

INFLUENCE OF SPIRULINA ON GROWTH AND GONADAL MATURATION IN GOLDFISH, *CARASSIUS AURATUS*

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ABSTRACT

The present study was carried out to find the influence of Spirulina on the growth and gonad maturation of goldfish, *Carassius auratus*. The gold fishes were stocked in troughs of 45 liters capacity and maintained with replicates. They were fed with diet supplemented with Spirulina at two concentrations viz., 10% (T₁₁) and 15% (T₁₂) and a control diet (basal diet) was also fed without Spirulina. The experiment was carried out for a period of 60 days. Sampling on the bio-growth parameters was done once in 10 days. At the end of feeding trial, fishes fed with Spirulina containing diet at 10% (T₁₁) level had higher growth (30.64 g) when compared to fishes fed with Spirulina containing diet at 15% level (28.56 g) and control feed (18.2 g). Histological sections were taken once in 30 days. On the 60th day of the experiment, the control group had less with immature ova but in fishes had well developed ovary with eggs in Secondary Vitellogenesis (SVO) stage as noticed in the histological sections of the ovary of T₁₁ and T₁₂ group. Therefore, 10% Spirulina containing diet is found optimum for growth and gonad maturation of goldfishes.

Key words: Spirulina, goldfish, feed additive, growth, gonad development

INTRODUCTION

Ornamental fish keeping is the second most popular hobby over the world next to photography (Das *et al.*, 2005). Particularly, goldfish is one of the common and preferred fish because of its colour and morphological characteristics (Sokolowska *et al.*, 1984). There is lack of knowledge about broodstock nutrition in ornamental industry. Proper nutrition is required for growth, maturation and reproduction of ornamental fishes. Generally ornamental fishes are fed with live feeds (James, 1998) but, the production of live feeds does not

satisfy the demand of ornamental fishes. Live feeds are known to enhance the gonadal maturation of fishes. Therefore, commercial broodstock feed incorporated with cheaply available feed additives must be established in the ornamental industry.

Spirulina is considered as an alternative non-conventional animal protein source that can be used as an additive in aquaculture feeds (Nagagawa and Gomez-Diaz, 1975). They are multicellular and filamentous blue green algae which is established as “wonderful future food source” in the International Association of Applied

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Microbiology (Sasson, 1997). Spirulina can satisfy the nutritional requirement of ornamental fish particularly goldfish because of their bio active components such as 55-70% protein, 5-6% lipid and vitamins (Phang *et al.*, 2000).

The inclusion level of 11% of raw Spirulina in the diet fed to Java Tilapia (*Oreochromis mossambicus*) improved the growth rate, specific growth rate and food conversion ratio when compared with control diet fed fishes (Chow and Woo, 1990). Vasudhevan & James (2011) reported that the dietary supplement of vitamin C along with Spirulina influenced the growth, gonadal development and colouration in gold fish due to interaction of Spirulina with vitamin C. They also reported that fishes in control group developed gonad on 60th day while in Spirulina fed fishes it was on 40th day. They observed four times higher gonad weight in female gold fishes when fed with the mix of Spirulina and vitamin C compared to control. Olvera-Novoa (1996) showed that inclusion of *S. maxima* in the diet has beneficial effects on the growth of tilapia (*Oreochromis mossambicus*) fry and it can be replaced up to 40% of the animal protein in the diet.

The present study aimed at finding the optimal level of Spirulina that can be incorporated in the feed to enhance growth and gonadal maturation of goldfish.

MATERIALS AND METHODS

Juveniles of goldfish, *Carassius auratus* with body weight ranging from 3 to 5 g were purchased from Dev's Aqua Farm, Keeranoor in Thoothukudi District and acclimatized for a period of 30 days with control feed (basal diet). Goldfishes with an average body weight of 5.79±0.91g were stocked at a density of 4 numbers per trough and maintained with replicates.

Spirulina flakes were purchased from a NGO at Nagercoil and ground well and sieved to

get uniformly sized particles for the experimental feed preparation (Jourdan, 2001). Spirulina was supplemented to the basal feed at two different concentrations viz., 10% (T₁₁) and 15% (T₁₂). The feed ingredients used and their percentage of inclusion is given in Table 1. A Control (C) feed was also prepared without addition of Spirulina. The experimental feeds were prepared according to Cho *et al.* (1985).

Before starting the feeding trial, the fishes were conditioned by starvation for one day and then the fishes were fed at 4% of their body weight per day with experimental diets. The feeding frequency of fishes was three times a day viz., 9 am, 1 pm and 5 pm. The fishes were subjected to observation of body weight once in 10 days for 60 days and the quantity of feed was then adjusted based on the weight of the fishes. Once in 30 days, one fish from each replicate was sacrificed to harvest the gonad for histological sectioning and observation.

Proximate composition such as moisture, crude protein, crude fibre, ether extract, total ash content and gross energy of experimental diets were determined by using standard analytical methods (AOAC, 1984) and given in Table 2. The feeds were isoproteineicous (32.69±1.38) and isocaloric (3948±47.14).

Bio-growth parameters such as growth, survival, feed consumption rate, mean weight gain (g), weight gain percentage and specific growth rate were estimated based on the body weight according to Olvera-Nova *et al.* (1996).

Sample fishes were sacrificed to observe Gonado Somatic Index (GSI) before starting the experiment. Further development of the gonad was observed at 30th and 60th day of the experiment. The GSI was calculated using the formula given by Billard *et al.* (1993). The fishes were dissected and gonads were taken out with the help of forceps (Gupta and Mullins, 2010). The collected gonads

were preserved in 10% formalin (Green *et al.*, 2012). The preserved gonads were then mounted in slides at a pathology lab. The histological sections of gonad were observed under microscope to study the development of gonads at 30 days an interval of during the experiment.

The collected data and the estimated parameters were subjected to 'F' test for finding out statistical significance. The growth parameters were detailed using Regression analysis and compared using ANOVA.

RESULTS AND DISCUSSION

Higher biomass of 30.64 g was observed in fishes fed with Spirulina containing diet at 10% level (T_{11}) while fishes fed with feed containing Spirulina at 15% (T_{12}) produced a biomass of 73.84 g as presented in Table 3. Fish fed with diet containing 10% Spirulina produced considerably higher body weight (3.82 ± 0.212 g) when compared with 15% Spirulina contained diet (3.57 ± 0.312 g). Feed conversions of fish fed with feeds containing Spirulina at 10% and 15% concentration were 35.42% and 33.09% respectively. This observation was in accordance with Teimouri *et al.* (2013) who reported higher growth in Rainbow Trout (*Oncorhynchus mykiss*) when fed with feed containing 7.5 to 10% Spirulina.

Growth of Sword tail (*Xiphophorus helleri*) was higher when fed with 8% Spirulina containing diet when compared to fishes fed with 1-3% Spirulina containing diet (James *et al.*, 2006). This result confirmed that certain concentration of Spirulina only can lead to significant growth in fishes. In the present study, higher growth performance was observed in fishes fed with feed containing 10% Spirulina. *S. platensis* is known to activate protein synthesis and is reported to increase the somatic growth in guppy (Dernekbasi *et al.*, 2010). Thus it is evident that Spirulina have a potential to be used as natural feed supplement for increasing fish growth.

Feed utilization by the fishes per day is depicted in Fig. 1. The observed and estimated growth parameters are given in Table 4. The bio-growth parameters of the experimental fishes indicate that the highest weight gain percentage in fishes was observed when fed with feed containing Spirulina at 10% ($65.92 \pm 2.89\%$), while the fishes in Control and T_{12} group had lesser weight gain percentage. The specific growth rate was also the highest ($0.843 \pm 0.26\%$) in fishes fed with diet containing 10% Spirulina (T_{11}).

In the present study, higher concentration of Spirulina (15%) in fish diet reduced the growth (3.57 ± 0.312 g) of gold fish. Similarly, Olvera-Novoa *et al.* (1998) reported that Spirulina can replace up to 20% of fish meal protein in diets of Tilapia (*O. mossambicus*) but it reduces growth and feed utilization at higher replacement levels. He also reported that generally plant protein have low dietary qualities and reduce the phosphorus availability which reduce the growth performance at high concentration.

Fishes fed with Spirulina showed a positive impact on growth performance when compared to Control fishes as depicted in Fig 4.11. Same result was obtained by James *et al.* (2006) who reported that Spirulina enhances growth by stimulating the production of enzymes that transport fats within the fish for growth instead of storage.

Use of Spirulina on gonad enhancement of *C. auratus*

Juvenile gold fish when fed with Spirulina at 10% and 15% concentration it showed considerable difference in the development of ovary on 30th and 60th day during the experimental period as presented in Table 5 and 6 respectively. The highest gonad weight (3.38 g) and GSI ($31.54 \pm 5.06\%$) was observed in fishes fed with 10% Spirulina containing diet on 60th day when compared to other experimental feeds (Table 6).

This was in accordance with James et al. (2006) who reported four times greater gonad weight in Swordtail when fed with 8% Spirulina diet may be due to greater availability of protein and gonad stimulatory substances. When Spirulina concentration is further increased in feeds, it is known to decrease feeding efficiency consequently reducing growth and gonad development.

Beresto (2001) reported that Spirulina has mucopolymer murein in its cell wall instead of cellulose which increases the palatability of the spirulina diet that can be easily digested by the digestive enzyme secreted by fish. This enhances feed consumption which directly increases the gonad development and fertility.

Goldfish belongs to asynchronous type of maturation in which ovary contains ova at various stages of development (Marza, 1938). In the present study also, the ovary of the experimental fishes exhibited ova at different developmental stages ranging from immature ova, chromatin Nucleolus stage, peri nucleolus stage, secondary vitellogenesis stage, and tertiary vitellogenesis stage. The histological sections of the ovary of goldfishes during 30th and 60th day of the experiment are shown in Fig. 2 and 3 respectively.

Fishes in the control group had only immature ova at the initial stage and chromatin nucleolus stage of ova during 30th and 60th day of experiment while fishes in other groups had developed ovary. This shows that feed additives had stimulants which helps the fishes to mature earlier (Rezvani *et al.*, 2011).

When fed with diet containing Spirulina at 15% level, ovary of the fishes showed early and

late Peri Nucleolus stage (PNS) on the 30th day, whereas it had Secondary Vitellogenesis (SVO) stage at the end of the 60th day. Thus, it can be understood that supplementation of Spirulina had stimulated the development of ovary.

Borowitzka, (1994) and Li and Qi, (1997) stated that Spirulina contains lipid which have a main role in the maturation of fishes. The essential lipid in Spirulina ranges from 1.3 to 15% of total lipids mainly constituting gamma linolenic acid which have main role in gonad maturation in fishes. This confirmed the result of the present study with Spirulina.

Yamamoto and Tagawa (1961) described the presence of ovarian tissues and irregular follicles as immature ova. In the present study, similar observations could be noticed in fishes fed with diet containing Spirulina at 10% (T₁₁) levels on 30th day of experiment.

CONCLUSION

From the present study, it can be concluded that Spirulina when included in the feed at 10% level can improve the performance of fish in terms of growth and gonad maturation when compared to inclusion level of 15% and Control. Hence, Spirulina can be incorporated in the feed at 10% level for higher growth and earlier maturation of fishes.

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Table 1: Feed ingredients and their percentage of inclusion in different treatments

| Sl.No | Ingredients | Control (C) | (T ₁₁) | (T ₁₂) (T ₁₂) |
|-------|-----------------------|-------------|--------------------|--|
| 1 | Ground nut oil cake | 20 | 20 | 20 |
| 2 | Rice bran | 50 | 40 | 35 |
| 3 | Wheat bran | 5 | 5 | 5 |
| 4 | Maize flour | 7.5 | 7.5 | 7.5 |
| 5 | Salt | 0.1 | 0.1 | 0.1 |
| 6 | Vitamin & mineral mix | 0.1 | 0.1 | 0.1 |
| 7 | Fish meal | 15 | 15 | 15 |
| 8 | Fish oil | 2 | 2 | 2 |
| 9 | Yeast | 0.1 | 0.1 | 0.1 |
| 10 | Vitamin E | 0.2 | 0.2 | 0.2 |
| 11 | Spirulina meal | 0 | 10 | 15 |
| | Total | 100 | 100 | 100 |

Table 2: Proximate composition of experimental feeds

| Sl. No | Proximate composition | C | T ₁₁ | T ₁₂ | Mean ± SD |
|--------|------------------------|-------|-----------------|-----------------|------------|
| 1 | Crude protein (%) | 32.43 | 32.76 | 32.88 | 32.69±0.23 |
| 2 | Crude fibre (%) | 6.28 | 4.25 | 4.69 | 5.073±1.06 |
| 3 | Ether Extract (%) | 4.32 | 5.52 | 5.75 | 5.196±0.76 |
| 4 | Gross energy (kcal/kg) | 3897 | 3990 | 3957 | 3948±47.14 |

Table 3: Feed consumption and conversion details of *C. auratus*

| Treatment | Total Initial Biomass (g) | Total Final Biomass (g) | Total Biomass produced (g) | Total feed given as dry matter (g) | Total fecal excretion as dry matter (g) | Total Feed Utilized (g) | Feed converted into flesh (%) |
|-----------------|---------------------------|-------------------------|----------------------------|------------------------------------|---|-------------------------|-------------------------------|
| CF | 46.20 | 54.4 | 18.2 | 108.38 | 40.90 | 67.88 | 26.81 |
| T ₁₁ | 46.48 | 77.12 | 30.64 | 125.24 | 37.42 | 86.5 | 35.42 |
| T ₁₂ | 45.28 | 73.84 | 28.56 | 128.94 | 43.00 | 86.3 | 33.09 |

Table 4: Bio- growth parameters of *C. auratus*

| Treatment | Inclusion level of Spirulina (%) | Initial mean weight (g) | Final mean weight (g) | Weight gain (g) | Weight gain (%) | SGR (%) |
|-----------------|----------------------------------|-------------------------|-----------------------|-----------------|-----------------|-------------|
| C | 0 | 5.826±0.563 | 8.05±0.213 | 2.23±0.079 | 38.27±4.97 | 0.538±0.479 |
| T ₁₁ | 10 | 5.81±1.11 | 9.64±3.02 | 3.82±0.212 | 65.92±2.89 | 0.843±0.260 |
| T ₁₂ | 15 | 5.66±0.866 | 9.23±1.85 | 3.57±0.312 | 63.07±3.61 | 0.816±0.450 |

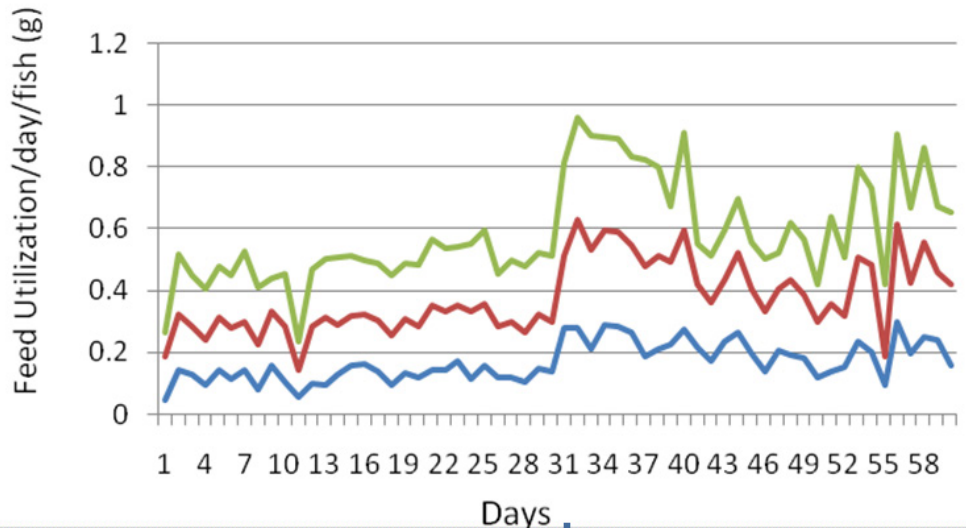
Table 5: Observations on the weight of the gonads of *C. auratus* on day 30

| Treatment | Weight of the fish (g) | | Weight of the gonad (g) | | GSI | | Mean GSI ± SD |
|-----------------|------------------------|------|-------------------------|-------|-------|-------|---------------|
| | R1 | R2 | R1 | R2 | R1 | R2 | |
| CF | 7.103 | 8.38 | 0.149 | 0.164 | 2.097 | 1.957 | 2.027±0.098 |
| T ₁₁ | 6.97 | 7.91 | 0.316 | 0.345 | 4.529 | 4.361 | 4.445±0.118 |
| T ₁₂ | 8.03 | 8.12 | 0.569 | 0.085 | 7.08 | 1.046 | 4.063±4.266 |

Table 6: Observations on the weight of the gonads of *C. auratus* on day 60 of the experiment

| Treatment | Weight of the fish (g) | | Weight of the gonad (g) | | GSI | | Mean GSI ± SD |
|-----------------|------------------------|------|-------------------------|------|-------|-------|---------------|
| | R1 | R2 | R1 | R2 | R1 | R2 | |
| CF | 9.78 | 9.83 | 0.39 | 0.44 | 3.98 | 4.47 | 4.22±0.34 |
| T ₁₁ | 9.62 | 9.87 | 3.38 | 2.76 | 35.13 | 27.96 | 31.54±5.06 |
| T ₁₂ | 9.235 | 9.43 | 2.11 | 2.32 | 22.84 | 24.60 | 23.75±1.24 |

Fig. 1: Feed utilization pattern in *C. auratus* fed with feed containing Spirulina at two inclusion levels



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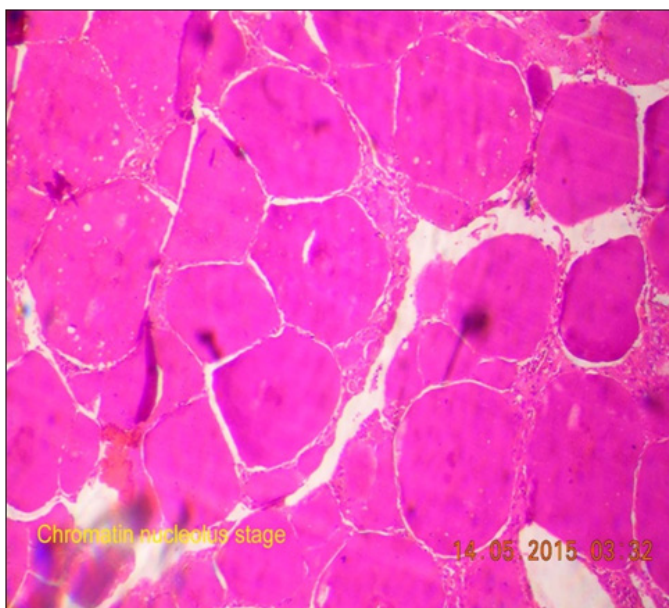


Fig. 2. Gonadal development at 30th day of the experiment in control fish

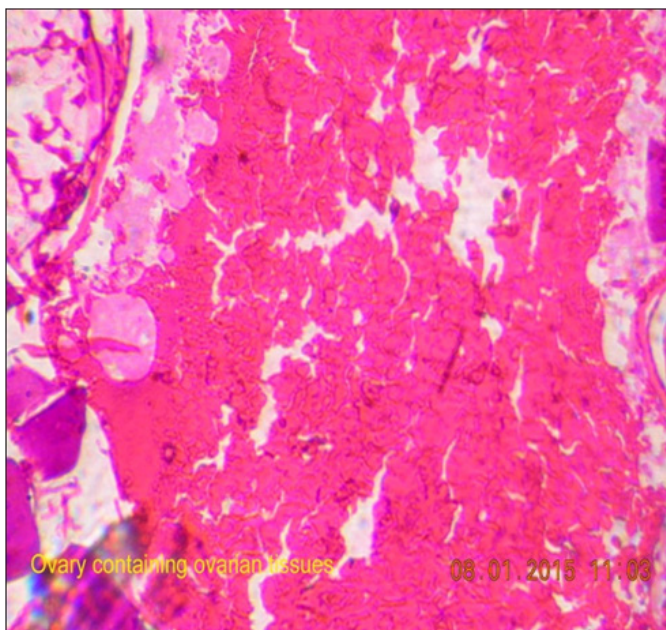


Fig. 3. Gonadal development at 60th day of the experiment in control fish

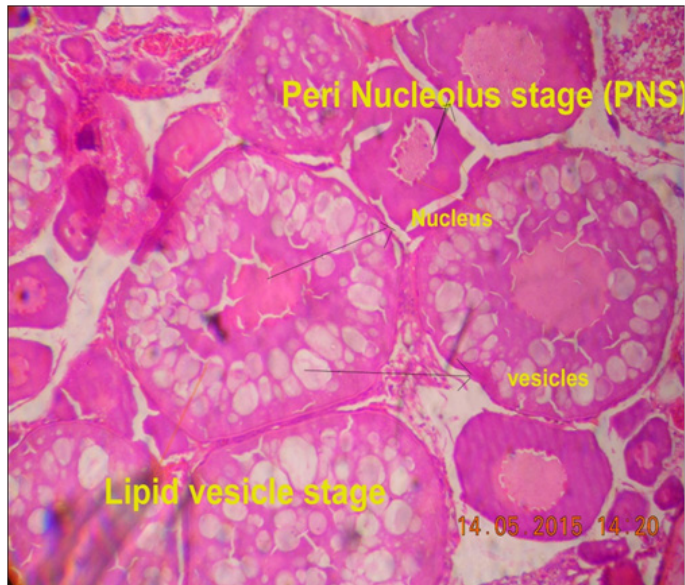


Fig. 4. Spirulina 10%- Gonad development at 30th day

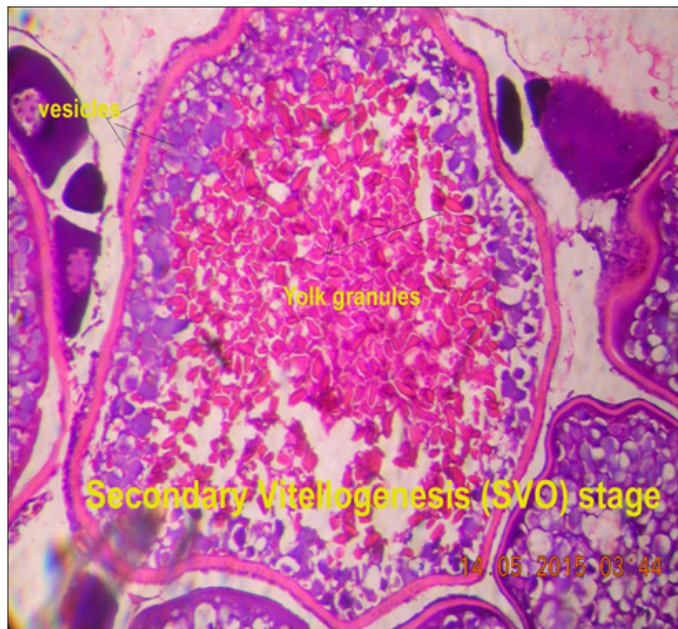


Fig.5. Spirulina 10%- Gonad development at 60th day

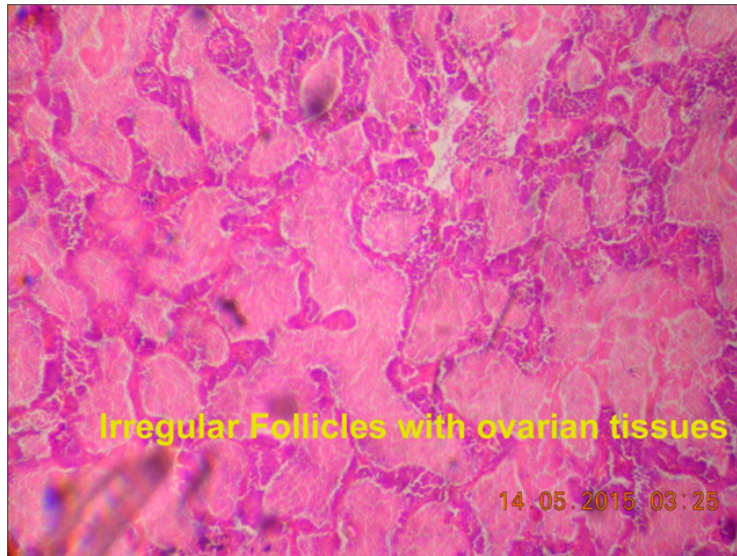


Fig. 6. Spirulina 15%- Gonad development at 30th day

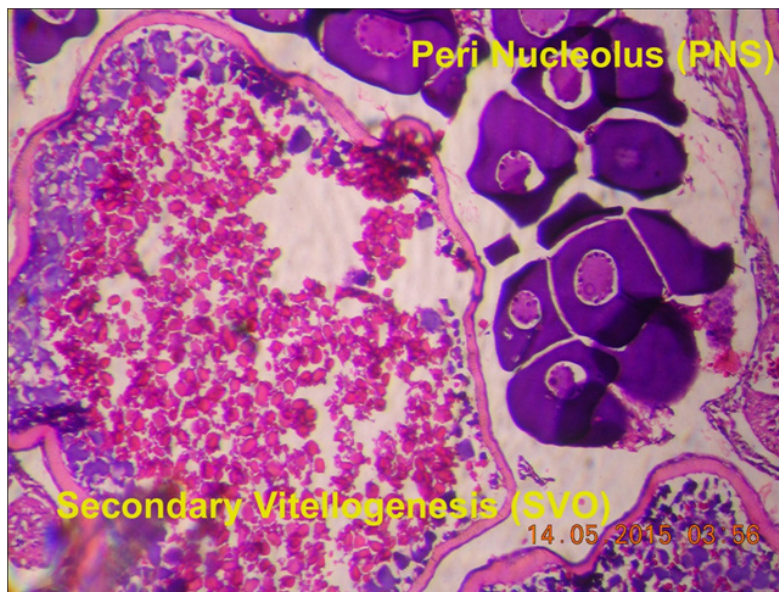


Fig. 7. Spirulina 15%- Gonad development at 60th day