

Role of Diethylstilbestrol on Growth of Green Chromide, *Eetroplus suratensis*

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Green chromide was cultured by feeding diets supplemented with five levels (1, 2, 3, 4 and 5 ppm) of diethylstilbestrol for 120 days in a brackish water pond and its growth performance was evaluated. Diethylstilbestrol at 2 ppm level produced significantly ($p < 0.01$) superior growth over the control. Feed consumption, conversion efficiency and nutrient digestibility were improved due to the supplementation of hormone in the diet. Gonadosomatic index was high in the fishes fed maximum dosage of diethylstilbestrol. The RNA/DNA ratio and protein contents in the muscle increased with the high growth rate. In green chromide, diethylstilbestrol stimulated protein synthesis and promoted growth through enhanced digestive enzymes activity and improved nutrient digestibility.

Key words : Diethylstilbestrol, growth, digestive enzymes, RNA/DNA ratio, *Eetroplus suratensis*.

Estrogens, produced by the ovary are responsible for sex function and have anabolic effects. A number of compounds are known to mimic the physiological effect of natural estrogens, although not related in chemical structure. One such synthetic hormone is diethylstilbestrol (DES) which possesses estrogenic and anabolic properties superior to those of natural estrogens. DES is widely used in livestock and poultry as a feed additive to improve milk and meat production. Aquaculturists have shown interest in developing estrogen-incorporated diets, which accelerate growth and lead to early maturation in fishes (Cowey *et al.*, 1973). Oral administration of DES to fish has been found to improve their growth (Satoh & Nimura, 1991; Sindhu, 1992; Jayaprakas & Sambhu, 1995; Basavaraja *et al.*, 1995). The present work was undertaken to standardise the optimum level of DES

required to induce maximum growth in green chromide, *Eetroplus suratensis*, a cichlid, widely distributed all over India and which has great aquaculture potential.

Materials and Methods

The study was conducted in a brackish water pond near Paravoor lake (84°6'N; 76°44', 76°48'E) for a period of 120 days. Fry of *E. suratensis* were collected from the lake using a cast net and stocked in nylon net hapa (1M²) 10 days prior to the experiment. Uniform size (3.64±0.21g) fry were randomly stocked (25 nos) in each hapa and three replicates of each treatment were maintained in a completely randomised design. Fish meal based supplementary diet having 40% protein was prepared (Table 1) and used for hormone incorporation. DES obtained from British Pharmaceuticals Pvt. Ltd., Bombay was

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Table 1. Proportions of ingredients of the diet of *Etroplus suratensis*

Ingredients	Proportion (g)	Protein (%)
Rice bran	13.24	1.15
Groundnut oil cake	36.76	18.49
Tapioca flour	13.24	0.29
Fish meal	36.76	20.07
Total	100.00	100.00

incorporated in the diet at dosages of 1 (T1), 2 (T2), 3 (T3), 4 (T4) and 5 ppm (T5). The 40% protein diet without hormone served as the control (T0). Feeding was done at the rate of 10% of the body weight daily which was split into two rations, one fed in the morning and the other in the afternoon. The quantity of the feed was readjusted fortnightly based on the growth of the fish. Water quality parameters like temperature, dissolved oxygen, pH and salinity were monitored biweekly adopting APHA (1992) procedures. The fishes were sampled once in a fortnight and minimum of 15 fishes were randomly collected for recording the total length and weight. On termination of the experiment, all the surviving fishes were collected and their individual length and weight were recorded. Samples of 10 specimens were dissected, weight of viscera, liver and

gonad were recorded and viscerosomatic index (VSI), hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated as follows.

Tissue index (%) = $\frac{\text{Weight of tissue}}{\text{Weight of fish}} \times 100$

The influence of DES on digestive enzymes viz., amylase, protease and lipase in the stomach and intestinal segments were assayed following the method of King (1965). Unit amylase activity was calculated as mg of maltose liberated during 10 min at 30°C. Protease activity was expressed as the amount of tyrosine liberated in 15 min and lipase activity was expressed as the amount of 0.025 N NaOH required to neutralise the fatty acids liberated during 18 h of incubation at 30°C. Total and specific activities of the digestive enzymes were calculated as follows.

Total activity =

Unit enzyme activity/g tissue

Specific activity =

Unit enzyme activity/mg protein

DNA and RNA contents of the muscle and liver were estimated by the method of Carlewis & Stone (1987). The residual hormone content in the serum of

Table 2. Analysis of covariance on the cumulative % increase in length and weight of *Etroplus suratensis* after each 15 day sampling

Treat ment	Length				Weight			
	Average growth (%)	f value	Rate of growth (%)	f value	Average growth (%)	f value	Rate of growth (%)	f value
T0	58.98		0.499		125.12		1.74	
T1	81.47		0.812		190.97		3.53	
T2	86.57		0.852		231.23		4.18	
T3	80.22	14.08**	0.781	3.71*	189.43	7.69**	3.41	12.30**
T4	70.97		0.702		177.64		2.85	
T5	66.35		0.603		135.73		2.04	

n = 15; * p, < 0.05; ** p < 0.01

hormone treated and control fishes was analysed by subjecting them to radioimmunoassay. For this, serum was collected by homogenizing the muscle tissues with chilled distilled water and the supernatant (serum) was collected by centrifuging the homogenate at 5000 rpm for 10 min. This serum was used to estimate the amount of DES present in it (RIA, CIS ORIS Industry, Calcutta). The technique of radioimmunoassay was based on the isotope dilution principle and the amount of hormone present was expressed as ng/ml.

A short term experiment was conducted in fibre glass tanks (50 litres) using 10 fish (3.5 ± 0.3 g) each in triplicate for 30 days to assess the feed intake, conversion efficiency, protein efficiency ratio and digestibility of protein and lipid (De Silva, 1989). Fishes were fed daily with the diet at 10% of the body weight for 30 days. The unconsumed feed and faecal matter were removed separately every day and the pooled faecal samples were analysed for nutrients (protein and lipid). Feed conversion efficiency and protein efficiency ratio were calculated as follows.

Feed conversion (FCE) (%) =

$$\frac{\text{Weight gain (g)}/\text{Feed consumed (g)} \times 100}{\text{Protein efficiency ratio (PER) (\%) =}}$$

Protein efficiency ratio (PER) (%) =

$$\frac{\text{Increase in body weight (g)}/\text{Protein consumed (g)} \times 100}{\text{Apparent nutrient (protein \& lipid)}}$$

Apparent nutrient (protein & lipid)

digestibility (%) =

$$\frac{(\text{Nutrient in feed} - \text{Nutrient excreta})/\text{Nutrient in feed} \times 100}{\text{Proximate analysis of fish muscle}}$$

Proximate analysis of fish muscle was carried out by standard methods (AOAC, 1990). Analysis of variance (ANOVA) (Snedecor & Cochran, 1968) was employed to find out the statistical significance in the final mean length and weight, feed utilisation and digestive enzyme levels. Duncan's multiple range test (Steel & Torrie, 1980) was also employed to compare the statistical difference between treatment means.

Results and Discussion

The mean water temperature, pH, dissolved oxygen and salinity were 24.5°C, 7.50, 6.12 mg/L and 29.7 ‰ respectively. The fortnightly average weight attained by the fishes are given

Table 3. Effect of DES on growth and survival of *Etroplus suratensis*

Parameter	f value	Treatment											
		T0		T1		T2		T3		T4		T5	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Initial length (cm)		5.51	0.35	5.01	0.25	5.31	0.30	5.50	0.31	5.41	0.25	5.31	0.53
Initial weight (g)		3.64	0.21	3.34	0.11	3.60	0.20	3.54	0.31	3.61	0.21	3.21	0.23
Final length (cm) #	2.03*	10.31 ^a	1.28	12.01 ^b	1.01	12.20 ^b	1.84	11.81 ^{ab}	0.83	11.22 ^{ab}	0.92	10.67 ^a	1.01
Final weight (g) #	12.32**	12.12 ^a	1.37	18.90 ^c	1.81	21.26 ^d	2.12	18.46 ^c	1.81	15.22 ^b	0.91	13.02 ^{ab}	0.71
Net weight gain (g)		8.48	0.84	15.56	1.81	17.66	2.12	14.92	2.00	11.61	1.83	9.71	1.83
S.G.R. (%)		1.00	0.01	1.37	0.04	1.47	0.03	1.35	0.06	1.19	0.02	1.02	0.01
Survival (%)		92.00	2.00	96.00	2.00	98.00	2.00	94.00	4.00	96.00	2.00	92.00	4.00

n = 25

* p<0.05 ** p<0.01

a, b, c, d - Means with the same superscript do not differ from each other (Duncan's multiple range test)

Table 4. Effect of DES on feed utilization by *Etroplus suratensis*

Parameter	f value	Treatment											
		T0		T1		T2		T3		T4		T5	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Feed consumption (g)	9.17**	18.88 ^a	1.11	19.31 ^a	1.84	23.24 ^c	3.71	19.01 ^a	2.00	20.61 ^{ab}	2.73	21.00 ^b	1.87
Assimilation (g)		14.67	2.02	14.87	1.28	18.23	0.93	14.01	1.87	15.71	2.00	15.93	1.87
Production (g)		5.01	0.68	5.93	0.45	7.00	0.72	6.71	0.87	6.40	0.41	5.42	0.53
Feed conversion efficiency (%)	10.12**	26.53 ^a	1.27	30.70 ^b	1.11	30.12 ^b	1.34	35.29 ^c	0.87	31.05	1.86	25.80 ^a	1.47
Feed assimilation efficiency (%)		77.70	3.34	77.00	2.79	78.44	1.31	73.69	2.81	73.79	3.14	75.85	3.00
P.E.R. (%)		12.52	3.05	14.82	1.01	17.50	0.86	16.77	1.88	16.00	1.81	13.55	1.00
A.P.D. (%)	7.11**	69.00	3.13	71.32	3.04	74.18	2.07	70.33	1.91	72.33	2.00	71.28	2.31
A.L.D. (%)	5.33**	83.33	2.92	81.24	2.18	88.15	1.31	85.33	1.89	84.58	1.82	83.99	2.09

P.E.R. - Protein Efficiency Ratio; A.P.D. - Apparent Protein Digestibility; A.L.D. - Apparent Lipid Digestibility

** $p < 0.01$

a, b, c, d - Means with the same superscript do not differ from each other (Duncan's multiple range test)

in Fig. 1. The cumulative percentage increase in length and weight of *E. suratensis* differed significantly ($p < 0.01$) from that of the control after every 15 day sampling (Table 2). Fishes that received 2 ppm DES exhibited significantly ($p < 0.01$) superior growth than any other treatments (Table 3). VSI and HSI were high in fishes fed 2 ppm hormone (Fig. 2). It is interesting to note that the GSI value increased ($p < 0.05$) with higher dosages of DES and the maximum value was for the group which received 5 ppm DES.

Details of the feed utilisation are presented in Table 4. Maximum feed

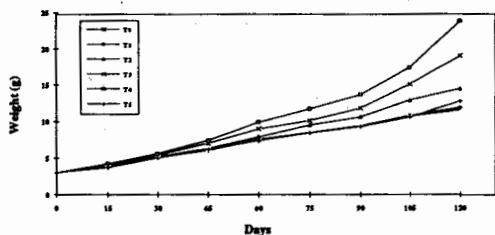


Fig. 1. Effect of diethylstilbestrol on the increase in weight of *Etroplus suratensis*.

intake, assimilation efficiency, protein efficiency ratio and nutrient (protein and lipid) digestibility were observed in fishes treated with 2 ppm DES. The maximum feed conversion efficiency was recorded in fishes fed with 3 ppm DES.

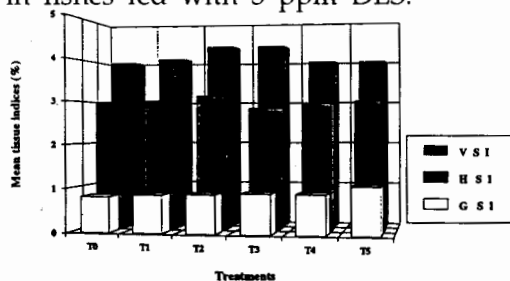


Fig. 2. Effect of diethylstilbestrol on tissue indices of *Etroplus suratensis*.

Data on the digestive enzyme activity are presented in Table 5. Total amylase, protease and lipase activities were high in the stomach and anterior intestinal segment of fishes fed 2 ppm DES. However lipase activity was comparatively lower than amylase and protease activities. Details regarding the nucleic acid content in the muscle and liver of experimental fishes are presented

Table 5. Effect of DES on digestive enzymes activity in the stomach and intestinal segments of *Etrophus suratensis*

Tissue	Enzyme Activity	f value	T0		T1		T2		T3		T4		T5	
			M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Amylase														
Stomach	T.A.	13.37**	389.13 ^a	2.83	439.21 ^b	3.01	532.11 ^e	4.59	475.81 ^d	3.82	463.31 ^c	4.61	428.19 ^b	3.01
	Sp.A	1.07 ^{NS}	5.79	0.82	6.29	2.34	6.24	0.11	5.95	0.31	5.80	0.82	5.43	0.62
Intestine-I	T.A.	12.11**	401.34 ^b	3.42	466.32 ^c	2.49	548.31 ^e	3.58	498.33 ^d	4.81	463.12 ^c	3.71	378.33 ^a	3.88
	Sp.A	0.89 ^{NS}	6.67	0.93	7.35	0.67	7.85	0.32	7.10	0.21	7.81	0.19	7.53	0.11
Intestine-II	T.A.	6.73**	318.19	3.11	333.67	2.87	394.28	1.87	363.18	3.01	348.19	2.01	325.63	3.01
	Sp.A	0.93 ^{NS}	6.46	0.71	6.51	0.22	7.02	0.18	7.34	0.21	7.21	0.29	6.87	0.12
Intestine-III	T.A.	6.71**	139.19	1.86	168.18	2.56	170.13	1.81	171.34	2.83	150.18	2.12	140.13	1.87
	Sp.A	1.02 ^{NS}	3.41	0.14	4.40	0.21	3.39	0.24	4.49	0.18	3.83	0.10	3.49	0.21
Protease														
Stomach	T.A.	3.31**	231.44 ^a	3.19	289.78 ^b	3.00	393.26 ^d	4.01	328.14 ^c	2.09	261.39 ^{ab}	3.03	248.33 ^a	3.01
	Sp.A	1.11 ^{NS}	3.44	0.08	4.15	0.09	4.61	0.31	4.09	0.56	3.27	0.41	3.15	0.19
Intestine-I	T.A.	5.82**	368.23 ^a	2.94	388.74 ^b	4.59	400.01 ^e	5.00	403.21 ^{cd}	3.87	396.17 ^{bc}	4.31	390.19 ^b	4.01
	Sp.A	0.82 ^{NS}	6.12	0.02	6.29	0.18	0.72	0.21	8.15	0.32	6.34	0.42	7.37	0.51
Intestine-II	T.A.	5.41**	241.39	1.28	273.13	2.08	308.26	4.00	304.71	3.93	286.39	4.01	260.18	2.01
	Sp.A	3.01*	4.90	0.09	5.33	0.01	5.49	0.51	6.16	0.71	5.95	0.62	5.49	0.66
Intestine-III	T.A.	6.12*	121.39	1.83	139.19	1.41	188.91	1.02	173.19	1.05	123.11	1.47	123.00	1.46
	Sp.A	1.02 ^{NS}	2.02	0.02	3.64	0.13	3.75	0.28	4.54	0.09	2.89	0.21	2.99	0.19
Lipase														
Stomach	T.A	3.11*	4.62	0.08	4.68	0.09	5.81	0.01	4.72	0.08	4.41	0.08	4.43	0.07
	Sp.A	0.86 ^{NS}	0.04	0.01	0.04	0.01	0.03	0.02	0.03	0.01	0.03	0.02	0.03	0.01
Intestine-I	T.A.	3.41*	5.62	0.01	5.71	0.04	7.87	7.07	5.61	0.01	5.67	0.05	5.51	0.09
	Sp.A	0.51 ^{NS}	0.04	0.01	0.13	0.01	0.03	0.03	0.04	0.01	0.04	0.01	0.03	0.02
Intestine-II	T.A.	0.43 ^{NS}	3.22	0.07	3.38	0.09	4.41	0.07	3.40	0.04	3.39	0.08	3.32	0.08
	Sp.A	0.21 ^{NS}	0.02	0.01	0.02	0.01	0.03	0.01	0.02	0.01	0.03	0.01	0.01	0.01
Intestine-III	T.A.	0.33 ^{NS}	1.20	0.07	1.28	0.04	1.33	0.04	1.24	0.08	1.30	0.07	1.21	0.07
	Sp.A	0.12 ^{NS}	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01

T.A. = Total activity; Sp.A = Specific activity

NS - Non significant; * p<0.05; ** p<0.01

a, b, c, d - Means with the same superscript do not differ from each other (Duncan's multiple range test)

in Fig. 3. Maximum levels of RNA and DNA and RNA/DNA ratio were observed in fishes of the T2 group which showed the highest growth. Proximate composition of fish muscle showed that the proportion of protein was highest for the T2 group (Table 6). Total lipid content was high in the fish given 2 ppm DES and low in the control. The residual DES content in the muscle of hormone treated and control fishes are presented in Table 7. Fishes treated with the maximum dosage (5 ppm) of hormone showed highest residual serum hormonal

content, followed by the 2 ppm group on the 120th day of the experiment. The residual serum hormone concentration dropped to the level comparable to that of control fishes 20 days after termination of the experiment.

The effect of DES in promoting growth appears to be dose dependent (Shreck & Fowler, 1982; Sower *et al.*, 1983; Sindhu, 1992). According to Basavaraja *et al.* (1989) 5 ppm DES brought about appreciable increase in growth of common carp. Similar results

Table 6. Effect of DES on body composition of *Etrophus suratensis*

Parameter	F value	Treatment					
		T0	T1	T2	T3	T4	T5
Moisture (%)	3.73*	60.11±2.11	63.72±3.12	53.17±1.87	58.73±2.84	16.71±1.85	69.72±1.87
Drymatter (%)	6.73**	39.89±1.08 ^{ab}	36.28±2.00 ^b	46.83±1.85 ^c	41.27±1.31 ^d	38.29±1.84 ^{bc}	30.28±2.00 ^a
Protein (%)	12.13**	62.13±1.72 ^a	64.73±2.09 ^{ab}	74.13±1.93 ^c	70.44±1.86 ^d	68.78±2.01 ^c	67.13±1.90 ^c
Lipid (%)	3.89*	2.89±0.08 ^a	2.94±0.03 ^{ab}	3.01±0.04 ^b	2.96±0.01 ^{ab}	2.38±0.08 ^a	2.91±0.07 ^a
Ash (%)	0.92 ^{NS}	3.66±0.57	3.81±0.48	3.92±0.34	4.02±0.29	3.83±0.72	3.61±0.31
NFE (%)	3.31*	31.32±0.87	28.52±0.29	18.94±0.81	22.58±1.12	25.01±1.12	26.35±0.97

Expressed on dry weight basis

NFE - Nitrogen free extract

NS - Non significant; * $p < 0.05$; ** $p < 0.01$

a, b, c, d - Means with the same superscript do not differ from each other (Duncan's multiple range test)

were also observed in rohu (Konda Reddy *et al.*, 1987) and tilapia (Sindhu, 1992). Nanjundappa & Varghese (1989) observed that 3 ppm DES enhanced growth rate in *Labeo rohita*, while 2.5 ppm had promoted better growth in Japanese eel. In the present study 2 ppm DES induced significantly high growth in *E. suratensis* over control. This finding clearly indicates that the influence of DES in promoting growth varies among species and its effect is dose dependent.

Higher levels of DES suppressed the growth rate in fishes (Shyama, 1987; Satoh & Nimura, 1991) which act as appetite depressor and adversely affect feed conversion efficiency (Nirmala & Pandian, 1983). In the present study also DES at levels higher than 2 ppm was not found to have any advantage. Hence it can be suggested that 2 ppm DES is the optimum dose required to promote maximum growth in *E. suratensis*.

Higher growth of fish under DES treatment can be correlated with better feed conversion efficiency, assimilation efficiency and nutrient digestibility (Sindhu, 1992; Sambhu & Jayaprakas, 1994). In the green chromide also similar

trend in feed utilisation was observed with 2 ppm DES.

Dietary administration of DES may bring about certain changes in the tissue indices of fishes (Nanjundappa & Varghese, 1989; Basavaraja *et al.*, 1989). In the present study high VSI and HSI were observed in fast growing fishes under 2 ppm DES. Lone & Matty (1983) observed that the changes in the tissue indices are more pronounced in fast

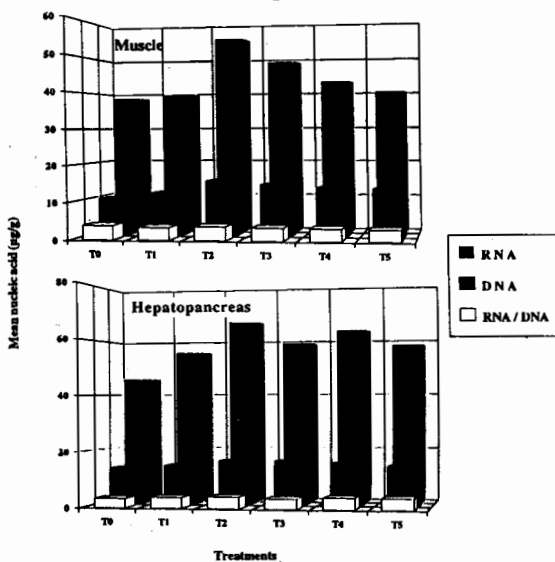


Fig. 3. Effect of diethylstilbestrol on nucleic acid content of *Etrophus suratensis*.

Table 7. Residual hormone content (ng/ml) in *Etroplus suratensis*

Treatment	Hormone dose (ppm)	Termination day			20th day		
		M	SD	f value	M	SD	f value
T0	Nil	2.10	0.02		2.11	0.02	
T2	2.0	2.31	0.04	6.71**	2.12	0.04	0.073 ^{NS}
T5	5.0	2.52	0.02		2.12	0.03	

**p<0.01, NS - Non significant

n = 5

growing fish and these indices are dependent upon weight rather than age of the fish. The increase in GSI with the higher dosages can be attributed to the stimulatory effect of DES on the development of gonad.

In the present investigation comparatively higher digestive enzyme (amylase, protease and lipase) activities were observed in the DES treated fishes, particularly in the fast growing fishes treated with 2 ppm DES. The exogenous growth promoters show the ability to stimulate gastro-intestinal hormones to act on the acinar cells to secrete digestive enzymes (Lone & Mathy, 1981). Hence it is suggested that the increased activity of digestive enzymes noticed in the green chromide was due to the incorporation of DES in the diet. The high nutrient digestibility observed in the DES treated fish can be attributed to the enhanced digestive enzyme activity.

The significantly high RNA and DNA contents observed in the present study is in agreement with the results reported by Lone & Matty (1982) in common carp. Bulow (1970) and Haines (1973) observed that with increased availability of food to the fish, there was an increase in RNA/DNA ratio (increased protein synthesis) in the liver and stomach with an overall increase in the weight of the fish. The high protein

content in the muscle of DES treated fishes may be due to the enhanced protein synthesis and better utilization of protein of the diet. Serum analysis on the 20th day after withdrawal of DES incorporated diet showed that the residual hormone present in the body on the termination of the experiment had been completely metabolised and eliminated (Table 7). Hence it is suggested that the DES treated fishes are fit for human consumption after 20 days of withdrawal of hormone supplemented diet.

The mechanism of action of estrogenic hormones in promoting growth is not well understood. The ingested hormones are digested in the gut of fishes (Pelissero & Sumpter, 1992). The small molecules are quite stable and are able to cross the gut wall much more easily than proteins. They can resist the low pH encountered in fish stomach (Western & Jennings, 1970). These compounds then stimulate the formation of steroid binding proteins (SBP) in the enterohepatic circulation (Pelissero & Sumpter, 1992). The enterohepatic circulation then conveys the SBP compounds directly to the liver (Adlercreutz *et al.*, 1987). In liver all estrogen like substances are able to act as stimulating agents. The liver is the major target organ since the hepatocytes contain mainly in their nuclei, proteins

which recognise estrogens, specifically the so called estrogen receptors (Smith & Thomas, 1990). When the estrogen-like compound is linked to the receptor it gets activated. The activated receptor can then react, probably as a dimer, coding for the appropriate protein. Estrogen not only regulates the transcription of specific genes, but also stabilizes mRNA (Mc Kenzie & Knowland, 1990).

The authors wish to record their sincere thanks to Prof. Dr. P. Natarajan, Head Department of Aquatic Biology and Fisheries and to Mr. M.A. Majeed Sahib at Mayyanad for the facilities provided.

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