

## FIELD APPLICATION OF ANABOLIC STEROIDS IN CARP SEED PRODUCTION

### II. REARING OF SPAWN TO FRY STAGE\*

BORA, U.,\*\*\* RAO, P.S., & VENKATESHVARAN, K.\*\*

*Central Fisheries Extension Training Centre (CIFE)*

*Kakinada - 533 007 A.P.*

### ABSTRACT

Two synthetic androgenic steroids, Ethylestrenol (17  $\beta$  - Hydroxy - 17  $\alpha$  ethyl - estr - 4 - en - 3 - one) and Stanozolol (17  $\beta$  - Hydroxy - 17  $\alpha$  - methyl - 5  $\alpha$  - androstano - (3, 2 - C) - pyrazole) were fed via diet at 3 ppm to the spawn of Rohu and Mrigal which were reared upto fry stage over a period of 15 days in earthen carp nurseries. Both hormones enhanced growth of spawn. A maximum of 25.78% increase in length and 25.69% increase in weight as compared to the controls has been recorded. Growth rate was recorded to be 0.8 mm & 2.48 mg/day (control), and 1.13 mm & 2.67 mg/day (Stanozolol treated) in case of Mrigal spawn; and 0.91 mm & 2.39 mg/day (control), 1.12 mm & 2.90 mg/day (Ethylestrenol treated), and 1.10 mm & 2.57 mg/day (Stanozolol treated) in case of Rohu spawn. A decrease in the values of Relative Condition Factor upon hormone administration was also noticed.

**Keywords :** anabolic steroids, carp nursery

### INTRODUCTION

Many workers have used steroid hormones and their derivatives to increase growth rate and feed conversion efficiency in various species of fish (Donaldson *et al.* 1979, Higgs *et al.*, 1982) and some amount of work has also been done in India (Deb and Varghese, 1988; Mogale, 1991; Omprakash; 1991; Venkateshvaran *et al.*, 1992). Despite these works, much remains to be explored on aspects of hormonal enhancement of the growth of the various life history stages of the Indian Major and Exotic carps. Moreover, most of these studies are *in vitro* and information on the

effectiveness of these compounds under actual field conditions are wanting. The present *in situ* study was therefore taken up to assess the anabolic activities of two synthetic androgenic hormones on carp spawn during the nursery phase.

### MATERIAL AND METHODS

#### i) Study Area :

The study was conducted during the induced breeding season (July-August, 1991) at the Freshwater Fish Farm of CIFE at Balabhadrapuram, Andhra Pradesh.

\* Based on a dissertation submitted by the Senior author.

\*\* Present Address : Central Institution of Fisheries Education, Versova, Mumbai-400 061.

\*\*\* Present Address : Dept. Fisheries (Assam), Mandhpur, Nagaon-782 001.

ii) Test Hormones :

Based upon their ready availability in the market in tablet form at inexpensive rates, and on their established anabolic properties, the two following hormones were selected and used :

a) Ethylestrenol : 17  $\beta$  - Hydroxy - 17  $\alpha$  - ethyl - estr - 4 - en - 3 - one. (Orabolin® of M/s Infar (India) Limited, Calcutta).

b) Stanozolol : 17  $\beta$  - Hydroxy - 17  $\alpha$  - methyl - 5  $\alpha$  - androstano - (3,2 - C) - pyrazole. (Menabol® of M/s CFL Pharmaceuticals Limited, Bombay).

iii) Test Animals : The spawn of Rohu, *Labeo rohita* and of Mrigal, *Cirrhina mrigala* were the test organisms which were reared upto fry stage in earthen nurseries over a period of 15 days.

iv) Experimental Design :

A total of 6 earthen nursery ponds, each measuring 20 m x 10 m (0.02 ha) and designated KNP 16, 20, 22, 23, and 25 were involved in the present study.

Details of stocking and artificial feeding are provided in Table 1. Replicates could

not be maintained due to constraints of training programme and commercial seed production activities of the farm.

Pre - and Post-stocking management procedures including artificial feeding were based on the CIFE Method (Venugopal and Venugopal, 1988). The hormonal tablets were finely powdered and premixed to the feed at 3 mg/kg of feed to achieve a uniform dosage of 3 ppm; this dosage was decided upon based on earlier works (Matty, 1985; Venkateshvaran *et al.*, 1993).

v) Water Quality:

Water quality parameters were monitored every morning and plankton population estimated every 5 days following standard methods (APHA, 1975):

vi) Growth studies and Relative Condition Factor:

Growth of spawn was measured on the 5th, 10th and 15th days from stocking and the fry harvested on the 15th day by repeated drag netting with 1/16" cotton drag net.

**Table 1 : Showing details of stocking and hormone treatment via artificial feed in the 6 nursery ponds under study.**

Pond No.	Species	No. Stocked in lakhs	Av. Size stocked	Date Stocked	Hormone Received	Date Harvested
16	Mrigal	1.2	7.0 mm - 1.5 mg	26.7.91	Ethylestrenol	9.8.91
20	Mrigal	1.2	7.0 mm - 1.5 mg.	26.7.91	Stanonolal	9.8.91
24	Mrigal	1.2	7.0 mm - 1.5 mg.	1.8.91	Nil	16.8.91
22	Rohu	2.0	5.0 mm - 1.5 mg.	19.7.91	Ethylestrenol	2.8.91
23	Rohu	2.0	5.0 mm - 1.5 mg	21.7.91	Stanozolal	4.8.91
25	Rohu	2.0	5.0 mm - 1.5 mg	22.7.91	Nil	5.8.91

The relative condition factor  $K_{TL}$  ( $K_{Total}$  Length) was computed separately for each 5 days set of length-weight data following Lagler (1956) and using the formula

$$K_{TL} = \frac{W}{n \cdot a.L}$$

## RESULTS

The observed range of variations in physico-chemical parameters of water is presented in Table 2. In all the ponds plankton sediment volumes, from 0.2 to 0.5 cc/50 a on day 0, rose to around 1.0 cc by the 5th day and remained between 1.0 and 1.5 cc during the subsequent period. *Anacystis* sp. dominated the phytoplankters while *Brachinus* sp. and *Keratella* sp. dominated the zooplankton population.

Results of the growth studies are presented in Table 3 as also Figs. 1 and 2. Table 4 presents the results of relative condition factor estimations.

## DISCUSSION

### i) Physico-chemical and biological parameters of water :

The fluctuations observed in the physico-chemical parameters of water are characteristic of eutrophicated tropical ponds associated with aquacultural activities (Stickney, 1979). The presence of large quantities of cyanophyceans and rotifers in the plankton biomass point towards the productive nature of the water bodies studied (George *et al.*, 1986).

### ii) Ethylestrenol :

Ethylestrenol has enhanced the growth of the spawn of both Rohu and Mrigal, with a higher daily growth increment (Table 3; Figs. 1 and 2). Growth as recorded on the 5th day was more in the control than in the ponds receiving treated feeds but the trend reversed subsequently. Ethylestrenol has been shown to enhance growth when fed via the diet in *Tilapia melanopleura* (Hutchinson and Campbell, 1964) in the

**Table 2 : Showing range of variations in physico-chemical parameters of water during the study period. Data pooled up for 6 ponds over a period of 15 days.**

Parameter	Mean with S.D.
Temperature	
Air	29.5 ± 2.0°C
Water	27.1 ± 2.2°C
Dissolved Oxygen Content	6.8 ± 2.4 ppm
Free Carbon Dioxide Content	4.8 ± 3.0 ppm
pH	8.4 ± 0.4
Total Alkalinity	134.3 ± 14.3 ppm CaCO <sub>3</sub>

Atlantic salmon and Rainbow trout at 2.5 ppm (Simpson, 1976a) and in the fry of Rohu, and Silver Carp at 3 ppm (Venkateshvaran *et al.* 1992) but it retarded growth of Catla fry at 3 ppm (Venkateshvaran *et al.* 1992). Matty (1985) also reported that ethylestrenol enhances growth in carps and salmonids.

iii) Stanozolol:

Stanozolol at 3 ppm in the present study enhanced growth of the spawn of both Rohu and Mrigal with better rate of daily growth increment; growth increment improved over the control only after the 5th day in this case also (Table 3, Fig. 1

and 2) Stanozolol had been shown to enhance growth in carps and salmonids by Matty (1985), in the gold fish *Carassius auratus* by Bulkley and Swihart (1973) and in the fry of Mrigal and Common Carp by Omprakash (1990).

Venkateshvaran *et al.*, (1992) reported growth retardation in the fry of Catla, Rohu, and Silver carp at dietary stanozolol levels of 3 ppm whereas the spawn of Rohu in the present case has grown better at the same dose. Anabolic responses in this case and in the case of Catla with Ethylestrenol may be size-related as the works of Simpson (1976 b) would suggest and further detailed investigations are needed in this regard.

**Table 3 : Showing growth of spawn in length (mm) and weight (mg) over a period of 15 days at intervals of 5 days, growth rate per day, and percentage of growth as compared to controls.**

**A : MRIGAL**

Pond	Hormone	0 Day	5 Day	10 Day	15 Day	Increment per day	% Growth over control
KNP 24	Nil (Control)	7.0 mm	11.0 mm	12.7 mm	19.0 mm	0.80 mm	-
		1.5 mg	10.0 mg	14.0 mg	38.6 mg	2.48 mg	-
KNP 16	E	7.0 mm	9.5 mm	16.8 mm	22.8 mm	1.05 mm	20.00
		1.5 mg	6.4 mg	41.5 mg	46.3 mg	2.99 mg	19.95
KNP 20	S	7.0 mm	9.0 mm	16.7 mm	23.9 mm	1.13 mm	25.78
		1.5 mg	6.1 mg	28.0 mg	41.5 mg	2.67 mg	7.51

**B : ROHU**

KNP 25	Nil (Control)	5.0 mm	12.0 mm	13.1 mm	18.7 mm	0.91 mm	-
		1.5 mm	12.0 mg	16.8 mg	35.8 mg	2.39 mg	-
KNP 22	E	5.0 mm	8.0 mm	15.0 mm	21.8 mm	1.12 mm	16.58
		1.5 mg	4.0 mg	42.0 mg	45.0 mg	2.90 mg	25.69
KNP 23	S	5.0 mm	9.0 mm	14.4 mm	21.5 mm	1.10 mm	14.97
		1.5 mg	5.0 mg	24.0 mg	40.0 mg	2.57 mg	11.73

E = Ethylestrenol, S = Stanozolol

*Cirrhina mrigala*

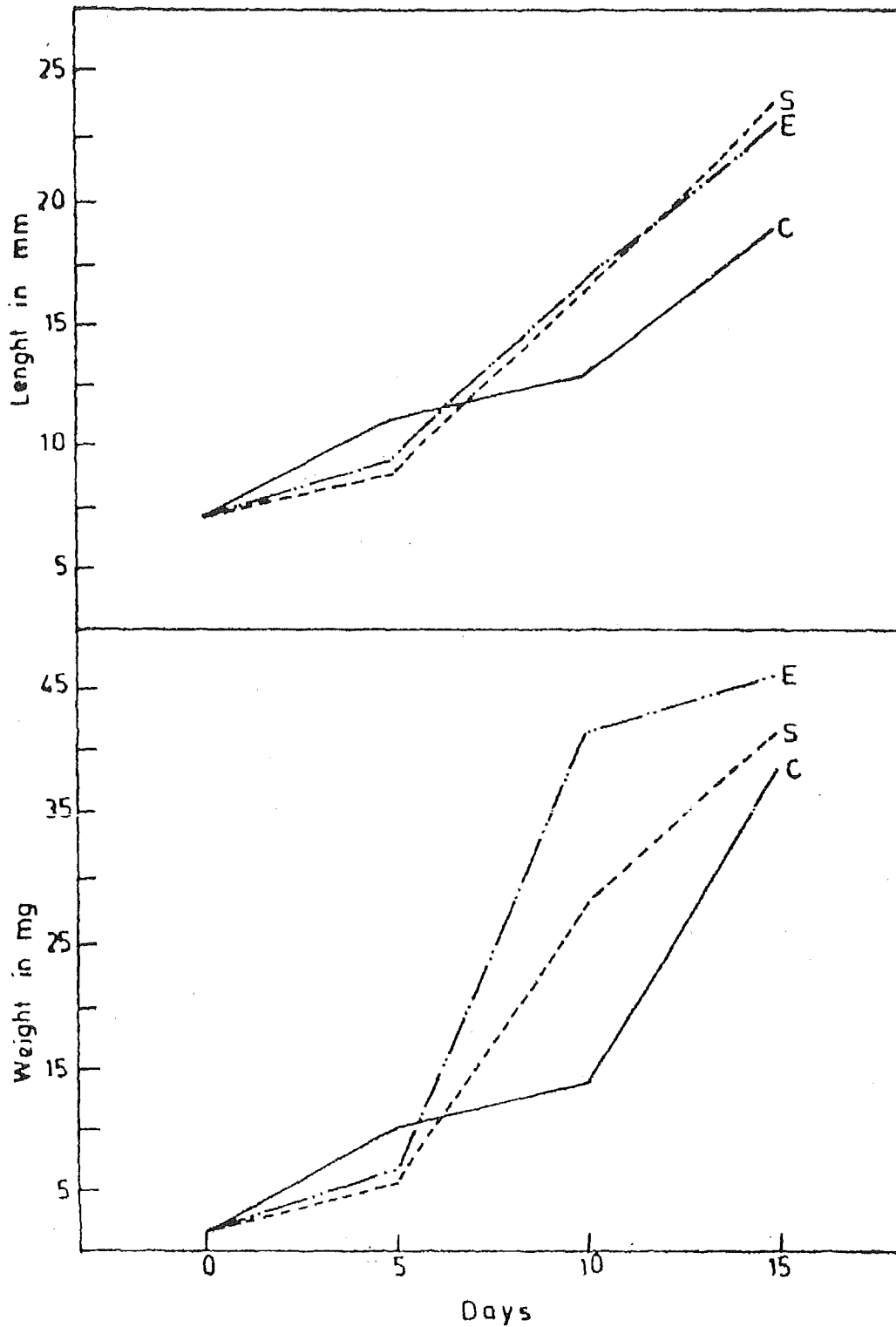


Fig. 1 : Showing growth of Mrigal spawn over a period of 15 days in the control pond and in ponds receiving hormone-treated feed.

C : Control; E : Ethylestrenol; S : Stanozolol

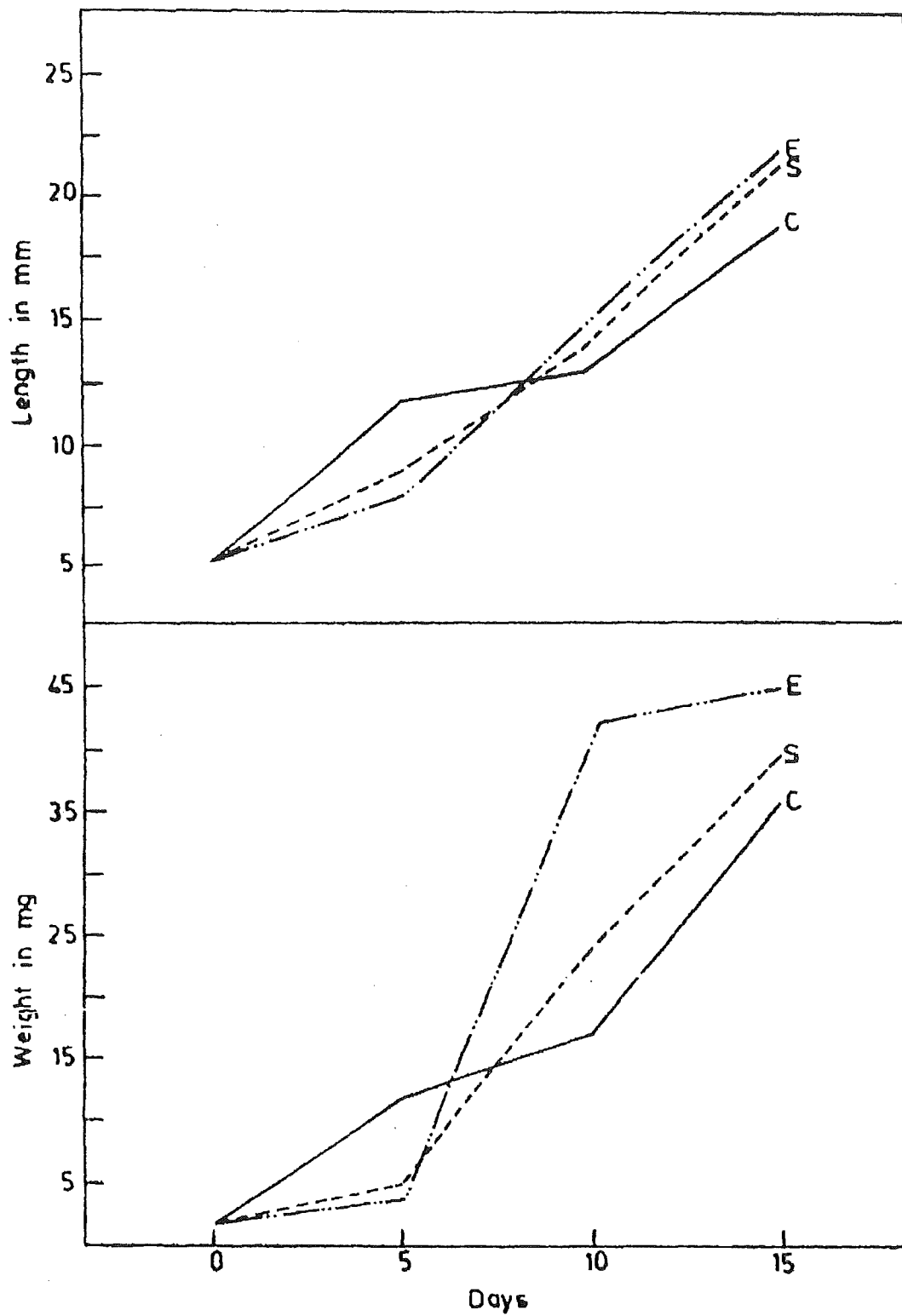
*Labeo rohita*

Fig. 2 : Showing growth of Rohu spawn over a period of 15 days in the control pond and in ponds receiving hormone-treated feed.

C : Control; E : Ethylestrenol; S : Stanazolol

**Table 4: Showing relative condition factor ( $K_{\text{Total Length}}$ ) of spawn over a period of 15 days computed on the 5th, 10th & 15th days.**

Pond	Species	Hormones	5 Days	10 Days	15 Days
KNP 24	MRIGAL	Nil	1.00	1.10	0.98
KNP 16	MRIGAL	E	0.98	0.95	0.90
KNP 20	MRIGAL	S	1.01	0.91	0.88
KNP 25	ROHU	Nil	0.97	1.01	1.01
KNP 22	ROHU	E	1.00	0.95	0.97
KNP 23	ROHU	S	0.99	0.88	0.82

E = Ethylestrenol, S = Stanozolol

iv) Relative condition factor :

A depression in the relative condition factor has been observed (Table 3) indicating increase in weight (= soft tissue growth) did not correspond to increase in length (= bone growth) as suggested by Matty (1985). No such depression was observed in an earlier study (Venkateshvaran *et al.* 1992). Fagerlund and McBride (1977) demonstrated statistically that the depression was not a direct effect of hormone treatment but rather the natural effect of increasing size.

v) Other considerations :

There was no organised effort to study any possible side-effects such as renal hypertrophy or depression of the hepatosomatic index since these side effects are reported to manifest only at levels of 8.3 ppm and above (Bulkley and Swihart, 1973).

It is customary to study the growth of fishes for varying periods upto 120 days or so subsequent to withdrawal of hormone

administration. Such a study could not be carried out due to commercial disposal of fry, etc.

As the fry are only to be restocked for further growing upto about 6 months, and are not meant for human consumption, the question of tissue residues was not looked into.

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