could be formulated using low-cost ingredients accessible in the concerned region.

2.5.1 Nutrient requirements

Nutrition has significant influence on growth and reproductive prospective of aquarium fish and several live feeds have been used for fish rearing. Fish reproductive performance is affected by the quantity and composition of dietary protein (Shim *et al*., 1989). Improvement of the egg and sperm quality of fish species can be carried out through the nutritional quality improvement of brood stock diets (Watanabe *et al.,* 1985 and Bromage *et al*., 1992). The short reproductive cycle of aquarium fishes requires continuous accessibility to right type of brood stock nutrition. An upgrading in brood stock nutrition and feeding has shown to improve both egg quality and seed production (Kithsiri *et al*., 2010). Gonadal maturation hangs on upon the quantity and quality of food (Mishra and Singh, 2002). For adequate breeding of fish in confined waters, feeding with appropriate high energy diets such as protein, vitamins and minerals are required. Eventually deprived brood stock nutrition will lead to the production of low-grade quality fry with little survival rate. It is normally believed that egg and larval quality are partly controlled by brood stock diet (Farahi *et al*., 2010). Early larval development is influenced heavily on yolk nutrients carried through the brood stock diet. Nutritious brood stock diet pedals the gonad weight, gonadosomatic index, sexual maturation and spawning frequency (Singh and Singh, 1979).

Earlier reports on the gross dietetic necessities of gouramis were restricted to the blue gourami, *Trichogaster trichopterus* and the dwarf gourami, *Colisa lalia* (Mohanta & Subramanian, 2007; Shim *et al* 1989; Zuanon *et al.,* 2013). Shim *et al.* (1989) presented that 35% feed protein levels improved growth rates, adult body mass, and the percentage of vitellogenic oocytes in *C. lalia*. Degani and Gur (1992) exhibited that the ideal dietary protein level for an additional wild tropical gourami, *T. leerii*, stretched between 26% and 36%. Mohanta and Subramanian (2007) informed that a feed composition of 35% protein, 8% lipid, and 382.24 kcal/100gm digestible energy (DE) was optimal for *Trichogaster trichopterus*. Zuanon *et al.* (2013) specified that 37% dietary protein was necessary for *Trichogaster lalius*.

Berenjestanaki *et al* (2014) estimated the effect of different lipid sources on growth and reproductive performance of the three-spot gourami, *T. trichopterus*. They found that fish diets, which contained 12% lipid, in which 50% of the lipid source could be replaced with vegetable oils, particularly canola and linseed, showed improved reproductive performance compared to feeds comprising only fish oil as the lipid source.

Formulations capable of improving the pigmentation of cultivated tropical fish have substantial application. There are numerous colour enhancing compounds like beta carotene, canthaxanthin, xanthophyll and astaxanthin. According to Fey and Meyers (1980) these pigment enhancers are usually provided in the feed at a rate of 0.05 percent of active ingredient.

The effect of dietary Spirulina level on the growth, fertility, colourations and leucocyte count in red sword tail, *X. helleri* was worhed upon by James *et al.* (2006). The effect of natural carotenoids in aqua feed to achieve bright colouration in ornamental fishes was observed by Gupta *et al.* (2007). The antioxidant and anti-inflammatory potential of selected *S. platensis* was demonstrated by Dartsch (2008). Culture, production and use of Spirulina as food for human and feed for domestic animals and fishes were reviewed by Habib *et al.* (2008). The effects of graded levels of *S. platensis* on the growth performance, feed utilization, non-specific immune response and resistance of Nile tilapia to *Aeromonas hydrophila* infection was reported by Tawwab *et al*. (2008). The interaction between Spirulina and different levels of vitamin E, on growth, gonad weight, reproduction and colouration in goldfish, *C. auratus was* investigated by James *et al.* (2009).

2.5.2 Nutritional interventions for growth performance

The effect of formulated plant feed with different protein concentration on growth performance in grass carp was observed by Nekoubin and Sudagar (2012). The efficacy of formulated diet with live feed on growth and survivability of hybrid cat fish (*Heterobranchus bidorsahis* X *H. longifilis*) fry was examined by Adekunle and Joyce (2014). Investigation on the dietary protein level and protein to energy ratio that support the best performance of juvenile top shell *Tectus niloticus* was carried out by Maria *et al.* (2014). The effect of formulated feeds on growth performance and colour enhancement in the fresh water gold fish, *C. auratus* was evaluated by Umaa Rani *et al.* (2014). The efficiency of cheap feed ingredients with algal meal in formulated diets for gold fish, *C. auratus* was studied by Rama *et al.* (2014). The growth performance, and feed conversion ratio of hybrid fingerlings (*Catla Catla* X *L. rohita*) fed on cotton seed meal, sunflower meal and bone meal was evaluated by Sahzadi *et al*. (2006). The effect of botanical additives on the growth and colouration of gold fish, *C.auratus* was studied by Ahilan *et al.* (2008). James *et al.* (2008) evaluated the effect of dietary vitamin E on growth, reproduction, enzyme activity and leucocyte count in *C.auratus*. The effect of various diets on growth and feed conversion ratio of Angel fish, *P. scalare* was examined by Sevel *et al.* (2009). The growth response of *L. rohita* fingerlings fed with different feeding regimes under intensive rearing was determined by Abid and Ahmed (2009). Vasudhevan *et al.* (2009) studied the effect of food quality on growth of the ornamental fish, Koi carp. Investigated of growth, feed conversion ratio and survival rate of *Huso huso* fingerlings in relation with different feeding times was done by Sudagar *et al.* (2011). Attempt was made by Dharmaraj and Dhevendaran (2011) to study the application of microbial carotenoids as a source of colouration and growth in case of ornamental fish, *X. helleri*.

It was suggested by Lim *et al*. (2003) that the live feeds used in marine food fish hatchery could be applied to freshwater ornamental fish culture to enhance their performance. The effect of live food organisms on the growth and maturation of gold fish, *C. auratus* was determined by Ahilan and Kumaran (2003). Kumar *et al.* (2008) suggested that live feeds are eagerly consumed, competently digested and provide the essential nutrients to support higher growth and health in fishes. The impact of live and artificial food on the length, weight of *Poecilia reticulate* was evaluated by Ingale and Charjan (2010). Salma *et al.* (2013) evaluated the use of fairy shrimp as live food for blue gourami, *T.trichopterus*. The efficacy of different commercially available feed and a live feed for better growth and coloration in ornamental fishes was studied by Mandal *et al* (2010). The efficacy of feeding with different live feeds and artificial feed on growth performance of black spot barb, *Punctius filamentosus* was evaluated by Saurabh *et al*. (2013). The growth and survival rate of adults and fingerlings of *P. sphenops* using live food (rotifer, cladoceran and copepod) and pelletilized feed was evaluated by Sumithra *et al.* (2014).

The effect of dietary supplementation with different rates of Spirulina on growth performance and feed conversion in guppy was determined by Dernekbasi *et al* (2010). The effect of feeding *S. platensis* on growth and carcass composition of hybrid red tilapia (*Oreochromis mossambicus* X *O.niloticus*) was studied by Ungsethaphand *et al*

(2010). The effect of feed containing various ratios of Spirulina on the growth of the Mekong giant cat fish was evaluated by Tongsiri *et al* (2010). The effect of dietary *S. platensis* on feeding parameters of blue gourami, *T. trichopterus* was studied by Rinna *et al* (2013).

2.5.3 Nutritional interventions for reproductive performance

The reproductive management of ornamental fish in aquaculture is explained by Roy (1996). The effect of various diets on spawning and egg quality in angel fish, *P. scalare* was studied by Degani and Yehuda (1996). Evaluation of the effect of brood stock nutrition on reproductive performance of some ornamental fish species was done by Izquierdo *et al.* (2001). The effect of different types of feed on growth and fecundity in *Betta splendens* was reported by James and Sampath (2002). James and Sampath (2003) studied the effect of different levels of dietary animal and plant proteins on growth, gonad weight and fecundity in *B. splendens.* The effect of feed quality on the growth, gonad development and fertility in the red swordtail fish, *X. helleri* was studied by James and Sampath (2004). Sotolu (2010) investigated the effect of varying dietary protein levels on the breeding performance of *C. gariepinus* brood stocks and fry growth rate. The effect of three types of feeds on the growth and reproductive performance in female guppy was evaluated by Kithsiri *et al.* (2010). The influence of temperature and brooder diet on reproduction of clownfish, *Amphiprion sebae* in captivity was determined by Dhaneesh *et al*. (2011). The effects of brood stock sex ratio on the reproductive performance of gold fish and also the growth performance of fry fed with different feeds in *C. auartus* was reported by Sharma *et al.* (2011).

The effect of fairy shrimp as live feed for reproductive performances and colour of fresh water ornamental fish prawns was determined by Masoud (2014). The effect of dietary Spirulina levels on the colourations, reproductive performance and growth of yellow tail chichlid was studied by Guroy *et al* (2012). Kasari *et al*. (2012) studied the effect of various diets including living earth worm, dried tubifex, dried gammarus and prepared granulated feed on the growth and reproductive performance of fresh water angel fish *P. scalare*.

2.5.4 Nutritional interventions for larval rearing

The effect of diet and feeding rate on growth, morphological development and behaviors of larval and juvenile gold fish, *C. auratus* was studied by Paulet (2003). The effect of different feeds of animal origin in composition with live plankton on growth and survival of *Wallago attu* larvae during hatchery rearing was evaluated by Sahoo *et al.* (2006). The impact of feeding made with decapsulated artemia eggs in the larval period of ornamental gold fish, *C. auratus* was examined by Hekimoglu *et al.* (2014). The rearing of *C. auratus* larvae with live feed such as *Brachionus plicatilis, Apocyclops dengizicus* and *Ceriodaphnia reticulate* was reported by Janakiraman and Altaff (2015). Evaluation of the effect of different diets on growth and survival rate of angelfish larvae using five different diets like Artemia, rotifer, Moina, Ceriodaphnia, egg custard and green water was done by Samiran and Ghosh (2015). The effect of different commercial diets on growth and survival of *Pterophyllum scalare* juveniles under controlled conditions was evaluated by Ulloa and Romero (2005). Comparative study on the survival rate, growth and body composition of *Oncorhynchus mykiss* larvae fed with live and artificial feed was done by Akbary *et al*. (2010). Faruque *et al*. (2010) studied the effect of different feeding rates on growth performance and survival of African cat fish (*C. gariepinus*) larvae with the newly hatched Artemia nauplii and artificial feed. Achiony *et al.* (2012) studied the effect of Artemia nauplii and formulated diet which ensure growth and survival of larvae and post larvae of *C. gariepinus*. Live feeds allow for better digestibility and are palatable to fish (Kolkovski, 2001). If well adapted, the larvae can thrive well on formulated diets which contain different nutrient composition. Several dry feed formulae have been examined as probable alternatives of live food for larval development (Appelbaum and Dor, 1978; Dabrowski, 1984). Several studies were carried out to investigate the possibility of replacing live food with manufactured diets from the onset of exogenous feeding (Jones *et al.,* 1993; Person *et al*., 1993). Degani (1990) reported that there was no significant difference in growth of larvae of *Trichogaster trichopterus* fed with commercial diets or a diet consisting of yeast and egg yolk or only yeast. The breeding and early development of the nest building gourami, *T. trichopterus* was determined by Bindhu *et al.* (2014).

In the present study, the growth and survival rate of larvae fed with live feed infusoria, boiled egg yolk and formulated feed was analyzed in dwarf gourami, *Colisa lalia*.

2.6 Water Quality

Fish are very sensitive to environmental factors, therefore as a primary preventive measure proper management of water quality should be done in ornamental fish breeding and culture. Any variations in the water quality parameters result in stress which disturbs the wellbeing, survival, reproduction and growth of the ornamental fishes. According to Rottman *et al.* (1991) temperature is an important factor for egg development and hatching. Factors that prompt spawning are water temperature, photo period, water quality such as dissolved oxygen, hardness, alkalinity, pH, total dissolved solids (TDS), salinity, flooding, water current, tide and cycles of the moon, weather cycles and spawning substratum (Rottman *et al.*, 1991). Aeration is essential but need to be moderated to prevent injury to the evolving embryos.

If eggs are infected with fungus or bacteria, then to avoid spread of infection, the bad eggs can have removed carefully without disturbing other eggs. Some type of disinfectant such as methylene blue or acriflavine is often used by the breeders, to prevent bacteria or fungus from contaminating the eggs. *Saprolegnia parasitica* often covers the unfertilized eggs or bad eggs, which turns opaque or white. As the fungus spreads quickly and can effortlessly destroy spawn, this can be very perilous (Roy, 1996).

Hails and Abdullah (1982) reported that under favourable environmental conditions, *T. trichopterus* displays an extended breeding period, with temperature and day length being essential reproductive indications. Axelrod *et al*. (1967) reported 18°C to be the minimum temperature for reproduction, whereas Degani (1989) and Cole *et al.* (1999) reported the minimum temperature to be 23°C. All researchers agree that the maximum temperature is 29°C. According to Reyes-Bustamante and Ortega-Salas, (2002) spawning is boosted in acidic water with a pH range of 5.5 - 6.5. Degani *et al*.

(1990) reported that growth rates are negatively affected by increased nitrite and nitrate.

Solis (1988) conveyed that growth, survival, distribution, behaviour and physiology of aquatic organism are affected by dissolved oxygen. Temperature is one of the most significant water quality parameter in aquaculture as it affects the metabolic rate of fish (IEPA, 2001). Bhatnagar and Devi (2013) reported that in the blood, Nitrite oxidizes hemoglobin to methaemoglobin, making the blood and gills brown and hampering respiration which also harms the nervous system, spleen, liver and kidneys of the fish, so nitrite can be named as an invisible killer of fish.

Ye *et al*. (2011) conveyed that the performance of fish larvae and juveniles is significantly affected by environmental factors such as temperature, salinity, dissolved oxygen, pH. The factors can be divided into biotic and abiotic factors. Feeding success and predation are believed to be the most important biotic factors affecting survival. Yet, a broad understanding of the significance of feeding success and the mechanisms leading it involves the incorporation of numerous factors in larval survival (Werner *et al*., 2001).

3. MATERIAL AND METHODS

3.1 Research Overview

Experimental Fish –The fish species used for the study is dwarf gourami, *Colisa lalia* (Hamilton, 1822). It belongs to class Actinopterygii, order Perciformes, suborder Anabantiformis and family Osphronemidae.

Procurement of fish - The fish was procured from fishermen of Tribeni, who collected the fish from the river area of Tribeni, Hooghly (District), West Bengal. Collection was done from their natural habitat from Ganga river basin of Tribeni (22.99°N 88.40°E), Hooghly area (i.e. divergence of three rivers, Yamuna, Ganga and Saraswati).

Experimental site – The studies and the experiments were carried out at two sites, ICAR-CIFE, Kolkata Centre and ICAR-CIFE, Mumbai. Collection, domestication, general biology and experiment on nutritional intervention on growth performance of dwarf gourami, *Colisa lalia* was carried out at ICAR-CIFE, Kolkata Centre within the period of September, 2014 to December, 2015. Later Feeding biology, Reproductive biology, experiment on nutritional intervention on reproductive performance and larval rearing of dwarf gourami, *Colisa lalia* were carried out at ICAR-CIFE, Mumbai, during the period July, 2018 to June, 2019.

3.2 Captive Rearing at ICAR-CIFE, Mumbai

Procurement and transportation of fish - The fish were procured from fishermen of Tribeni, Hooghly, West Bengal, who collected the fishes from the river. The fishes were then packed in polythene bags with oxygen packing and then again packed in cardboard boxes. These fishes were transferred to Mumbai by air through cargo.

Acclimatization and maintenance of fish - The fish were carefully transferred to well aerated cemented rectangular tanks of 2500 L capacity tank, after a salt dip treatment.

Fish used for the study were advanced fingerlings of *Colisa lalia* (Hamilton, 1822) with an average weight of 1.13 g, ranging from 0.72 – 1.62 g and average length of 4.03 cm, ranging from 3.4 – 4.6 cm. The stock was acclimatized and maintained under captive condition at stocking density 1fish per 2 litre water. The height of the water level in the tanks was kept around 40 – 45 cm, as the species is air breather. Regular siphoning and 20% water exchange was also carried out.

Feeding of the experimental animal - Commercial feed was used for feeding purpose. Brand name of the feed was Taiyo. The feed was in form of floating pellets of size 2 mm diameter. The major ingredients of the feed were fish meal, wheat meal, yeast, germ meal, vegetables, spirulina, shrimp meal, vitamin and minerals. The proximate composition of the feed is 30% Crude Protein, 2% Crude Fat, 5% Crude fibre, 9% Crude Ash. Feeding was done twice a day @ *adlibitum*.

Studies carried out - During the captive rearing phase at ICAR-CIFE, Mumbai, study of the feeding biology and reproductive biology was carried out from July 2018 to June 2019.

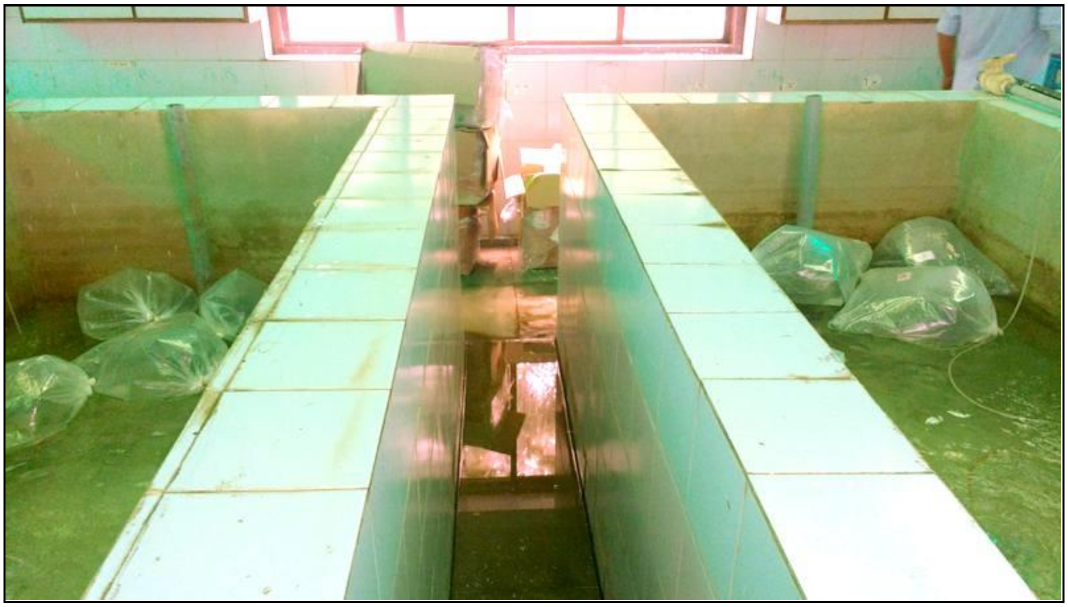

Plate1: Acclimatization of dwarf gourami, *Colisa lalia*

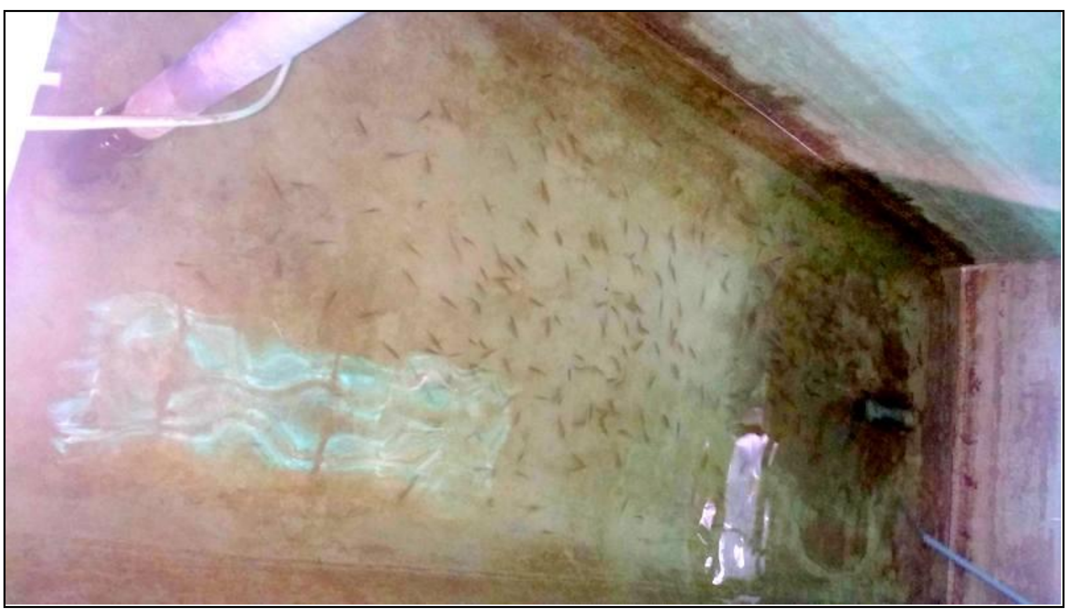

Plate2: Captive rearing of dwarf gourami, *Colisa lalia*

3.3 General Biology

3.3.1 Morphometric study

A sample size of 100 fish was taken of length ranging from 3.5 – 4.7 cm and weight in range of 1.02 – 1.98 g and preserved in 10% formalin solution. Length of the following morphometric characters was measured using a mm vs cm scale. The measurements were taken to nearest round figure. The parameters studied can be referred in Plate 3 and are as follows:

1. Total length (TL) - Maximum elongation from both the ends is measured.
2. Standard Length (SL) - Measurement from the tip of the snout or premaxilla to the base of the caudal fin (hypural joint), where a groove forms, is measured.
3. Predorsal Length (PDL) - Distance from anterior most part of the body to the first dorsal fin ray is measured.
4. Preanal length (PAL) - Distance from anterior most part of the body to the first anal fin ray is measured.
5. Prepectoral length (PPL) - Distance from anterior most part of the body to the base of the pectoral fin is measured.
6. Prepelvic / ventral length (PVL) - Distance from anterior most part of the body to the base of the pelvic fin is measured. The pair of pelvic fins in this fish is long and thin, modified to antenna like structures.
7. Head length (HL) - Distance between the snout or premaxilla & the posterior most edge of the opercular bone is measured.
8. Body Depth (BD) - The distance between the dorsal & ventral surface at the deepest point is measured.
9. Eye Diameter (ED) - Distance between the anterior & posterior rims of the eye in the longitudinal axis is measured.
10. Snout Length (SnL) - Distance from anterior most part of the body to the front margin of the eye orbit is measured.

3.3.2 Meristic count

A sample size of 50 fish was taken, and the following meristic characters are counted, using a hand magnifying glass and needle:

1. Dorsal Spines (DS) – The hard spines present at the anterior portion of dorsal fin, are counted.
2. Dorsal Soft Rays (DSR) – The soft rays present behind the dorsal spines are counted.
3. Anal spines (AS) - The hard spines present at the anterior portion of anal fin, are counted.
4. Anal Soft Rays (ASR) - The soft rays present behind the anal spines are counted.

3.3.3 Statistical analysis for morphomeristics

The mean, standard deviation, minimum range, maximum range and range difference were calculated of all the ten morphometric characters. Correlation Coefficient, R^2 value and Regression Equation were worked out in comparison to total length (TL). Relationships were analyzed using a standard linear regression expression $Y = a + b X$, where 'Y' is the dependent variable, 'X' is the independent variable (TL), 'a' is the constant (Y intercept) and 'b' (slope) is the regression coefficient, were fitted for all the variables for different localities. The goodness of fit of the relationship between the variables was derived from the coefficient of correlation.

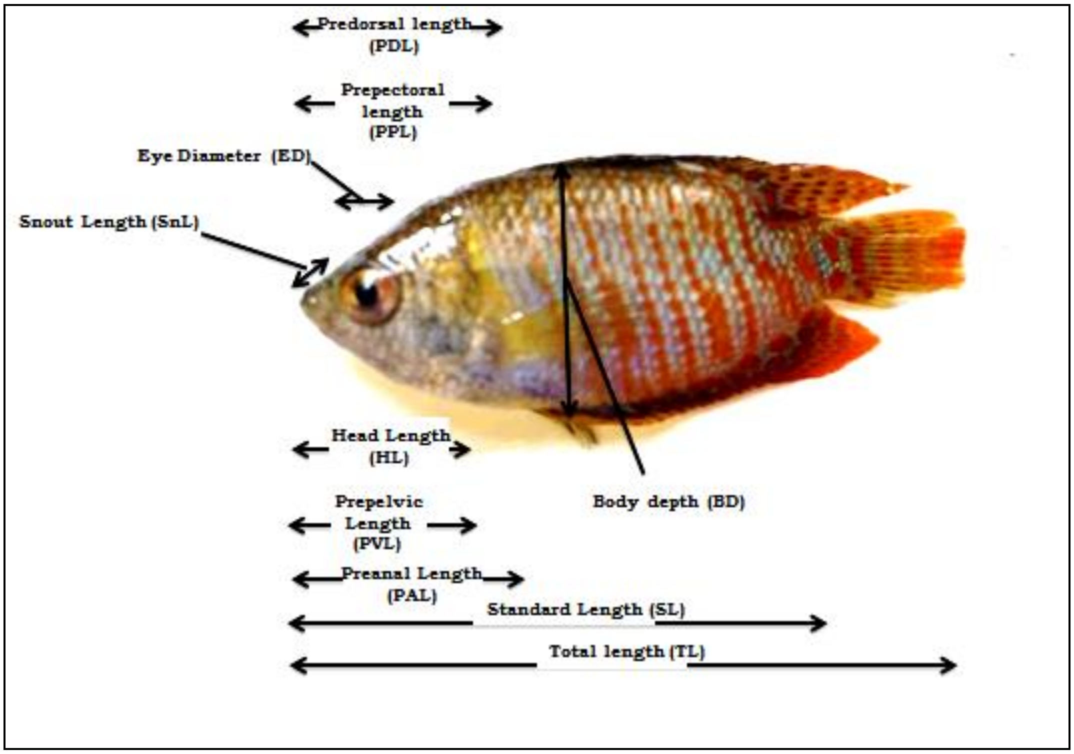

Plate 3: Morphometric measurements of dwarf gourami, *Colisa lalia*

3.4 Feeding Biology

3.4.1 Relative gut length

The characterization of fish as carnivore, herbivore or omnivore is done by using the Relative gut length (RGL) as the main morphological variable. A sample size of 10 fish were taken every month and dissected. Before dissection the total length of the fish was taken, and then later the gut length was taken (Plate 4 and Plate 5). Relative gut length (RGL) was calculated by the following formula.

$$\text{Relative gut length (RGL)} = \frac{\text{Gut length (cm)}}{\text{Total lenght(cm)}}$$

3.4.2 Gastro-somatic index

Feeding intensity of fish was determined as the Gastro-somatic Index, a numerical value. A sample size of 10 fish was taken every month and dissected (Plate 4 and Plate 5). The body weights and gut weight were measured by using an electronic balance. Gastro-somatic index (GaSI) was calculated by the following formula.

$$\text{GaSI} = \frac{(\text{Weight of the gut})}{(\text{Weight of the body})} \times 100$$

Plate 4: Half empty intestine of dwarf gourami, *Colisa lalia*

Plate 5: Empty intestine of dwarf gourami, *Colisa lalia*

3.5 Reproductive Biology

3.5.1 Sexual dimorphism

Randomly few adult fishes were collected from the stock and kept in a transparent aquarium and their size, colouration, fins shape and body shape was observed and recorded. The observations were carried out for the entire captive rearing period, so as to find the differences in the characters in breeding and non-breeding period. A couple of fishes were dissected for sex confirmation.

3.5.2 Gonadosomatic index (GSI)

Monthly samplings of 30 fish were carried out from the captive culture stock from July, 2018 to June, 2109. The gonadosomatic index, abbreviated as GSI is a tool for measuring the sexual maturity of animals in correlation to ovary development and testes development. It is the calculation of the gonad mass as a proportion of the total body mass. It is represented by the formula

$$\mathbf{GSI = [gonad\ weight / total\ body\ weight] \times 100}$$

3.5.3 Histological analysis of gonadal development

Monthly samplings of 10 fish were carried out from the captive culture stock from July, 2018 to June, 2019. The gonads were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) for histological examination. The samples were then processed using a graded series of alcohol, embedded in paraffin wax and the sections of 5µm thickness were cut using a microtome and spread on slides, the sections thus obtained were stained with Delafield's haemotoxylin – Eosin stain (Gray, 1964).

3.5.4 Breeding behaviour

Male and female fish were segregated for fifteen days. They were given Tubifex as feed in *adlibitum* during these fifteen days. Then breeding setups were arranged by taking one adult male and one adult female in each aquarium. The breeding setup was left undisturbed and observations were made and recorded without disturbing the breeding pair.

3.5.5 Fecundity

Absolute fecundity is the total number of eggs that are likely to be spawned in one spawning period. Matured female fishes were dissected, and then ovaries were carefully removed from the body, and put into 10% neutral buffered formalin (NBF) for fixation. Then the eggs were loosened from the ovary by shaking the tube and counted under microscope to find out fecundity.

3.5.6 Ova diameter

Ova diameter of spawned oocyte was found out by using scale in Nikon phase contrast microscope.

3.5.7 Ichthyoplankton

The study encompassed the morphological changes in the oocyte and larval stages by using Optical/Light Microscope and Phase contrast Microscope.

3.6 Evaluation of effect of nutritional intervention on growth performance of dwarf gourami, *Colisa lalia.*

3.6.1 Site of the experiment

The experiments were conducted over period of 90 days from September, 2016 to December, 2016 at the wet laboratory of ICAR-Central Institute of Fisheries Education (CIFE), Kolkata Centre. Subsequently, the laboratory work was carried out in the different analytical laboratories of Kolkata Centre.

3.6.2 Procurement and acclimatization of experimental fish

Fish used for the experiment were advanced fingerlings of *Colisa lalia* (Hamilton, 1822) of average length 3.26 (± 0.20) cm and average weight 0.93 (± 0.09) g. The fish were procured from fishermen of Tribeni, Hooghly, West Bengal, who collected the fish from the river. The fish were carefully transferred to well aerated rectangular tanks of 1000 L capacity (240 X 66.25 X 56 cm3) after a salt dip (20-25ppt) treatment. The stock was acclimatized under aerated conditions for about 15 days in the same tank and feeding was done with commercial feed.

3.6.3 Experimental design, set-up & fish maintenance

Two forty (240) advanced fingerlings of *Colisa lalia* were randomly distributed in four distinct experimental groups such as T1, T2, T3 and T4, with triplicate in each following a completely randomized design (CRD). The experiment was conducted for a period of 90 days in the wet laboratory of ICAR-CIFE, Kolkata Centre. The setup consisted of 12 FRP tanks of 200 L capacity (70 X 52.5 X 46.25 cm^3) capacity, covered with perforated lids to prevent the fishes from jumping out during rearing period. The tanks were initially washed and filled with potassium permanganate solution (4 ppm) and were left overnight. The tanks were flushed out on the very next day and were thoroughly washed with clean water. Based on design each tank containing 150 L Bidhan Nagar Municipality supplied water was stocked with 20 fishes with average initial body weight ranging from 0.93 (± 0.09) g. The total volume of the water in each tank was maintained at 150 L throughout the experimental period. Round the clock aeration was provided in each tank. The aeration pipe in each tub was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tanks. The tanks were provided with hideouts for fish. The experimental conditions were kept same throughout the trial period. The fishes were starved overnight before taking the body weight.The experimental tanks were cleaned manually and siphoning was done in order to remove the remaining fecal matter every morning. Everyday 20% water was exchanged. This was carried out throughout the experimental period of 90 days.

Table 1: Experimental Design for Growth Performance

T1 (T1R1,T1R2,T1R3)	- **Feed A (Control Feed)**
T2 (T2R1,T2R2,T2R3)	- **Feed B (Control feed + 2 % supplementary mix)**
T3 (T3R1,T3R2,T3R3)	- **Feed C (Control feed + 4 % supplementary mix)**
T4 (T4R1,T4R2,T4R3)	- **Live feed (*Tubifex*)**

Plate 6: Experimental site at ICAR – CIFE, Kolkata

3.6.4 Formulation of experimental diets

Ingredients and additives of interest such as fish meal (FM), soyabean meal (SBM), groundnut cake (GNC), de oiled rice bran (DRB), wheat flour, starch powder, soya oil (ω-3 fatty acid source), vitamin-mineral premix, carboxymethyl cellulose (CMC) as binder and supplementary diet mix were considered for feed formulation as shown in the table below. Supplementary diet mix was prepared by simple mixing of Refined lecithin (phopholipid), α Tocopheral acetate (Vitamin E), Celin (L-ascorbic acid) (Vitamin C), Spirulina powder and L-tryptophan (amino acid) by weight as given in the formulation table for Supplementary diet mix.

Table 2: Formulation of Experimental diets

Ingredients	Composition (%)		
	T1 (Feed A)	T2 (Feed B)	T3 (Feed C)
Fish meal	25	25	25
Soybean meal	22	22	22
GNOC	15	15	15
De-oiled Rice bran	20	18	16
Wheat flour	10	10	10
Starch powder	3	3	3
Soya oil	2	2	2
Vitamin and mineral*	1	1	1
Carboxy methyl cellulose	2	2	2
Supplementary diet mix	0	2	4
Total	100	100	100

*Composition of vitamin-mineral mixture (Minamil) (quantity/kg): Vitamin A, 20,00,000 IU; Vitamin D3, 4,00,000 IU;; Vitamin E, 320 mg; Vitamin B2, 1.2 g; Vitamin B6, 0.4 g; Vitamin B12, 4 mg; Calcium Pantothenate, 1.2 g; Nicotinamide, 8 g; Choline Chloride, 60 g; Vitamin K3, 0.40 g; Calcium, 320 g; Phosphorus, 20 g; Manganese, 12 g; Iodine, 0.40 g; Iron, 3.2 g; copper, 1 g; Cobalt, 0.2 g; Selenium,10 mg; Zinc, 8 g.

Table 3: Formulation for Supplementary diet mix

INGREDIENTS	Composition (%)
Refined lecithin	40
α Tocopheral acetate	1.5
Celin (L-ascorbic acid)	2.5
Spirulina powder	50
L-tryptophan	6
Total	100

3.6.5 Preparation of experimental diets

As per formula all the ingredients were grinded, weighed and mixed well (except oils and additives) and dough was formed with the addition of distilled water. The dough was steam cooked in pressure cooker for 20-30 minutes followed by cooling the dough. After cooling, vitamin-mineral premix, feed supplement/additive mixture and carboxymethyl cellulose (CMC) were mixed with the soy oil and then that is mixed thoroughly in the dough. Pellets of 1.5 mm diameter were prepared by hand pelletizer and dried at room temperature under air of ceiling fan for some time and kept in hot air oven at 60^{o}C till complete drying (<12% moisture). After drying and cooling the pellets were packed in airtight polythene zipper bags, labelled properly and stored at 4^{o}C until further use. During feeding the pellets strands were broken into smaller size.

3.6.6 Experimental feeding

Feeding of fish fingerlings for each group of T1, T2, T3 and T4 was done with respective diets on satiation basis twice daily at early morning (6 am) and evening (6 pm) throughout the feeding trial of 90 days. Fishes of T4 were fed with only *Tubifex* worm.

3.6.7 Proximate analysis of formulated diets and live food

Proximate analysis of formulated feed and live feed were performed as per the standard method of AOAC (1995) on dry matter basis.

3.6.7.1 Moisture

The moisture content of the experimental diets and live food (tubifex worm) was determined by taking a known weight of the sample in the Petri dish and drying it in a hot air oven at 70-80 ^{0}C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula

$$\textbf{Moisture}\ (\%) = \frac{\textbf{Wet weight of sample} - \textbf{Dried Weight of sample}}{\textbf{Wet weight of sample}} \times \mathbf{100}$$

3.6.7.2 Crude protein (CP)

Nitrogen content of the dried experimental diets and live food (*tubifex* worm) was estimated by semi-automated nitrogen analyzer (Pellican, Kelplus-KES06L, Chennai, India) followed by titration. The nitrogen content was calculated by the following formula.

$$\textbf{Nitrogen}\ (\%) = \frac{\mathbf{V\ X\ 0.0014\ X\ D}}{\mathbf{A\ X\ W}} \times \mathbf{100}$$

Where, V = Volume of N/10 H_2SO_4 used during titration; 0.0014 factor is used because 1 ml of N/10 H_2SO_4 contains 0.0014 g N; D = Dilution Factor (i.e. total volume made); A = Aliquot (diluted sample) taken for distillation; W = Weight of the sample taken for digestion.

The crude protein percentage was obtained by multiplying the Nitrogen percentage with a factor of 6.25 as follows.

$$\textbf{Crude protein}\ (\%) = \mathbf{N2}\ (\%)\ \mathbf{X\ 6.25}$$

3.6.7.3 Ether extract (EE) or crude lipid

Ether extract of dried experimental diets, carcass tissue and live food (*tubifex* worm) was estimated by lipid extraction system (Socsplus-SCS06R, Pellican, Chennai, India) using petroleum ether (Boiling point 40-60 ^{0}C) as the solvent. The calculation was made as follows.

$$\textbf{Ether extract (\%)} = \frac{\textbf{(Weight of extraction beaker + lipid) − Weight of empty beaker}}{\textbf{Weight of the sample}} \times \textbf{100}$$

3.6.7.4 Total ash (TA)

Ash content of the dried experimental diets, carcass tissue and live food (*tubifex* worm) was estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 550 ^{0}C for 2 hours after reaching the temperature. The calculation was done as follows:

$$\textbf{Total Ash (\%)} = \frac{\textbf{Weight of crucible + ash − Weight of empty crucible}}{\textbf{Weight of the sample}} \times \textbf{100}$$

3.6.7.5 Total carbohydrate (TC)

The total carbohydrate (TC) of the dried carcass tissue and live food was calculated by subtracting the total percentage of other nutrients from 100 (Hasting,1969).

$$\textbf{TC (\%)} = \textbf{100} - (\textbf{CP\%} + \textbf{EE\%} + \textbf{TA\%})$$

3.6.7.6 Gross energy (GE)

Gross energy of the experimental diets was calculated as per standard physiological fuel values according to the following formula.

$$\mathbf{GE\ (kcal/100g) = Protein(\%)x5.4 + Lipid(\%)x9.2 + Carbohydrate\ (\%)x4.4}$$

3.6.8 Growth performance

Sampling for growth was done at after 90 days to assess the body weight and length of the fishes. Fishes were starved overnight before taking the length and weight. The weight was taken in an electronic balance. The growth performance was assessed using the following formulae.

3.6.8.1 Percent weight gain

The percentage weight gain was calculated using the following formula.

$$\mathbf{Weight\ gain\ (\%) = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100}$$

3.6.8.2 Specific growth rate (SGR)

The specific growth rate was calculated by the following formula.

$$\mathbf{SGR\ \% = \frac{Log_e\ Final\ weight - Log_e\ Initial\ weight}{Number\ of\ days} \times 100}$$

3.6.8.3 Length gain

The length gain was calculated by the following formula.

$$\mathbf{Length\ gain\ (cm) = Final\ length - Initial\ length}$$

3.6.8.4 Survival rate

At the end of the experiment, all the experimental tanks were dewatered and the number of the experimental fish in each tank was counted and the survival rate (%) was calculated by the following formula.

$$\text{Survival}(\%) = \frac{\textbf{Total number of harvested fish}}{\textbf{Total number of stocked fish}} \times 100$$

3.7 Evaluation of effect of nutritional intervention on reproductive performance of dwarf gourami, C*olisa lalia*.

3.7.1 Site of the experiment

The experiment was conducted at the wet laboratory of old campus of ICAR-Central Institute of Fisheries Education (CIFE), Mumbai. The duration of the experiment was from August 2018 to October 2018. The experiment was carried out for 60 days, later breeding trials were conducted for one month. Subsequently, the laboratory work was carried out in the laboratories of ICAR-CIFE, Mumbai.

3.7.2 Procurement and acclimatization of experimental fish

Fish used for the experiment were advanced fingerlings of *Colisa lalia*(Hamilton, 1822) with an average weight of 1.13 g, ranging from 0.72 – 1.62 g and average length of 4.03 cm, ranging from 3.4 – 4.6 cm.The fish were procured from fishermen of Tribeni, Hooghly, West Bengal, who collected the fishes from the river. The fishes were then packed in polythene bags with oxygen packing and then again packed in cardboard boxes. These fish were transferred to Mumbai by air through cargo. The fish were carefully transferred to well aerated rectangular tanks of 2000 L capacity after a salt dip treatment. The stock was acclimatized under aerated conditions in the same tanks and maintained in captive conditions.

3.7.3 Experimental design, set-up & fish maintenance

Two forty (240) advanced fingerlings of *Colisa lalia* were randomly distributed in four distinct experimental groups, keeping males and females separate for each treatment, such as T1, T2, T3 and T4, with triplicate in each following a completely randomized design (CRD). The experiment was conducted for a period of 90 days in the wet laboratory of old campus of ICAR-CIFE, Mumbai. The setup consisted of 24 glass aquariums of 27 L capacity (20 X 45 X 30 cm3) capacity, during the experimental period. The aquariums were initially washed and filled with potassium permanganate solution (4 ppm) and were left overnight. The aquariums were flushed out on the very next day and were thoroughly washed with clean water. Two forty fishes were randomly distributed in 4x2 (male and female) distinct experimental groups with triplicate each. Based on design each tank containing bore well water was stocked with 10 fish with initial body weight ranging from 1.2 – 1.4 g. The total volume of the water in each tank was maintained at 25 Lthroughout the experimental period. Round the clock aeration was provided in each tank. The aeration pipe in each tub was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tanks. The experimental conditions were kept same throughout the trial period. The experimental aquariums were cleaned manually and siphoning was done in order to remove the fecal matter at every day morning. Everyday 20% water was exchanged. This was carried out throughout the experimental period of 60 days.

3.7.4 Formulation and preparation of experimental diets

The formulation of the feed, which gave best result in the previous growth experiment and the formulation for control diet, was taken into consideration and prepared in the nutrition division of ICAR-CIFE, Mumbai. Therefore the formulation for Feed A and Feed C as given in the Table 2, was used for the preparation of the Control diet and the targeted experimental diet. As per formula all the ingredients were ground, weighed and mixed well (except oils and additives) and dough was formed with the addition of distilled water. The dough was steam cooked in pressure cooker for 20-30 minutes followed by cooling the dough. After cooling of the dough vitamin-mineral premix, feed supplement/additive mixture and carboxymethyl cellulose (CMC) were mixed with the soy oil and then that is mixed thoroughly in the dough. Pellets of 1.5 mm diameter were prepared by pelletizer machine and dried at room temperature under air of ceiling fan for some time and kept in hot air oven at 60^oC till complete drying (<12% moisture). After drying and cooling the pellets were packed in airtight polythene zipper bags, labelled properly and stored at 4^oC until further use. During feeding the pellets strands were broken into smaller size.

Table 4: Experimental Design for Reproductive Performance

T1	**Male (T1R1,T1R2,T1R3)**	**Control feed**
	Female (T1R1,T1R2,T1R3)	
T2	**Male (T2R1,T2R2,T2R3)**	**Feed C**
	Female (T2R1,T2R2,T2R3)	
T3	**Male (T3R1,T3R2,T3R3)**	**Live feed (*Tubifex*)**
	Female (T3R1,T3R2,T3R3)	
T4	**Male (T4R1,T4R2,T4R3)**	**Combination (Feed C + *Tubifex*)**
	Female (T4R1,T4R2,T4R3)	

Plate 7: Experimental site at ICAR – CIFE, Mumbai

Plate 8: Preparation of experimental diets of dwarf gourami, *Colisa lalia*

Plate 9: *Tubifex* for feeding purpose of dwarf gourami, *Colisa lalia*

3.7.5 Experimental feeding

Feeding of fish fingerlings for each group of T1, T2, T3 and T4 was done with respective diets on satiation basis twice daily at morning (9 am) and evening (6 pm) throughout the feeding trial of 90 days. Fishes of T4 were fed with *Tubifex* worm at the evening and formulated feed in the morning.

3.7.6 Reproductive performance

3.7.6.1 Gonadosomatic index

The gonadosomatic index, abbreviated as GSI, is the calculation of the gonad mass as a proportion of the total body mass. It is represented by the formula

$$\mathbf{GSI} = \frac{\mathbf{Gonad\ weight}}{\mathbf{Total\ body\ weight}} \times \mathbf{100}$$

3.7.6.2 Spawning Success (%)

The spawning success was calculated after the breeding trials of every treatment. Spawning Success (%) was calculated by using the formula given

$$\mathbf{Spawning\ Success\ \%} = \frac{\mathbf{No.\ Spawning\ success}}{\mathbf{No.\ of\ Spawning\ Trials}} \times \mathbf{100}$$

3.7.6.3 Fertilization (%)

The fertilization rate was calculated after the eggs are fertilized, few hours after the spawning activity. The fertilized egg is marked with a clear and transparent colour, whereas the unfertilized egg is characterized by its feculent white colour. The Fertilization Rate was calculated by using the formula given

$$\mathbf{Fertilization\ \%} = \frac{\mathbf{No.\ of\ eggs\ fertilized}}{\mathbf{No.\ of\ eggs\ in\ the\ batch}} \times \mathbf{100}$$

3.7.6.4 Hatching (%)

Hatching % can be calculated by using the formula given

$$\text{Hatching \%} = \frac{\text{No. of eggs hatched}}{\text{No. of eggs in the batch}} \times 100$$

3.8 Analysis of larval rearing of dwarf gourami, *Colisa lalia*

3.8.1 Site of the Experiment

The experiments were conducted at the wet laboratory of ICAR-Central Institute of Fisheries Education (CIFE), Mumbai.

3.8.2 Experimental design, set-up & maintenance

Newly hatched larvae of *Colisa lalia* were utilized for the present study. The experiment was conducted for 30 days. It was a completely randomized design. The larvae were reared in three types of experimental diet; Live feed (Infusoria), formulated diet (Feed C), Boiled egg yolk. The experiment was conducted in triplicates, with stocking density of 100 larvae per 10 L water. Feeding was done after 3 days of hatching. Gentle aeration was supplied in the experimental tanks. Daily water exchange rate of 20% was carried out during the experimental period. During larval rearing, dead larvae and waste was siphoned off every day to avoid any means of stress.

Table 5: Experimental Design for Larval Rearing

T1 (T1R1,T1R2,T1R3)	**Live feed (Infusoria)**
T2 (T2R1,T2R2,T2R3)	**Formulated feed (Feed C)**
T3 (T3R1,T3R2,T3R3)	**Boiled egg yolk**

3.8.3 Formulation and preparation of experimental diets

3.8.3.1 Infusoria

Infusoria was cultured using banana pealings and filtered water in 27 liters aquarium, covered with cloth from above to prevent flies and mosquitoes but allow passage of air. To get a regular supply of infusoria, regular harvesting was done and drops of milk were added regularly.

3.8.3.2 Formulated feed

The formulation of the feed, which gave best result in the previous growth experiment was taken into consideration and prepared in the nutrition division of ICAR-CIFE, Mumbai. Therefore the formulation for Feed C (4% supplementary mix) as given in the Table 2, was used for the preparation of the targeted experimental diet. As per formula all the ingredients were ground, weighed and mixed well (except oils and additives) and dough was formed with the addition of distilled water. The dough was steam cooked in pressure cooker for 20-30 minutes followed by cooling the dough. After cooling of the dough vitamin-mineral premix, feed supplement/additive mixture and carboxymethyl cellulose (CMC) were mixed with the soy oil and then that is mixed thoroughly in the dough. Pellets of 1.5 mm diameter were prepared by pelletizer machine and dried at room temperature under air of ceiling fan for some time and kept in hot air oven at $60^{o}C$ till complete drying (<12% moisture). After drying and cooling the pellets were packed in airtight polythene zipper bags, labeled properly and stored at $4^{o}C$ until further use. The pellets strands were grounded to powdered form for feeding purpose.

3.8.3.3 Boiled egg yolk

Egg was boiled, and then the yolk was used to make the yolk emulsion with water, which was used for feeding purpose.

3.9 Water Quality Monitoring

Water quality monitoring was done for both the experimental sites. Few selected parameters of water such as water temperature, pH, dissolved oxygen (DO), total alkalinity (TA), and hardness were measured following the standard methods (APHA, 1998). Water quality parameters were analyzed for three months, while rearing at ICAR-CIFE, Kolkata. Water quality parameters were analyzed for the duration of one year, while rearing and spawning at ICAR-CIFE, Mumbai.

3.9.1 Temperature

Water temperature was measured on spot using a mercury thermometer having 0.5 ^{0}C accuracy. Temperature was recorded in the noon hours at around 1.00 p.m.

3.9.2 pH

The pH of water samples was measured using universal pH indicator to monitor the pH of test water.

3.9.3 Dissolved oxygen

For estimation of dissolved oxygen content of water, the samples were collected with all necessary precautions. Winkler's method was followed for estimation of dissolved oxygen (APHA, 1998). The value was expressed in ppm.

3.9.4 Alkalinity

It was estimated by Titration method (APHA, 1998). Fifty ml of sample was taken in a conical flask, to it 2-3 drops of phenolphthalein indicator was added. When the sample becamepink, it was titrated with 0.02 N sulphuric acid until the pink colour just disappeared. Thequantity of acid used was recorded. Then 2-3 drops of methyl orange indicator was added and titrated against 0.02 N sulphuric acid. The end point was orange to wine red. The volume of sulphuric acid used was recorded and alkalinity was determined. The value was expressed in ppm.

3.9.5 Hardness

Hardness is the measure of total concentration of divalent metallic cations like calcium, magnesium, ferrous and strontium and is expressed in mg/l. It was estimated by Titrationmethod (APHA, 1998). For testing hardness, 50 ml of the sample water was taken in a conical flask. To it 1.5 ml ammonia buffer and 3 drops of Erichrome Black-T indicator was added. Then it was titrated against standard EDTA solution till the sample water colour changed from winered to blue. Volume of the EDTA solution used was recorded, and hardness was determined. The value was expressed in ppm.

3.10 Statistical Analysis

MS Excel program was used for finding Correlation and Regression. One way ANOVA was used to compare the means, followed by DUNCANS Multiple range test to determine the significant difference ($p<0.05$) between the means by using SPSS (Version 22).

4. RESULTS

4.1 General Biology

4.1.1 Morphometric studies

Ten morphometric characters were measured, having Mean value for Total length (TL) = 4.06, Standard Length (SL) = 3.25, Predorsal Length (PDL) = 1.42, Preanal length (PAL) = 1.57, Prepectoral length (PPL) = 1.15, Prepelvic / ventral length (PVL) = 1.17, Head length (HL) = 1.12, Body Depth (BD) = 1.51, Eye Diameter (ED) = 0.30 and Snout Length (SnL) = 0.28. Table 6 given below gives information about standard deviation (SD), Maximum (Max) limit, Minimum (Min) limit and their range differences.

Correlation Coefficient, Regression Equation and R^2 Value were found out of nine morphometric characters in relation with Total Length (TL), shown in Table 7. Highest correlation coefficient was observed in case of Standard length (SL) where R^2 value was 0.8642 and lowest in case of Eye Diameter (ED) where R^2 value was 0.0333. Figure 1, 2, 3, 4, 5, 6, 7, 8 and 9 shows graphical representation of the relationship of Standard Length (SL), Predorsal Length (PDL), Preanal length (PAL), Prepectoral length (PPL), Prepelvic / ventral length (PVL), Head length (HL), Body Depth (BD), Eye Diameter (ED) and Snout Length (SnL) in relation to total length of *Colisa lalia*.

Correlation Coefficient, Regression Equation and R^2 Value were also found out of Eye Diameter (ED) and Snout Length (SnL) in relation with Head Length (HL) and R^2 was found out to be $R^2 = 0.0375$ and $R^2 = 0.0539$ respectively (Table8). Figure 10 and 11 shows graphical representation of the relationship of two morphometric characters Eye Diameter (ED) and Snout Length (SnL) in relation to head length of *Colisa lalia*.

Table 6: Morphometrics of dwarf gourami, *Colisa lalia*

Morphometric Characters	Mean (cm)	SD	Min (cm)	Max (cm)	Range Difference
Total length (TL)	4.06	0.21	3.5	4.7	1.2
Standard Length (SL)	3.25	0.18	2.8	3.7	0.9
Predorsal Length (PDL)	1.42	0.11	1.2	1.7	0.5
Preanal length (PAL)	1.57	0.11	1.3	1.8	0.5
Prepectoral length (PPL)	1.15	0.07	1.0	1.3	0.3
Prepelvic / ventral length (PVL)	1.17	0.07	1.0	1.4	0.4
Head length (HL)	1.12	0.07	1.0	1.3	0.3
Body Depth (BD)	1.51	0.10	1.3	1.8	0.5
Eye Diameter (ED)	0.30	0.02	0.3	0.4	0.1
Snout Length (SnL)	0.28	0.04	0.2	0.3	0.1

N = 100, SD: Standard Deviation

Table 7: Correlation of Morphometric Characters in comparison with total length of dwarf gourami, *Colisa lalia*

In Comparison with Total Length (TL)	Correlation Coefficient	R^2 Value	Regression Equation
Standard Length (SL)	0.93	$R^2 = 0.8642$	$y = 0.8108x + 0.0403$
Predorsal Length (PDL)	0.47	$R^2 = 0.2213$	$y = 0.2412x + 0.4413$
Preanal length (PAL)	0.63	$R^2 = 0.3919$	$y = 0.3285x + 0.2352$
Prepectoral length (PPL)	0.55	$R^2 = 0.2999$	$y = 0.1919x + 0.3705$
Prepelvic / ventral length (PVL)	0.45	$R^2 = 0.2055$	$y = 0.1602x + 0.5196$
Head length (HL)	0.53	$R^2 = 0.2769$	$y = 0.1699x + 0.4284$
Body Depth (BD)	0.59	$R^2 = 0.3438$	$y = 0.2783x + 0.3788$
Eye Diameter (ED)	0.18	$R^2 = 0.0333$	$y = 0.0191x + 0.2274$
Snout Length (SnL)	0.31	$R^2 = 0.0953$	$y = 0.0621x + 0.0261$

Table 8: Correlation of Eye Diameter and Snout Length in comparison with head length of dwarf gourami, *Colisa lalia*

In Comparison with Head Length (HL)	Correlation Coefficient	R^2 Value	Regression Equation
Eye Diameter (ED)	0.19	$R^2 = 0.0375$	$y = 0.0627x + 0.2347$
Snout Length (SnL)	0.23	$R^2 = 0.0539$	$y = 0.1448x + 0.1165$

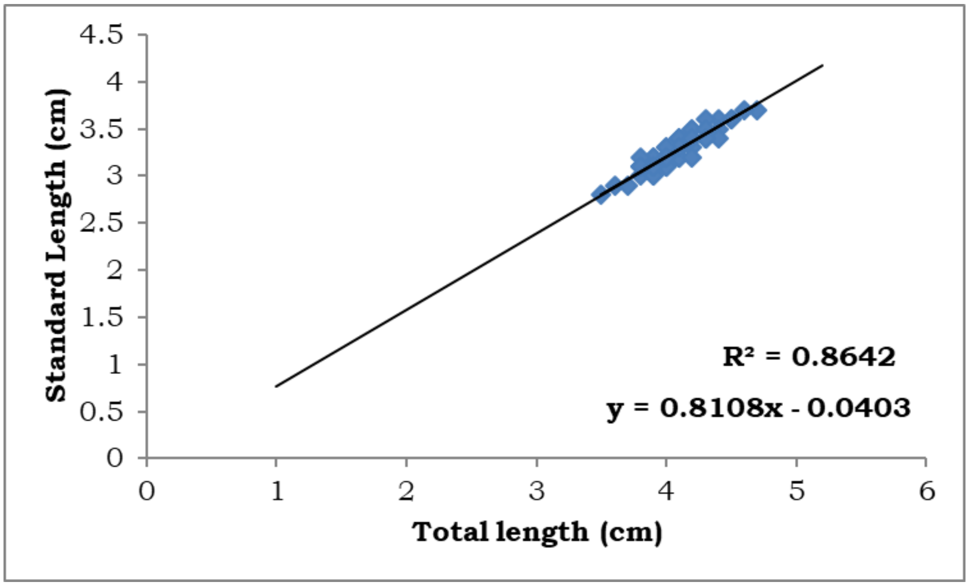

Fig 1: Standard Length in relation to Total length of dwarf gourami, *Colisa lalia*

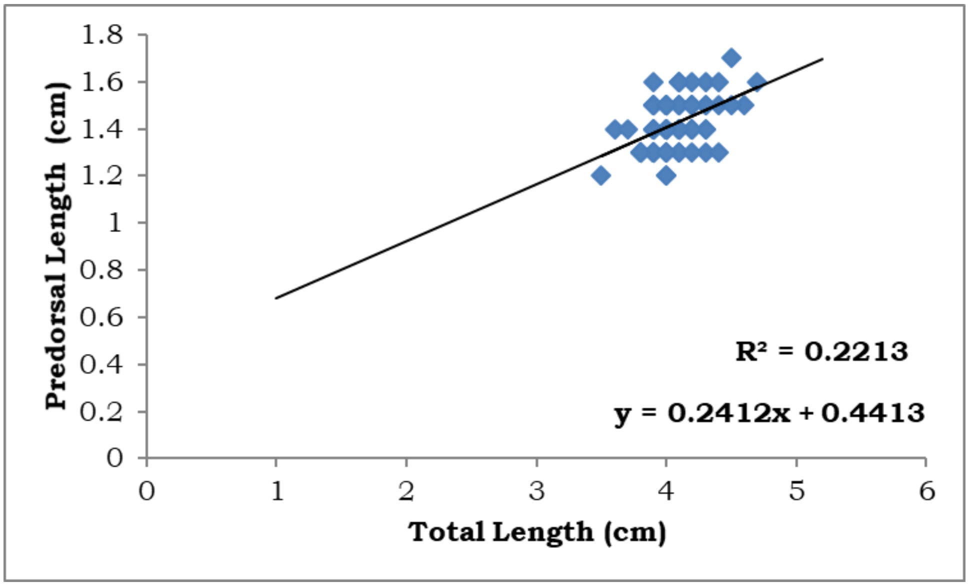

Fig 2: Predorsal Length in relation to Total length of dwarf gourami, *Colisa lalia*

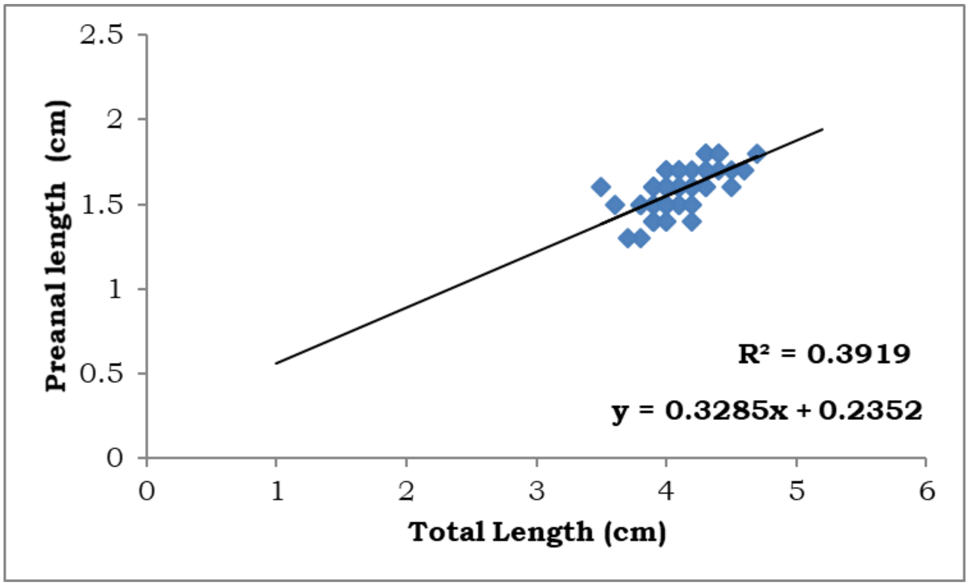

Fig 3: Preanal Length in relation to Total length of dwarf gourami, *Colisa lalia*

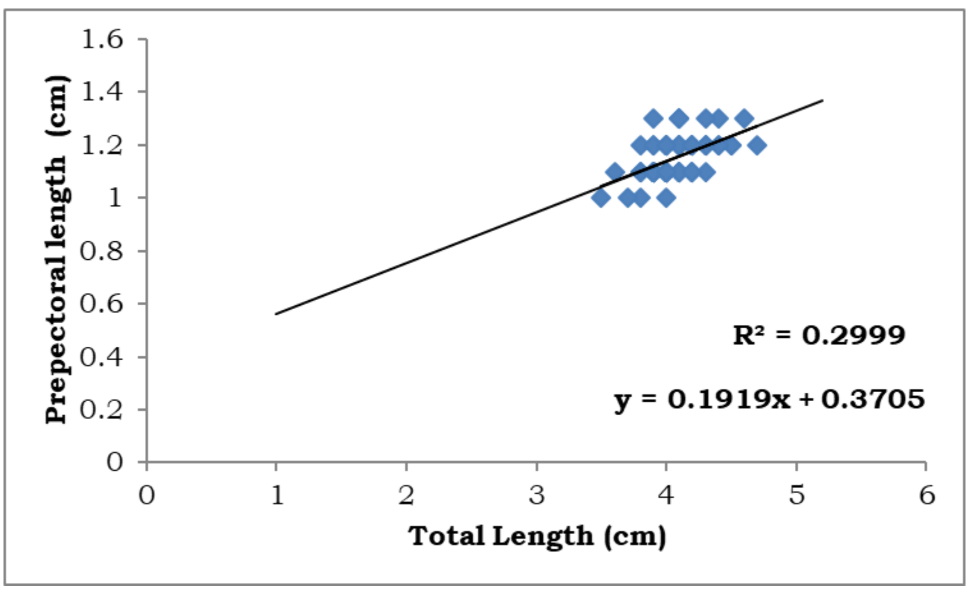

Fig 4: Prepectoral Length in relation to Total length of dwarf gourami, *Colisa lalia*

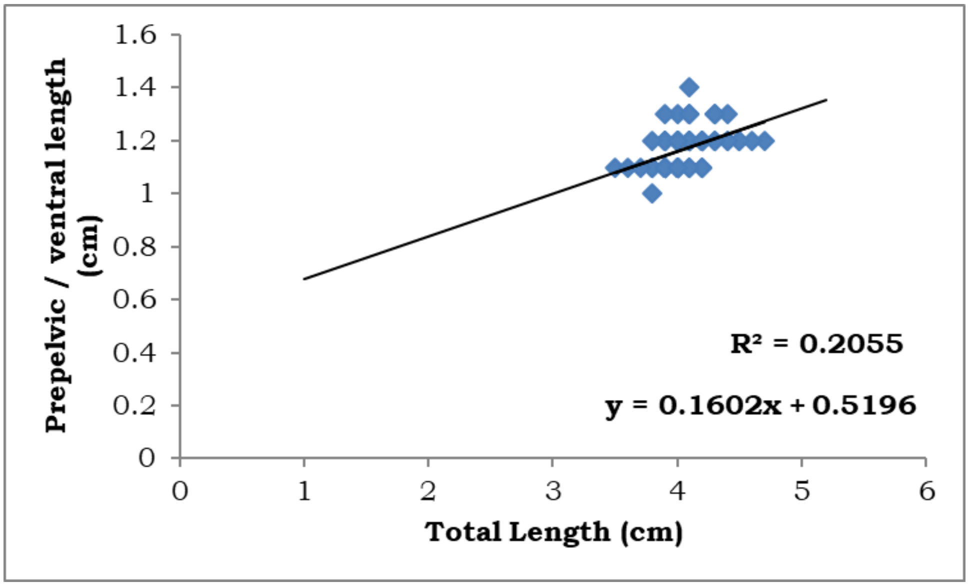

Fig 5: Prepelvic / Ventral Length in relation to Total length of dwarf gourami, *Colisa lalia*

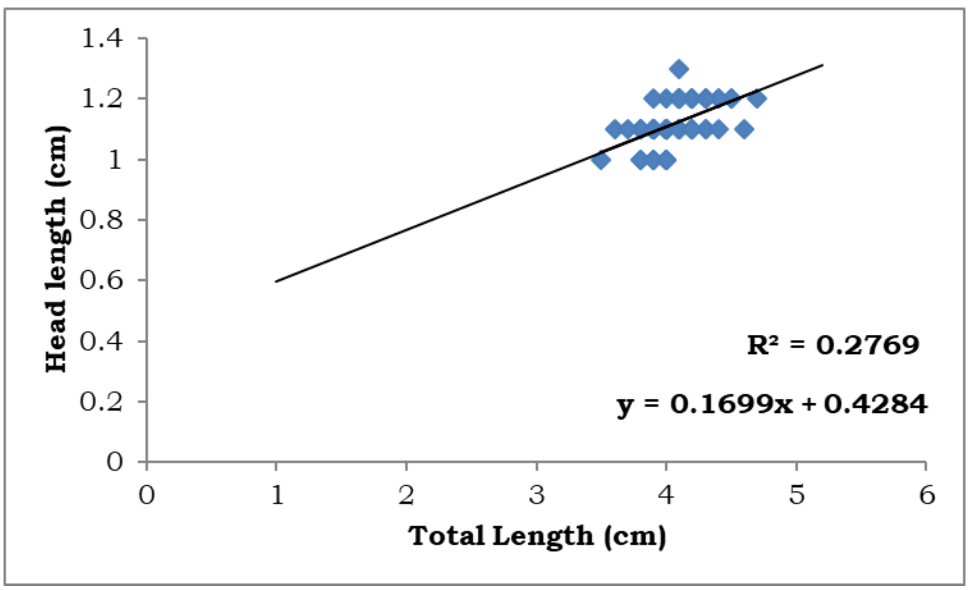

Fig 6: Head Length in relation to Total length of dwarf gourami, *Colisa lalia*

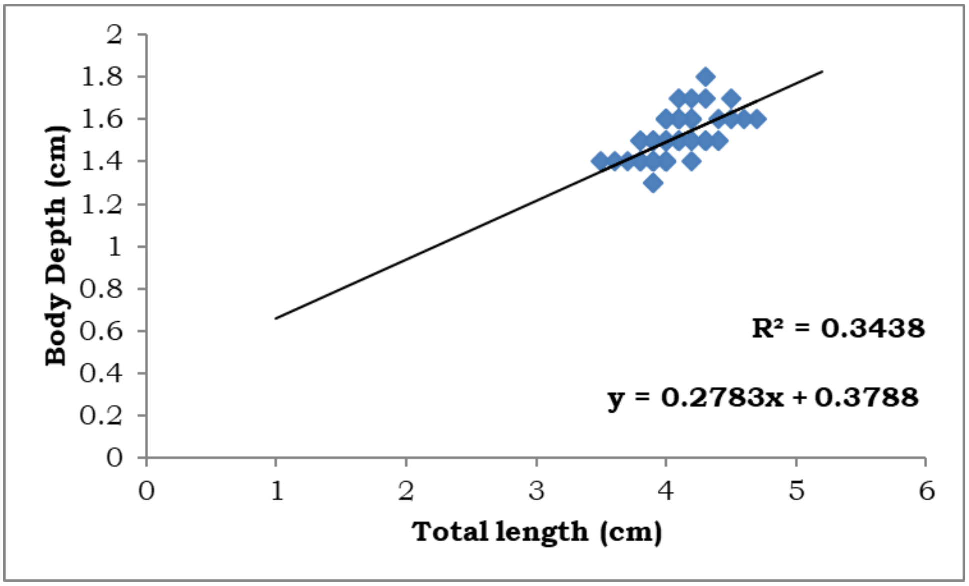

Fig 7: Body Depth in relation to Total length of dwarf gourami, *Colisa lalia*

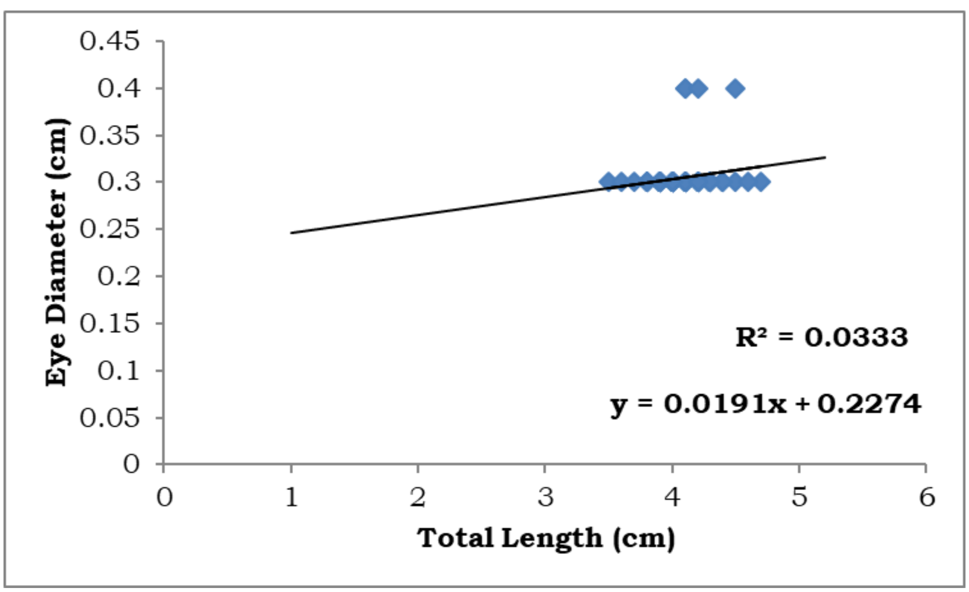

Fig 8: Eye Diameter in relation to Total length of dwarf gourami, *Colisa lalia*

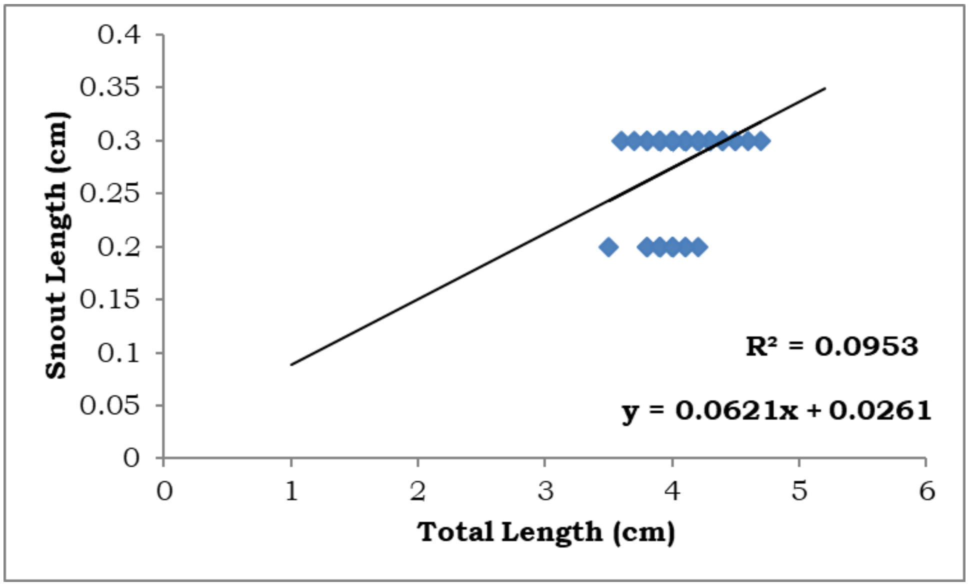

Fig 9: Snout Length in relation to Total length of dwarf gourami, *Colisa lalia*

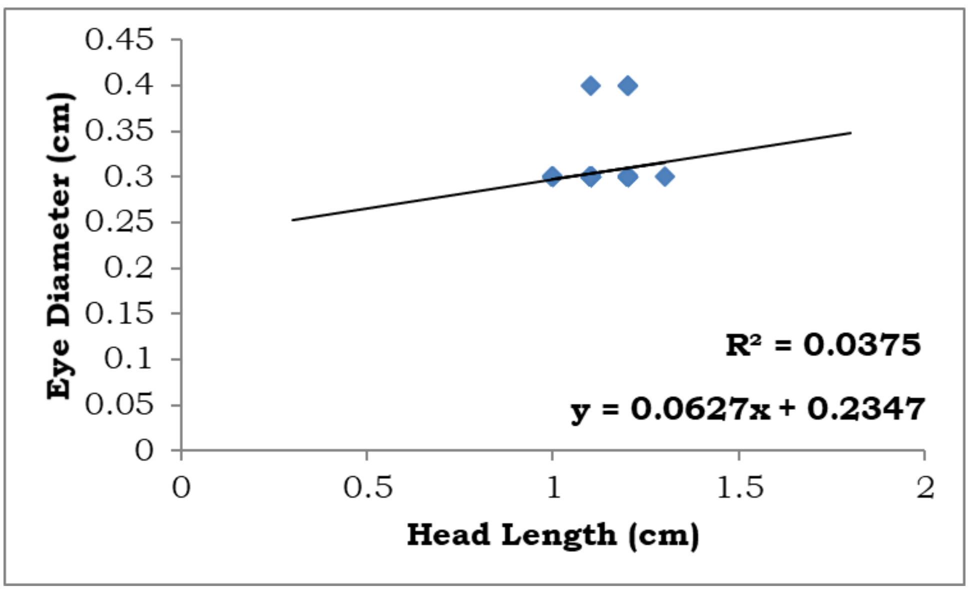

Fig 10: Eye Diameter in relation to Head length of dwarf gourami, *Colisa lalia*

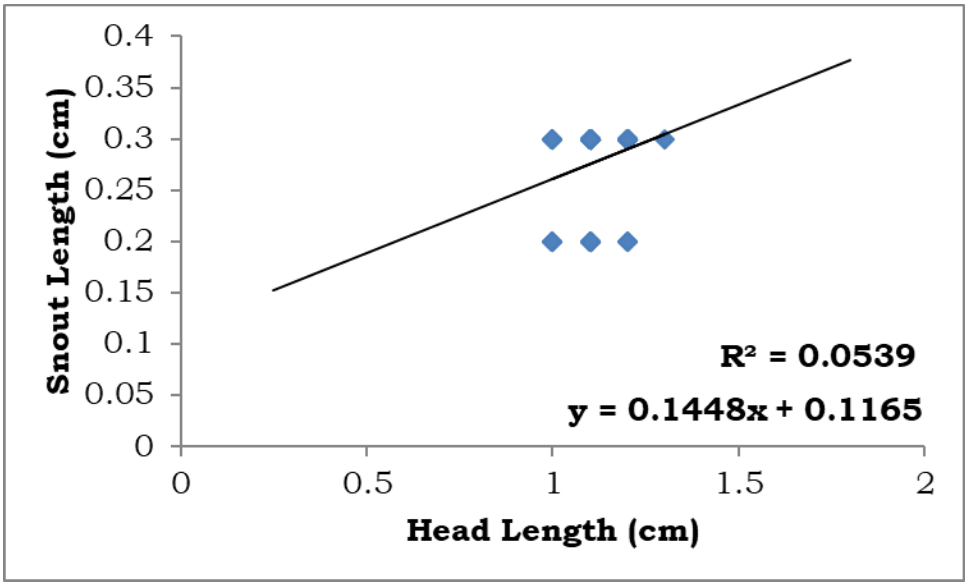

Fig 11: Snout Length in relation to Head length of dwarf gourami, *Colisa lalia*

4.1.2 Meristic studies

Variations in the meristic characters of *Colisa lalia* are given in Table 9. This table gives information regarding the range and mode of Dorsal Spines (DS), Dorsal Soft Rays (DSR), Anal spines (AS), Anal Soft Rays (ASR), Pelvic Fin (V), Pectoral Fin (PcF), Caudal Fin. Based on the observation, we can get the fin formula of this fish D. XV/8; P.8; A. XVII/15; V. 2; C. 14

Table 9: Variations of Meristic Characters of dwarf gourami, *Colisa lalia*

Meristic Characters	Range	Mode
Dorsal Spines (DS)	15 – 17	15
Dorsal Soft Rays (DSR)	7 – 9	8
Anal spines (AS)	16 – 19	17
Anal Soft Rays (ASR)	14 -17	15
Pelvic Fin (PF)	2 – 2	2
Pectoral Fin (PcF)	8 – 10	8
Caudal Fin	14 – 16	14

4.2 Feeding Biology

4.2.1 Relative gut length

The Relative Gut Length of dwarf gourami, *Colisa lalia* was found out to be 2.29 ± 0.42 (standard deviation) with a range of 1.43 – 2.9 (Table 10). The fish sample taken for study had mean total length of 4.28 ± 0.26 cm having a range between 3.8 – 4.9 cm. The mean gut length of the fishes was 9.78 ± 1.92 cm, having a range of 6 – 12.5 cm.

Correlation Coefficient, R^2 Value and Regression Equation were also taken out of Gut Length and Relative Gut Length in comparison with Total Length (TL). Gut Length showed 0.341317 correlation coefficient and R^2 value was 0.1165 in comparison to TL. Relative Gut Length showed 0.034974 correlation coefficient and R^2 value was 0.0012 in comparison to TL (Table 11)

Table 10: Relative Gut Length of dwarf gourami, *Colisa lalia*

n=33	Total length (cm)	Gut length (cm)	Relative Gut Length
Average	4.28 ± 0.26	9.78 ± 1.92	2.29 ± 0.42
Range	3.8 – 4.9	6 – 12.5	1.43 – 2.91

Table 11: Correlation between Gut Length and RGL with Total Length of dwarf gourami, *Colisa lalia*

In Comparison with Total Length (TL)	Correlation Coefficient	R^2 Value	Regression Equation
Gut Length	0.341317	$R^2 = 0.1165$	$y = 2.5238x - 1.0143$
Relative Gut Length	0.034974	$R^2 = 0.0012$	$y = 0.0567x + 2.0432$

4.2.2 Gastro-somatic index

Gastro-somatic index (GaSI) of dwarf gourami, *Colisa lalia* was found out for 12 months from July, 2018 to June 2019. The GaSI of the male dwarf gourami found for the month of July 2018, August 2018, September 2018, October 2018, November 2018, December 2018, January 2019, February 2019, March 2019, April 2019, May 2019 and June 2019 are 1.98 ± 0.06, 2.17 ± 0.07, 2.42 ± 0.04, 2.62 ± 0.07, 2.88 ± 0.14, 3.11 ± 0.11, 2.80 ± 0.10, 2.60 ± 0.07, 2.53 ± 0.07, 2.37 ± 0.07, 2.02 ± 0.05 and 1.88 ± 0.03 respectively. There was significant difference ($p<0.05$) observed between the GaSI of male dwarf gourami in all the twelve months. Highest value of GaSI in male was observed in the month of December 2018 having the value of 3.11 ± 0.1. Lowest value of GaSI was observed in the month of June 2019, having the value of 1.88 ±0.03. Significant difference ($p<0.05$) in both length and weight was observed in the male dwarf gourami in all the twelve months.

The GaSI of the female dwarf gourami found for the month of July 2018, August 2018, September 2018, October 2018, November 2018, December 2018, January 2019, February 2019, March 2019, April 2019, May 2019 and June 2019 are 1.28 ± 0.12, 2.90 ± 0.05, 3.68 ± 0.07, 4.07± 0.06, 4.46 ± 0.08, 4.75 ± 0.13, 4.20 ± 0.20, 3.93 ± 0.15, 3.58 ± 0.04, 3.43 ± 0.17, 2.26 ±0.05b and 1.20 ±0.07 respectively. There was significant difference ($p<0.05$) observed between the GaSI of female dwarf gourami in all the twelve months. Highest value of GaSI in female was observed in the month of December 2018 having the value of 4.75 ± 0.13. Lowest value of GaSI was observed in the month of June 2019, having the value of 1.20 ± 0.07. Significant difference ($p<0.05$) in weight was observed, but no significant difference ($p>0.05$) is observed in the length of female gourami in all the twelve months.

Table 12 and 13 indicates the monthly variations of Gastro-somatic Index in both male and female dwarf gourami, *Colisa lalia,* along with the length, weight and range of GaSI for individual month. Values are presented as Mean ± SE. The superscript letter indicates significant difference, $P<0.05$. Fig. 12 and 13 shows the graphical presentation of monthly variations of Gastro-somatic Index in male and female dwarf gourami, *Colisa lalia* respectively.

Table 12: Monthly variations of Gastro-somatic Index in male dwarf gourami, *Colisa lalia*

Month	Length (cm)	Weight (g)	GaSI	Range of GaSI
July 2018	4.44 ±0.07^{ab}	1.56±0.01^{ab}	1.98 ±0.06^{ab}	1.79 - 2.10
August 2018	4.52±0.06^{c}	1.66±0.05^{d}	2.17 ±0.07^{bc}	1.98 - 2.38
September 2018	4.52±0.04^{c}	1.66±0.02^{d}	2.42 ±0.04^{d}	2.34 - 2.55
October 2018	4.38±0.07^{ab}	1.70±0.01^{d}	2.62 ±0.07^{de}	2.40 - 2.79
November 2018	4.26±0.10^{a}	1.64±0.02^{cd}	2.88 ±0.14^{fg}	2.43 - 3.29
December 2018	4.4±0.10^{ab}	1.53±0.03^{a}	3.11 ±0.11^{g}	2.83 - 3.38
January 2019	4.3±0.05^{a}	1.53±0.01^{a}	2.80 ±0.10^{ef}	2.65 - 3.20
February 2019	4.54±0.02^{c}	1.58±0.00^{abc}	2.60 ±0.07^{fe}	2.45 - 2.87
March 2019	4.42±0.08^{ab}	1.69±0.02^{d}	2.53 ±0.07^{d}	2.31 - 2.68
April 2019	4.52±0.04^{c}	1.68±0.03^{d}	2.37 ± 0.07^{cd}	2.11 - 2.50
May 2019	4.56±0.04^{c}	1.63±0.01^{bcd}	2.02 ±0.05^{ab}	1.88 - 2.16
June 2019	4.54±0.05^{c}	1.57±0.01^{abc}	1.88 ±0.03^{a}	1.79 - 1.96

Values are presented as Mean ± Standard Error. The superscript letter indicates significant difference, $P<0.05$.

Table 13: Monthly variations of Gastro-somatic Index in female dwarf gourami, *Colisa lalia*

Month	Length (cm)	Weight (g)	GaSI	Range of GaSI
July 2018	4.38 ±0.08^{c}	1.45±0.07^{bcd}	1.28 ±0.12^{a}	0.82 - 1.53
August 2018	4.06 ±0.12^{a}	1.45±0.01^{bcd}	2.90 ±0.05^{c}	2.74 - 3.01
September 2018	4.16 ±0.09^{abc}	1.46±0.00^{bcd}	3.68 ±0.07^{de}	3.51 - 3.90
October 2018	4.10 ±0.10^{ab}	1.33±0.03^{a}	4.07±0.06^{f}	3.90 - 4.23
November 2018	4.18 ±0.04^{abc}	1.35±0.02^{ab}	4.46 ±0.08^{gh}	4.23 - 4.66
December 2018	4.24 ±0.05^{abc}	1.39±0.00^{abc}	4.75 ±0.13^{h}	4.46 - 5.07
January 2019	4.22 ±0.09^{abc}	1.37±0.03^{abc}	4.20 ±0.20^{fg}	3.67 - 4.84
February 2019	4.16 ±0.09^{abc}	1.41±0.03^{abcd}	3.93 ±0.15^{ef}	3.54 - 4.34
March 2019	4.10 ±0.08^{ab}	1.45±0.08^{bcd}	3.58 ±0.04^{d}	3.49 - 3.66
April 2019	4.10 ±0.07^{ab}	1.46±0.01^{bcd}	3.43 ±0.17^{d}	2.75 - 3.74
May 2019	4.22 ±0.04^{abc}	1.49±0.02^{cd}	2.26 ±0.05^{b}	2.12 - 2.38
June 2019	4.34 ±0.06^{bc}	1.51±0.01^{d}	1.20 ±0.07^{a}	0.92 - 1.32

Values are presented as Mean ± SE. The superscript letter indicates significant difference, P<0.05.

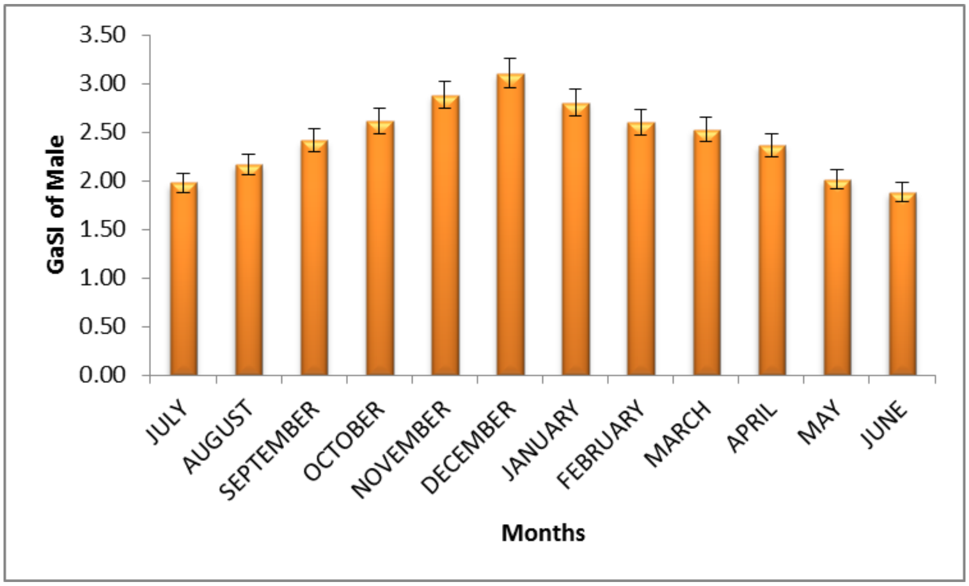

Fig. 12: Monthly variations of Gastro-somatic Index in male dwarf gourami, *Colisa lalia*

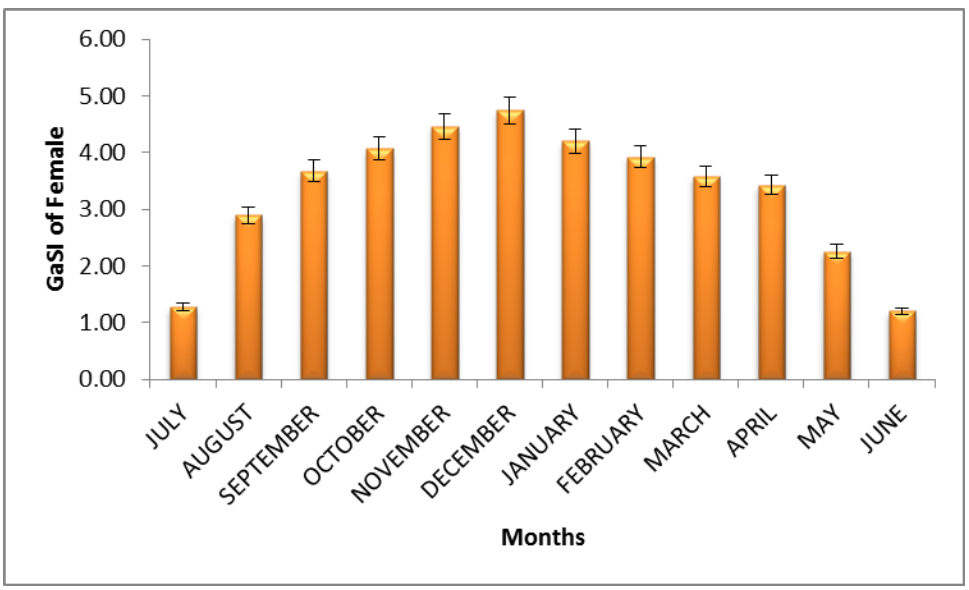

Fig. 13: Monthly variations of Gastro-somatic Index in female dwarf gourami, *Colisa lalia*

4.3 Reproductive Biology

4.3.1 Sexual dimorphism

Sexual dimorphism is observed between both the sexes of dwarf gourami, *Colisa lalia.* The male dwarf gourami is more colourful and attractive than the female dwarf gourami. The colours become more vibrant during the breeding season. The detail comparison between the two sexes is given in the Table 14 below. The picture of both male and female dwarf gourami is provided below in the Plate 10 and 11, which will make the comparison more clear.

During breeding season, a matured male can be identified by seeing the blue patch of colour at the ventral region between the opercula and the pectoral fin. This blue patch is not seen in immature males. During the breeding season, the female can be identified by the enlarged fat abdomen.

Table 14: Sexual dimorphic characters of male and female dwarf gourami, *Colisa lalia*

Characters	**Male**	**Female**
Colour of the body	Translucent blue colour with alternating vertical diagonal strips of red and dark orange bands.	Dull silvery blue to grey colour.
Colour of fins	Colourful as body	Yellowish grey
Body size	Comparatively larger	Smaller
Abdominal shape	Slender and sleek.	Only in breeding season, enlarged fat abdomen.
Dorsal fin	Pointed	Curved
Anal fin	Pointed	Curved

Plate 10: Male dwarf gourami, *Colisa lalia*

Plate 11: Female dwarf gourami, *Colisa lalia*

4.3.2. Gonadosomatic index (GSI)

Gonadosomatic index (GSI) of dwarf gourami, *Colisa lalia* was found out for 12 months from July 2018 to June 2019. The GSI of the male dwarf gourami found for the month of July 2018, August 2018, September 2018, October 2018, November 2018, December 2018, January 2019, February 2019, March 2019, April 2019, May 2019 and June 2019 are 0.563 ± 0.01, 0.482 ± 0.02, 0.361 ± 0.01, 0.330 ± 0.01, 0.305 ± 0.00, 0.314 ± 0.01, 0.352 ± 0.01, 0.392 ± 0.01, 0.413 ± 0.00, 0.463 ± 0.02, 0.528 ± 0.02 and 0.585 ± 0.01 respectively. There was significant difference ($p<0.05$) observed between the GSI of male dwarf gourami in all the twelve months. Highest value of GSI in male was observed in the month of June 2019 having the value of 0.585 ± 0.01. Lowest value of GSI was observed in the month of November 2018, having the value of 0.305 ± 0.00. Significant difference ($p<0.05$) in both length and weight was observed in the male dwarf gourami in all the twelve months.

The Gonadosomatic Index of the female dwarf gourami found for the month of July 2018, August 2018, September 2018, October 2018, November 2018, December 2018, January 2019, February 2019, March 2019, April 2019, May 2019 and June 2019 are 5.92 ± 0.17, 5.41 ± 0.05, 4.36 ± 0.05, 3.88 ± 0.21, 2.97 ± 0.02, 3.44 ± 0.05, 3.70 ± 0.02, 4.05 ± 0.05, 4.50 ± 0.31, 5.23 ± 0.09, 5.50 ± 0.06 and 6.08 ± 0.14 respectively. There was significant difference ($p<0.05$) observed between the GSI of female dwarf gourami in all the twelve months. Highest value of GSI in male was observed in the month of June 2019 having the value of 6.08 ± 0.14. Lowest value of GSI was observed in the month of November 2018, having the value of 2.97 ± 0.02. Significant difference ($p<0.05$) in weight is observed, but no significant difference ($p>0.05$) is observed in the length of female gourami in all the twelve months.

Table 15 and 16 indicates the monthly variations of Gonadosomatic Index in male and female dwarf gourami, *Colisa lalia* respectively, along with the length, weight and range of GSI for individual month. Values are presented as Mean ± SE. The superscript letter indicates significant difference, $P<0.05$. Fig. 14 and 15 shows the graphical presentation of monthly variations of Gonadosomatic Index in male and dwarf gourami, *Colisa lalia* respectively.

Table 15: Monthly variations of GSI in male of dwarf gourami, *Colisa lalia*

Month	Length (cm)	Weight (g)	GSI	Range of GSI
July 2018	4.44 ± 0.07^{ab}	1.56 ± 0.01^{ab}	0.563 ± 0.01^{fg}	0.52 - 0.58
August 2018	4.52 ± 0.06^{c}	1.66 ± 0.05^{d}	0.482 ± 0.02^{e}	0.42 - 0.52
September 2018	4.52 ± 0.04^{c}	1.66 ± 0.02^{d}	0.361 ± 0.01^{bc}	0.35 - 0.37
October 2018	4.38 ± 0.07^{ab}	1.70 ± 0.01^{d}	0.330 ± 0.01^{ab}	0.30 - 0.35
November 2018	4.26 ± 0.10^{a}	1.64 ± 0.02^{cd}	0.305 ± 0.00^{a}	0.30 - 0.31
December 2018	4.4 ± 0.10^{ab}	1.53 ± 0.03^{a}	0.314 ± 0.01^{a}	0.28 - 0.34
January 2019	4.3 ± 0.05^{a}	1.53 ± 0.01^{a}	0.352 ± 0.01^{b}	0.33 - 0.39
February 2019	4.54 ± 0.02^{c}	1.58 ± 0.00^{abc}	0.392 ± 0.01^{cd}	0.38 - 0.44
March 2019	4.42 ± 0.08^{ab}	1.69 ± 0.02^{d}	0.413 ± 0.00^{d}	0.40 - 0.43
April 2019	4.52 ± 0.04^{c}	1.68 ± 0.03^{d}	0.463 ± 0.02^{e}	0.42 - 0.52
May 2019	4.56 ± 0.04^{c}	1.63 ± 0.01^{bcd}	0.528 ± 0.02^{f}	0.49 - 0.56
June 2019	4.54 ± 0.05^{c}	1.57 ± 0.01^{abc}	0.585 ± 0.01^{g}	0.57 - 0.63

Values are presented as Mean ± SE. The superscript letter indicates significant difference, $P<0.05$.

Table16: Monthly variations of GSI in female of dwarf gourami, *Colisa lalia*

Month	Length (cm)	Weight (g)	GSI	Range of GSI
July 2018	4.38 ± 0.08^{c}	1.45 ± 0.07^{bcd}	5.92 ± 0.17^{g}	5.48 - 6.43
August 2018	4.06 ± 0.12^{a}	1.45 ± 0.01^{bcd}	5.41 ± 0.05^{f}	5.27 - 5.56
September 2018	4.16 ± 0.09^{abc}	1.46 ± 0.00^{bcd}	4.36 ± 0.05^{de}	4.19 - 4.45
October 2018	4.10 ± 0.10^{ab}	1.33 ± 0.03^{a}	3.88 ± 0.21^{c}	3.16 - 4.45
November 2018	4.18 ± 0.04^{abc}	1.35 ± 0.02^{ab}	2.97 ± 0.02^{a}	2.90 - 2.99
December 2018	4.24 ± 0.05^{abc}	1.39 ± 0.00^{abc}	3.44 ± 0.05^{b}	3.24 - 3.53
January 2019	4.22 ± 0.09^{abc}	1.37 ± 0.03^{abc}	3.70 ± 0.02^{bc}	3.63 - 3.75
February 2019	4.16 ± 0.09^{abc}	1.41 ± 0.03^{abcd}	4.05 ± 0.05^{cd}	3.90 - 4.15
March 2019	4.10 ± 0.08^{ab}	1.45 ± 0.08^{bcd}	4.50 ± 0.31^{e}	4.14 - 5.75
April 2019	4.10 ± 0.07^{ab}	1.46 ± 0.01^{bcd}	5.23 ± 0.09^{f}	5.03 - 5.45
May 2019	4.22 ± 0.04^{abc}	1.49 ± 0.02^{cd}	5.50 ± 0.06^{f}	5.36 - 5.69
June 2019	4.34 ± 0.06^{bc}	1.51 ± 0.01^{d}	6.08 ± 0.14^{g}	5.88 - 6.62

Values are presented as Mean ± SE. The superscript letter indicates significant difference, $P<0.05$.

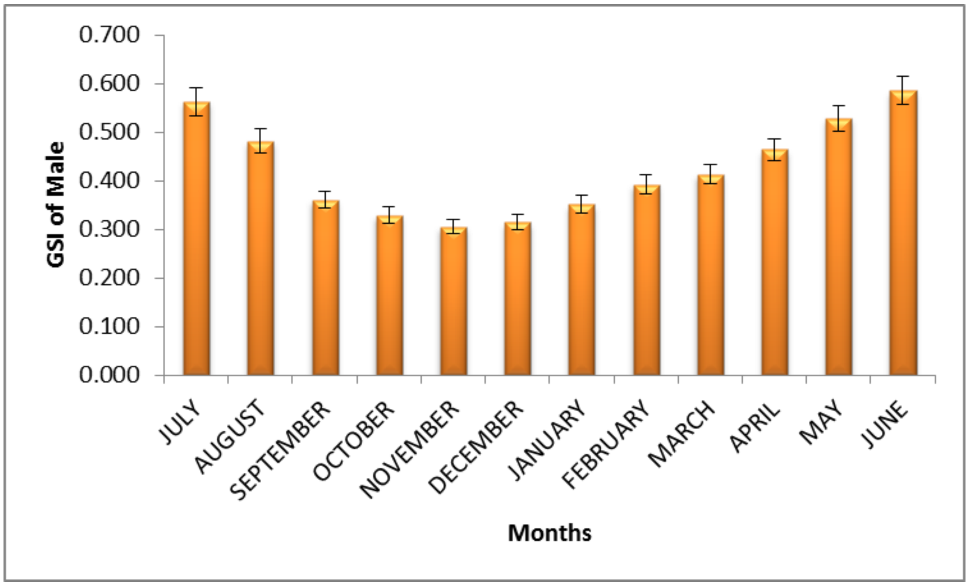

Fig. 14: Monthly variations of Gonadosomatic Index in male dwarf gourami, *Colisa lalia*

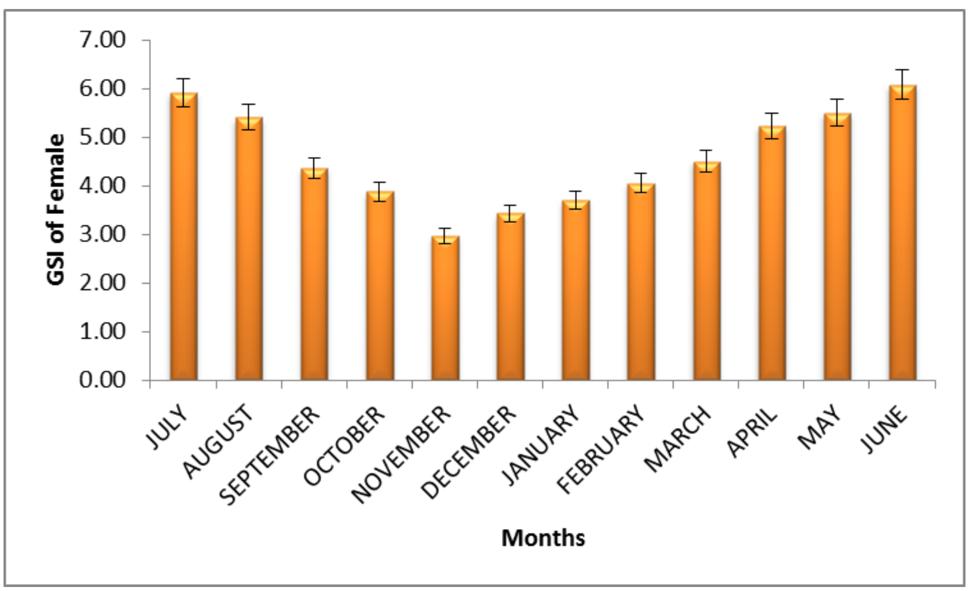

Fig. 15: Monthly variations of Gonadosomatic Index in female dwarf gourami, *Colisa lalia*

4.3.3 Gonad morphology

In the present study, it was found that the testis of *Colisa lalia* was paired and unequal in length throughout the year. The testis is asymmetrical in shape. Both the testis are attached all along to each other but were not free. It was lying ventral to the kidney and dorsal to the alimentary canal. It was remaining attached to the body wall by means of a thin layer of membrane called Mesorchium.

The colour of the testis was varying according to the maturation of testis and with the rhythm of seasonal cycle. During non-breeding season of the testis became creamy white in colour. With the start of breeding season, the colour of testis turns creamy white to yellowish or pinkish red.

The ovaries are elongated and paired sac-like structures and lies in the abdominal cavity, ventral to the kidneys. The anterior parts of the two ovaries are free but their posterior parts are united together into one. The pair remains attached to the body wall by means of the Mesovarium.

It was observed that the colour of the ovaries was changing according to the maturation of ovaries and with the rhythm of seasonal cycle. During non-breeding season, the ovaries were pale, dirty white in colour or opaque and light yellow in colour. During the breeding season the ovary became bright yellow.

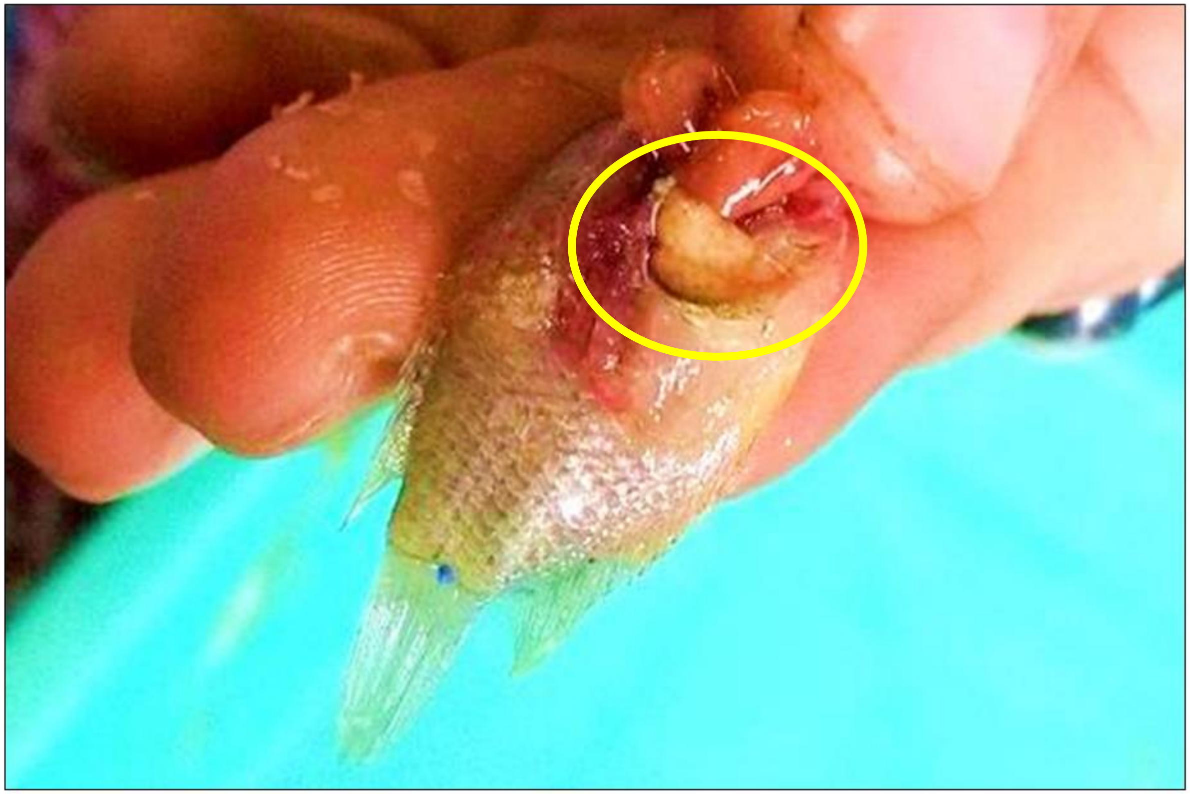

Plate 12: Ovary of dwarf gourami, *Colisa lalia*

4.3.4. Histological analysis of gonadal development

The gonadal development was studied in both the male and female dwarf gourami, *Colisa lalia,* in captive rearing conditions from June 2018 to July 2019.

4.3.4.1 Testis

As the testis dwarf gourami, *Colisa lalia* too small, the asymmetric structure of it of can be confirmed from the histological observations (Plate 13). Leydig cells were seen in all stages. Intertubular tissue was thick and muscle bundles were also seen On the basis of histological study of testis of *Colisa lalia*, 5 phases of testicular cycles were observed during the study period.

Developing I Phase Testis- Testis is small but can be easily identified. In this phase the concentration of the primary Spermatocyte is more and few spermatids were seen in the lumen. Germinal epithelium is continuous throughout (Plate 14).

Developing II Phase Testis- In this phase the concentration of primary spermatocytes has decreased, as they have developed into secondary spermatocytes. The lumen of the seminiferous tubules is getting packed with spermatids. Germinal epithelium is continuous throughout. Spermatogenesis in different phases is seen. Secondary spermatogonia developing in cysts are observed. Each cyst shows same developmental stage. Several cysts are seen with primary and secondary spermatogonia (Plate 15).

Spawning Capable Phase Testis - In this phase primary spermatocytes are not visible. There are fewer Secondary Spermatocytes visible in the Germinal Epithelium. The lumen of the Seminiferous Tubules is packed with Spermatids. Discontinuous germinal epithelium is seen (Plate 16).

Actively Spawning Phase Testis- In this phase only spermatids and spermatozoa could be found. We could see that the germinal epithelium was discontinuous in all lobules throughout testes (Plate 17).

Spent Phase Testis - In this phase we can find testes were slender and flaccid. Germinal epithelium lies vacant with few residual spermatozoa present in lumen of lobules and in sperm ducts (Plate 18).

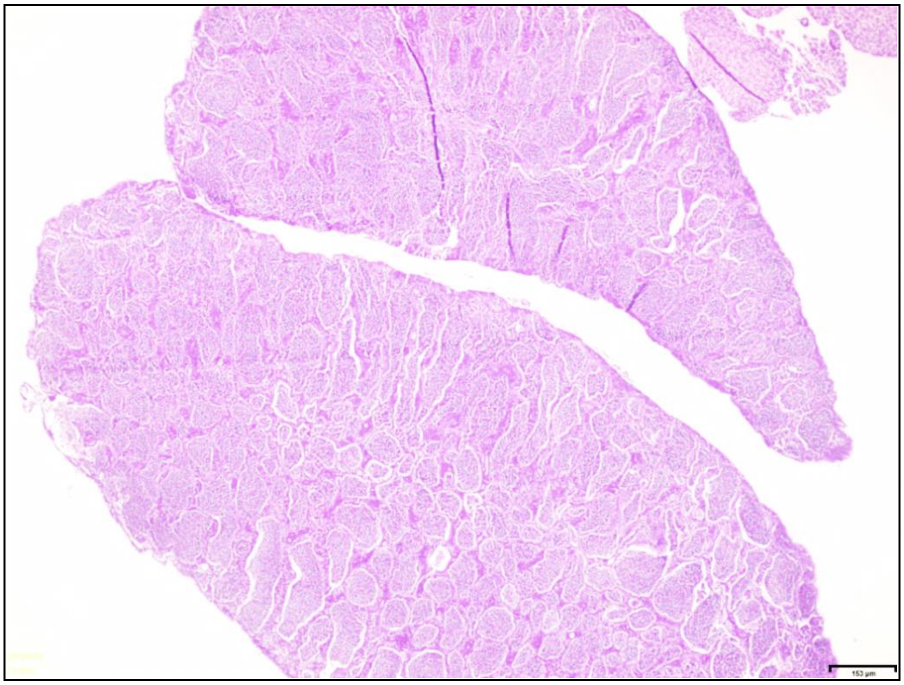

Plate 13: Asymmetrical structure of the testes (4.2x) in the male dwarf gourami, *Colisa lalia*

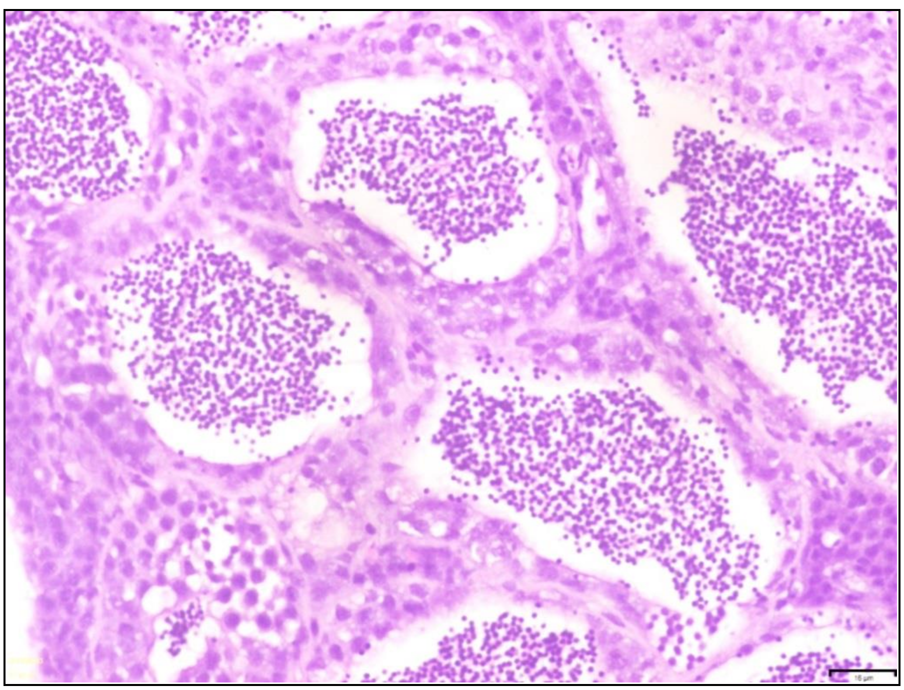

Plate 14: Developing I Phase Testis (40x)

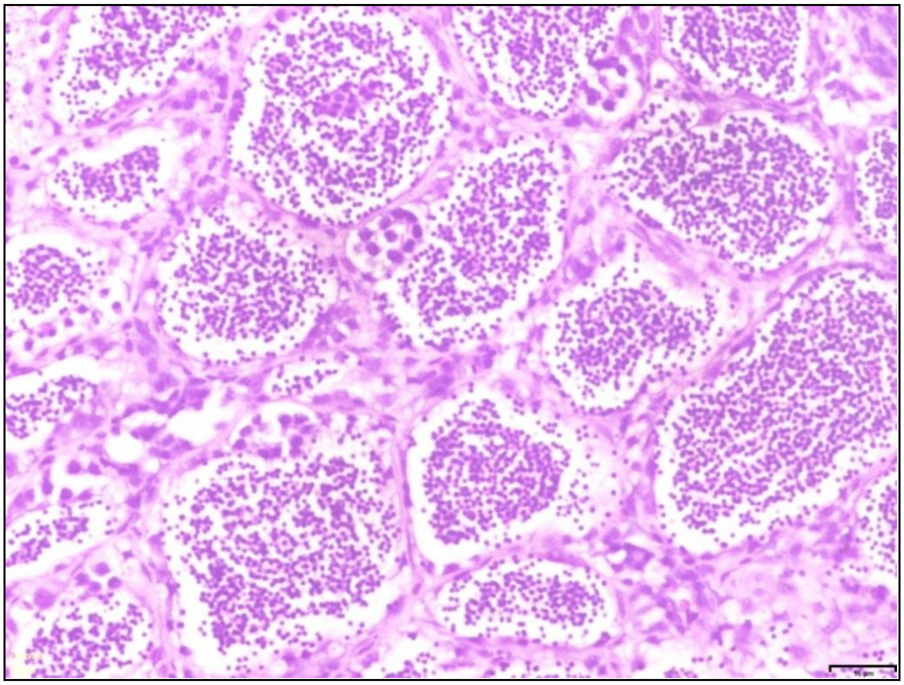

Plate 15: Developing II Phase Testis (40x)

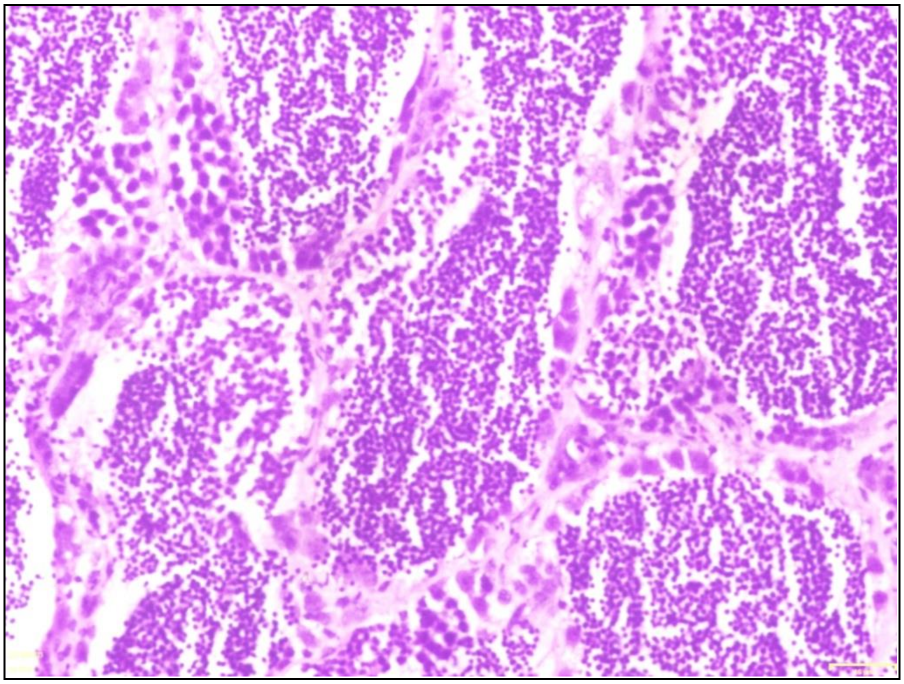

Plate 16: Spawning Capable Phase Testis (40x)

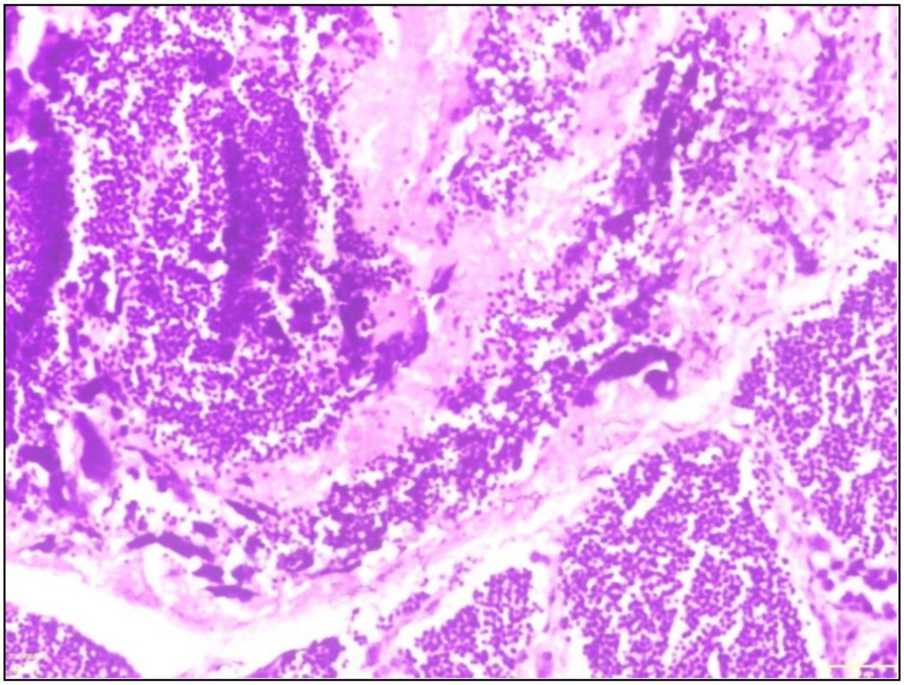

Plate 17: Actively Spawning Phase Testis (40x)

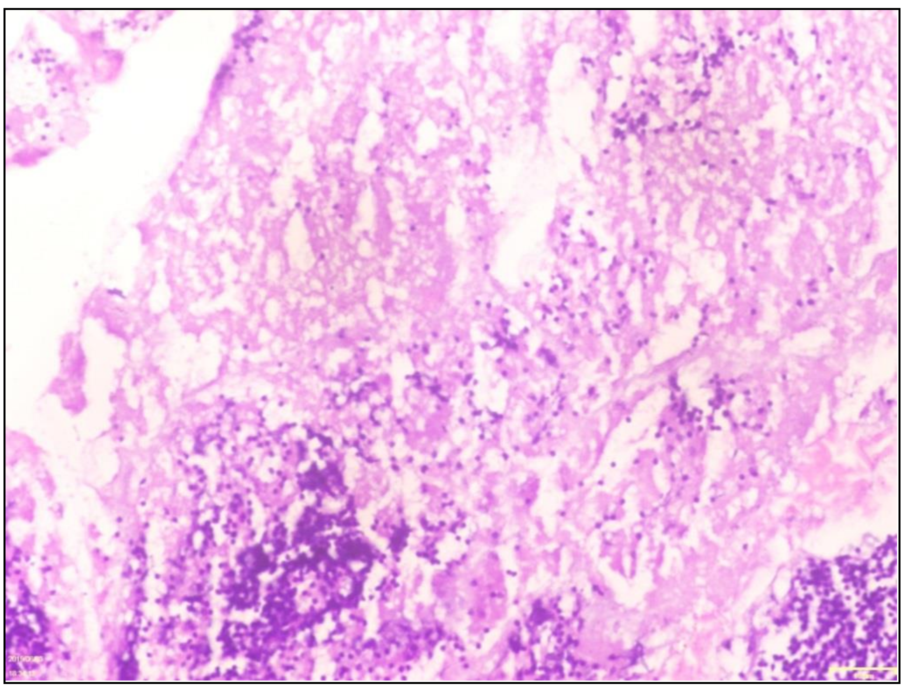

Plate 18: Spent Phase Testis (40x)

4.3.4.2 Ovary

. On the basis of histological study of ovaries of *Colisa lalia*, 4 phases of cycles were observed during the study.

Immature Phase Ovary - In this phase we can find ovaries are small in size and the percentage of ovarian tissue is more with oogonia, primary growth oocyte and very few perinucleolar oocytes. Developing oogonia were seen in the ovigerous lamellae. Distinct tunica albuginea was seen (Plate 19).

Developing I Phase Ovary - In this phase we can find enlargement of the ovary and the percentage of ovarian tissue has decreased with few primary growth oocyte and large number of perinucleolar oocyte (Plate 20).

Developing II Phase Ovary - In this phase we can find the ovaries have grown to bigger size with large number of perinucleolar oocyte and the appearance of cortical alveolar oocyte. In the sections there was no evidence of any vitellogenic oocyte as vittelogenesis has not commenced (Plate 21).

Spawning Capable Phase Ovary- In this phase we can find secondary vitellogenic oocyte with yolk granules, yolk vesicles and germinal vesicle. Well-developed follicular membrane was observed (Plate 22). The free ends of a spawning capable phase ovary possess oocytes of many stages including primary vitellogenic oocyte, secondary vitellogenic oocyte, perinucleolar oocyte and cortical alveolar oocyte. Spawning Capable Phase Ovary shows asynchronous continuous oocyte development with oocytes in all stages of development (Plate 23).

Although the entry into the spawning capable phase is defined as the presence of vitellogenic oocytes but the batch spawners in this phase can have oocytes in any stage of vitellogenesis, including primary and secondary vitellogenic oocytes. Thus from the above observations it could be concluded that the ovary of dwarf gourami, *Colisa lalia is* of asynchronous type.

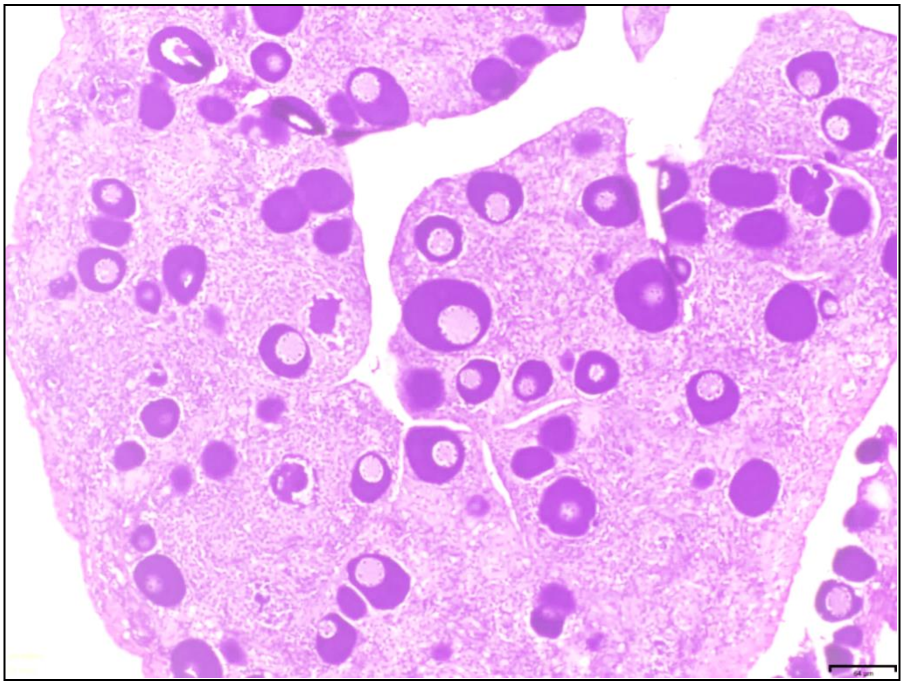

Plate 19: Immature Phase Ovary (10x)

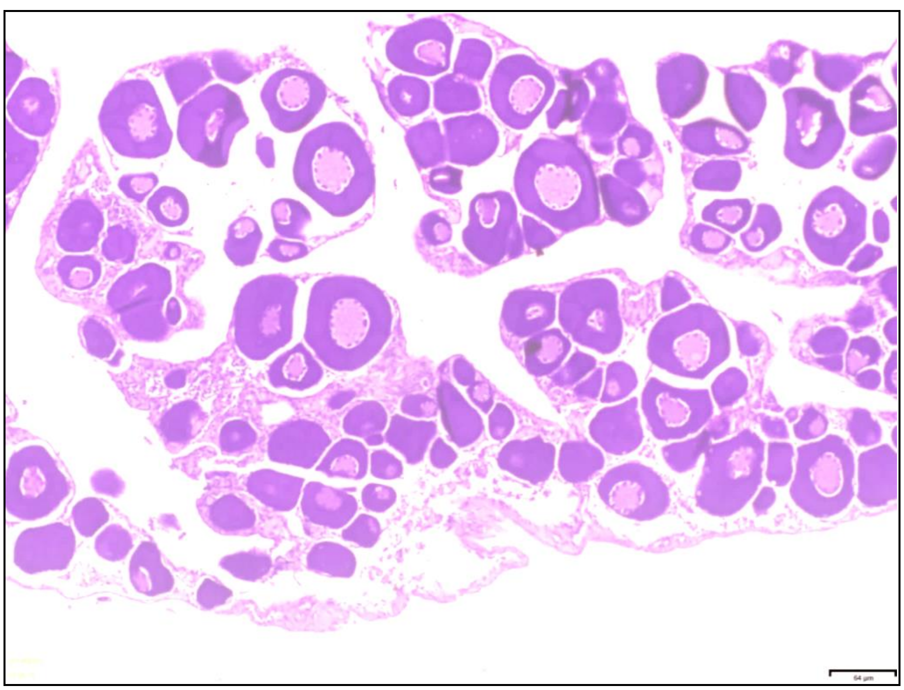

Plate 20: Developing Phase I Ovary (10x)

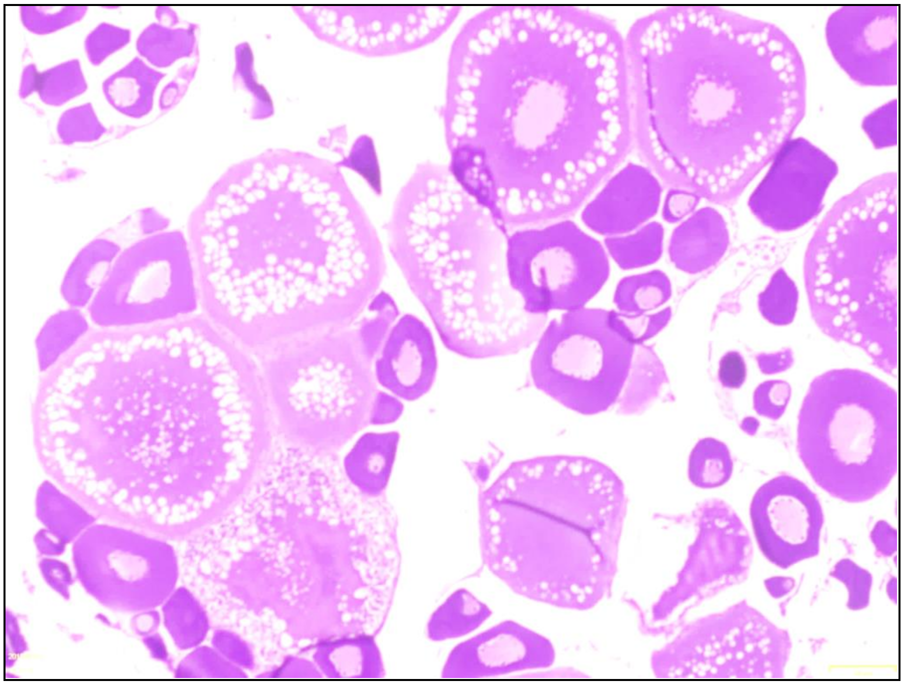

Plate 21: Developing Phase II Ovary (10x)

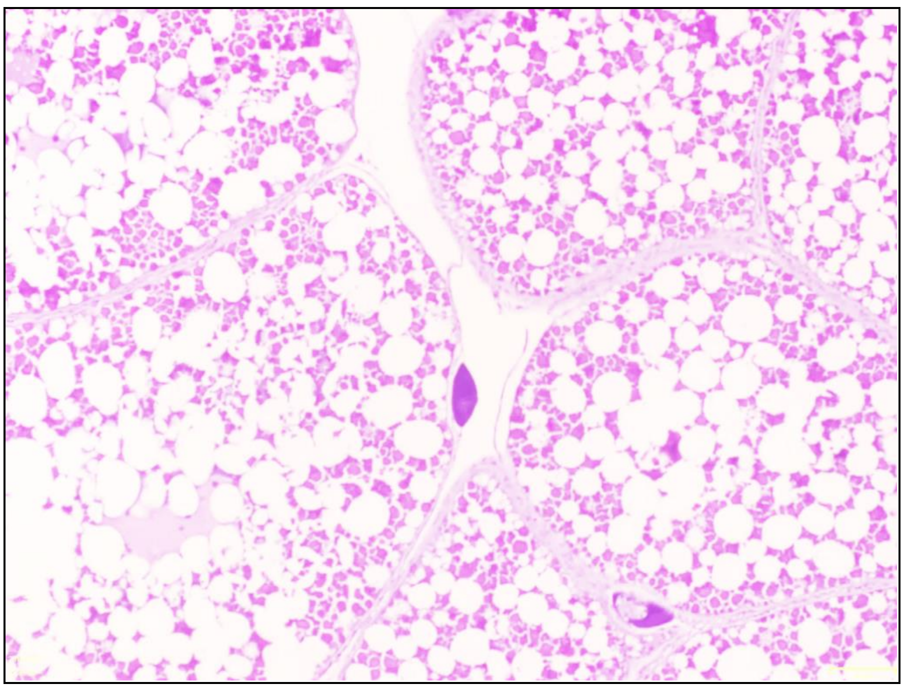

Plate 22: Spawning Capable Phase Ovary (10x)

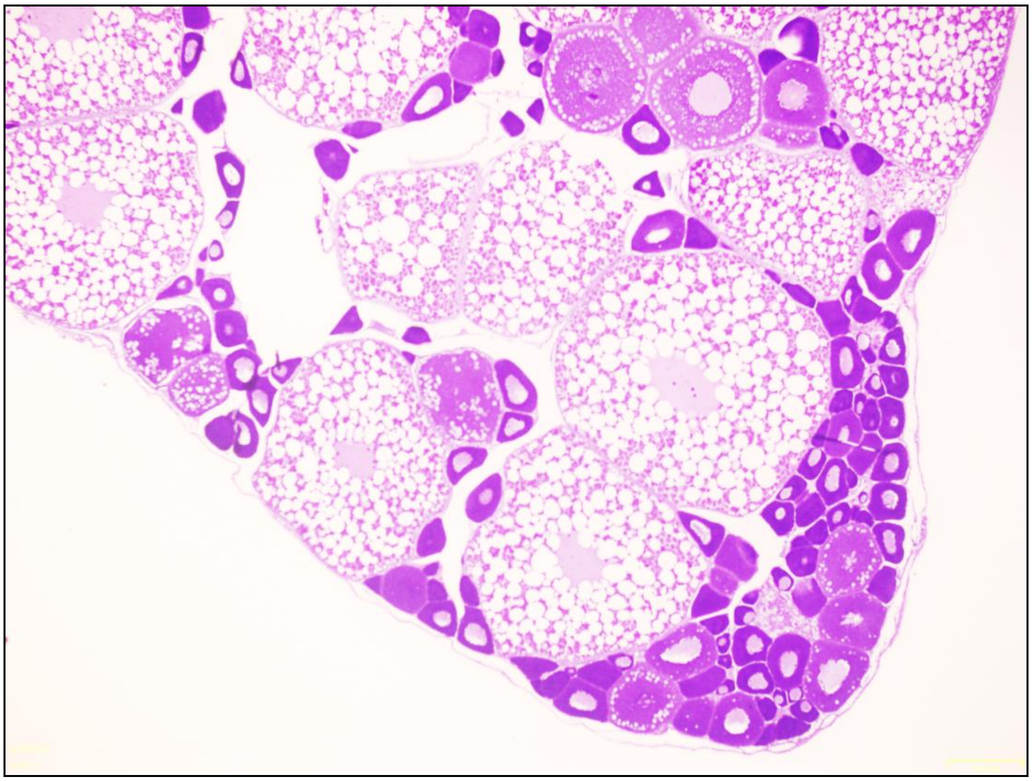

Plate 23: Spawning Capable Phase Ovary (4.2x)

4.3.5 Breeding behavior

In this study, breeding setup for the dwarf gourami is arranged, to observe the breeding behavior. Initially both the sexes were segregated for around fifteen days, and were fed with live feed, tubifex worm in *adlibitum*. Accumulated bubbles (Plate 24) were observed in the tanks of male, and the bellies of the females seemed bulky. This gave the hint that they are ready to breed.

Breeding set up was arranged (Plate 25) by keeping a single male in an aquarium of dimension 1.5 x1.5 x 1′ and providing that with a floating piece of plastic sheet (Plate 26) which can hold the bubbles of bubble nest. Six replicates of this breeding setup was arranged, for better observation. In the breeding setups 1:1 male and female sex ratio was maintained.

After the bubble nest is made by the male dwarf gourami, gravid female was introduced to the tank (Plate 27). Chasing activity was observed (Plate 28) for that particular day. On the early second day courtship behavior was observed (Plate 29) followed by the spawning activity. Male wrapped his body around the female and turned female body upwards so that female's belly turns upwards to facilitate the released eggs to attach the prepared nest. After successful achievement male curved his body along with the female and separated the spawned eggs from the female. Simultaneously the male fertilized the eggs. It was observed that the spawning activity was occurring in intervals of few minutes.

After spawning, the female was removed from the tank. It was observed that the scattered eggs were collected by the male and were guarded till hatching. Parental care was performed by the male till hatchlings were obtained. Hatchling were observed (Plate 30) in the bubble nest on the third day, *i.e.,* around 24 – 26 hours of the spawning activity. Parental care was still observed in the male gourami. After the fourth day of spawning, three males were removed from the 6 replicates. Therefore three replicates were having the male in it and three replicates were not having the male in it. Cannibalism was observed in the males, after the yolk sac was absorbed in the hatchlings.

Table 17: Breeding behavior of dwarf gourami, *Colisa lalia*

Days Count	Breeding Behavior
Day 0	Breeding set up was arranged with single male in the tank with floating plastic substrate (Plate 26).
Day 1	Bubble nest was built by the male below the floating plastic substrate (Plate 24).
Day 3	Female was introduced (Plate 27). Chasing activity was observed (Plate 28).
Day 4	Courtship behavior was observed, followed by spawning activity. Parental care observed by the male, after spawning (Plate 29).
Day 5	Hatchlings were observed in the bubble nest. Parental care still observed by the male (Plate 30).
Day 7 - 8	Cannibalistic activity was observed by male dwarf gourami.

Plate 24: Bubbles produced by male dwarf gourami, *Colisa lalia*

Plate 25: Breeding set up of dwarf gourami, *Colisa lalia*

Plate 26: Breeding set up with substrate to hold the bubble nest

Plate 27: Breeding set up with female introduced after bubble nest made by male

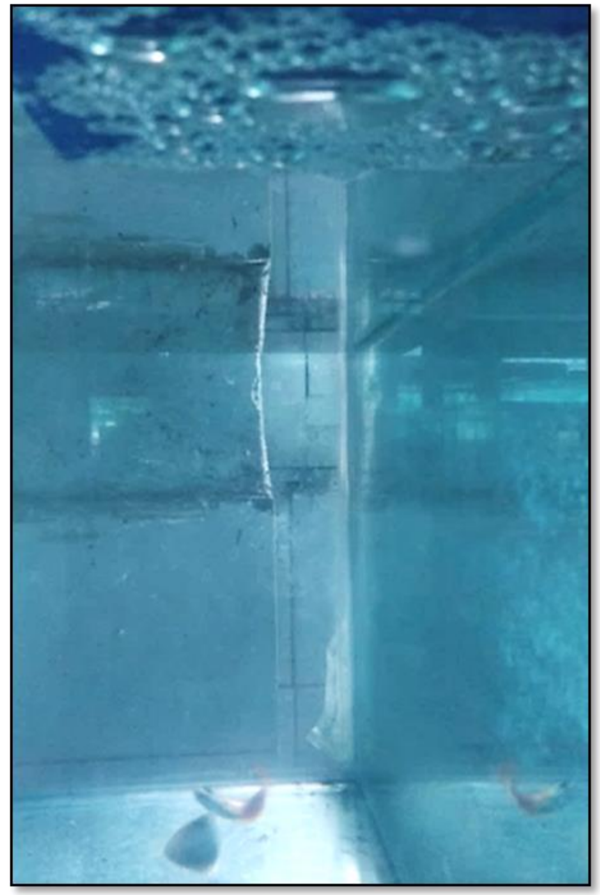

Plate 28: Chasing activity of male and female

Plate 29: Courtship behavior displayed

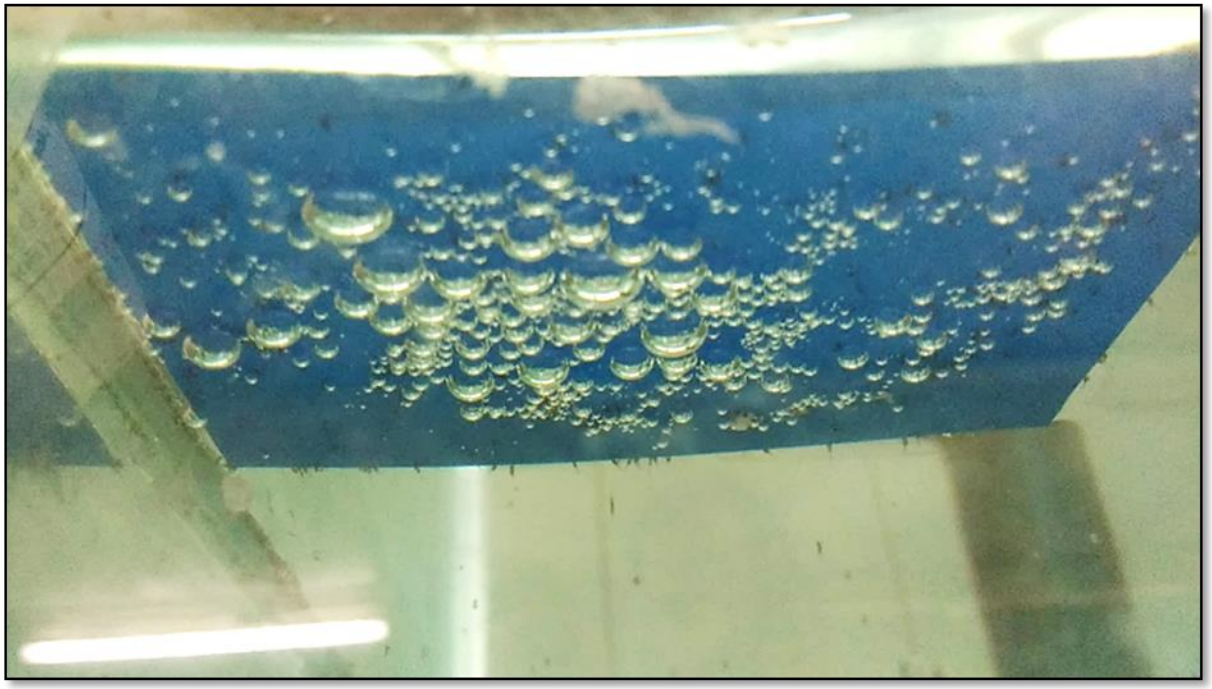

Plate 30: Hatchlings in the bubble nest after breeding activity

4.3.6 Fecundity

Pre-spawning absolute fecundity of dwarf gourami, *Colisa lalia* was found out to be 439 as mean and 48.74 as standard deviation and the range was 372 – 502. The average, SD, minimum and maximum value of length, weight and ovary weight is given in the table 18 below. Correlation of Length, Body weight and gonad weight is given in Table 19. Graphical representation of the relationship of fecundity with body weight along with R^2 value of 0.191 is shown in the Figure 16. Graphical representation of the relationship of fecundity with gonad weight along with R^2 value of 0.588 is shown in the Figure 17.

Table 18: Pre- spawning Absolute Fecundity of dwarf gourami, *Colisa lalia*

N=30	Length of fish (cm)	Weight of fish (g)	Weight of ovary (g)	Fecundity
Average	4.44	1.53	0.09	439
SD	0.11	0.07	0.01	48.74
Min	4.30	1.42	0.08	372
Max	4.60	1.71	0.11	502

Table 19: Correlation of Length, Body weight and gonad weight in comparison with Fecundity of dwarf gourami, *Colisa lalia*

In Comparison with Absolute Fecundity	Correlation Coefficient	R^2 Value	Regression Equation
Body Length	0.325966447	$R^2 = 0.1063$	y = 141.7x - 190.23
Body Weight	0.437027367	$R^2 = 0.191$	y = 287.45x - 1.6253
Gonad Weight	0.767295753	$R^2 = 0.5888$	y = 4232.3x + 69.311

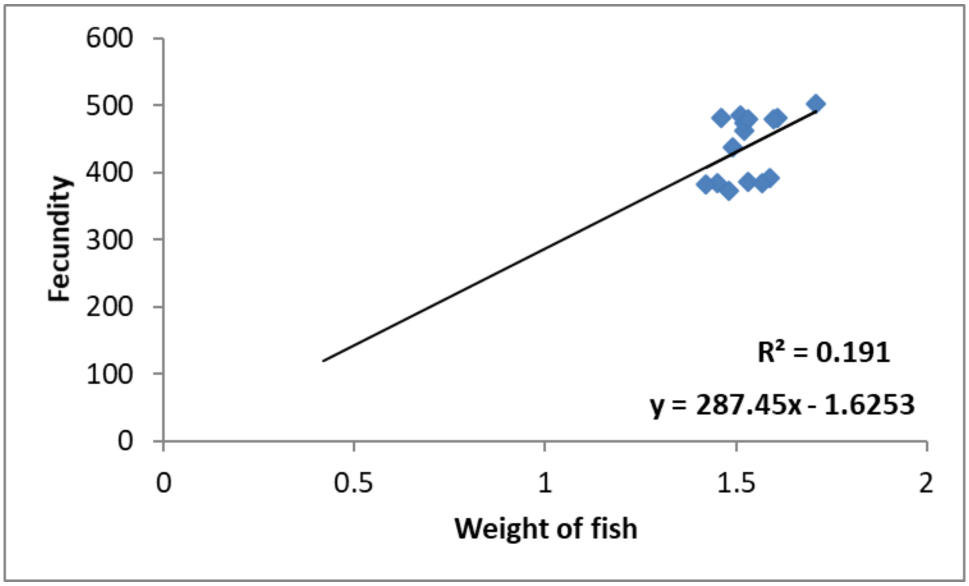

Fig 16: Variation in fecundity with body weight of *Colisa lalia*

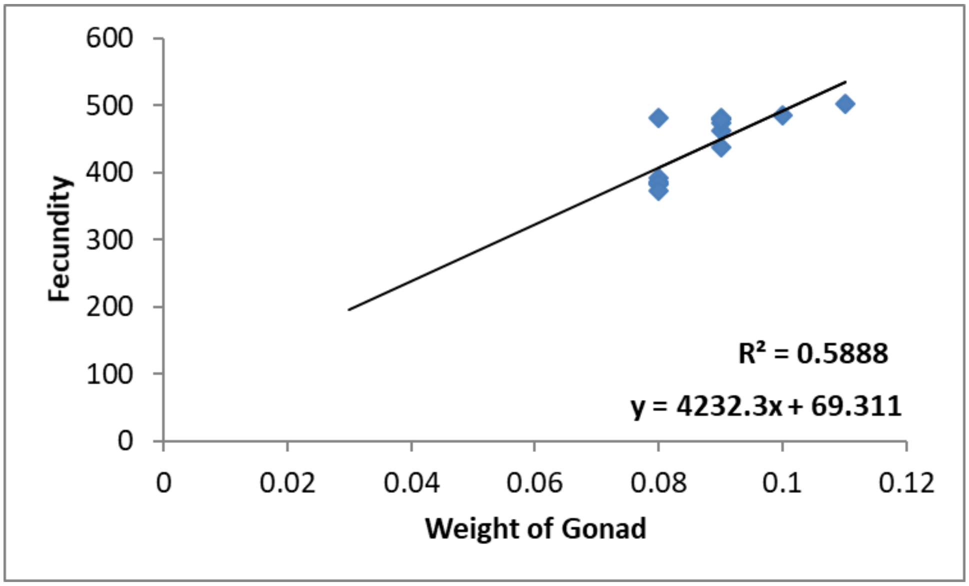

Fig 17: Variation in fecundity with gonad weight of *Colisa lalia*

4.3.7 Ova Diameter

The mean ova diameter observed was found to be 0.265 mm, with 0.003 standard deviation and range of 0.271 - 0.261mm. The appearance and size of the ova can also be observed from the picture obtained by the phase contrast microscope and the scale given in Plate 31.

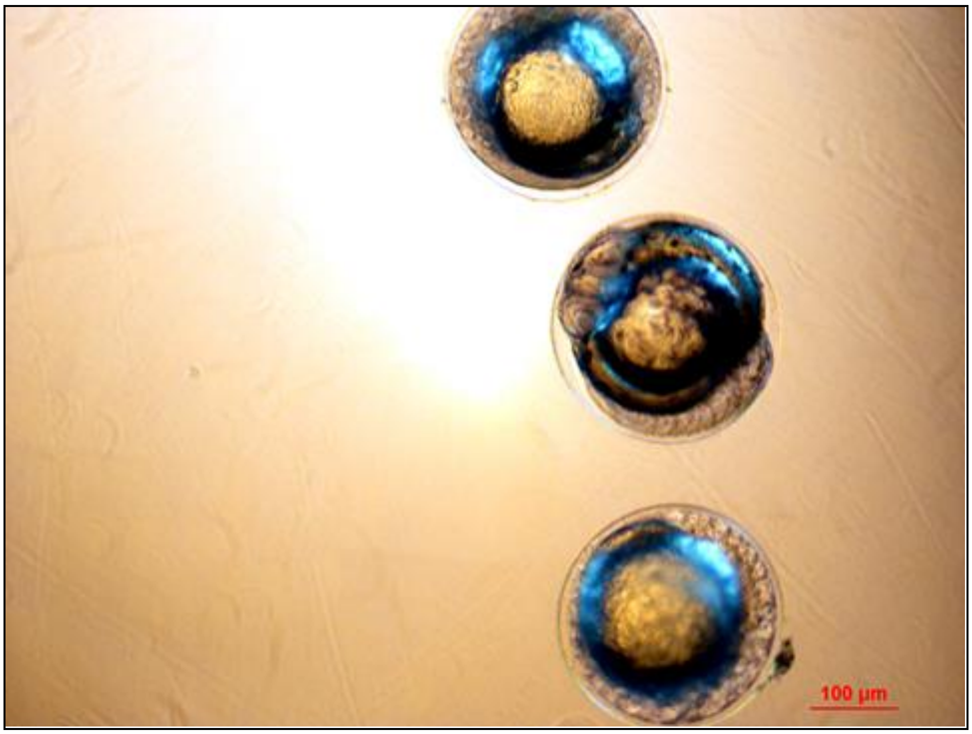

Plate 31: Ova diameter of dwarf gourami, *Colisa lalia*

4.3.8 Ichthyoplankton

Study of the icthyoplankton, *ie.*, eggs and larvae of dwarf gourami, *Colisa lalia* was done after the spawning activity. The study was confined to the morphological changes occurring in the egg stages and larval stages. In the photograph taken by the phase contrast microscope, we can also find the bluish and yellowish colour pigmentation at the very early embryo stage of *Colisa lalia* (Plate 32).

Plate 32: Embryo before hatching and just hatched larvae of dwarf gourami, *Colisa lalia*

4.3.8.1 Egg stage

The egg stage is mainly confined from spawning to hatching phase. The fertilized egg of dwarf gourami is transparent and golden in colour, unlike the unfertilized egg which is opaque. Soon after fertilization there is perivitelline space formation (Plate 33). Cell division starts soon after 10 minutes of fertilization process, with cytoplasmic circulation. The morphological changes while staring of the gastrulation process is shown in Plate 34. The gastrula phase of egg of dwarf gourami is shown in Plate 35. Embryonic body formation is shown in Plate 36, where the head formation along with optic vesicles is seen. During this phase, heart beat is also observed. Process of Hatching is shown in Plate 37, where it is seen that the egg envelop is pushed by the fully grown up embryo at its posterior end of tail. Just hatched hatchling (Plate 38) is obtained on the next day of spawning. The details are given in table 20. The incubation period for eggs to hatch is around 24 – 25 hours.

Table 20: Egg stages of dwarf gourami, *Colisa lalia*

Egg Stage	Time Duration	Description
Fertilized egg	25 -30 min	Non adhesive and optically transparent. Activation of cytoplasm movement. Clear distinction between perivitelline space and egg-yolk (Plate 33).
Initiation of Gastrula phase	45 – 55min	Excessive cell movements (involution, convergence and extension) (Plate 34).
Gastrula phase	6 – 7 hr	Three layers of germ-cells produced, looks like hollow cup-shaped structure (Plate 35).
Embryonic body Formation	17-19 hr	Embryo body is formed. Heart beat and twitching movement observed (Plate 36).
Removal of egg envelope	24-25 hr	The embryo pushes the egg envelope. Lots of twitching movement observed (Plate 37).
Just Hatched Larvae	24-25 hr	No twitching movement, due to exhaustion of hatching. Only cytoplasmic circulation and heart beat observed.

Plate 33: Fertilized egg

Plate 34: Initiation of Gastrula phase

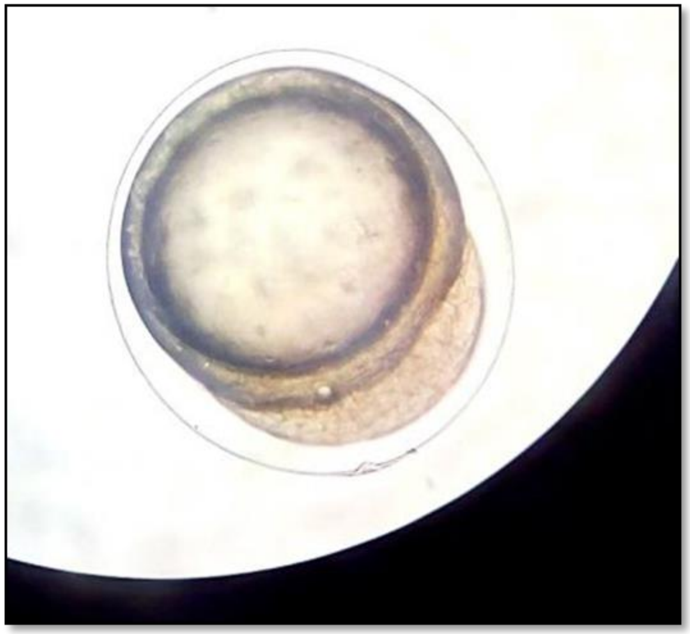

Plate 35: Gastrula phase

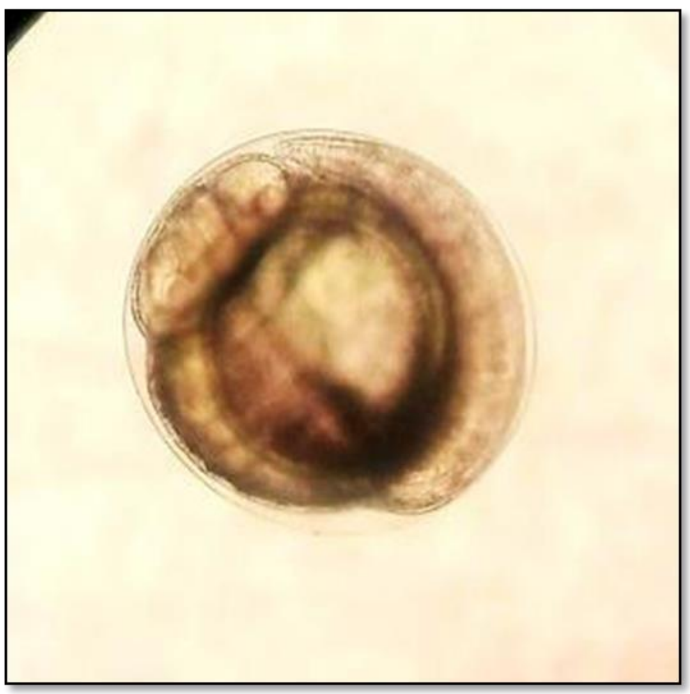

Plate 36: Embryonic body Formation

Plate 37: Removal of egg envelope

Plate 38: Just Hatched Larvae

4.3.8.2 Larval stage

Larvae of dwarf gourami are produced after 24 – 25 hours of spawning. In this present study observations are taken for fifteen days. The descriptions of the developmental stages of the larvae are presented in the table 21 and their features are demonstrated in plates given below.

Table 21: Larval stages of dwarf gourami, *Colisa lalia*

Larval stage	Description
Newly hatched larvae	In just hatched larvae, we can find the optic vesicles, myotome, heart, yolk sac. There is no locomotion done by the larvae. Oil globules are visible. Circulation of fluid is observed (Plate 39)
One day old larvae	In one day old larvae, we can find eye bulbs, myotome, and heart. Yolk sac is reduced. The body is transparent but pigmentation can be found (Plate 40).
Two days old larvae	In two days old larvae, we can find dark eye bulbs, mouth lining, nostrils, pectoral fins, alimentary canal, myotome, heart. Yolk sac reduced than before. There is fast movement observed in the larvae (Plate 41).
Five days old larvae	In five days old larvae, we can find eyes with pupil, mouth opening, nostrils, and developed pectoral fin. Yolk sac is pushed upwards. Jaws become stronger. The notochord becomes segmented. It moves very fast (Plate 42).
Ten days old larvae	In ten days old larvae, we can find air bladder. With the development of muscles and bones the larvae is becoming opaque. There is a lot of pigmentation. Yolk sac is almost completely absorbed (Plate 43).
Fifteen days old larvae	In fifteen days old larvae, we can find the different organs. Caudal rays are well developed in the caudal fin (Plate 44).

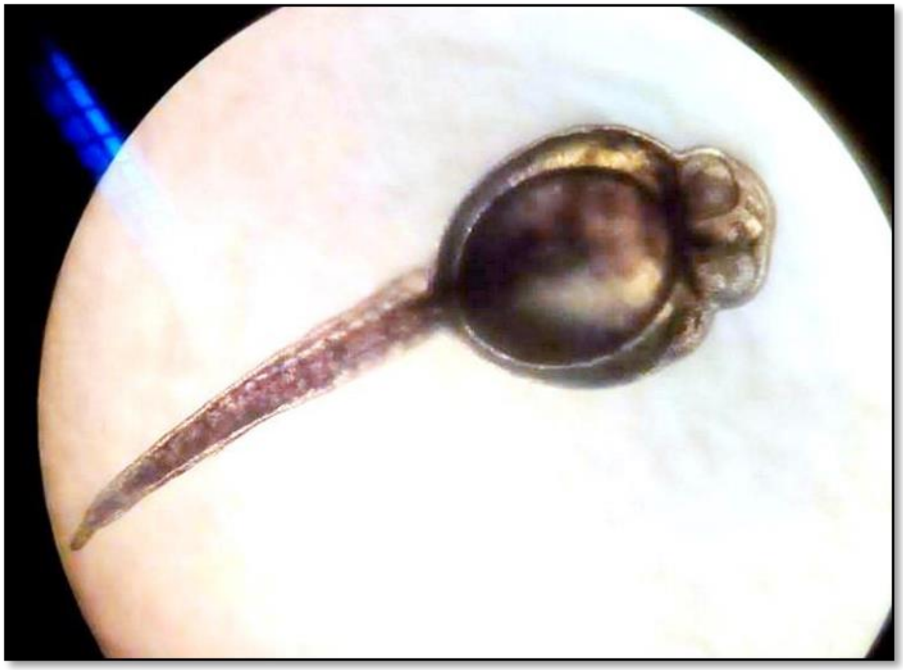

Plate 39: Newly hatched larvae

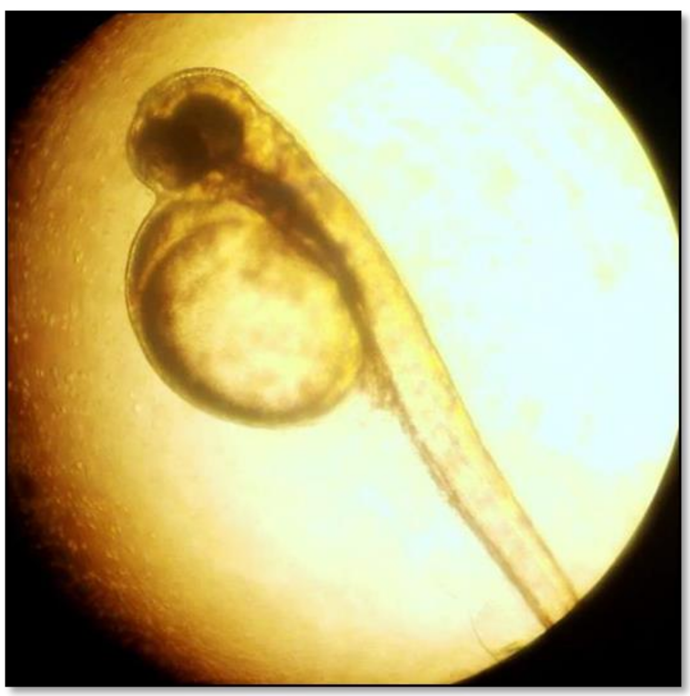

Plate 40: One day old larvae

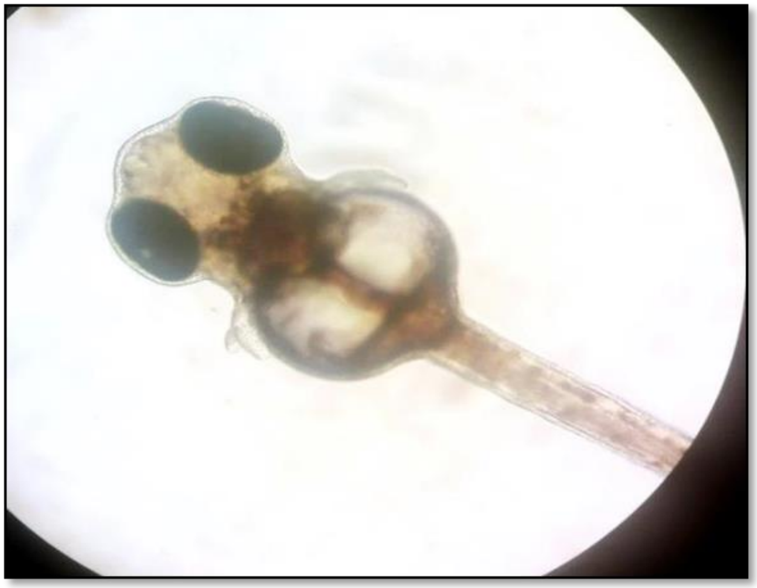

Plate 41: Two days old larvae

Plate 42: Five days old larvae

Plate 43: Ten days old larvae

Plate 44: Fifteenth day larvae

4.4 Evaluation of the effect of nutritional intervention on growth performance of dwarf gourami, *Colisa lalia*

4.4.1 Proximate analysis of formulated diets and live food

The proximate compositions of the feed used in the experiments are given in the table 20 given below. The moisture, protein, ether extract, ash, carbohydrate and energy content of Feed A, i.e., the control feed is 10.22%, 37.83%, 5.32%, 9.05%, 47.80% and 463.57 kcal/100gm respectively. The moisture, protein, ether extract, ash, carbohydrate and energy content of Feed B, i.e., the control feed added with 2% supplementary mix is 10.06%, 38.15%, 6.16%, 9.04%, 46.65% and 467.96 kcal/100gm respectively. The moisture, protein, ether extract, ash, carbohydrate and energy content of Feed C, i.e., the control feed added with 4% supplementary mix is 9.9%, 38.47%, 7.00%, 9.03%, 45.50% and 472.36 kcal/100gm respectively. The moisture, protein, ether extract, ash, carbohydrate and energy content of live feed i.e., *Tubifex* is 86.32%, 62.56%, 15.34%, 6.37%, 15.73% and 548.164kcal/100gm respectively. (Table 22)

Table 22: Proximate composition of experimental diets for dwarf gourami, *Colisa lalia*

Diet	Feed A (T1)	Feed B (T2)	Feed C (T3)	Tubifex (T4)
Moisture (%)	10.21 ± 0.05	10.08 ± 0.03	9.830 ± 0.06	86.40 ± 0.08
Protein (%)	37.44 ± 0.36	38.19 ± 0.03	38.45 ± 0.03	62.52 ±0.04
Ether Extract (%)	5.21 ± 0.11	6.19 ± 0.02	7.00 ± 0.00	15.35 ±0.03
Ash (%)	9.05 ± 0.01	9.02 ± 0.02	9.03 ± 0.00	6.38 ±0.01
Carbohydrate (%)	48.30 ± 0.47	46.61 ± 0.03	45.52 ± 0.03	15.75 ±0.03
Energy (kcal/100gm)	462.62 ± 0.92	468.22 ±0.23	472.34 ± 0.02	548.16 ±0.08

4.4.2 Growth performance and survivality

Growth performance and survivability of dwarf gourami, *Colisa lalia* (Hamilton, 1822) over a time period of three months and subjected to four treatments with different feeds are given below in the table 23. A significant difference (P<0.05) was observed between the treatments in terms of length gain, weight gain, weight gain %, specific growth rate (SGR) and survival rate.

4.4.2.1 Length gain

Size variation in fish length was observed in all the treatments as shown in table 23. The length gain achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.56 ± 0.03 cm, 0.62 ± 0.05 cm, 0.78 ± 0.02 cm and 1.03 ± 0.04 cm respectively. The highest value of length gain of 1.03 ± 0.04 cm was observed in Treatment 4, which was fed with live feed, Tubifex. The lowest value of length gain of 0.56 ± 0.03 cm was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference (P<0.05) was observed in length gain in Treatment 3 and Treatment 4, but no significant difference (P>0.05) was observed in length gain in Treatment 1 and Treatment 2 (Table 23). Figure 18 show the graphical representation.

4.4.2.2 Weight gain

Size variation in fish weight was observed in all the treatments as shown in table 23. The weight gain achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.41 ± 0.06 g, 0.63 ± 0.05 g, 1.00 ± 0.01 g and 1.12 ± 0.02 g respectively. The highest value of weight gain of 1.12 ± 0.02 g was observed in Treatment 4, which was fed with live feed, Tubifex. The lowest value of weight gain of 0.41 ± 0.06 g was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference (P<0.05) was observed in weight gain in Treatment 1 and Treatment 2, but no significant difference (P>0.05) was observed in weight gain in Treatment 3 and Treatment 4(Table 23). Figure 19 show the graphical representation.

4.4.2.3 Weight gain percentage

The weight gain percentage achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 43.90 ± 6.88 %, 67.67 ± 4.97%, 108.03 ± 0.99% and 120.75 ± 1.63% respectively. The highest value of weight gain percentage of 120.75 ± 1.63 % was observed in Treatment 4, which was fed with live feed, Tubifex. The lowest value of weight gain percentage of 43.90 ± 6.88 % was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference ($P<0.05$) was observed in weight gain percentage in Treatment 1 and Treatment 2, but no significant difference ($P>0.05$) was observed in weight gain percentage in Treatment 3 and Treatment 4 (Table 23). Figure 20 show the graphical representation.

4.4.2.4 Specific growth rate

The specific growth rate achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.40 ± 0.05, 0.57 ± 0.03, 0.81 ± 0.01, 0.88 ± 0.01 respectively. The highest value of SGR of 0.88 ± 0.01 was observed in Treatment 4, which was fed with live feed, Tubifex. The lowest value of SGR of 0.40 ± 0.05 was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference ($P<0.05$) was observed in SGR in Treatment 1 and Treatment 2, but no significant difference ($P>0.05$) was observed in SGR in Treatment 3 and Treatment 4 (Table 23). Figure 21 show the graphical representation.

4.4.2.5 Survival rate %

The survival rate % achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 96.67 ± 1.67, 96.67 ±3.33, 96.67 ±1.67 and 98.33 ±1.67 respectively. No significant difference ($P>0.05$) was observed in survival rate in all the four treatments (Table 23). Figure 22 show the graphical representation of survival rate % of *Colisa lalia* in the four treatments.

Table 23: Growth performance due to different experimental diets of dwarf gourami, *Colisa lalia*

Treatment	Length gain (cm)	Weight gain (g)	Weight gain %	SGR	Survival Rate %
T1 (Feed A)	0.56 ± 0.03^{a}	0.41 ± 0.06^{a}	43.90 ± 6.88^{a}	0.40 ± 0.05^{a}	96.67 ± 1.67^{a}
T2 (Feed B)	0.62 ± 0.05^{a}	0.63 ± 0.05^{b}	67.67 ± 4.97^{b}	0.57 ± 0.03^{b}	96.67 ± 3.33^{a}
T3 (Feed C)	0.78 ± 0.02^{b}	1.00 ± 0.01^{c}	108.03 ± 0.99^{c}	0.81 ± 0.01^{c}	96.67 ± 1.67^{a}
T4 (Tubifex)	1.03 ± 0.04^{c}	1.12 ± 0.02^{c}	120.75 ± 1.63^{c}	0.88 ± 0.01^{c}	98.33 ± 1.67^{a}

Values are presented as Mean ± SE. The superscript letter indicates significant difference, $P<0.05$.

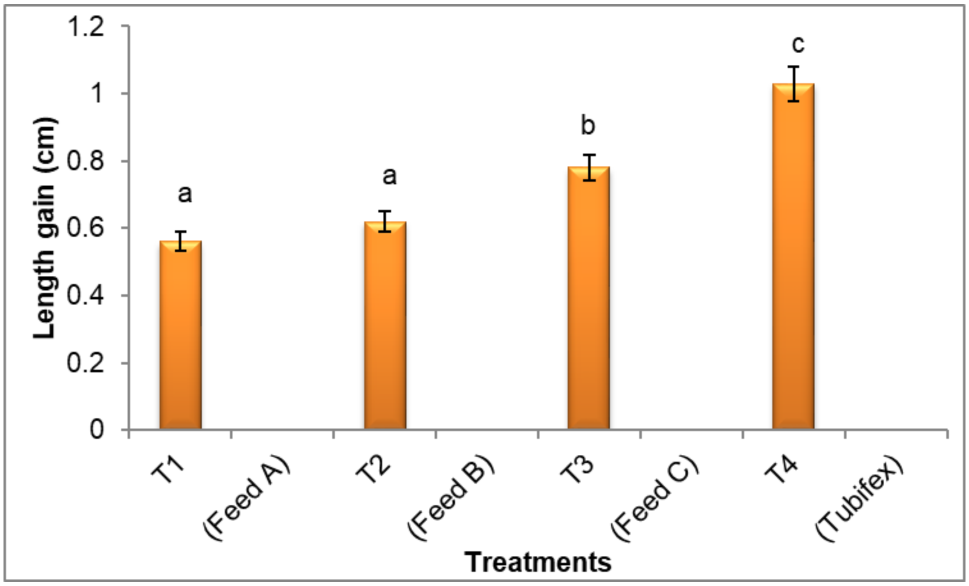

Fig 18: Length gain (cm) of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

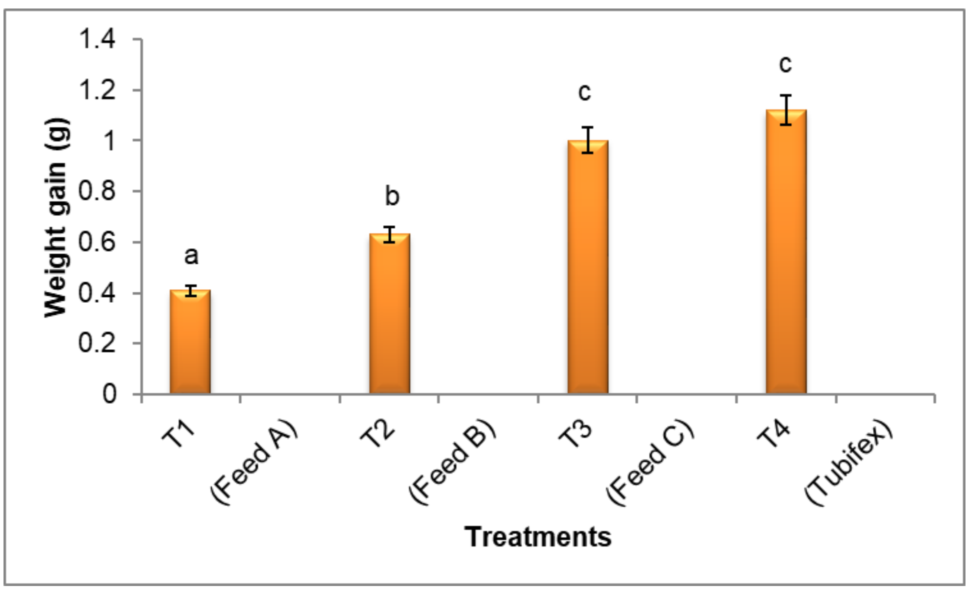

Fig 19: Weight gain (g) of *Colisa lalia.* Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

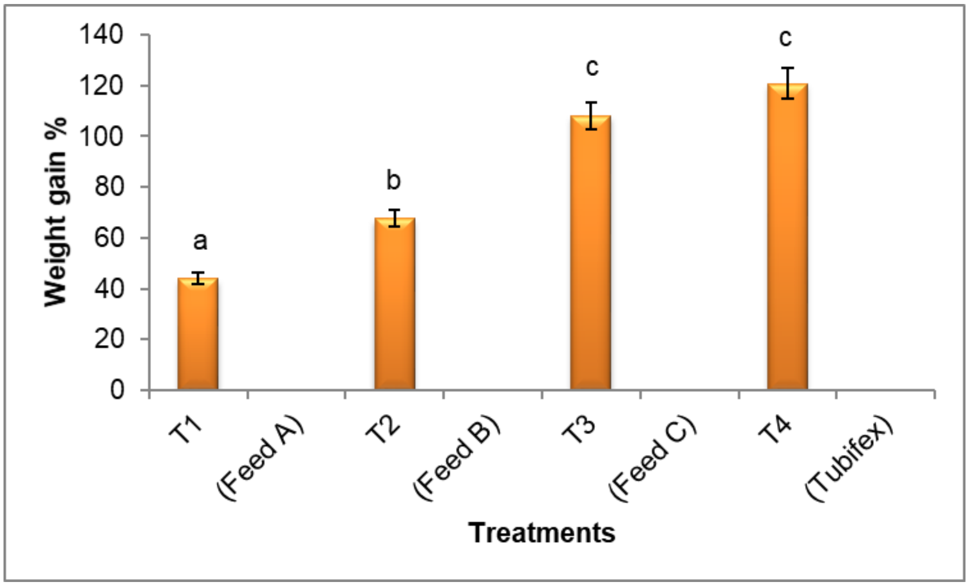

Fig 20: Weight gain % of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

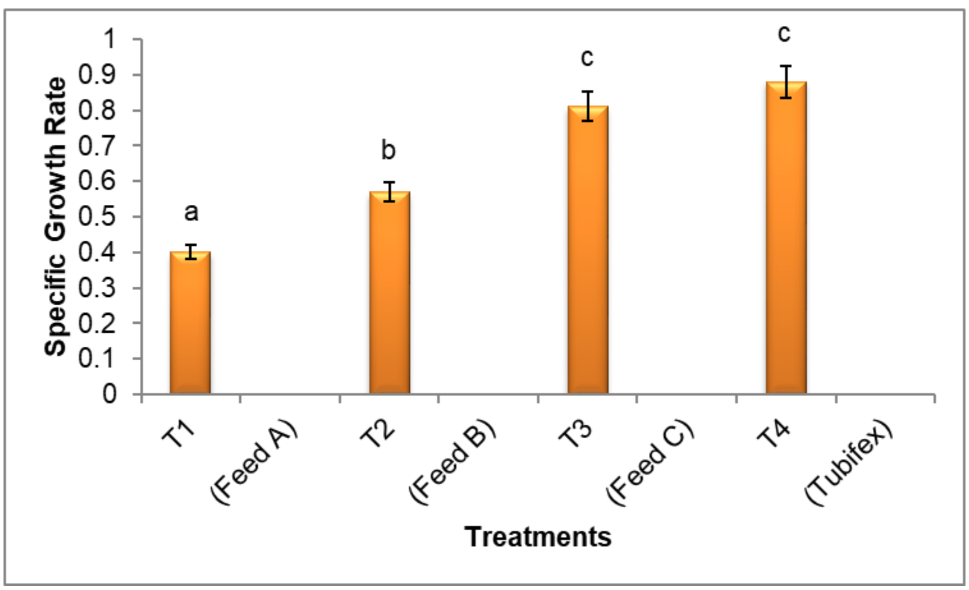

Fig 21: SGR of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

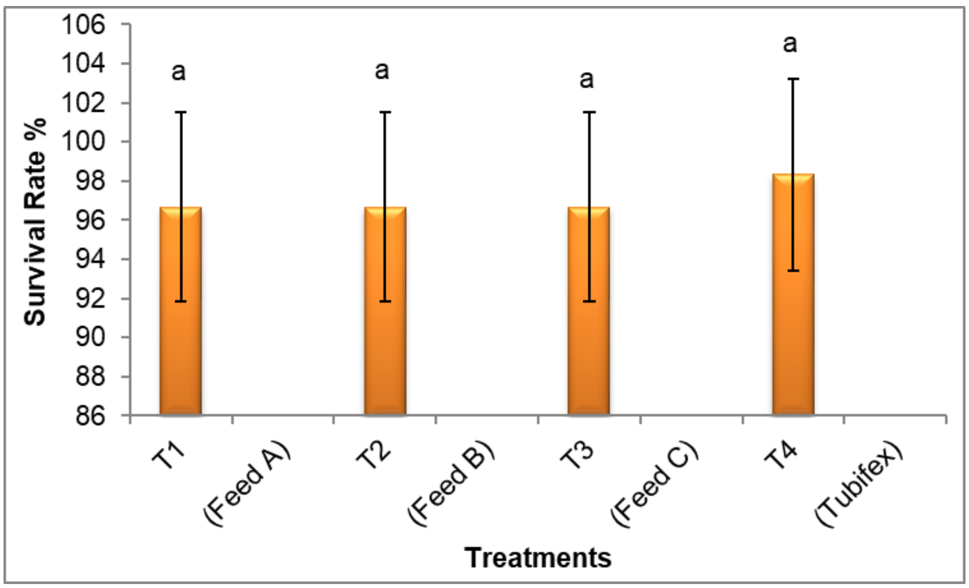

Fig 22: Survival rate % of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05)

4.5 Evaluation of the effect of nutritional intervention on reproductive performance of dwarf gourami, *Colisa lalia*

Reproductive performances of both male and female dwarf gourami, *Colisa lalia*, under different treatments are presented in the table 24 and table 25 given below. Their initial weight in all the treatments was in the range 1.2 – 1.4 g. A significant difference (P<0.05) was observed between the parameters.

4.5.1 Gonad weight of male dwarf gourami

The gonad weight found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.006 ± 0.000 g, 0.007 ± 0.000g, 0.008 ± 0.000 g and 0.009 ± 0.000 g respectively. The highest value of gonad weight of 0.009 g was observed in Treatment 4, which was fed with Combination diet (Feed C + Tubifex). The lowest value of gonad weight 0.006 g was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference (P<0.05) was observed in male gonad weight in all the treatments (Table 24). The length and weight of the respective fishes are given in the Table 24.

4.5.2 Gonad weight of female dwarf gourami

The gonad weight found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.061 ± 0.000 g, 0.079 ± 0.000 g, 0.082 ± 0.000 g and 0.090 ± 0.000 g respectively. The highest value of gonad weight of 0.09 g was observed in Treatment 4, which was fed with Combination diet (Feed C + Tubifex). The lowest value of gonad weight 0.061 g was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference (P<0.05) was observed in female gonad weights in all the treatments (Table 24). The length and weight of the respective fishes are given in the Table 24.

4.5.3 Gonadosomatic index of male dwarf gourami

The Gonadosomatic Index of male dwarf gourami found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.390 ± 0.008, 0.462 ± 0.002, 0.519 ± 0.007 and 0.538 ± 0.006 respectively. The highest value of GSI of 0.538 was observed in Treatment 4, which was fed with Combination diet (Feed C + Tubifex). The lowest value of gonad weight 0.390 was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference ($P<0.05$) was observed in GSI of male dwarf gourami in all the treatments (Table 24). Figure 23 shows the graphical representation of GSI of male *Colisa lalia* in all the four treatments.

4.5.4 Gonadosomatic index of female dwarf gourami

The Gonadosomatic Index of female dwarf gourami found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 4.154 ± 0.010, 5.310 ± 0.007, 5.403 ± 0.016 and 5.720 ± 0.013 respectively. The highest value of GSI of 5.72 was observed in Treatment 4, which was fed with Combination diet (Feed C + Tubifex). The lowest value of gonad weight 4.154 was observed in Treatment 1, which was fed with control feed (Feed A). Significant difference ($P<0.05$) was observed in GSI of female dwarf gourami in all the treatments (Table 24). Figure 24 shows the graphical representation of GSI of female *Colisa lalia* in all the four treatments.

4.5.5 Spawning success

The spawning success of dwarf gourami found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0 %, 0%, 100% and 100% respectively. Spawning success of 100 % was observed only in Treatment 3 and Treatment 4, which was fed with only live feed (Tubifex) and Combination diet (Feed C + Tubifex) respectively. No spawning was observed in Treatment 1 and Treatment 2, which was fed with Control diet (Feed A) and formulated diet (Feed C) (Table 25). Fig 25 shows the graphical representation of spawning success% in the four treatments.

4.5.6 Fertilization %

The fertilization % of dwarf gourami found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0%, 0%, 65.33 ± 1.76%, 70.67 ± 0.67% respectively. As no spawning was observed in Treatment 1 and Treatment 2, which was fed with Control diet (Feed A) and formulated diet (Feed C), there was no eggs spawned, thus no fertilization was there. Highest fertilization % of 70.67 % was observed in Treatment 4, which was fed with Combination diet (Feed C + Tubifex). Significant difference (P<0.05) was observed in Fertilization % in Treatment 3 and Treatment 4 (Table 25). Fig 25 shows the graphical representation of fertilization% in the four treatments.

4.5.7 Hatching %

The Hatching % of dwarf gourami found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0%, 0%, 35.33 ± 1.76% and 42.00 ± 1.15% respectively. As no spawning was observed in Treatment 1 and Treatment 2, which was fed with Control diet (Feed A) and formulated diet (Feed C), there were no eggs spawned, thus neither fertilization, nor hatching was observed. Highest hatching % of 42 % was observed in Treatment 4, which was fed with Combination diet (Feed C + Tubifex). Significant difference (P<0.05) was observed in hatching % in Treatment 3 and Treatment 4 (Table 25). Fig 25 shows the graphical representation of hatching% in the four treatments.

Table 24: Gonadosomatic index in different experimental diets of dwarf gourami, *Colisa lalia*

Parameters		T1	T2	T3	T4
Length (cm)	Male	4.51 ± 0.03[a]	4.48 ± 0.01[a]	4.60 ± 0.02[b]	4.63 ± 0.02[b]
	Female	4.17 ± 0.01[a]	4.23 ± 0.02[b]	4.23 ± 0.02[b]	4.27 ± 0.02[b]
Weight (g)	Male	1.56 ± 0.01[a]	1.59 ± 0.02[b]	1.63 ±0.01[c]	1.71 ± 0.01[d]
	Female	1.47 ± 0.01[a]	1.48 ± 0.01[b]	1.53 ± 0.02[c]	1.58 ±0.01[d]
Gonad weight (g)	Male	0.006 ± 0.000[a]	0.007 ± 0.000[b]	0.008 ± 0.000[c]	0.009 ± 0.000[d]
	Female	0.061 ± 0.000[a]	0.079 ± 0.000[b]	0.082 ± 0.000[c]	0.090 ± 0.000[d]
GSI	Male	0.390 ± 0.008[a]	0.462 ± 0.002[b]	0.519 ± 0.007[c]	0.538 ± 0.006[d]
	Female	4.154 ± 0.010[a]	5.310 ± 0.007[b]	5.403 ± 0.016[c]	5.720 ± 0.013[d]

Values are presented as Mean ± SE. The superscript letter indicates significant difference, P<0.05.

Table 25: Breeding success in different experimental diets of dwarf gourami, *Colisa lalia*

Parameters		T1	T2	T3	T4
Length	**Male**	4.40 ± 0.06^{a}	4.53 ± 0.03^{a}	4.53 ± 0.03^{a}	4.53 ± 0.03^{a}
	Female	1.53 ± 0.01^{a}	1.57 ± 0.01^{b}	1.61 ± 0.01^{c}	1.69 ± 0.01^{d}
Weight	**Male**	4.13 ± 0.03^{a}	4.17 ± 0.03^{a}	4.23 ± 0.03^{a}	4.23 ± 0.03^{a}
	Female	1.43 ± 0.01^{a}	1.46 ± 0.00^{b}	1.52 ± 0.01^{c}	1.53 ± 0.01^{c}
Spawning Success (%)		0	0	100	100
Fertilization %		0	0	65.33 ± 1.76^{a}	70.67 ± 0.67^{b}
Hatching %		0	0	35.33 ± 1.76^{a}	42.00 ± 1.15^{b}

Values are presented as Mean ± SE. The superscript letter indicates significant difference, $P<0.05$.

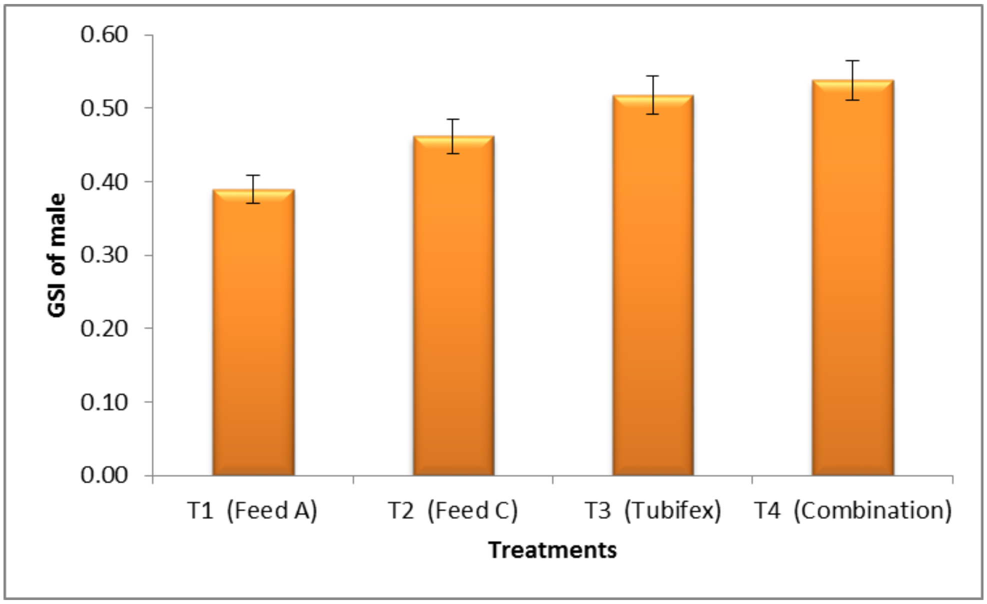

Fig 23: Gonadosomatic Index of male in different experimental diets of *Colisa lalia.* Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

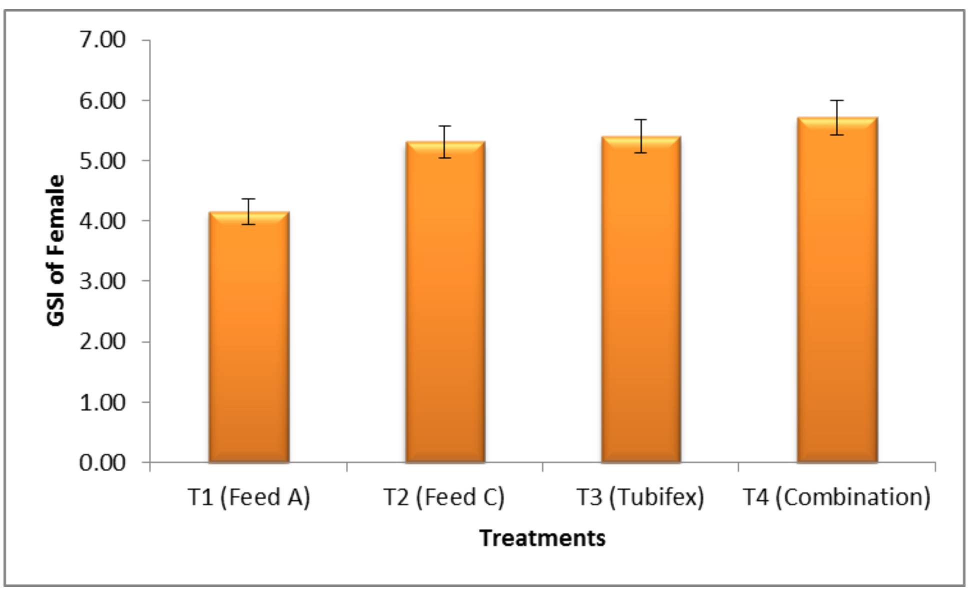

Fig 24: Gonadosomatic Index of female in different experimental diets of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

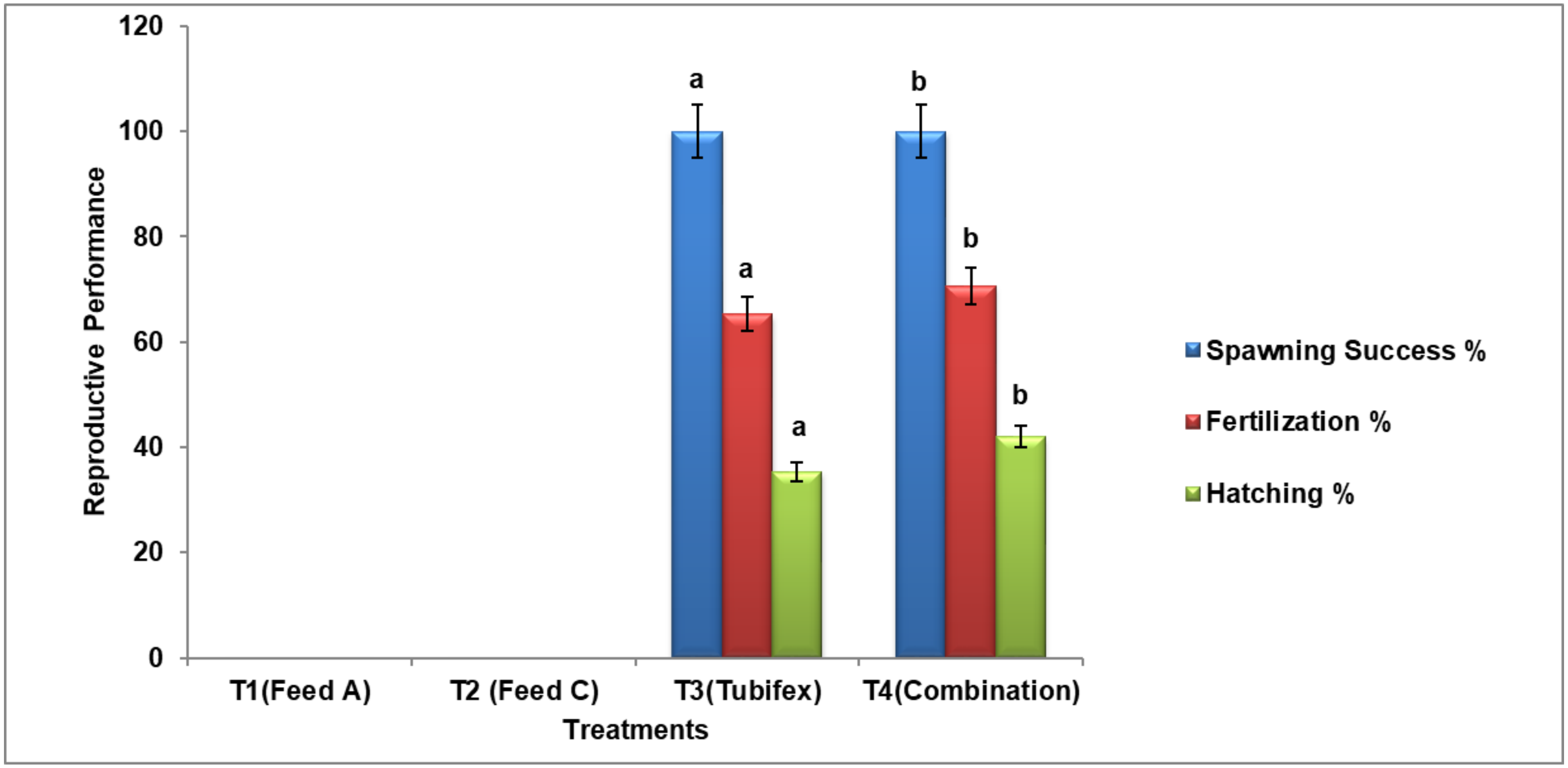

Fig 25: Spawning success%, fertilization% and hatching% in the four treatments of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

4.6 Analysis of larval rearing of dwarf gourami, *Colisa lalia*

Larval growth performance and survivability of dwarf gourami, *Colisa lalia* (Hamilton, 1822) over a time period of thirty days and subjected to three treatments with different feeds are given below in the Table 26. A significant difference (P<0.05) was observed between the treatments in terms of length gain and survival rate.

4.6.1 Length gain

Size variation in larval length was observed in all the treatments as shown in Table 26. The length gain achieved in Treatment 1, Treatment 2 and Treatment 3 are 10.54 ± 0.07 mm, 5.84 ± 0.07mm and 7.71 ± 0.12 mm respectively. The highest value of length gain of 10.54 ± 0.07 mm was observed in Treatment 1, which was fed with live feed, Infusoria. The lowest value of length gain of 5.84 ± 0.07 mm was observed in Treatment 2, which was fed with Feed C. Significant difference (P<0.05) was observed in length gain in all the treatments (Table 26). Figure 26 shows the graphical representation.

4.6.2 Survival Rate %

The survival rate % achieved in Treatment 1, Treatment 2 and Treatment 3 is 66.33 ± 0.88 %, 52.33 ± 2.19% and 34.00 ± 1.15% respectively. The highest value of survival rate of 66.33 ± 0.88 % was observed in Treatment 1, which was fed with live feed, Infusoria. The lowest value of survival rate % of 34.00 ± 1.15% was observed in Treatment 3, which was fed with boiled egg yolk. Significant difference (P<0.05) was observed in survival rate % in all the treatments (Table 26). Figure 27 shows the graphical representation.

Table 26: Growth performance of larvae due to different types of diet in dwarf gourami, *Colisa lalia*

Treatment	Length gain (mm)	Survival Rate %
T1	10.54 ± 0.07^{b}	66.33 ± 0.88^{a}
T2	5.84 ± 0.07^{c}	52.33 ± 2.19^{b}
T3	7.71 ± 0.12^{a}	34.00 ± 1.15^{c}

Values are presented as Mean ± SE. The superscript letter indicates significant difference, P<0.05.

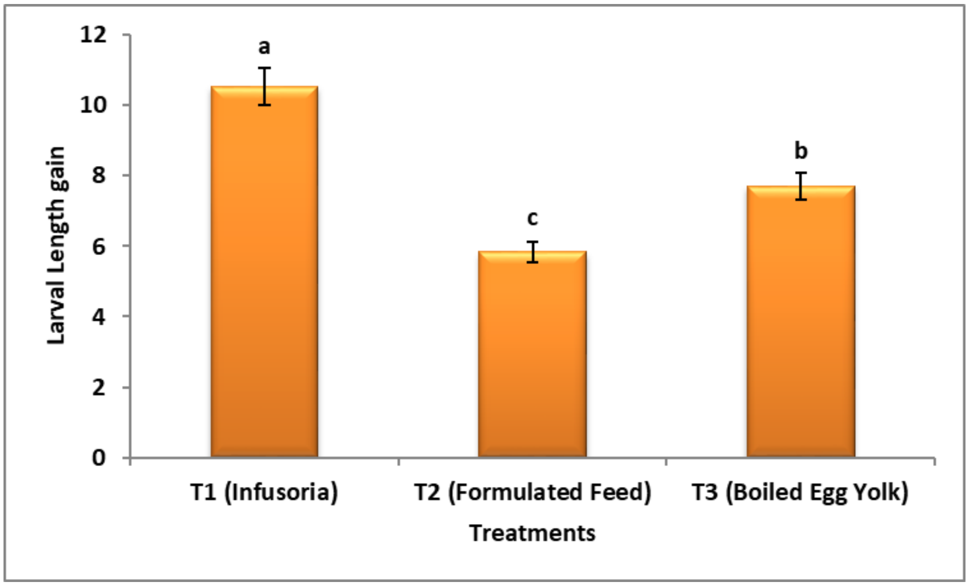

Fig 26: Larval length gain in three treatments of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

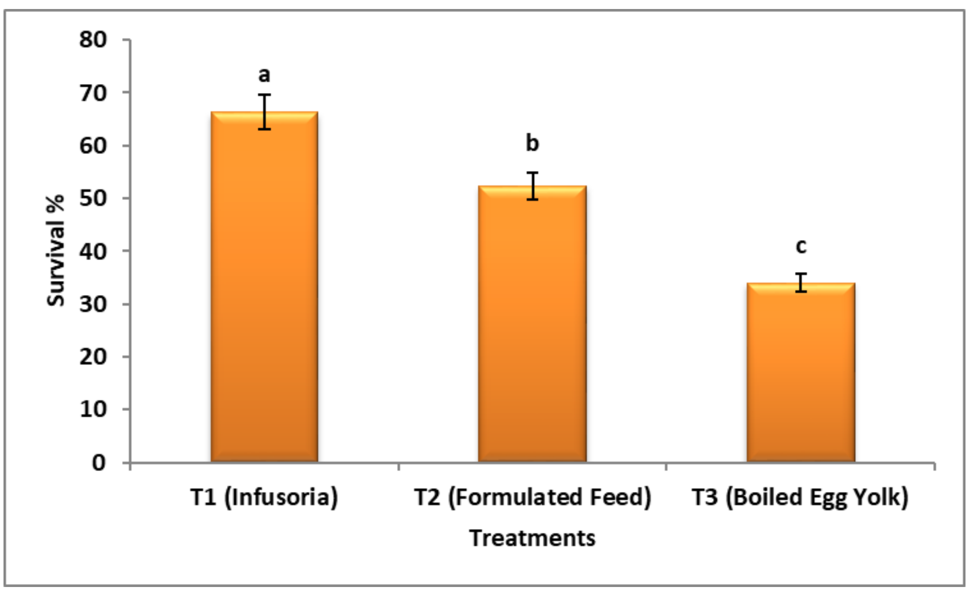

Fig 27: Larval survival rate in three treatments of *Colisa lalia*. Values are presented as Mean ± SE. Treatments with different superscripts indicate significant differences (P<0.05).

4.7 Water quality parameters

As there are two experimental sites, water quality parameters of both the sites were analyzed. At ICAR-CIFE, Kolkata Centre the water quality parameters were analyzed for the four treatments of the experiment for growth performance through nutritional interventions. Municipality supply water was used, after overnight conditioning, in the rearing experiment. It was observed that there was no significant difference among the treatments in the four treatments with respect to temperature, pH, Dissolved oxygen, Hardness and Total Alkalinity. The temperature remained in a suitable range of 24 – 25 ^{o}C, for the experimental 90 days. Both alkalinity and hardness remained in the range of 139 – 140 ppm, with almost neutral pH of 7. The dissolved oxygen concentration was also at a good level of 7 ppm in all the four treatments. The details are given in the table 27.

Water quality parameters for rearing and spawning at ICAR-CIFE, Mumbai were analyzed for twelve months from July 2018 to June 2019. The available bore well water was used for the rearing and spawning activities at Mumbai. There was no significant difference observed in all the parameters analyzed for 12 months, except the temperature which remained within a range of 20 – 28^{o}C, having seasonal fluctuations. Hardness was in the range of 224.00 - 231.50 ppm, Total alkalinity in the range 176.75 - 185.25 and pH in the range 7.13 – 7.25. Dissolved oxygen range was in range of 5.13 - 5.38 ppm. The monthly details of the water quality parameters are given below in the table 28.

Table 27: Water quality parameters for rearing at ICAR-CIFE, Kolkata

Treatments	Temperature (^oC)	pH	Dissolved O_2 (ppm)	Hardness (ppm)	Total Alkalinity (ppm)
T1	25.00±0.41	7.67 ± 0.09	7.57 ± 0.23	139.00 ± 1.00	139.00 ± 3.58
T2	24.88±0.25	7.70 ± 0.12	7.73 ± 0.13	139.33 ± 0.33	139.00 ± 2.58
T3	24.88±0.25	7.63 ± 0.15	7.70 ± 0.15	139.33 ± 1.20	139.00 ± 2.58
T4	24.88±0.25	7.70 ± 0.12	7.67 ± 0.18	139.00 ± 0.58	138.67 ± 2.67

Table 28: Water quality parameters for rearing at ICAR-CIFE, Mumbai

MONTHS	Temperature (^{o}C)	pH	Dissolved O_2 (ppm)	Hardness (ppm)	Total Alkalinity (ppm)
JULY	24.28 ±0.17	7.25±0.29	5.23±0.05	226.00±1.63	179.00±0.82
AUGUST	24.30±0.14	7.13±0.25	5.25±0.06	224.00±2.16	179.25±1.26
SEPTEMBER	24.78±0.10	7.13±0.25	5.28±0.05	224.00±0.82	185.25±1.50
OCTOBER	27.65±0.13	7.25±0.29	5.13±0.05	228.00±1.41	183.50±1.91
NOVEMBER	26.50±0.08	7.13±0.25	5.15±0.06	226.25±1.26	178.50±0.58
DECEMBER	26.05±0.37	7.25±0.29	5.28±0.05	226.75±0.96	178.75±0.50
JANUARY	20.48±0.17	7.13±0.25	5.38±0.05	227.50±1.00	184.75±1.50
FEBRUARY	24.60±0.08	7.13±0.25	5.30±0.00	228.25±0.50	183.50±1.91
MARCH	25.63±0.10	7.25±0.29	5.23±0.05	231.50±0.58	178.50±0.58
APRIL	26.60±0.26	7.13±0.25	5.23±0.05	229.50±0.58	176.75±3.40
MAY	27.18±0.33	7.25±0.29	5.18±0.05	228.00±0.82	183.75±1.89
JUNE	23.05±0.48	7.13±0.25	5.35±0.06	226.25±0.50	182.75±0.96
RANGE	**20.48 - 27.65**	**7.13 -7.25**	**5.13 - 5.38**	**224.00 - 231.50**	**176.75 - 185.25**

5. DISCUSSION

The present study was carried out to strengthen the breeding and propagation of dwarf gourami, *Colisa lalia*. Market demand of this species is based on its availability at that particular area where the market is located. As the work has been carried out with this fish at Kolkata (East of India) and Mumbai (West of India), it is observed that due to plenty of natural production at the rivers of eastern India its cost is less there, whereas the cost is quite high in western part. *Colisa lalia* have a good market outside India and the cost is around US$ 5 – US$ 6 per unit of fish. The export market is purely based upon the wild collection, which can cause the depletion of this indigenous variety in future. The study of the biology of *Colisa lalia* gives idea regarding its feeding and reproductive aspects. The experiments based on the nutritional interventions gives an idea regarding the type of feed it requires for rearing and breeding practices of the adult dwarf gourami. Development in larval rearing practices for ornamental fish larvae is considered as an urgent necessity as there is a demand for ornamental fish seed supply. The experiment on the nutritional interventions for larval rearing strengthens us to rear the larvae for the future market of dwarf gourami, *Colisa lalia*.

5.1 General Biology

5.1.1 Morphometrics

Ten morphometric characters were measured of dwarf gourami, *Colisa lalia*. Their Mean ± (SD) value are as follows; Total length (TL) 4.06±0.21, Standard Length (SL) 3.25±0.18, Pre-dorsal Length (PDL) 1.42±0.11, Pre-anal length (PAL) 1.57±0.11, Pre-pectoral length (PPL) 1.15±0.07, Pre-pelvic / ventral length (PVL) 1.17±0.07, Head length (HL) 1.12±0.07, Body Depth (BD) 1.51±0.10, Eye Diameter (ED) 0.30±0.02, Snout Length (SnL) 0.28±0.04. These measurements denote that dwarf gourami,*Colisa lalia,* has small size oval body, which is strongly compressed.

Correlation Coefficient, Regression Equation and R^2 Value were found out of nine morphometric characters in relation with Total Length (TL). Highest correlation coefficient (0.93), R^2 value² of 0.8642 for regression equation y = 0.8108x + 0.0403 was observed in case of Standard length (SL) and lowest in case of Eye Diameter (ED) where correlation coefficient and R^2 value were 0.18 and 0.0333 for regression equation y = 0.0191x + 0.2274. There is a positive correlation between all the characters. This means when one character increases in size, the other morphometric characters too increases in size, but the frequency of growth rate among them varies. This result shows that there is allometric growth pattern in dwarf gourami, *Colisa lalia.* With the increase in TL only the SL increases, other morphometric characters do not show any remarkable increase. The ED remains within the range of 0.3 - 0.4 cm for any length of the fish. This implies that there is hardly any remarkable change in the eye diameter with the growth of the fish. In *Tor putitora* Bhatt *et al.* (1998) reported eye diameter as a least correlated variable.

Correlation Coefficient, Regression Equation and R^2 Value were also found out of Eye Diameter (ED) and Snout Length (SnL) in relation with Head Length (HL).Correlation Coefficient value was 0.19 and R^2 value was 0.0375 for regression equation y = 0.0627x + 0.2347 for ED. Correlation Coefficient value was 0.23 and R^2 value was 0.0539 for regression equation y = 0.1448x + 0.1165 for SnL. This denotes that although there is positive correlation, but very less change in length in the ED and SnL occurs with respect to the increase in the HL.

Similar morphometric studies were conducted on banded gourami, *Colisa fasciata*, by Akter *et al*. (2016). Similar work on morphometric and meristic study was carried out by Dube and Dubey (1986-87) on *Tor tor* from Narmada River.

5.1.2 Meristics

The meristic count ranges of dwarf gourami, *Colisa lalia* are Dorsal Spines (DS) 15 – 17, Dorsal Soft Rays (DSR) 7 – 9, Anal spines (AS) 16 – 19, Anal Soft Rays (ASR) 14 -17, Pelvic Fin (V) 2 – 2, Pectoral Fin (P) 8 – 10, Caudal Fin (C)14 – 16. Fin Formula of dwarf gourami, *Colisa lalia* was formed by taking the mode values; D. XV/8; P.8; A. XVII/15; V. 2; C. 14. Similar observations are reported by Shafi and Quddus (1982),Talwar and Jhingran (2001) and Rahman (2005) in *Colisa lalia*.

In the present study there is variation in the meristic count, which may be due to the variations in the size of the fish. There are many reports on differences in meristic characters in many fishes such as *Pterophyllum scalare* (Bibi *et al.,* 2008), *Crossocheilus latius latius* (Brraich and Akhter, 2015), *Pseudobagrus ichikawai* (Watanabe, 1998), *Nematalosa nasus* (AlHassan, 1987).

5.2 Feeding Biology

5.2.1 Relative gut length

The Relative Gut Length of dwarf gourami, *Colisa lalia* was found out to be 2.29 ± 0.42 (standard deviation) with a range of 1.43 – 2.9. The fish sample taken for study had mean total length of 4.28 ± 0.26 cm having a range of 3.8 – 4.9 cm. The mean gut length of the fish was 9.78 ± 1.92 cm, having a range of 6 – 12.5 cm. The result denotes that *Colisa lalia* is omnivorous in nature, which feeds on both plant and animal nutrients. This result is supported by Sahu (2017). Bhatti (1943) and Rao (2014) reported it as carnivorous fish, feeding on mosquito larvae.

Correlation Coefficient, R^2 Value and Regression Equation were also found out of Gut Length and Relative Gut Length in comparison with Total Length (TL). Gut Length showed 0.341317 correlation coefficient and R^2 value was 0.1165 in comparison to TL. Relative Gut Length showed 0.034974 correlation coefficient and R^2 value was 0.0012 in comparison to TL. Positive correlation is observed between GL and RGL with TL, but it is very weak and linear regression is not found among them. This result denotes that the GL changes according to the TL to some extent, but there is least change of RGL in relation to the TL. Khongngain *et al.* (2017) carried out similar studies on *Trichogaster faciata*, and reported similar results.

5.2.2 Gastro-somatic index

Gastro-somatic index (GaSI) of dwarf gourami, *Colisa lalia* was found out for 12 months from July, 2018 to June 2019, for both male and female. The GaSI of the male dwarf gourami found for the month of July 2018, August 2018, September 2018, October 2018, November 2018, December 2018, January 2019, February 2019, March 2019, April 2019, May 2019 and June 2019 are 1.98 ± 0.06, 2.17 ± 0.07, 2.42 ± 0.04, 2.62 ± 0.07, 2.88 ± 0.14, 3.11 ± 0.11, 2.80 ± 0.10, 2.60 ± 0.07, 2.53 ± 0.07, 2.37 ± 0.07, 2.02 ± 0.05 and 1.88 ± 0.03 respectively, and for female are 1.28 ± 0.12, 2.90 ± 0.05, 3.68 ± 0.07, 4.07± 0.06, 4.46 ± 0.08, 4.75 ± 0.13, 4.20 ± 0.20, 3.93 ± 0.15, 3.58 ± 0.04, 3.43 ± 0.17, 2.26 ±0.05b and 1.20 ±0.07 respectively. There was significant difference ($p<0.05$) observed between the GaSI of both male and dwarf gourami in all the twelve months.

Highest value of GaSI in both male and female was observed in the month of December 2018 having the value of 3.11 ± 0.1 and 4.75 ± 0.13 respectively. Lowest value of GaSI was observed in the month of June 2019, having the value of 1.88 ±0.03 and 1.20 ± 0.07 for male and female respectively. Significant difference ($p<0.05$) in both length and weight was observed in the male dwarf gourami in all the twelve months. Significant difference ($p<0.05$) in weight was observed, but no significant difference ($p>0.05$) is observed in the length of female gourami in all the twelve months.

From the observations it can be concluded that the feeding intensity of *Colisa lalia* is more in the winter months, highest in December. The feeding intensity is less in pre monsoon months and lowest in June, at the captive conditions of Mumbai. This denotes that *Colisa lalia,* being a tropical fish, needs warmer temperature for gonadal maturation and breeding purposes. Therefore during the warmer season, the species have a low feeding intensity. In the colder months the species have higher feeding intensity, to recover the loss after reproductive activities carried out during the warmer months.

Khongngain *et al.* (2017) carried out similar studies on *Trichogaster faciata*, and reported similar results. Chaturvedi and Saksena (2013) studied GaSI of *Mystus cavasius* and reported maximum value in the month of January and minimum during the month of June which has resemblance with the present study. Dewan and Saha (1979) reported that Tilapia species showed low feeding intensity during the month of

February to June. Mushahida-Al-Noor *et al*. (2013) found maximum number of empty stomachs in the month of June, in *Rita rita*.

5.3 Reproductive Biology

5.3.1 Sexual dimorphism

For successful induced breeding, the identification of sex is quite necessary. The study of secondary sexual characteristics is helpful for segregation of sex and other biological studies (Rath, 2000). In the present study, *Colisa lalia* was observed from June 2018 to July 2019 to understand the changes in the external morphology for identification of sex during breeding and non-breeding season. During the period of investigation the secondary sexual characters like colour of the body, colour of fins, body size, abdominal shape, dorsal and anal fin of the fish were observed.

Colour of the body in male is translucent blue with alternating vertical diagonal strips of red and dark orange bands. During breeding season, a matured male can be identified by seeing the blue patch of colour at the ventral region between the opercula and the pectoral fin. This blue patch is not seen in immature males. The fins are as colourful as the body. Body size is comparatively larger than the female, with slender and sleek abdomen. Dorsal and anal fins are pointed.

Colour of the body in female is dull silvery blue to gray colour. Colour of fins is yellowish grey. Body size is comparatively smaller than male. Enlarged fat abdomen can be observed, but only in breeding season. Dorsal and Anal fins are curved.

Similar studies on *Colisa fasciata* were carried out by Swarup *et al.* (1972), Das and Kalita (2006) and Dehadri *et al.* (1973). The earlier workers Mookerjee and Mazumdar (1946), Dehadrai *et al.* (1973) and Khan (2004) had identified the sex of fish on the basis of their colour. *Colisa lalia* is an ornamental fish, which has an attractive colour pattern. The colour pattern of male and female is completely different from each other and maintained throughout the year in a constant manner which helps us to identify the sex during breeding and non-breeding season. Therefore, in the present study, the colour pattern of the fish was found to be a suitable criterion for identification of sex.

5.3.2 Gonadosomatic Index

Gonadosomatic index (GSI) of dwarf gourami, *Colisa lalia* was found out for 12 months from July 2018 to June 2019. The GSI of the male dwarf gourami found for the month of July 2018, August 2018, September 2018, October 2018, November 2018, December 2018, January 2019, February 2019, March 2019, April 2019, May 2019 and June 2019 are 0.563 ± 0.01, 0.482 ± 0.02, 0.361 ± 0.01, 0.330 ± 0.01, 0.305 ± 0.00, 0.314 ± 0.01, 0.352 ± 0.01, 0.392 ± 0.01, 0.413 ± 0.00, 0.463 ± 0.02, 0.528 ± 0.02 and 0.585 ± 0.01 respectively and for female are 5.92 ± 0.17, 5.41 ± 0.05, 4.36 ± 0.05, 3.88 ± 0.21, 2.97 ± 0.02, 3.44 ± 0.05, 3.70 ± 0.02, 4.05 ± 0.05, 4.50 ± 0.31, 5.23 ± 0.09, 5.50 ± 0.06 and 6.08 ± 0.14 respectively .

There was significant difference ($p<0.05$) observed between the GSI of male dwarf gourami in all the twelve months. Highest value of GSI in male was observed in the month of June 2019 having the value of 0.585 ± 0.01. Lowest value of GSI was observed in the month of November 2018, having the value of 0.305 ± 0.00. Significant difference ($p<0.05$) in both length and weight was observed in the male dwarf gourami in all the twelve months. There was significant difference ($p<0.05$) observed between the GSI of female dwarf gourami in all the twelve months. Highest value of GSI in male was observed in the month of June 2019 having the value of 6.08 ± 0.14. Lowest value of GSI was observed in the month of November 2018, having the value of 2.97 ± 0.02. Significant difference ($p<0.05$) in weight is observed, but no significant difference ($p>0.05$) is observed in the length of female gourami in all the twelve months.

The GSI of the fish increases with the maturation of fish, being highest during the period of peak maturity and declining after spawning (Le Cren, 1951; Verma, 1997 and Khanna, 2002). The similar fact was also observed in the present investigation. The GSI of *Colisa lalia* was in an increasing trend from March to June and reached its peak in June. On the other hand, the GSI value was declining abruptly from August and continued up to November-December. Then it again showed an increasing trend of variation from January to June.

The length of breeding period is thus extremely variable, and some species spawn only once and others twice, while still others may spawn several times during the year (Khanna, 2002). Based on GSI, several authors reported the spawning season which differ from species to species of fishes. Spawning takes place between March to June in *Mystus seenghala* (Sathyanesan, 1962), from April to September in *Clarias batrachus* (Lehri, 1967), from July to September in case of *Channa punctatus* (Belsare, 1962), from June to August *Channa gachua* (Khanna and Sanwal, 1971) and *Anabas testudineus* (Das, 2002).

Quasim and Qayyum (1961) reported breeding season of *Puntius sarana* extended from late June to early September, with peak June-August in northern India. Borah (2000) observed the spawning season of *P. sarana* was during April to July, with a peak during May to June in Assam. It might be attributed to the onset of monsoon in Assam and West Bengal as well as environmental factors like photoperiod and temperature.

In the present observation at the captive conditions of Mumbai, the GSI of male *Colisa lalia* is more than 0.5 in May, June and July and in case of female the GSI is more than 5 from April to August. These sequential changes in GSI value indicated that the spawning season of *Colisa lalia* can be considered between April to August with a peak period during May to July. However Sutradhar *et al.* (2016) reported peak spawning season of *Colisa lalia* during May-June.

Khan (2004) has reported that, the breeding season of *Colisa fasciata* from April to July. Mitra *et al.* (2007) have reported March to October as breeding season of *C. fasciata* in West Bengal. Das and Kalita (2006) have documented April to August for *C. fasciata* in Assam.

5.3.3 Gonad morphology

The testes are paired but asymmetrical and attached to each other. In the present study the colour of testis was varied from pale white to creamy white or pinkish red due to enhanced blood circulation. The size of the testis is comparatively more in breeding season in comparison to the non-breeding season. Colour variation is seen in the testis during breeding season and non-breeding season (Pandey, 1987; Besra, 1997 and Ziauddin, 2002).

The telostean testis is usually paired and attached to the dorsal wall of the body cavity by a thin layer of mesorchia. They are either fused along the entire length (Khanna and Pant, 1966) or completely separated (Dixit and Agarwal, 1974) or fused at the posterior region only (Rai, 1965; Sanwal and Khanna, 1972; Bisht, 1974; Pandey and Mishra, 1981). In the present study, it was found that the testis of *Colisa lalia* was paired and asymmetric throughout the year even during breeding season. They are fused along their posterior end to form a common spermatic duct which is same as the earlier findings on Anabas (Das, 2002), *Colisa lalia* (Banu and Bhakta, 1985) and *Colisa fasciatus* (Khan, 2004).

In the present observation, the ovary of *Colisa lalia* was paired and attached to the dorsal wall of the body cavity by a short thick mesovarium. The two ovaries lie close to each other. Anteriorly, each of them has its end free, while at the posterior end, it extends into a thick-walled oviduct. Similar observation was made by Khanna (1996) and Verma (1997).

In the present study, the colour of ovary of *Colisa lalia* became less bright during non-breeding phase because of empty lobulation of the ovary. On the other hand, blood supply to the ovary is reduced due to less content of ova. It is with the agreement of the observation of Agarwal (1996) that the colour of the ovaries becomes dirty yellowish during this season. During breeding season, the colour of ovary became bright yellow or pinkish-yellow due to maximum blood supply to the ovary for nourishment of eggs. The ovarian wall of the fish became thin and the eggs with its contents are visible with a yellow colour.

5.3.4 Histological study of gonadal development

In the present study of histology of testis of *Colisa lalia*, 5 phases of testicular cycles were observed during the study period of one year; Developing I Phase, Developing II Phase, Spawning Capable Phase, Actively Spawning Phase and Spent Phase Testis. The phases are described as per the standard terminology given by Brown-Peterson *et al*., 2011. The Primary spermatocytes undergo mitotic division to form secondary spermatocytes. The life of secondary spermatocytes is relatively shorter and they rapidly divide to form spermatids. One primary spermatocyte after one complete mitotic division produces four spermatids. The spermatids transform into mature spermatozoa by reorganization of nucleus, cytoplasmic components and acquire flagellum for mobility (Nagahama, 1983; Agarwal, 1996).

In the present study of histology of ovary of *Colisa lalia*, 4 phases of ovarian cycles were observed during the study period of one year; Immature Phase,Developing I Phase, Developing II Phase and Spawning Capable Phase ovary. The phases are described as per the standard terminology given by Brown-Peterson *et al*., 2011. Histological observation of ovaries showed the different stages of ova in various quantities in turn of the ovarian cycle. The germ cells or oogonia were found in the ovigerous lamellae and originate from germinal epithelium. The oogonia undergo proliferation through meiotic division and are known as oocytes, which enter to a period of growth. By the process of oogenesis, oocyte becomes a ripe egg inside the follicle. The development of the primary spermatogonia into secondary spermatogonia is very rapid as the histology revealed very few primary spermatogonia in the sections.

The occurrence of the particular phase of the gonads was not confined to a particular month of the year. Mixed stages were found in different individuals in particular month. In case of male dwarf gourami, *Colisa lalia* higher percentage of matured gonads were found between May to July and a little lesser percentage of matured gonads are found in the months before and after the above described months. Similar observations are also found in the case of ovary of female dwarf gourami, *Colisa lalia.* This implies that this particular species needs warm environment for gonadal maturation and breeding. According to Shim *et al*. (1987) in temperate regions dwarf gourami takes 8 to 12 months to mature but in tropical regions dwarf gourami, *Colisa lalia* can breed all year round with newly hatched fry reaching sexual maturity in as little as four months.

From the present histological analysis of the gonads of dwarf gourami, *Colisa lalia,* in the captive conditions of Mumbai, it can be concluded that this species can breed throughout the year through temperature manipulation and nutritional interventions. The presence of multiple oocyte stages found in the free end of the ovary and presence of multiple sperm stages in the testis, proves it to be batch spawner. According to Sadovy (1996) iteroparous batch spawners demonstrate different oocyte recruitment patterns. These patterns are affected in part by oocyte developmental rates and their relationship to metabolic rates and water temperature.

According to Hunter and Goldberg (1980) many batch spawners in warm water habitats exhibit continuous oocyte recruitment, repeatedly recruiting oocytes from primary growth to secondary growth, thus increasing their fecundity and enhancing their ability to spawn over an stretched time period and these species are considered to have indeterminate fecundity. The present study also corroborates with the earlier observation.

5.3.5 Breeding behavior

Floating plastic substrate was provided to support the bubble nest as dwarf gourami, *Colisa lalia* is a bubble nest builder. Chasing activity was observed after the female was introduced to the breeding setup with bubble nest. After the chasing activity, courtship behaviour was observed, followed by spawning activity. Parental care was observed by the male, not by the female, after spawning, as the male deposited the eggs in the bubble nest and chased away the female from the bubble nest. Present observations are similar to the observations of Saha *et al*. (2017) in *Colisa lalia*.

In this present study the incubation period was recorded 24-25 hours. Mitra *et al* (2006) reported incubation period to be 28-30 hours in honey gourami, *Colisa sota*. According to Bindu *et al.* (2014) the hatching period of *Trichogaster trichopterus* was reported to be 24 hours at water temperature of 26-27 ^{0}C which was similar to the present observation. According to Burman *et al.* (2013) the incubation period was varied from 24-30 hours in *Colisa faciatus*.

Parental care was still observed by the male for the hatchlings too, but not for a longer period. Cannibalistic activity was observed by male dwarf gourami later, when there is development of movement in the hatchlings.

5.3.6 Fecundity

Fecundity is defined as “The number of ova that are likely to be laid by a fish during spawning season”. The numbers of eggs produced by a fish differ in different species, and depends upon the size and age of the fish. Fecundity may also differ in different races of the same species (Khanna, 2002). The role of fecundity has been acknowledged by the fishery biologists to understand the population of fishes, as fecundity is one of the decisive factors in the formation of the new year-class which ensure the replacement of the stock (Khan *et al*., 2001).

In the present study, the average pre-spawning absolute fecundity of *Colisa lalia* in the captive conditions was 439 ± 49 for 4.44± 0.11 cm length and 1.53± 0.07 g weight of fish. Results differ from the findings of Sutradhar *et al.* (2016), who reported fecundity of 1024 to 1351 in 1.24 g to 1.81 g weight of *Colisa lalia*.

In my study, absolute fecundity of *Colisa lalia* in the captive conditions has the highest correlation with the gonad weight (0.8), followed by the body weight (0.4) and least correlation with the body length (0.3). Linear regression coefficient was found only between fecundity and gonad weight. This denotes that the increase in the gonad weight has more impact on the increase of absolute fecundity of *Colisa lalia*, in captive conditions. Similar observations were reported by Bhuiyan *et al.* (1995) and Khan (2004) in *Colisa fasciata*.

5.3.7 Ova diameter

In the present study, the mean ova diameter observed was found to be 0.265 mm, with 0.003 standard deviation and range of 0.271 - 0.261mm. The size of the ova diameter increases from its origination as oogonia to the matured egg, which can be fertilized and embryo can be formed. This is a proof of the histological study carried out of the different phases of the ovary. The size which I am describing is of the matured water hardened spawned egg diameter. Barman *et al*., (2013) carried out similar work on *Colisa fasciatus,* and reported similar results.

5.3.8. Egg stages

After 25 -30 min of spawning, non-adhesive and optically transparent fertilized egg is observed with clear distinction between perivitelline space and egg-yolk. Cytoplasm movement could also be observed. After 45 – 55 min early Gastrula phase could be observed where involution, convergence and extension were occurring. Completion of the gastrula phase took a longer period of time around 6 – 7 hr. In the Gastrula phase three layers of germ-cells are produced and the egg looks like hollow cup-shaped structure. After 17-19 hr embryonic body formation occurred, giving rise to pharyngula stage, where embryo body is formed. Heart beat and twitching movement observed. After 24-25 hr lots of twitching movement is observed as the embryo pushes the egg envelope and removes it and comes out as a hatchling. In just hatched larvae no movement is observed, due to exhaustion of hatching. Only cytoplasmic circulation and heart beat observed.

Saha *et al*. (2017) reported similar observations in embryonic study on *Colisa lalia.* Barman *et al.* (2013) and Islam *et al.* (2017) reported similar observations in embryonic study on *Colisa fasciatus.* Bindu *et al.* (2014) reported similar observations in embryonic study on *Trichogaster trichopterus.*

5.3.9. Larval stages

In newly hatched larvae, we can find optic vesicles, myotome, yolk sac, heartbeat and oil globules. There is no locomotion done by the larvae but there is circulation of fluid. In one day old larvae, we can find eye bulbs, myotome, and heart. Yolk sac is reduced. The body is transparent but pigmentation can be found. In two days old larvae, we can find dark eye bulbs, mouth lining, nostrils, pectoral fins, alimentary canal, myotome, heart. Yolk sac reduced than before. There is fast movement observed in the larvae. In five days old larvae, we can find eyes with pupil, mouth opening, nostrils, and developed pectoral fin. Yolk sac is pushed upwards. Jaws become stronger. The notochord becomes segmented. It moves very fast. In ten days old larvae, we can find air bladder. With the development of muscles and bones the larvae is becoming opaque. There is a lot of pigmentation. Yolk sac is almost completely absorbed. In fifteen days old larvae, we can find the different organs. Caudal rays are well developed in the caudal fin.

Saha *et al*., (2018) reported similar observations in larval development of *Colisa lalia.* Barman *et al* (2013) reported similar observations in larval development of *Colisa fasciatus.* Bindu *et al.* (2014) reported similar observations in larval development of *Trichogaster trichopterus.*

5.4 Growth Performance

The length gain achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.56 ± 0.03 cm, 0.62 ± 0.05 cm, 0.78 ± 0.02 cm and 1.03 ± 0.04 cm respectively. No significant difference was observed in Treatment 1 and Treatment 2, but significant difference was observed in Treatment 3 and Treatment 4. Treatment 4, which was fed with live feed, *Tubifex* have highest value of length gain. The protein content of Tubifex is more than 60%, which may be the reason of better length gain.

The weight gain achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.41 ± 0.06 g, 0.63 ± 0.05 g, 1.00 ± 0.01 g and 1.12 ± 0.02 g respectively. The weight gain percentage achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 43.90 ± 6.88 %, 67.67 ± 4.97%, 108.03 ± 0.99% and 120.75 ± 1.63% respectively. The specific growth rate achieved in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.40 ± 0.05, 0.57 ± 0.03, 0.81 ± 0.01, 0.88 ± 0.01 respectively.

Significant difference was observed in weight gain, weight gain percentage and specific growth rate in Treatment 1 and Treatment 2, but no significant difference was observed in weight gain in Treatment 3 and Treatment 4. Treatment 3 and Treatment 4 gave best results in weight gain, weight gain percentage and specific growth rate, which shows that formulated feed with 4% supplementary diet mix can bring similar effect as live feed in dwarf gourami, *Colisa lalia*.

Experimental diets used in all the treatments were isonitrogenous and isocaloric, except T4, which was fed with live feed. The major difference in the formulated feeds was the addition of supplementary diet mix in 2 % and 4% in T2 and T3, which constituted of refined lecithin (40%), α Tocopheral acetate (1.5%), Celin (L-ascorbic acid) (2.5%), Spirulina powder (50%)and L-tryptophan (6%).

Using dried Spirulina as a feed supplement, several studies have been conducted (Chow and Woo, 1990; Watanabe *et al*., 1990). According to Belay *et al*., (1996) spirulina is considered as a rich source of protein, vitamins, minerals, essential amino acids, and fatty acids (gamma - linolenic acid) and antioxidant pigments such as carotenoids. According to Takeuchi *et al*., (2002) spirulina is also effective as an immunomodulatory. Spirulina is a biomass of cyanobacteria having more than 55% protein content. In the present study, there is 2 % incorporation of spirulina in T3, which gave us better results in growth. Baksi *et al.,* (2017) reported similar results in *Colisa lalia*, through incorporation of spirulina powder in feed at the dose of 2g/kg. Jana *et al* (2014) reported similar results in *Pangasius sutchi*, through incorporation of spirulina powder in feed at the dose of 5%.

Javadi and Montajami (2013) reported that addition of spirulina on the feed formulation affects positively on the growth and survival of angel fish. Saroch *et al.*, (2012) reported that combination of spirulina in the feed considerably improved the growth in terms of specific growth rate, weight gain and survival rate of the fingerlings of *Catla catla* than fed with fish meal based control diet.

Refined lecithin is dietary phospholipid, which helps in growth and survival. In the present study, there is 1.6 % incorporation of lecithin in T3, which gave us better results in growth. Atar *et al.* (2009) reported similar results in fry of *Oreochromis niloticus*, through incorporation of lecithin in feed at the dose of 2 %. Hung *et al*., (2003) reported similar results in juvenile Atlantic salmon, through incorporation of lecithin in feed at the dose of 4g/kg.

Other purified components *viz.,*α Tocopheral acetate, Celin (L-ascorbic acid) and L-tryptophan have their importance.αTocopheral acetate is vitamin E which is essential for fertility and immunity. Celin is vitamin C which is essential as anti-stresser and is important in the nutrition of both broodstock and larvae. L-tryptophan is an essential amino acid which is the only precursor of serotonin, which is a neuro transmitter responsible for reducing stress and aggression. Thus L-tryptophan helps in increasing survival rate and improves growth.

According to Rezvani *et al*., (2011) use of live feed in aquaculture increases the absorption of protein resulting enhanced growth and survival percentage of fish. Although the formulated feed with 4 % supplementary mix gave better growth results, the best results was found in T4, fed with live feed, *Tubifex.* In similar research conducted, it is reported by Saha and Patra (2013) that *Tubifex* gave the best result for *Colisa lalia* for growth performance. Hossen *et al*. (2014) reported better growth of *Trichogaster fasciata* using live feed *Tubifex sp*. Kasiri *et al*. (2012) also reported similar results in angelfish, *Pterophyllum scalare*, with live feed *Tubifex.*

5.5 Reproductive Performance

According to Manissery *et al.,* (2001) and Muchlisin *et al.,* (2006) nutrition is play a major role in the reproductive performance of fish. According to Izquierdo *et al*., (2001) broodstock nutrition is an important factor which governs egg production and larval survival rate. According to Watanabe *et al*. (1990) nutrition has intense consequence on gonadal growth and fecundity. Washburn *et al*. (1990) observed that maximum growth, egg production and hatchability were witnessed in fish fed with 35% protein diets. Shim and Chua (1986) reported that the female fish fed with 30-40% dietary protein and 9-10.5% lipid gained the maximum ovary weight, GSI and number of yolky oocytes. Ling *et al*. (2006) stated the importance of the dietary protein (30%) and lipid (12%) on growth and reproductive performance of female swordtails.

James *et al*. (2006) revealed that the fishes fed with Spirulina diet (30%) performs higher feed consumption, growth, fertility, coloration and leucocyte count in the ornamental red sword tail, *X. helleri*. He proposed that 30% of Spirulina can be replaced in fishmeal to produce low cost nutritionally balanced feed which can boost reproductive performance in ornamental fishes. Kasiri *et al*. (2012) reported significant increase in the gonadosomatic index (GSI), fecundity and hatchability of fresh water angelfish when fed with the combination of live earth worm, dried tubifex, dried gammarus and prepared granulated feed. According to Ahilan and Kumaran (2003) live feeds are the best feed in ornamental fishes which is used as a better alternative feed in producing good quality eggs, increasing fertilization, hatching and survival rates in ornamental fishes.

The testis weight of dwarf gourami, *Colisa lalia* found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.006 ± 0.000 g, 0.007 ± 0.000g, 0.008 ± 0.000 g and 0.009 ± 0.000 g respectively. The Gonadosomatic Index of male dwarf gourami, *Colisa lalia* found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.390 ± 0.008, 0.462 ± 0.002, 0.519 ± 0.007 and 0.538 ± 0.006 respectively.

The ovary weight of dwarf gourami, *Colisa lalia* found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 0.061 ± 0.000 g, 0.079 ± 0.000 g, 0.082 ± 0.000 g and 0.090 ± 0.000 g respectively. The Gonadosomatic Index of female dwarf gourami, *Colisa lalia* found out at the end of 60 days experiment in Treatment 1, Treatment 2, Treatment 3 and Treatment 4 are 4.154 ± 0.010, 5.310 ± 0.007, 5.403 ± 0.016 and 5.720 ± 0.013 respectively.

The best results in both male and female were obtained in Treatment 4, which was fed with Combination diet (Feed C + *Tubifex*). Feed C is the formulated feed prepared with 4% supplementary diet mix, followed by Treatment 3, which was fed with only live feed *Tubifex*. As it is known that dwarf gourami, *Colisa lalia* has omnivorous feeding habit therefore the combination diet gives better results.Kasiri *et al*., (2012) observed similar results in GSI of angelfish, *Pterophyllum scalare*. Farahi *et al*., (2010) reported similar results in hatchability of angelfish, *Pterophyllum scalare.*

Spawning success was observed only in Treatment 3 and Treatment 4, which was fed with only live feed (Tubifex) and Combination diet (Feed C + *Tubifex*) respectively. No spawning was observed in Treatment 1 and Treatment 2, which was fed with formulated feeds. Conclusion can be drawn from this that dwarf gourami, *Colisa lalia* relies on some natural food or live feed for inducement of spawning. Similar results were reported by Das and Kalita (2006) in *Colisa fasciata*.

As no spawning was observed in Treatment 1 and Treatment 2, which was fed with Control diet (Feed A) and formulated diet (Feed C), there was no eggs spawned, thus no fertilization and hatching was there. The fertilization % of dwarf gourami found out at the end of 60 days experiment in Treatment 3 and Treatment 4 are 65.33 ± 1.76%, 70.67 ± 0.67% respectively. The Hatching % in Treatment 3 and Treatment 4 are 35.33 ± 1.76% and 42.00 ± 1.15% respectively. Treatment 4 fed with Combination diet gives better results in fertilization and hatching.

Productivity of the broodstock is the most significant constraint on commercial production of fish (Sharma *et al*., 2011). Several factors influence reproduction in fishes, *viz.,*environmental conditions, genetic factors and feed nutrition (Bromage, 1995, Dzikowski *et al*., 2001, Mehrad and Sadagur, 2010).

Kolkoski *et al*., (1997) reported that the addition of live feed with a formulated feed enhances the efficiency of formulated feed by promoting the assimilation and deposition of dietary nutrients in Sea bass.

The breeding success in live feed treatment (T3) and the combination treatment (T4) may be due to the nutrients present in the live feed. Possibly live feed (*Tubifex*) carry some micro elements which are essential for the secretion of gonadotrophic hormone (GTH). According to Patino and Thomas (1990) and Degani and Boker (1992) live feed might affect steroid production which is essential for oogenesis and reproductive cycle.The present study demonstrated that the use of formulated feed along with life feed enhances breeding performance.

5.6 Larval Rearing

The length gain achieved in Treatment 1, Treatment 2 and Treatment 3 are 10.54 ± 0.07 mm, 5.84 ± 0.07mm and 7.71 ± 0.12 mm respectively. The highest value of length gain of 10.54 ± 0.07mm was observed in Treatment 1, which was fed with live feed, Infusoria, followed by Treatment 3, which was fed with boiled egg yolk emulsion. The lowest length was observed in Treatment 2, which was fed with formulated feed (Feed C).

In case of larval rearing the survival rate plays an important role. Survival rate % achieved in Treatment 1, Treatment 2 and Treatment 3 is 66.33 ± 0.88 %, 52.33 ± 2.19% and 34.00 ± 1.15% respectively. The highest value of survival rate of 66.33 ± 0.88 % was observed in Treatment 1, which was fed with live feed, Infusoria, followed by Treatment 2, which was fed with formulated feed (Feed C). The lowest survival rate was observed in Treatment 3, which was fed with boiled egg yolk.

Das and Kalita (2006) achieved similar results in the feeding of larval stages of *Trichogaster fasciata*. Similar results were observed by Degani (1991), in blue gourami, *Trichogaster trichopterus*. Similar results were observed by Patra & Ghosh (2015), in freshwater Angelfish, *Pterophyllum scalare*. Nagano *et al.,* (2000) also demonstrated the importance of ciliates as initial food for first-feeding *Epinephelus septemfasciatus* for both growth and survival.

Live feed is an important factor in larval rearing of a number of fish species (Wolnicki *et al*., 2003; Wang *et al.,* 2005 and Girri *et al*., 2002). The necessity of huge amount of live feed in aquaculture and mainly in ornamental fish culture is essential to rear its larvae from endogenous to exogenous feeding phases. Providing appropriate live feed through this phase is vital which governs the survival rate (Altaff and Janakiraman, 2013). One of the major responsible factors for failure in larval growth and survival is malnutrition (Luis *et al*., 2010).

5.7. Water Quality Parameters

At ICAR-CIFE, Kolkata Centre the water quality parameters were analyzed for the four treatments of the experiment for growth performance through nutritional interventions. Municipality supply water was used, after overnight conditioning, in the rearing experiment. It was observed that there was no significant difference among the treatments in the four treatments with respect to temperature, pH, Dissolved oxygen, Hardness and Total Alkalinity. The temperature remained in a suitable range of 24 -25 ^{0}C, for the experimental 90 days. Both alkalinity and hardness remained in the range of 139 – 140 ppm, with almost neutral pH of 7. The dissolved oxygen concentration was also at a good level of 7 ppm in all the four treatments.

Water quality parameters for rearing and spawning at ICAR-CIFE, Mumbai were analyzed for twelve months from July 2018 to June 2019. The available borewell water was used for the rearing and spawning activities at Mumbai. There was no significant difference observed in all the parameters analyzed for 12 months, except the temperature which remained within a range of 20 – 28^{0}C, having seasonal fluctuations. Hardness was in the range of 224.00 - 231.50 ppm, Total alkalinity in the range 176.75 - 185.25 and pH in the range 7.13 – 7.25. Dissolved oxygen range was in range of 5.13 – 5.38 ppm, as it was bore well water.

Developmental rates of fish embryos, larvae and young juveniles are affected significantly by water temperature (Romboug, 1997; Bjornsson*et al*., 2001 and Von *et al.*, 1996). Fish shows an increase in growth, feeding, metabolic rate, food digestion, absorption and appetite with the increase of water temperature, under optimal water temperature conditions (Sun *et al*., 2005 and Zhang *et al.,* 2001). Marilyn (1993) reported that in India, for normal growth of fishes the optimal temperature range is between 28^0C - 30^0C.

Various developmental phases in the life cycle of fish are influenced by environmental factors (Koumoundouros, 2001). Larval phase is one of the most sensitive phases that are affected by change of environmental conditions (Bailey and Houde, 1989). Physico-chemical parameters of water such as temperature, pH, dissolved oxygen, total hardness, alkalinity and ammonia level should be within the optimum range of freshwater ornamental fish production (Chapman, 2000). In the present investigation, water quality parameters of all the experimental tanks were within the optimum range of fish production indicated that water quality did not cause any stress in the experimental fish during the experimental period. Ornamental fishes are highly adaptable in culture condition and capable of living in wide range of environment (Chapman, 2000). The hardness of water from which many ornamental fishes originate can vary from 5 to 20 mg/l to over 300 mg/l CaCO3 (Chapman, 2000). Several species of aquarium fishes (*Mollienesia latipinna, M. senops, Xiphophorus maculatus*) are well adapted in hard water environment and grew faster than in low water hardness. Bhatnagar and Devi (2013) suggested that the water quality criteria for water fishery to get high yield should be in the range of 20-30^0C temperature; 6.5-9 pH and 5mg/l of DO. In the present study too the temperature, pH and dissolved oxygen content of water was within this range, at both the experimental sites.

6. SUMMARY

India is the main abode of the range of ornamental fishes available in the subcontinent. There is a huge market for gourami in the ornamental fish industry. There is variety of gourami available in nature and some species can grow quite large, and are unsuitable for the general hobbyist. Thus, smaller varieties of gouramis are often kept in home aquaria and one of them is dwarf gourami, *Colisa lalia.* This particular variety has high foreign demand, but its export market mainly depends upon wild collection. The sole dependence on wild catch has a very depleting effect on the species in future. Breeding and rearing coupled with market demand are the factors that encourage venturing into this sector. With a view to above, the present research work entitled “Biology, captive maturation and breeding of indigenous ornamental fish, dwarf gourami, *Colisa lalia* (Hamilton, 1822)” was undertaken with the following objectives

- To analyse the feeding and reproductive biology of dwarf gourami, *Colisa lalia.*
- To evaluate the effect of nutritional intervention on growth and reproductive performance of dwarf gourami, *Colisa lalia*.
- To analyze the larval rearing of dwarf gourami, *Colisa lalia.*

To accomplish the above objectives the general biology, feeding biology and reproductive biology of the *Colisa lalia* were studied. Three experiments were also conducted based upon nutritional interventions to decide the best feed for growth performance, reproductive performance and larval rearing.

General biology

Ten morphometric characters were measured of dwarf gourami, *Colisa lalia*. Their Mean ± (SD) value are as follows; Total length (TL) 4.06±0.21 cm, Standard Length (SL) 3.25±0.18 cm, Predorsal Length (PDL) 1.42±0.11 cm, Preanal length (PAL) 1.57±0.11 cm, Prepectoral length (PPL) 1.15±0.07 cm, Prepelvic / ventral length (PVL) 1.17±0.07 cm, Head length (HL) 1.12±0.07cm, Body Depth (BD) 1.51±0.10 cm, Eye Diameter (ED) 0.30±0.02 cm, Snout Length (SnL) 0.28±0.04 cm. There is a positive correlation between all the characters but there is allometric growth pattern found in dwarf gourami, *Colisa lalia.* The increase in TL has greater impact on SL, in comparison to other lengths. The ED remains within the range of 0.3 - 0.4 cm for any length of the adult fish. These measurements denote that dwarf gourami, *Colisa lalia,* has small size oval body, which is strongly compressed. Fin Formula of dwarf gourami, *Colisa lalia* was formed by taking the mode values of the fins; D. XV/8; P.8; A. XVII/15; V. 2; C. 14.

Feeding biology

The Relative Gut Length of dwarf gourami, *Colisa lalia* was found out to be 2.29 ± 0.42 (SD) with a range of 1.43 – 2.9. The Gut Length changes according to the Total Length to some extent, but there is least change of RGL in relation to the TL. It denotes that *Colisa lalia* is omnivorous in nature, which feeds on both plant and animal nutrients.

Gastro-somatic index (GaSI) of dwarf gourami, *Colisa lalia* was found out for 12 months from July, 2018 to June 2019, for both male and female. There was significant difference ($p<0.05$) observed between the GaSI of both male and female dwarf gourami in all the twelve months. Highest value of GaSI in both male and female was observed in the month of December having the value of 3.11 ± 0.1 and 4.75 ± 0.13 respectively. Lowest value of GaSI was observed in the month of June, having the value of 1.88 ±0.03 and 1.20 ± 0.07 for male and female respectively. This denotes that the feeding intensity of *Colisa lalia* is more in the winter months and less in pre monsoon months in the captive conditions of Mumbai.

Reproductive biology

In the present study, *Colisa lalia* was observed from June 2018 to July 2019. Colour of the body in male is translucent blue with alternating vertical diagonal strips of red and dark orange bands. During breeding season, a matured male can be identified by seeing the blue patch of colour at the ventral region between the opercula and the pectoral fin. This blue patch is not seen in immature males. The fins are as colourful as the body. Body size is comparatively larger than the female, with slender and sleek abdomen. Dorsal and anal fins are pointed. Colour of the body in female is dull silvery blue to gray colour. Colour of fins is yellowish grey. Body size is comparatively smaller than male. Enlarged fat abdomen can be observed, but only in breeding season. Dorsal and Anal fins are curved. The colour pattern of the fish was found to be a suitable criterion for identification of sex for captive breeding purpose.

There was significant difference ($p<0.05$) observed between the Gonadosomatic index (GSI) of both male and female dwarf gourami in all the twelve months. Highest value of GSI in male was observed in the month of June having the value of 0.585 ± 0.01. Lowest value of GSI was observed in the month of November, having the value of 0.305 ± 0.01. Highest value of GSI in female was observed in the month of June having the value of 6.08 ± 0.14. Lowest value of GSI was observed in the month of November, having the value of 2.97 ± 0.02. In the present observation at the captive conditions of Mumbai, the GSI of male *Colisa lalia* is more than 0.5 in May, June and July and in case of female the GSI is more than 5 from April to August. These sequential changes in GSI value indicates that the spawning season of *Colisa lalia* can be considered between April to August with a peak period during May to July, in the captive conditions of Mumbai.

The testis of *Colisa lalia* is paired and asymmetric throughout the year even during breeding season. They are fused along their posterior end to form a common spermatic duct. The ovary of *Colisa lalia* is paired and attached to the dorsal wall of the body cavity by a short thick mesovarium. The two ovaries lie close to each other. Anteriorly, each of them has its end free, while at the posterior end, it extends into a thick-walled oviduct. The colour of the ovary became less bright during non-breeding phase because of empty lobulation of the ovary. During breeding season, the colour of ovary became bright yellow or pinkish-yellow due to maximum blood supply to the ovary for nourishment of eggs. The ovarian wall of the fish became thin and the eggs with its contents are visible with a yellow colour.

In the histology study of testis of *Colisa lalia*, 5 phases of testicular cycles were observed during the study period of one year; Developing I Phase, Developing II Phase, Spawning Capable Phase, Actively Spawning Phase and Spent Phase Testis.

In case of ovary, 4 phases of ovarian cycles were observed during the study period of one year; Immature Phase, Developing I Phase, Developing II Phase and Spawning Capable Phase ovary. The occurrence of the particular phase of the gonads was not confined to a particular month of the year. Mixed stages were found in different individuals in particular month. In case of male dwarf gourami, *Colisa lalia* higher percentage of matured gonads were found between May to July and a little lesser percentage of matured gonads are found in the months before and after the above described months. Similar observations are also found in the case of ovary of female dwarf gourami, *Colisa lalia.* This implies that this particular species needs warm environment for gonadal maturation and breeding. It can also be concluded that this species can breed throughout the year through temperature manipulation and nutritional interventions. The presence of multiple oocyte stages found in the free end of the ovary and presence of multiple sperm stages in the testis, proves it to be batch spawner.

For breeding purpose it needs some floating plastic substrate to hold the bubble nest. Chasing activity was observed after the female was introduced to the breeding setup with bubble nest. After the chasing activity, courtship behavior was observed, followed by spawning activity. Parental care was observed by the male not the female, after spawning, as the male deposited the eggs in the bubble nest and chased away the female from the bubble nest. The incubation period was recorded 24-25 hours. Parental care was still observed by the male for the hatchlings too, but not for a longer period. Cannibalistic activity was observed by male dwarf gourami later, when there is development of movement in the hatchlings.

In the present study, the average pre-spawning absolute fecundity of *Colisa lalia* in the captive conditions was 439 ± 49 for 4.44± 0.11 cm length and 1.53± 0.07 g weight of fish. Highest correlation was observed between fecundity and gonad weight (0.8), rather than fecundity with body weight (0.4) and body length (0.3). This denotes that the increase in the gonad weight has more impact on the increase of absolute fecundity of *Colisa lalia*, in captive conditions.

In the present study, the mean ova diameter observed was found to be 0.265 mm, with 0.003 standard deviation and range of 0.271 - 0.261mm. The size of the ova diameter increases from its origination as oogonia to the matured egg, which can be fertilized and embryo can be formed. This is a proof of the histological study carried out of the different phases of the ovary. The size which I am describing is of the matured water hardened spawned egg diameter.

After 25 -30 min of spawning, non-adhesive and optically transparent fertilized egg is observed with clear distinction between perivitelline space and egg-yolk. Cytoplasm movement could also be observed. After 45 – 55 min early Gastrula phase could be observed where involution, convergence and extension were occurring. Completion of the gastrula phase took a longer period of time around 6 – 7 hr. In the Gastrula phase three layers of germ-cells are produced and the egg looks like hollow cup-shaped structure. After 17-19 hrs embryonic body formation occurred, giving rise to pharyngula stage, where embryo body is formed. Heart beat and twitching movement observed. After 24-25 hr lots of twitching movement is observed the embryo pushes the egg envelope and removes it and comes out as a hatchling. In just hatched larvae no movement is observed, due to exhaustion of hatching. Only cytoplasmic circulation and heart beat observed.

In newly hatched larvae, we can find optic vesicles, myotome, yolk sac, heartbeat and oil globules. There is no locomotion done by the larvae but there is circulation of fluid. In one day old larvae, we can find eye bulbs, myotome, and heart. Yolk sac is reduced. The body is transparent but pigmentation can be found. In two days old larvae, we can find dark eye bulbs, mouth lining, nostrils, pectoral fins, alimentary canal, myotome, heart. Yolk sac reduced than before. There is fast movement observed in the larvae. In five days old larvae, we can find eyes with pupil, mouth opening, nostrils, and developed pectoral fin. Yolk sac is pushed upwards. Jaws become stronger. The notochord becomes segmented. It moves very fast. In ten days old larvae, we can find air bladder. With the development of muscles and bones the larvae is becoming opaque. There is a lot of pigmentation. Yolk sac is almost completely absorbed. In fifteen days old larvae, we can find the different organs. Caudal rays are well developed in the caudal fin.

Experiment 1 - Evaluation of the effect of nutritional intervention on growth performance of dwarf gourami, *Colisa lalia*

In the 90 days experiment, the experimental diets used in the treatment 1, 2 and 3 were isonitrogenous and isocaloric, except T4, which was fed with livefeed, Tubifex. The major difference in the formulated feeds was the addition of supplementary diet mix in 0%, 2 % and 4% in T1, T2 and T3 respectively, which constituted of refined lecithin (40%), α Tocopheral acetate (1.5%), Celin (L-ascorbic acid) (2.5%), Spirulina powder (50%) and L-tryptophan (6%).

The Highest length gain (1.03 ± 0.04 cm) was achieved in Treatment 4, which was fed with live feed Tubifex. The protein content of Tubifex (60%) may be is the reason of better length gain. Highest weight gain (1.12 ± 0.02 g), weight gain percentage (120.75 ± 1.63%) and specific growth rate (0.88 ± 0.01) was achieved in Treatment 4 fed with live feed, *Tubifex*, followed by Treatment 3 with no significant difference observed in weight gain, weight gain percentage and specific growth rate in Treatment 3 and Treatment 4. Treatment 3 and Treatment 4 gave best results which show that formulated feed with 4% supplementary diet mix can bring similar effect as live feed, *Tubifex* for growth performance in dwarf gourami, *Colisa lalia*.

Experiment 2 - Evaluation of the effect of nutritional intervention on reproductive performance of dwarf gourami, *Colisa lalia*

In the 60 days experiment, the experimental diets used in the treatment 1 and 2 were isonitrogenous and isocaloric, with inclusion of the supplementary diet mix of 0 % and 4% in formulated feed. As 4% supplementary mix formulated feed gave good growth performance, that particular diet was used to check its impact in reproductive performance. Treatment 3 was fed with live feed, Tubifex. Treatment 4 was fed with combination diet, including live feed Tubifex and 4% supplementary mix formulated feed.

In male dwarf gourami, *Colisa lalia,* highest gonad weight (0.009 ± 0.000 g) and Gonadosomatic index (0.538 ± 0.006) was observed in Treatment 4, fed with combination diet, followed by T3, T2 and T1, with significant difference between all the treatments. In female dwarf gourami, *Colisa lalia,* highest gonad weight (0.090 ±

0.000g) and Gonadosomatic index (5.720 ± 0.013) was observed in Treatment 4, fed with combination diet, followed by T3, T2 and T1, with significant difference between all the treatments.

When all the treatments were subjected to breeding trials, spawning success was observed only in Treatment 3 and Treatment 4, which was fed with only live feed (Tubifex) and Combination diet (Feed C + *Tubifex*) respectively. No spawning was observed in Treatment 1 and Treatment 2, which was fed with formulated feeds. Conclusion can be drawn from this that dwarf gourami, *Colisa lalia* relies on some natural food or live feed for inducement of spawning.

As no spawning was observed in Treatment 1 and Treatment 2, there was no eggs spawned, thus no fertilization and hatching was there. The fertilization % of dwarf gourami found out at the end of 60 days experiment in Treatment 3 and Treatment 4 are 65.33 ± 1.76%, 70.67 ± 0.67% respectively. The Hatching % in Treatment 3 and Treatment 4 are 35.33 ± 1.76% and 42.00 ± 1.15% respectively. Treatment 4 fed with Combination diet gives better results in fertilization and hatching.

The breeding success in livefeed treatment (T3) and the combination treatment (T4) may be due to the nutrients present in the live feed. Possibly live feed (*Tubifex*) carry some micro elements which are essential for the secretion of gonadotrophic hormone (GTH). The present study demonstrated that the use of formulated feed along with life feed enhances the captive breeding performance of dwarf gourami, *Colisa lalia*.

Experiment 3 – Analyze the effect of nutritional intervention on larval rearing of dwarf gourami, *Colisa lalia*

In the 30 days experiment, the experimental diets used in the treatment 1, 2 and 3 are live feed Infusoria, 4% supplementary mix formulated feed and boiled egg yolk. The highest length gain (10.54 ± 0.07 mm) was observed in Treatment 1, which was fed with live feed, Infusoria, followed by Treatment 3, which was fed with boiled egg yolk emulsion. The lowest length gain (5.84 ± 0.07mm) was observed in Treatment 2, which was fed with formulated feed. This may be due to the reason that the nutrients of the live feed, Infusoria, are better digested and utilized in comparison to boiled egg yolk and formulated feed, by the larvae of dwarf gourami, *Colisa lalia*.

The highest survival rate (66.33 ± 0.88 %) was observed in Treatment 1, which was fed with live feed, Infusoria, followed by Treatment 2, which was fed with formulated feed. The lowest survival rate (34.00 ± 1.15%) was observed in Treatment 3, which was fed with boiled egg yolk. There was significant difference observed in all the three treatments. This may be due to depletion of water quality, though enough care was taken and available nutrients. In treatment 3, the egg yolk emulsion depleted the water quality much faster, in comparison to the treatment 2, fed with formulated feed.

Conclusion

Dwarf gourami, *Colisa lalia* is a small, oval, strongly compressed fish having an allometric growth pattern. Among both the sexes male is colourful and attractive in comparison to female and juveniles. It is omnivorous in feeding habit with high feeding intensity in the winter months and least in pre monsoon months. Its peak breeding season is between May to July. Parental care is observed by the male till the larvae hatches out. The movement in larvae induces cannibalistic nature in the male. Multiple breeding can be practiced through nutritional interventions. Presence of dietary phospholipids, vitamin E, vitamin C, Spirulina powder and essential amino acids in the formulated diets enhances the performance of *Colisa lalia,* but live feed has an important role in broodstock and larval rearing. In the present study it is observed that feeding the broodstock with live feed, *Tubifex,* induces spawning activities. But the combination diet of live feed and formulated diet improves the overall reproductive performance. In case of larval rearing Infusoria gave best results.

Breeding Protocol of dwarf gourami, *Colisa lalia*

Sex wise segregation of matured fish (Male ≥ 4.5 cm; Female ≥4cm)

⇓

Adlibitum feeding with live *Tubifex* for fifteen days

⇓

Breeding set up (1:1). Introduction of female after bubble nest is prepared by male under substrate

⇓

Removal of female after spawning

⇓

Removal of male after hatching

⇓

Mild aeration to be provided to the hatchlings

⇓

Feeding with *Infusoria* from the third day to the larvae

⇓

Feeding with boiled egg yolk emulsion + *Infusoria* after fifteen days, with proper water quality maintenance

⇓

Fry to be fed with commercial feed (carotenoid)

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Appendices

%	Percentage
FAO	Food and Agriculture Organization
^0C	Degree centigrade
$	Dollar
Mg	Milligram
@	At the rate of
SD	Standard Deviation
ppm	Parts per million
cm	Centimeter
L / l / lit	Litre
g	Gram
mm	Millimeter
µm	Micrometer
hr	Hours
ml	Millilitre
T	Treatment
IU	International Unit
^0C	Degree Celsius
ANOVA	Analysis of variance
SE	Standard Error
SPSS	Statistical Package for Social Science
µg	Microgram
AOAC	The Official Methods of Analysis of AOAC INTERNATIONAL
N	Normality
i.e.	that is
‘	Inch
min	minute
R^2	Coefficient of determination
$<$	Less than
$>$	More than
AM	Ante meridiem
PM	Post meridiem
viz	videlicet