

**EFFECTS OF THIOUREA ON THE TESTICULAR
STEROIDOGENESIS AND SECONDARY SEXUAL
CHARACTERS OF A TROPICAL FRESHWATER
FISH *COLISA FASCIATUS* (BLOCH & SCHN.)**

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Markandey Misra and Kamleshwar Pandey (1985) Effects of thiourea on the testicular steroidogenesis and secondary sexual characters of a tropical freshwater fish *Colisa fasciatus* (Bloch & Schn.) *Bull. Inst. Zool., Academia Sinica* 24(1): 85-94. Chemosterilising properties of 300 ppm thiocarbamide (thiourea) solution has been tested on a nonedible fish, *Colisa fasciatus* which largely shares the food of fry and fingerlings of economically important fish species. After 4 weeks of the treatment a significant ($p < 0.001$) decline has been recorded in the number and size of the interstitial Leydig cells of their testis. Regressive changes thus observed in the interstitial Leydig cells are preceded by the gradual diminution of the secondary sexual character (SSC) i.e. the size of the dorsal and ventral fins and nuptial colouration. These changes though appear from the second week of treatment yet its manifestation in the SSC starts in the later weeks.

Thiourea is a known chemosterilant being extensively used in the control of insect pests (Richard Mercier, 1977; Richmond, 1978; and Matolin, 1979) and also on certain gastropod snail Singh and Agarwal (1983). But so far no effort has been made to study the effect of thiourea (thiocarbamide) on the testicular steroidogenesis of any harmful fish species, which voraciously consume the food of fry and fingerlings of economically important fishes. *Colisa fasciatus* treated with 0.03% (300 ppm) thiourea solution for four weeks display significant ($p < 0.001$) decline in number and size of the interstitial Leydig cells

in their testes. It has been observed that the regressive changes in the interstitial cells are followed by the gradual diminution of the secondary sexual characters i.e. the size of dorsal and ventral fins and nuptial colouration. Such changes are though marked from the second week of treatment in the interstitial Leydig cells yet its manifestation in the secondary sexual characters (SSC) takes place in the later weeks.

Pickford and Atz (1957), Dodd (1960), Hoar (1965), Loftis *et al.* (1966) and Pandey (1969) have shown that the secondary sex characters develop under the influence of gonadal steroids which are regulated by the

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hypophysis. Hoar *et al.* (1967) and Wiebe (1968) have recorded regressive changes in the secondary sex character in the stickleback after methallibure treatment. Similar results were also obtained by Pandey (1970) on the adult and juvenile *Poecilia reticulata*.

In contrast to the limited informations by the above investigators who have studied the effect of methallibure, the effect of thiourea particularly on the testicular steroidogenesis and secondary sexual characters has not been investigated in detail. Thiourea is another compound which exercises similar effect to that of methallibure in blocking the production of pituitary gonadotropin (Pandey, 1970). Owing to the general variation, it has been thought worthwhile to study the effect of thiourea on the testicular steroidogenesis and concomitant changes in the secondary sexual characters of a tropical freshwater fish *Colisa fasciatus*. The present study, therefore, incorporates the chemosterilisation property of thiourea and also its effect on the interdependent characteristics, of male *Colisa fasciatus*, the secondary sexual characters. To the best of our knowledge this study forms the first report of its type in tropical fish.

MATERIALS AND METHODS

Swarup *et al.* (1972) have described the following features of sexual dimorphism in adult *Colisa fasciatus*.

- (1) Males have thicker lips (both upper and lower) covered with numerous very well developed papillae than females.
- (2) In males the soft posterior parts of dorsal and anal fins form large conical lobes. In females, on the contrary soft part forms somewhat rounded lobes.
- (3) Males are brighter, more colourful and attractive than females, more so during breeding season (Body colour in addition to the broad dark stripes shows silvery-bluish background over which bright orange streaks appear).

Therefore, only male fishes were collected mostly from 'Ramgarh lake', Gorakhpur and

brought alive to the laboratory, Fishes were acclimatized to the laboratory conditions for three weeks prior to the commencement of the experiments and were fed with a ground mixture of prawn and wheat flour in the ratio of 1 : 2. Changes in secondary sexual characters were noted at the intervals of one, two, three and four weeks. Owing to its labile nature, however, the first SSC (papillae on lips) was not considered in this study.

Eighty specimens were immersed in 0.03% (300 ppm) thiourea solution for four weeks, changing the solution twice a week. Controls were kept in ordinary tap water which was also changed twice a week like that of the experimentals. Both the groups of fishes were equally fed half an hour before changing the water of aquaria. Groups of fifteen fishes were sacrificed at regular weekly intervals (for four weeks) and small pieces from the anterior, middle and posterior regions of the testes were used for histological and histochemical studies.

Following fixatives were employed to fix testicular parts:

- (1) Bouin's fixative (both aqueous and alcoholic)
- (2) Picro-mercurio-formal (Pandey, 1979)
- (3) Baker's formal calcium

Routine histological and histochemical procedure were adopted. Tissues fixed in Baker's formaldehyde calcium were dehydrated in three changes of acetone, each of 1/2 h duration and placed directly in molten paraffin wax and sections were stained in Sudan Black B to localize lipids.

In the present study mean and standard error ($M \pm SE$) were calculated for various testicular parameters. Student's 't' test (Campbell, 1974) was used to test significant difference used between experimental and their respective control groups at $p < 0.05$ or less.

RESULTS

Experimental (First week)

The interlobular septa or stroma was measured $11.24 \pm 0.54 \mu\text{m}$ average in thickness.

The number of interstitial Leydig cells was $6.3 \pm 0.11\%$ with an average diameter of $2.9 \pm 0.06 \mu\text{m}$ (Table 1). These cells possess sudanophilic granular cytoplasm with a nucleus in the centre.

The average length of the dorsal and anal fins was measured $4.69 \pm 0.02 \text{ cm}$ and $4.71 \pm 0.02 \text{ cm}$ respectively (Table 2). Body colour was observed bright with deep orange streaks on shining silvery-bluish background.

Control

The interlobular septa were $11.35 \pm 0.54 \mu\text{m}$ average in thickness. The interstitial Leydig cells in the interstices have been recorded $6.0 \pm 0.19\%$ and measuring $2.70 \pm 0.06 \mu\text{m}$ average in diameter (Table 1). Their cytoplasm is also granular and sudanophilic with a centrally situated nucleus.

The average length of dorsal and ventral fins was $4.70 \pm 0.02 \text{ cm}$ and $4.72 \pm 0.02 \text{ cm}$ respectively (Table 2) and body colour was bright.

It was observed that one week of thiourea treatment did not bring any significant change.

Experimental (Second week)

The thickness of interlobular septa shows an increase from $11.24 \pm 0.34 \mu\text{m}$ to $12.50 \pm 0.62 \mu\text{m}$ average in thickness from the previous week. There is also a reduction in the number from $6.3 \pm 0.11\%$ to $5.4 \pm 0.15\%$ and size from $2.90 \pm 0.06 \mu\text{m}$ to $2.73 \pm 0.07 \mu\text{m}$ of interstitial Leydig cells (Table 1). The cytological features of Leydig cells are more or less similar to those observed in the first week of treatment.

A decline in the average length of the dorsal and ventral fins (from $4.69 \pm 0.02 \text{ cm}$ to $4.64 \pm 0.04 \text{ cm}$ and from $4.76 \pm 0.02 \text{ cm}$ to $4.66 \pm 0.03 \text{ cm}$ respectively) was recorded (Table 2) which was, however, not significant. The nuptial colouration of body was though dwindling yet bright.

Control

The average thickness of interlobular septa was recorded $11.00 \pm 0.53 \mu\text{m}$ having $6.0 \pm 0.26\%$ interstitial Leydig cells. An increase in the size of Leydig cells has been recorded (from $2.70 \pm 0.06 \mu\text{m}$ to $2.80 \pm 0.06 \mu\text{m}$ average in diameter) (Table 1).

TABLE 1
Showing weekly changes in the thickness of interlobular septa, per cent number of the germ cells and Diameter and per cent number of interstitial Leydig cells of *Colisa fasciatus* following treatment with 0.03% (300 ppm) thiourea solution

Observations Time (weeks)	No. of SMC (%)	No. of PS (%)	No. of SS (%)	No. of Sptd/Sptz (%)	No. of IC (%)	Diameter of IC (μm)	
First	Experimental	16.2 ± 0.48	15.0 ± 0.40	14.5 ± 0.56	48.0 ± 0.49	6.3 ± 0.11	2.90 ± 0.06
	Control	13.6 ± 0.41	15.2 ± 0.57	15.2 ± 0.55	50.0 ± 0.52	6.0 ± 0.19	2.70 ± 0.06
Second	Experimental	16.0 ± 0.56	17.0 ± 0.46	17.6 ± 0.47	44.0 ± 0.56	5.4 ± 0.15	2.73 ± 0.07
	Control	13.9 ± 0.39	13.2 ± 0.40	14.9 ± 0.50	52.0 ± 0.38	6.0 ± 0.26	2.80 ± 0.06
Third	Experimental	15.5 ± 0.56	20.0 ± 0.47	20.9 ± 0.52	39.0 ± 0.44	$4.6 \pm 0.13^{***}$	$2.30 \pm 0.09^{***}$
	Control	14.0 ± 0.38	12.0 ± 0.45	14.9 ± 0.59	53.0 ± 0.52	6.1 ± 0.10	2.83 ± 0.03
Fourth	Experimental	15.1 ± 0.55	23.0 ± 0.60	24.9 ± 0.59	33.0 ± 0.47	$4.1 \pm 0.21^{***}$	$2.10 \pm 0.08^{***}$
	Control	14.2 ± 0.45	10.6 ± 0.49	14.0 ± 0.45	55.0 ± 0.41	6.2 ± 0.17	2.90 ± 0.13

Weekly Cytometric changes in the testes (Mean \pm SE) of ten replicates of *Colisa fasciatus* following thiourea treatment.

*** Significantly different from the corresponding control at $p < 0.001$.

Abbreviations: SMC=Sperm mother cell, PS=Primary spermatocyte, SS=Secondary spermatocyte, Sptd=Spermatid, Sptz=Spermatozoa, IC=Interstitial Leydig cell.

TABLE 2
Showing weekly changes in the secondary sexual characters of *Colisa fasciatus* following treatment with 0.03% (300 ppm) thiourea solution.

Observations Time (Weeks)	Average Length of Dorsal Fins (cm)	Average Length of Ventral Fins (cm)	Nuptial Colouration	
First	Experimental	4.69±0.02	4.71±0.02	###
	Control	4.70±0.02	4.72±0.02	###
Second	Experimental	4.64±0.04	4.66±0.03	##
	Control	4.70±0.02	4.72±0.02	###
Third	Experimental	4.56±0.04***	4.53±0.04***	++
	Control	4.71±0.01	4.73±0.02	###
Fourth	Experimental	4.43±0.04***	4.45±0.03***	+
	Control	4.72±0.02	4.74±0.02	###

Weekly changes in the secondary sexual characters (Mean±SE) of Ten replicates of *Colisa fasciatus* following thiourea treatment.

*** Significantly different from the corresponding control at $p < 0.001$.

The average length of dorsal and ventral fins and colouration of the body were similar to that of the preceding week.

It may be inferred that in spite of some decline in per cent number and size of interstitial Leydig cells there is no significant change in the secondary sexual characters even after two weeks of thiourea treatment.

Experimental (Third week)

A significant ($p < 0.001$) increase in the thickness of interlobular septa was recorded (from $11.24 \pm 0.54 \mu\text{m}$ to $14.15 \pm 0.59 \mu\text{m}$ average in thickness) while the interstitial Leydig cells show significant ($p < 0.001$) decline in their number (from $6.3 \pm 0.11\%$ to $4.6 \pm 0.13\%$) and size (from $2.90 \pm 0.06 \mu\text{m}$ to $2.30 \pm 0.09 \mu\text{m}$ average in diameter) (Table 1). The degradation of the cytoplasm and pycnosis of the nucleus of the Leydig cells started.

A further decline in the length of dorsal and ventral fins was observed (from 4.69 ± 0.02 cm to 4.56 ± 0.04 cm and from 4.71 ± 0.02 cm to 4.53 ± 0.04 cm respectively) (Table 2). Nuptial colouration loses its brightness and becomes dull.

Control

The $10.40 \pm 0.28 \mu\text{m}$ thick interlobular

septa contain an increasing number (from $6.0 \pm 0.19\%$ to $6.1 \pm 0.10\%$) of interstitial Leydig cells of $2.83 \pm 0.03 \mu\text{m}$ average in diameter (Table 1) with identical cytological features to that of the previous week.

Slight increase in the length of dorsal and ventral fins was also recorded from 4.70 ± 0.02 cm to 4.71 ± 0.01 cm and from 4.72 ± 0.02 cm to 4.73 ± 0.02 cm respectively (Table 2) in this week. The brightness of body colour persists.

After three consecutive weeks of thiourea treatment a marked reduction in the number and size of the interstitial Leydig cells was observed. Their secretory function was also adversely affected and a significant decline in the size dorsal and ventral fins has been recorded.

Experimental (Fourth week)

The interlobular septa show further significant ($p < 0.001$) increase in the thickness (from $11.24 \pm 0.54 \mu\text{m}$ to $17.45 \pm 1.11 \mu\text{m}$). The interstitial Leydig cells were marked with a significant ($P < 0.001$) decline in their number (from $6.3 \pm 0.11\%$ to $4.1 \pm 0.21\%$) and size (from $2.90 \pm 0.06 \mu\text{m}$ to $2.10 \pm 0.08 \mu\text{m}$ average in diameter) in this week (Table 1). Hypotrophy and hypoplasia along with the nuclear pycnosis of the Leydig cells are quite distinct.

Further decline in the length of dorsal

and ventral fins (from 4.69 ± 0.02 cm to 4.43 ± 0.04 cm and from 4.71 ± 0.02 cm to 4.45 ± 0.03 cm respectively) was recorded (Table 2). The nuptial colouration was observed fading.

Control

The interlobular septa or stroma ($10.00 \pm 0.41 \mu\text{m}$ average) contain an increased number and size (from $6.0 \pm 0.19\%$ to $6.2 \pm 0.17\%$ and from $2.70 \pm 0.06 \mu\text{m}$ to $2.90 \pm 0.13 \mu\text{m}$ average in diameter respectively) of interstitial Leydig cells (Table 1). Their cytoplasm is more granular with prominent central nucleus.

Further increase in the average length of dorsal and ventral fins has been noted (from 4.70 ± 0.02 cm to 4.72 ± 0.02 cm and from 4.72 ± 0.02 cm to 4.74 ± 0.02 cm respectively) in this week (Table 2). The brilliance of the body colour is maintained.

At the expiry of the 4th week of thiourea treatment it may be inferred that thiourea exercises inhibitory influence on the secretory activity of Leydig cells thereby suppressing the development of SSC. Evidently there is a significant ($p < 0.001$) decline in the size of the dorsal as well as ventral fins along with the fading nuptial colouration.

DISCUSSION

Role of Methallibure and Thiourea in blocking the gonadotropin secretion in the adult teleosts are almost identical. Pandey (1970) has demonstrated that both methallibure thiourea effectively block the initiation of spermatogenesis in juvenile and adult guppies. However, there is no unequivocal opinion regarding the exact modus operandi of these afore-mentioned chemicals. It has been variously argued that the effect of thiourea on the inhibition of spermatogenesis is by and large, through suppressing the synthesis of gonadotropic hormone from the hypophysis and the degree of action also depends upon the various ages and the reproductive activities of fishes (Scott, 1953; Pickford and Atz 1957; Matty, 1960; Grosso, 1961; Wiebe, 1968; Leatherland, 1969 and Pandey 1970).

Workers who have attempted to record the action of thiourea or methallibure on the spermatogenesis of fishes have not shown much concern with the steroid synthesizing sites in the testes. However, Pandey (1970) while studying the role of Methallibure has mentioned that there are no change in either Sertoli cells or interstitial cells and that regressive changes are comparatively less marked than those occur after hypophysectomy. But on the other hand, Wiebe (1968) recorded an atrophy in these cells after Methallibure treatment.

Hoar *et al.* (1967) and Wiebe (1968) have recorded a decrease in the gonadosomatic indices of the adult *Carasius auratus*, *Gasterosteus aculeatus* and *Cymatogaster aggregata* which was later on confirmed by Pandey (1970) in an adult guppy. However, Pandey (1970) has recorded 3.4% (16% in control) of testes area comprising of spermatogonia spermatocytes and intact spermatophores respectively in methallibure treated specimens. These observations thus differ from that of the Wiebe (1968) who has recorded 96% of the lobule area containing spermatogonia and spermatocytes (only 10% in the control) and spermatophores were altogether wanting. Such a difference has been neither attributed to the duration of the treatment and, therefore, related to the fact that guppy is an ovoviviparous monthly breeder whereas the sea perch is a seasonally breeding viviparous teleost. There are, however, certain differences between the present findings with those of the earlier workers. The thiourea treated *Colisa fasciatus* shows a decline in the number of sperm mother cells ($15.10 \pm 0.55\%$) and also the spermatids/spermatozoa ($33.0 \pm 0.47\%$), whereas the number of primary spermatocytes ($23.00 \pm 0.60\%$) and secondary spermatocytes ($24.90 \pm 0.59\%$) increases (Table 1). These observations thus are in greater conformity with that of the Pandey (1970) so far the sperm mother cell (spermatogonia) are concerned and not with those of the Wiebe (1968).

The aforementioned differences in the

Colisa fasciatus with that of the *Cymatogaster aggregata* and *Poecilia reticulata* point that they are chiefly because of the fact that these fishes are annual breeder. Similar species specificity has also been demonstrated by Paget *et al.* (1961) Schmidt-Elmendorff *et al.* (1962), Walpole (1965), Call and Barker (1967), Hemsworth *et al.* (1968) in mammal species studied by them.

Changes initiated in the steroid synthesizing cellular sites under the influence of thiourea or Methallibure has not been demonstrated in detail. Wiebe (1968) has noted that the

interstitial cells of Leydig and the columnar epithelium (Sertoli) border cells atrophy after methallibure treatment. Contrary to it, Pandey (1970) has not found any regressive change in the structure of the Sertoli cells and interstitial cells of *Poecilia reticulata*. Structural and statistical analysis of the interstitial Leydig cells in *Colisa fasciatus* demonstrate revealing changes. In view of the highly scanty work, the present observations are thus exceedingly interesting and the data may be further utilized for an altogether different angle of interpretation regarding the combined

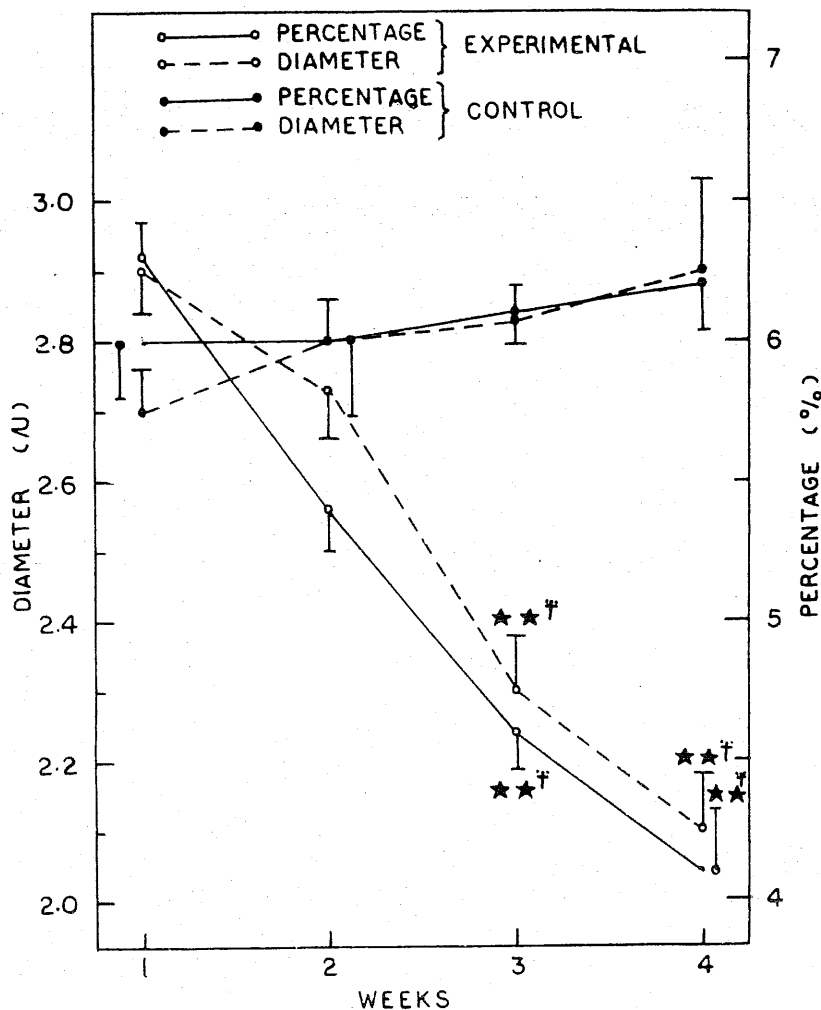


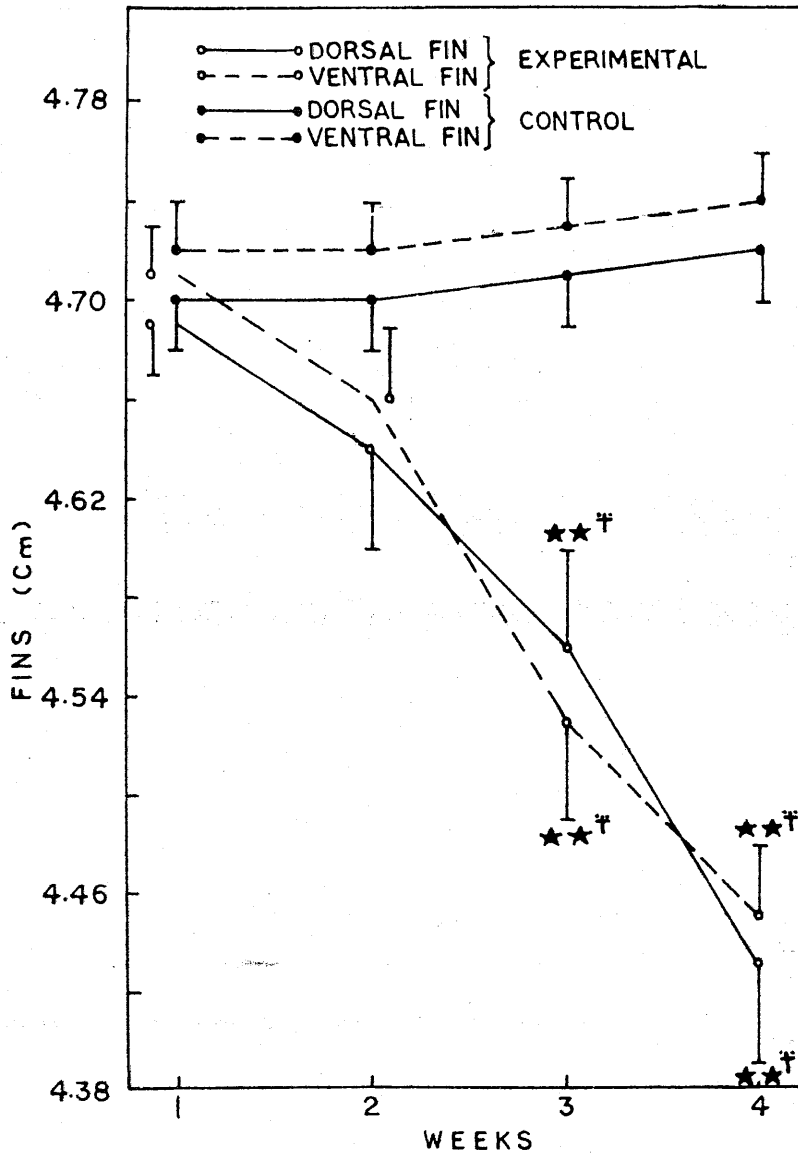
Fig. 1. Graph showing weekly changes in diameter and per cent number of interstitial Leydig cells in the testes of *Colisa fasciatus* following treatment with 0.03% (300 ppm) thiourea solution.

*** Significantly different from the corresponding control at $p < 0.001$.

gonadal function (i. e. production of germ cells and control of secondary sexual characters).

The diameter of the interstitial Leydig cells shows a reduction from $2.90 \pm 0.06 \mu\text{m}$ to $2.10 \pm 0.08 \mu\text{m}$ ($2.90 \pm 0.13 \mu\text{m}$ in control) in *Colisa fasciatus* are highly significant ($p < 0.001$) data. Similarly the number of interstitial cells in *Colisa fasciatus* $6.30 \pm 0.11\%$ to $4.10 \pm 0.21\%$ ($6.20 \pm 0.17\%$ in control) also reveals

the type of changes they undergo during the thiourea treatment. Additionally the hypotrophy and regressive changes including degranulation and vacuolization point a stressful secretory burden over the interstitial Leydig cells in maintaining the spermatogenesis up to a certain level. These observations favour the changes studied in the interstitial Leydig cells by Wiebe (1968) while they differ



[Fig. 2. Graph showing weekly changes in secondary sexual characters (Dorsal and ventral fins) in *Colisa fasciatus* following treatment with 0.03% (300 ppm) thiourea solution.

*** Significantly different from the corresponding control at $p < 0.001$.

from the observations made by Pandey (1970).

Role of gonadal steroid in the differentiation of secondary sexual characters has been reported by several workers (Pickford and Atz (1957); Dodd (1960); Hoar (1965); Lofts *et al.* (1966); Pandey (1966). The cellular parameters considered regarding the interstitial cells in *Colisa fasciatus* indicate their positive involvement in the development and maintenance of secondary sexual characters. Thiourea treated *Colisa fasciatus* show significant ($P < 0.001$) reduction in its dorsal and ventral fins along with the gradual decline of the nuptial colouration. Regression of the secondary sexual character has also been reported in the stickleback (Hoar *et al.* 1967), sea perch (Wiebe, 1968) and adult guppy (Pandey, 1970) after methallibure treatment though degree of such changes were not taken into consideration.

The present observations and statistical analysis reveal that thiourea is a potent chemical and can sterilise even fishes. It adversely affects their testicular steroidogenesis which is even externally apparent in a low grade differentiation of SSC (Figs. 1 and 2). This can be used to sterilise groups of such harmful fishes and release them in their natural habitat as in the sterile male release technique recommended for the insect pests (Borkovec, 1976).

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硫脲對一種熱帶淡水魚 (*Colisa fasciatus* Bloch and Schn) 之睪丸類固醇生成及第二性徵之影響

MARKANDEY MISRA and KAMLESHWAR PANDEY

Colisa fasciatus 是一種非食用性之魚類，唯其為多種具有經濟價值魚苗的食料競食者。本實驗之目的在利用硫脲 (Carbamide, thiourea) 以作為其化學性抗生殖藥物。

以 300 百萬分之一 (ppm) 濃度之硫脲處理四週後，發現其睪丸間質萊氏細胞 (Leydig cells) 的大小及數目有明顯 ($p < 0.001$) 的縮小及減少。而在發現萊氏細胞變化之前，於處理後第二週，其第二性徵包括背鰭、腹鰭大小及求偶之顏色變化在往後的數週內即已漸漸的失去。

