Effect of thyroxine on larval survival, growth and morphology in *Betta splendens*

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Abstract- Effect of different levels of thyroxine on larval growth (Eltroxine (Glaxo) 0.1mg) such as 0, 0.001, 0.005, 0.01, 0.02 and 0.04 ppm on larval survival, growth and morphology were study by thyroxine immersion method. Thyroxine levels increased the larval development, morphometry and growth of *B. splendens* increased in fry reared upto 0.005 ppm T4 and thereafter they significantly ($P < 0.05$) declined. Air bladder began to develop on day 14 in all treatments and the length of the air bladder was high (0.624 mm) in fry reared with 0.01 ppm T4 as compared to other treatments. Intensity and appearance of melanophores in the body surfaces (head, trunk, caudal fin and dorsal fins) were related to time and T4 concentrations. The skin of fry was transparent upto 14 days in fry reared in controland0.001 ppm T4 and transparent nature became opaque and moreopaque related to T4 concentrations and time. Thyroxine influenced the growth parameters (mean body weight and length) in *B. splendens* fry. Based on the present study, it is observed that, 0.01 ppm was to maximize the melanophore formation, air bladder development food utilization parameter and growth in *B. splendens* fry.

Keywords: Thyroxine, larval survival, growth, melanophores, airbladder, *B. splendens/*

INTRODUCTION

The endocrine hormones play a central role in the regulation of growth and nutrient utilization in fish (Mac-Kenzie *et al.,* 1988). Survival, growth and development of carp larvae (*Cyprinus carpio*) increased with increasing salinity of the ambient medium from fresh water to sea water and in an optimum concentration of thyroxine (Lam and Sharma, 1985). There is an increased rate of feeding, conversion and conversion efficiency with an increased concentration of thyroxine upto 0.025 ppm and beyond this level a significant reduction of these parameters were observed in *Oreochromis mossambicus* (James and Sampath, 1994). Similar observations were made in *Labeo rohita* (Pandey *et al.,* 2004), *Heteropneustes fossilis* (Nayak *et al.,* 2003a). Apart from egg / larval immersion studies, injection of T3 (triiodothyronine to mother fish prior to spawning, enhanced larval survival and growth in striped bass, *Merone saxatilis* (Brown *et al.,* 1988). Similar results were reported by Ayson and Lam (1993) in rabbit fish, *Siganusguttatus.* Newly hatched larvae of Indian major carp (*Catla catla* Ham.) exposed to thyroxine (2.6 ppm) and cortisol (0.1 ppm) and found faster development of larvae by accelerating yolk absorption, initial gut formation, swim bladder development and survival rate (Nayak *et al.,* 2000a).The addition of L Thyroxine in the diet of the red sea breamresulted the increase in growth rate, appetite and activities of intestinal andhepatic enzymes and thereby increased the efficiencies of digestion and absorption of it (Woo *et al.,* 1991). It also been shown to control metamorphosis in fish (Miwa and Inui, 1987; Lam, 1994; Tay *et al.,* 1994). Previous studies elicited the anabolic effects of thyroxine on many fish species (Brown *et al.,* 1988; Brown and Millward, 1983; Reddy and Lam, 1991) and there is paucity of information on the effect of thyroxine on the fry and larva of *Betta splendens.* Hence, the present investigation has been undertaken to study the effect of thyroxine on embryonic development and morphometric aspects in *B.splendens* fry.

MATERIALS AND METHODS

Three days old active *B. splendens* fry were collected from laboratory bred brooders. They were divided into 5 groups corresponding to five thyroxine concentrations viz., 0, 0.001, 0.005, 0.010 and 0.02 ppm respectively. Triplicates were maintained for each group. Each group comprised 20 fry. The different concentrations of thyroxine were prepared from medically available Eltroxin (Glaxo) tablets, each containing 0.1 mg of the hormone. A tablet was ground in a mortar, mixed with distilled water, stirred and filtered. The process was repeated several times. The filtrate was made up to 25 ml with distilled water and (Lam, 1980) the solution gave a concentration of 0.1 ppm. From this stock solution, the desired concentrations (0, 0.001, 0.005, 0.01 and 0.02 ppm) were prepared by suitably diluting the above hormone solution with freshwater. The hormones media were renewed daily. Gentle aeration was provided. Larvae were fed three times a day. The best performed feeding schedule i.e. mixture / rotation of feeding was followed. The experiment was terminated in 28 days. Mortality rate and morphometric study were observed. Daily fluctuations in temperature were noted.

RESULTS

Larval survival

Fry treated in 0.005 and 0.01 ppm T4 elicited the 100% survival and it gradually declined with increasing the T4 concentrations . Fry treated in control and high concentration of T4 (0.04 ppm) showed the low survival (93%) as compared to fry treated with 0.005 and 0.001 ppm T4.

Larval growth and development

The dorsal fin rays appeared in fry on day 14 in all thyroxine treatments and the no. of dorsal fin rays were more (12 nos.) in fry subjected to 0.005 –0.04 ppm T4 as compared to fry reared in 0 and 0.001 ppm T4 (8 nos.) during the experiment (Table1). The number of caudal finrays was low (8-12) in fry reared in control at early

rearing period (14 days) and it was14 inthyroxine treated fry. Air bladder begun to develop in fry on day 14 in controland thyroxine treated fries. The length of the air bladder was high (0.624 mm)in fry treated with 0.01 ppm T4 and it significantly declined to 0.398 ($t = 12.55$; $P < 0.01$), 0.457 ($t = 3.33$; $P < 0.05$), 0.497 ($t = 4.70$; $P < 0.01$) and 0.527 mm ($t = 3.24$; $P < 0.05$) in fry treated with 0, 0.001, 0.005 and 0.02 ppm T4 respectively on day 28 (Table 1 and Plate 1). Melanophores wereappeared in the skin of fry on day 8 and the intensity and appearance of melanophores in the body surfaces (head, trunk, caudal and dorsal fins) were time and T4 concentrations dependent (Table 1; Plate 2). Similar trend was observed in the skin of *B. splendens* fry also. The skin of fry was transparent upto 14th day in fry reared in 0 and 0.001 ppm T4 and transparent nature became opaque and more opaque related to T4 concentrations and rearing period (Table 1; Plate 3). Thyroxine hormone influenced the growth parameters in *B. splendens* fry. The growth parameters were increased with an increase in thyroxine concentrations from 0 to 0.01 ppm T4 and thereafter it significantly ($P < 0.05$) declined. For instance, the mean body weight of *B. splendens* fry was 43, 76, 111, 152, 76 and 70 mg wet weight reared in 0, 0.001, 0.005, 0.01 and 0.04 ppm T4 respectively on day 28 (Table 2; Plate 1 - *5.6). Duncan multiple range test showed that MBW, MB Land length of air bladder between thyroxine concentrations differed significantly ($P<0.05$) with better values in fry treated in 0.01 ppm. Similar results were also obtained in other parameters (See Tables 1 – 2). As there rearing period increases, the chosen growthparameters were also increased. Two-way ANOVA test revealed that, thyroxine concentrations and rearing period elicited the significant ($P < 0.01$) impact on growth parameters like MBW, MBL, head size, and length of air bladder (Table 3).

DISCUSSION

Fry of *B. splendens* treated with thyroxine hormones (0.005 and 0.01 ppm) enhanced the survival as compared to control.Similarly, thyroxine hormones enhanced the larval survival in tilapia, rabbit fish, striped bass, walleye, gold striped amberjack and carp (Lam, 1980; Lam and Sharma, 1985; Brown *et al.*, 1988; 1989; Miwa *et al.*, 1992; Ayson and Lam, 1993; Hey and Farrar, 1996; Tachihara *et al.*, 1997). Mean body weight and length and caudal fin length were higher in T4 treated fries than in controland it suggests that T4 induces the early development of fish young ones. There was some evidence that thyroxine hormones may also stimulate embryonic and larval development (Lam and Loy, 1985; Lam and Sharma, 1985; Nugegoda and Lam, 1994). Nayak and Thomas (2000) observed length increment in climbing perch larvae by immersing the eggs in 0.05 ppm of T4 right from the time of fertilization. Similarly, elongation of caudal fin after egg immersion treatment was noticed in tilapia larvae (Reddy and Lam,1992a), which supports the present study. Nugegoda and Lam (1994) observed that immersion of fertilized eggs in 0.010 ppm T3 for 40 hrs was effective in enhancing larval survival in *Oreochromis mossambicus*. The present study also showed that, number of dorsal and caudalfin rays were induced by thyroxine. Reddy and Lam (1992a) observed a fast development of fin rays in early larvae of *Oreochromis mossambicus* and accelerated growth of caudal fin by theexogenous supply of thyroxine. Immersing they olksac larvae of *O. mossambicus* in 0.05 ppm T4 significantlyaccelerated the differentiation and growth of all fins (Reddy and Lam, 1992a),supports the observation made in the present investigation. The differentiation of paired and unpaired fins was fast in zebra fish received the thyroxine exogenously (Brown, 1997).

Length of the air bladder was high in 0.01 ppm T4 treated fry as compared to other concentrations and in control. Zairin *et al.* (2000) found that the exogenous T4 treatments accelerated the swim bladder formation in larvae of marble goby. Houndry (1993) observed that the formation andinflation of swim bladder by there gulatory action of thyroid hormones supports the present observation. Higher concentration of thyroxine (0.01, 0.01 and 0.04 ppm) induced the melanophores formation from 14th day onwards in body parts than lower concentrations of thyroxine (0.001 and 0.005 ppm) and control. This indicates that higher concentrations of T4 are required form elanisation in *B. splendens* fry. This may be due to the hormonal control of melanophores dispersal. The exogenous supply of T4hormones considerablely enhanced the pigmentation in the larvae of marblegoby (Zairin *et al.*, 2000). According to Abbott (1996), neural and endocrine agents exert their effects upon the melanophore by causing changesin the amount of cyclic AMP in the cell. While explaining the adrenergic control of *Betta* melanophores, Neuroscience Laboratory 120 L of California suggested that pigment dispersion is activated by increase in cAMP levels where as aggregation occurs when cAMP levels are reduced. Cyclic AMP activate cAMP dependent Protein Kinase (PKA) and phosphorylation of many targets and there by dispersion of pigment granules. If the adenylatecyclase is inhibited on exposure to norepinephrine, cAMP levels drop, PKA is inhibited, and the pigment granules aggregate in themelanophores.

B. splendens skin was transparent on early rearing period and lowT4 concentration (0.001 ppm) and it became opaque and more opaque in relation to T4 concentrations (0.005 –0.04 ppm) and time (14 days). The transparent larvae of *Chanos chonos* changed in toopaque on day 15 when they treated with
0.5 ppm *L. thyroxine* (Lam*et al.*, 1985). Nacario (1983) found that thyroxine caused thickening of the epidermis in yolk sac larvae and fry of *Sarotherodonniloticus*.

The present study revealed that *B. splendens* fry treated with 0.01ppm T4 elicited the better growth parameters than other T4 concentration. It suggests that 0.01 ppm is considered as optimum dose for *B. splendens* fry. The same optimum dose was obtained in larvae of *Cyprinus carpio* (Lam and Sharma, 1985) and *Carassius auratus* (Reddy and Lam, 1992b), supports the present study. To maximise the growth and survival in *Anabas testudineus* larvae

was found to be 0.005 ppm T4 (Pradhan, 1997; Nayak and Thomas, 2000); 0.005 ppm for *O. mossambicus* (Reddy and Lam, 1992a); 0.025 ppm for *O. mossambicus* (James and Sampath, 1994), 1 ppm for *Mystus vittatus* (Muniandy, 1990). Comparing the present study with earlier works it issuggested that the optimum dose may be species specific which differs in different species.

High growth observed in 0.01 ppm may be due to the increased basal metabolism, glucose disposal and oxidation (Muller *et al.,* 1988) which in turn increased the appetite and food intake resulted in good growth. Sambhu and Jayaprak as (1997) reported that, increased RNA and DNA contents and RNA / DNA ratio in thyroxine treated *E. suratensis* which was also found to simulate protein synthesis and to promote growth and thereby increase the feed conversion efficiency and more grow that 0.05 ppm thyroxine level. In addition, T4 treatment decreased the excretion of metabolites and increase nitrogen retention in tissues (Garg, 2007) also another reason for higher growth.

In the current study, higher dose of thyroxine (0.04 ppm) caused marked reduction in growth parameters in *B.splendens.* Honma (1955) reported that thyroid hormone at higher concentration cause tissue breakdown and loss of weight in fishes. Excessive exogenous supply of thyroid hormone interferes with feedback mechanism causing decrease in synthesis and release of TSH from pituitary resulting in growth reduction (Rangneker and Latey, 1971; Muniandy, 1990). Donaldson *et al.,* (1979) found that higher doses of steroid hormones exert deleterious effects on various organs and cumulatively cancel the growth promoting effects.

CONCLUSION

The present study concludes that, 0.01 ppm is an optimumosefor fry to maximize the growth, melanophore formation and air bladder development.

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Table 1. Effect of thyroxine concentrations on morphometric measurements In *Betta splendens* fry.

Each value is the mean (X ± SD)performance of three observations.

Rearing period (days)	T4 concentrations (ppm)					
	0	**0.001**	**0.005**	**0.010**	**0.020**	**0.040**
	No. of doral fin rays					
0	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND
14	8.00±0.00	8.00±0.00	10.30±1.51	12.00±0.00	12.00±0.00	12.00±0.00
21	8.00±0.00	8.00±0.00	12.00±0.00	12.00±0.00	12.00±0.00	12.00±0.00
28	8.00±0.00	8.00±0.00	12.00±0.00	12.00±0.00	12.00±0.00	12.00±0.00
	No. of caudal fin rays					
0	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00
7	11.00±0.00	12.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00
14	12.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00
21	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00
28	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00	14.00±0.00
	Length of air bladder (mm)					
0	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND
14	^{a}0.018±0.003	^{b}0.042±0.003	^{b}0.050±0.002	^{d}0.088±0.006	^{c}0.078±0.003	^{c}0.068±0.003
21	^{a}0.071±0.033	^{b}0.325±0.015	^{c}0.357±0.006	^{d}0.409±0.013	^{d}0.410±0.009	^{d}0.410±0.009
28	^{a}0.398±0.003	^{b}0.457±0.006	^{c}0.497±0.007	^{d}0.624±0.038	^{c}0.517±0.029	^{c}0.510±0.013
	Melanophores					
0	-	-	-	-	-	-
7	Melanophores are seen in patches in mid dorsal region of head only	Uniformly scattered in head & trunk not in C.F. and D.F.	Uniformly scattered in head & trunk including C.F. and D.F.	Uniformly scattered in head and trunk including C.F. & D.F.	Uniformly scattered in head and trunk including D.F.	Uniformly scattered in head and trunk including C.F.

14	Melanophores found in head and trunk	Found in head and trunk	Found in head and trunk	More cells in head and trunk	Intensified	Intensified
21	Lesser	More	More intensified	Intensified	Intensified	Intensified
28	More intensified under high power, invisible to eye	Invisible to eye	Invisible to eye	Visible to eye, both caudal anal fins	Visible in both fins	Visible in both fins
	Nature of skin					
0	Transparent	Transparent	Transparent	Transparent	Transparent	Transparent
7	Transparent	Transparent	Transparent	Transparent	Transparent	Transparent
14	Transparent	Transparent	Partial opaque	Opaque	Opaque	Opaque
21	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
28	Opaque	Opaque	More opaque	More opaque	More opaque	More opaque

Student's 't' test – Length of air bladder (on day 28)

0 Vs 0.010 : t = 12.55 ; P < 0.01, 0.010 Vs 0.001 : t = 3.33 ; P < 0.05

0.010 Vs 0.005 : t = 4.70; P < 0.01

0.010 Vs 0.020 : t = 3.24; P < 0.05

Table 2. Effect of thyroxine concentrations on mean body weight (mg wetwt.) and length, standard length, caudal fin length and head size (mm) in *Betta splendens)* fry. Each value is the mean (X ± SD) ofthree observations.

Rearing period (days)	T4 Concentrations (ppm)					
	0	**0.001**	**0.005**	**0.010**	**0.020**	**0.040**
	Mean body weight (MBW)					
0	^{a}1.08±0.04	^{a}1.08±0.04	^{a}1.08±0.04	^{a}1.08±0.04	^{a}1.08±0.04	^{a}1.08±0.04
7	^{a}10.01±0.04	^{a}12.00±1.79	^{b}16.00±3.10	^{c}25.80±2.04	^{ab}13.30±1.51	^{ab}13.50±1.64
14	^{a}22.30±0.16	^{b}39.30±0.07	^{d}58.30±0.93	^{e}70.80±0.92	^{c}43.40±0.91	^{b}38.30±0.34
21	^{a}32.30±1.53	^{b}64.50±1.67	^{c}82.50±1.17	^{d}108.30±1.30	^{b}62.80±1.99	^{b}61.70±1.50
28	^{a}42.50±2.07	^{c}75.50±2.95	^{d}111.30±3.01	^{e}152.00±3.95	^{c}76.20±3.37	^{a}70.30±1.03
	Mean body length (MBL)					
0	^{a}3.25±0.07	^{a}3.25±0.07	^{a}3.25±0.07	^{a}3.25±0.07	^{a}3.25±0.07	^{a}3.25±0.07
7	^{a}5.39±0.04	^{b}6.50±0.15	^{c}7.80±0.26	^{d}8.50±0.05	^{b}6.80±0.41	^{a}5.70±0.16
14	^{b}8.24±0.16	^{b}8.50±0.18	^{c}9.20±0.18	^{c}9.50±0.25	^{a}7.80±0.21	^{a}7.50±0.15
21	^{a}9.83±0.15	^{b}11.80±0.14	^{c}13.80±0.15	^{d}17.30±0.26	^{c}14.00±0.09	^{b}12.05±0.09
28	^{a}12.20±0.20	^{c}17.50±1.13	^{c}18.0±1.10	^{d}19.80±0.26	^{c}17.80±0.10	^{b}15.50±0.12
	Mean standard length					
0	3.06±0.08	3.06±0.08	3.06±0.08	3.06±0.08	3.06±0.08	3.06±0.08
7	^{a}5.08±0.04	^{b}6.46±0.02	^{c}7.37±0.23	^{d}8.07±0.01	^{b}6.43±0.38	^{a}5.39±0.50
14	^{b}7.28±0.16	^{b}7.40±0.16	^{b}7.60±0.16	^{b}7.50±0.25	^{a}5.80±0.20	^{a}5.50±0.14
21	^{b}8.45±0.15	^{c}9.50±0.05	^{e}11.30±0.12	^{f}13.30±0.25	^{d}10.20±0.03	^{a}8.13±0.01
28	^{a}9.60±0.20	^{c}14.13±0.57	^{b}13.00±1.09	^{c}14.80±0.26	^{b}12.80±0.01	^{c}9.50±0.12
	Mean caudal fin length					
0	0.19±0.01	0.19±0.01	0.19±0.01	0.19±0.01	0.19±0.01	0.19±0.01
7	^{a}0.31±0.01	^{bc}0.40±0.03	^{c}0.43±0.03	^{b}0.43±0.03	^{b}0.37±0.03	^{a}0.31±0.02
14	^{a}0.96±0.01	^{b}1.10±0.02	^{c}1.60±0.02	^{d}2.00±0.01	^{d}2.00±0.01	^{d}2.00±0.01
21	^{a}1.38±0.01	^{b}2.30±0.19	^{c}2.50±0.03	^{e}4.00±0.01	^{d}3.80±0.06	^{de}3.92±0.10
28	^{a}2.60±0.01	^{b}4.00±0.01	^{c}5.00±0.01	^{c}5.00±0.01	^{c}5.00±0.10	^{d}6.00±0.04
	Mean head size					
0	0.29±0.007	0.29±0.007	0.29±0.007	0.29±0.007	0.29±0.007	0.29±0.007
7	^{a}0.31±0.010	^{bc}0.40±0.025	^{cd}0.43±0.027	^{d}0.47±0.013	^{b}0.38±0.036	^{b}0.37±0.032
14	^{a}0.99±0.003	^{b}2.00±0.004	^{b}2.00±0.005	^{c}3.00±0.002	^{c}3.01±0.011	^{c}3.02±0.014
21	^{a}2.00±0.004	^{b}3.00±0.002	^{b}3.00±0.001	^{c}4.00±0.001	^{d}4.52±0.324	^{e}5.00±0.002
28	^{a}3.00±0.009	^{b}4.03±0.026	^{c}5.05±0.047	^{c}5.08±0.019	^{c}5.08±0.019	^{d}6.00±0.002

Values (mean ±SD) with different superscript in the same row are significantlydifferent (P<0.05)

Table 3. Two-way ANOVA for growth parameters of *Betta splendens* fry inrelation to thyroxine levels and rearing period.

Source of variation	SS	df	MS	F-value	P-value
Mean body weight					
Between rearing period	2066.20	14	147.59	105.21	P < 0.01
Between thyroxineconcentrations	224.72	5	44.94	32.04	P < 0.01
Error	98.20	70	1.40		
Total variation	2389.12	89			
Mean standard body length					
Between rearing period	927.08	14	66.22	66.23	P < 0.01
Between thyroxineconcentrations	144.84	5	28.97	28.97	P < 0.01
Error	69.99	70	1.00		
Total variation	1141.91	89			
Mean caudal fin length					
Between rearing period	249.89	14	17.85	57.38	P < 0.01
Between thyroxineconcentrations	26.15	5	5.23	16.81	P < 0.01
Error	21.78	70	0.31		
Total variation	297.81	89			
Mean head size					
Between rearing period	271.20	14	19.37	69.19	P < 0.01
Between thyroxineconcentrations	25.46	5	5.09	18.19	P < 0.01
Error	19.60	70	0.28		
Total variation	316.26	89			
Length of air bladder					
Between rearing period	3.69	14	0.26	86.28	P < 0.01
Between thyroxineconcentrations	0.15	5	0.03	9.55	P < 0.01
Error	0.21	70	0.003		
Total variation	4.05	89			

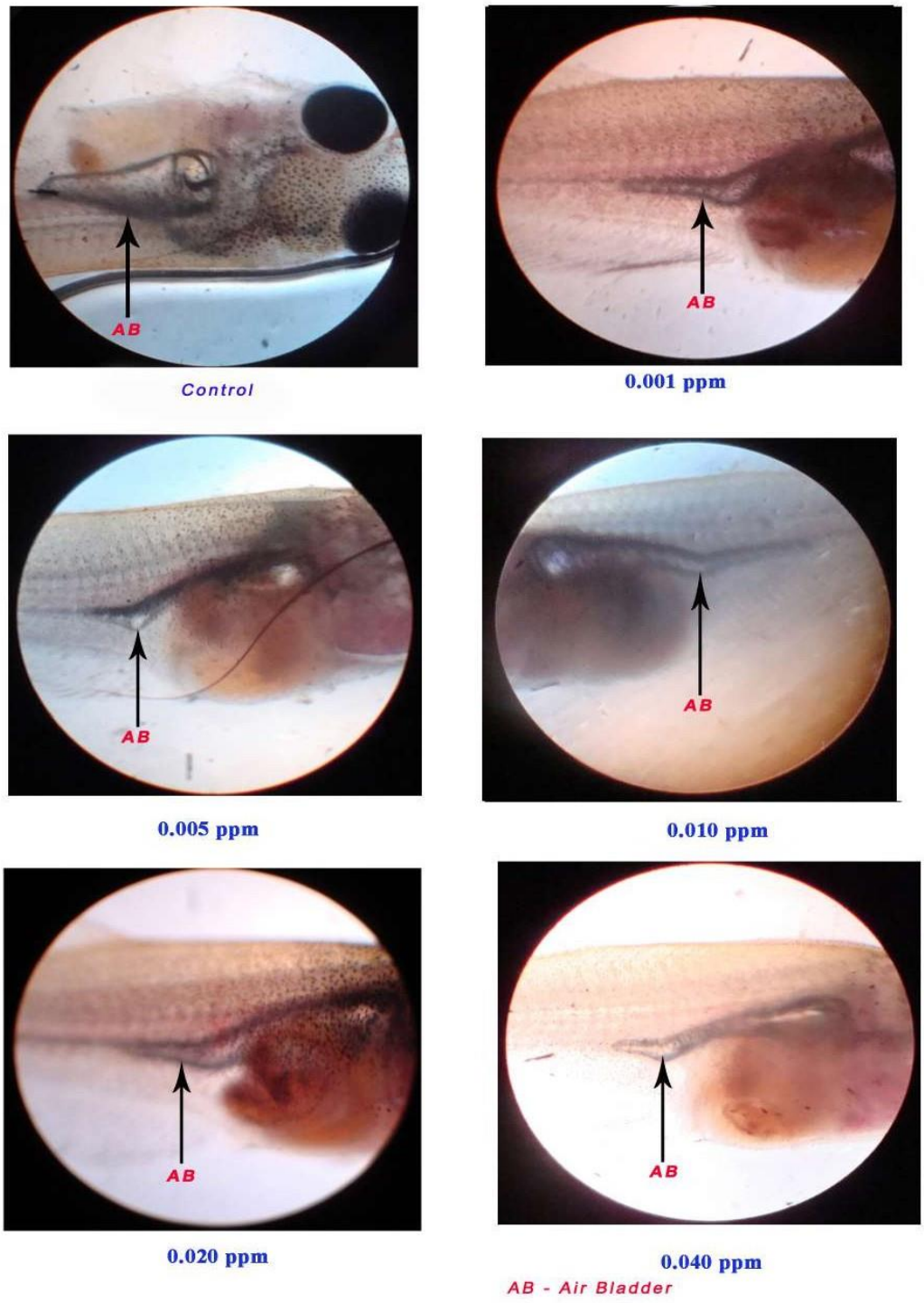


Plate 1. Effect of thyroxine on development of air bladder in *Betta splendens* fry on day 14.

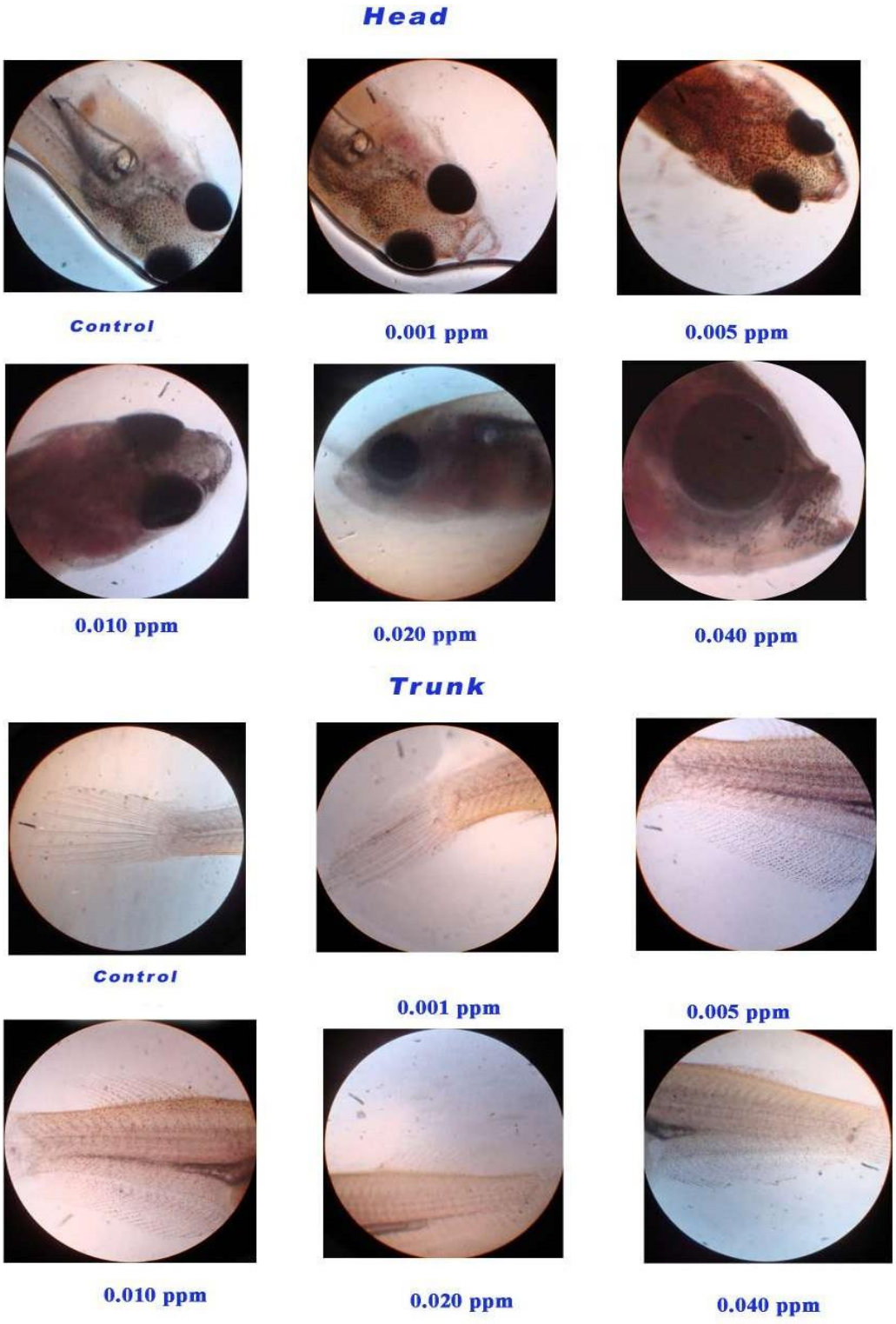
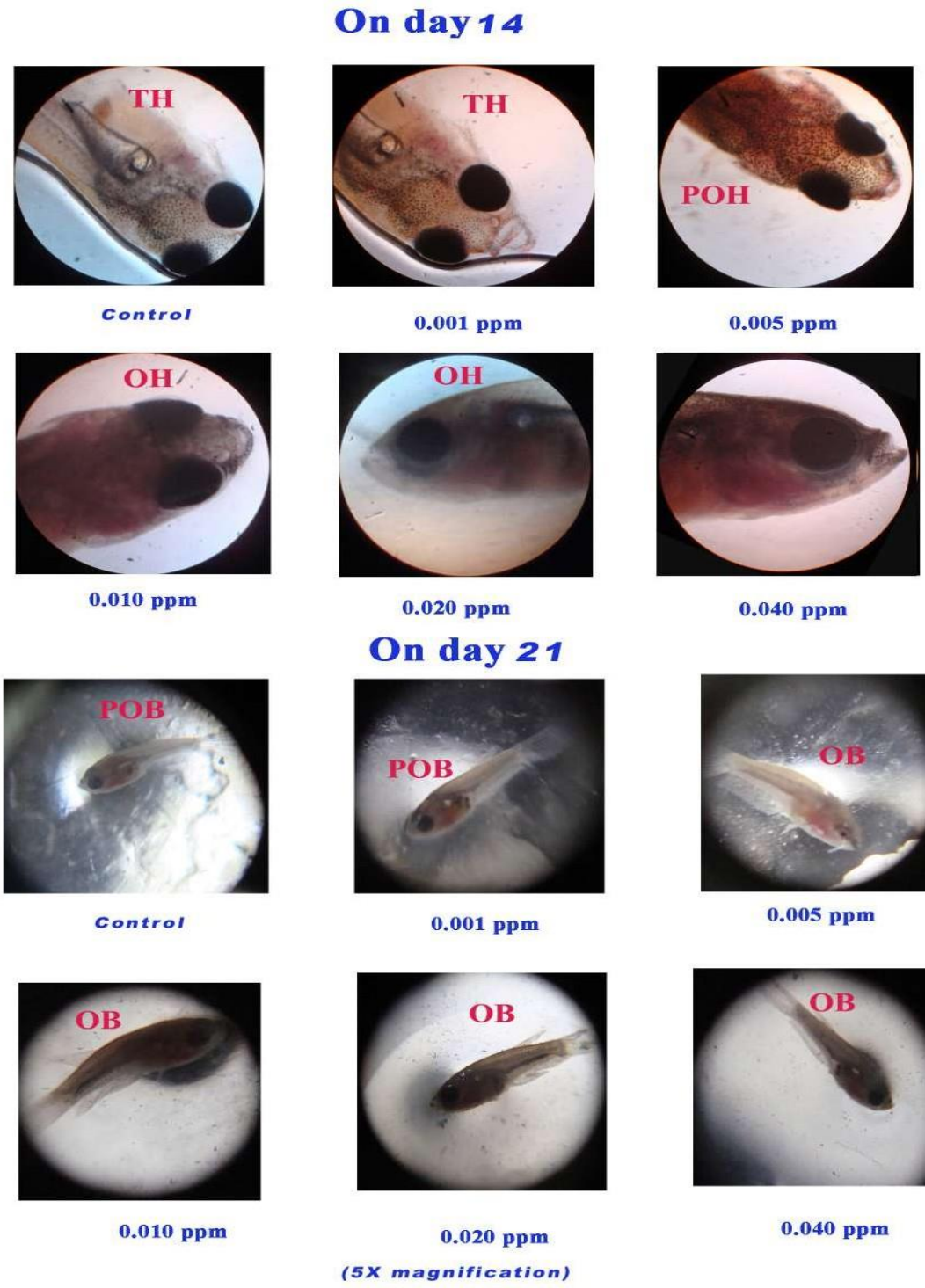


Plate 2. Effect of thyroxine on distribution of melanophores in *Betta splendens* fry on day 14.



TH - Transparent head; POH - Partial obaque head; OH - Obaque head;
POB - Partial obaque body; OB - Obaque body

Plate 3. Effect of thyroxine on development of body texture in *Betta splendens* fry on day 14 and 21.