CHAPTER IV

ENVIRONMENTAL EFFECTS ON GENES
INTRODUCTION

No precise information is available on the influence of photoperiod on the breeding cycle of Indian teleosts. It has often been debated whether photoperiod has any influence at all on the breeding cycle of fishes in the tropical regions where, the light cycle changes seasonally by only 2-3 hours.

With regard to the nature of the photoperiod it is now generally accepted that long or increasing day length provides the initial stimulus for gonadal recrudescence (Henderson, 1963; Saxena and Anand, 1977; Whitehead and Bromage, 1980; Bromage et al., 1982). However, there is less agreement regarding the possible photoperiodic control of the later stages of development. According to Henderson (1963) and Bromage et al. (1982b) long days early in the cycle followed by short days are the most stimulatory for reproductive function. Additionally, long photoperiod later in the cycle delays the time of spawning (Allison, 1951; Shiraishi and Fukuda, 1966; Skarphedinson et al., 1982). However, spawning can occur even under cycles of constant long photoperiods (Henderson, 1963; Bromage et al., 1982b). The trouts are able to spawn under conditions of constant long photoperiod in short days and 12 L : 12 D (Whitehead et al., 1978; Whitehead and Bromage, 1980).
According to De Vlaming (1972), the daylength or photoperiod is of prime importance in salmonids (Henderson, 1963; Whitehead and Bromage, 1980; Bromage et al., 1982; Elliott et al., 1984). In Cyprinid and perciform fishes, both photoperiod and temperature are found to be significant.

Experiments on the interplay between photoperiod and temperature in relation to reproduction have been carried out by a number of investigators (Hubbs and Strawn, 1957; Yoshioka, 1962, 1963; Weibe, 1968; Hyder, 1970; Kaya and Hasler, 1972; De Vlaming, 1972, 1975; Sundararaj and Vasal, 1976; Saxena and Anand, 1977; Breton et al., 1977; Gillet et al., 1978; Jonathan and Taylor, 1983; Montes and Peter, 1983).

In the present study, the effect of long and short photoperiods on the reproductive biology of *Garra* has been studied and compared to results obtained from the study of the reproductive cycle under natural photoperiods in the environment.

**Observations**

(a) **Effect of long photoperiod on the gonads:**

Long photoperiod experiments were set up in winter season (January to February) for forty five days at a time when the natural day length is short and the temperature of water is 18±2°C.

During this period the normal ovaries contain mostly
immature oocytes, i.e., early and late chromatin nucleolus stages and early and late perinucleolus stages (Fig. 80). The values of gonoosomatic index are low (Table 3 and Histogram 1). The experiment was conducted under artificially maintained long photoperiod (L 23 h; D 1 h). The results indicated a marked change in the histological structure of ovaries. The ovaries contained a large number of maturing oocytes with the initiation of vitellogenesis in some. Mature yolky oocytes are also present besides early stages of oogenesis. Mitotic activity becomes very rapid and rate of oogenesis is enhanced (Fig. 81). There is an increase in the gonoosomatic index when compared to controls (Table 3). This shows that long photoperiod in winter brings the ovaries near to the pre-spawning and spawning periods.

In case of male fishes the normal testis contains mostly immature spermatocytes. It contains mainly spermatogonia and spermatocytes (Fig. 88). Morphologically the testes are long thread-like structures. The values of gonoosomatic index are low (Table 3). The testes of experimental fishes showed a marked change in the histological structure. The testes contained large number of spermatocytes, few spermatogonia and very few spermatids (Fig. 89). The values of gonoosomatic index showed an increase when compared to the controls. This shows that long photoperiod brings the testes near to the spawning period in the month of January-February.
The ascorbic acid content in the gonads of control and experimental fishes show differences. The ascorbic acid content is lower in the experimental fishes (Table 4 and Histogram 2).

(b) Effect of short photoperiod on the gonads:

Short photoperiod experiments were set up in summer season (April to May) for forty five days when the natural day length is long and the temperature of the water is high (26°C). The experiments were set up under artificially maintained short photoperiod (11 h : 13 h).

The spawning period of the fish begins in May and lasts till August. The results of these experiments show that there is a remarkable change in the condition of the ovaries when compared to the controls. The control ovaries contain mostly mature oocytes and corpora atretica. The early stages of oocytes particularly oogonia are absent (Fig. 82). In experimental fishes, the oocytes cease to develop further and remain as such. It means further development stops. The ovaries contain mainly early and late nucleolar stages, early and late yolk vesicle stages while the mature oocytes are totally absent (Fig. 83). The values of gonosomatic index are not significantly different when compared to controls (Table 3 and Histogram 1). This suggests that the effect of short photoperiod stops further development of oocytes in this fish.

In case of male fish the spawning period begins in May and lasts till September. The results of these experiments show
that there is no remarkable change in the condition of testes when compared to the controls. The testis of control and experimental fishes mainly consists of spermatids and spermatozoids (Figs. 86, 89, 90 and 91), but the values of gonosomatic index increase not significantly in the experimental fishes when compared to controls (Table 3 and Histogram 1).

The ascorbic acid content in the gonads of control and experimental fishes do not show much differences, however, it is lower in the experimental fishes but not significantly low (Table 4 and Histogram 2).

DISCUSSION

There is now considerable evidence indicating that the pineal gland inhibits gonadal development. Melatonin is synthesized in the pineal and the rate of synthesis is inhibited by continuous light. Thus the stimulatory effect of long photoperiod may be the result of reduced melatonin synthesis. Very little information is available linking the pineal with gonadal function in fishes (Fenwick, 1977; Urasaki, 1972, 1973; De Vlaming et al., 1974; De Vlaming, 1975; Suraya et al., 1976; Sundararaj and Keshavanath, 1976; De Vlaming and Vodicnik, 1977; Saxena and Anand, 1977; Montela and Piter, 1980; Jonathan and Taylor, 1983).

Histological analysis showed that the constant light regime delayed the processes of both vitellogenesis and spermatogenesis
(Bourlier and Billard, 1984). Similar results have been obtained in salmonid fishes, viz., *Oncorhynchus nerka*, *O. rhodurus*, *Salvelinus fontinalis* and *Salmo gairdneri* (Shiraiishi and Fukuda, 1966); in *Salvelinus fontinalis* (Allison, 1951; Hazard and Eidy, 1951) and *Salmo salar* (Lundqvist, 1980).

A majority of the investigators are of the opinion that the long photoperiod brings the gonads to an advanced stage of spawning in fishes. Some of the most recent observations are those of Whitehead and Bromage (1980) in trout, Bromage *et al.* (1982) and Skarphauscha *et al.*, in rainbow trout, Bromage *et al.* (1982b) and Sumpter *et al.* (1984) in trout.

In the present study, the long photoperiod *L 23 : D 1* experiments were conducted in winter when the fish are in the pre-spawning period and when the natural day length is short. Long photoperiods at this time of the reproductive cycle brings the gonads nearer to the spawning period, i.e., maturation is enhanced. Similar results have been shown by Harrington (1956, 1957, 1959a) in two fishes, viz., *N. bifrenatus* and *Enneacanthus obscus* in which pre-spawning period could be advanced by long photoperiod. Harrington (1957, 1959) has also reported that if long photoperiodic regime is started during the post-spawning period of the fish it is not effective as the condition of gonads do not come near to spawning. The acceleration of ovarian recrudescence in the catfish brought about by exposure to long photoperiod during the preparatory
period was reported by Sundararaj and Sehgal (1970a, b). The results in Barra suggest that the long photoperiod will be effective in the responsive period of the reproductive cycle and the spawning can be advanced only in the pre-spawning period.

In the present study the artificially maintained long photoperiod (L 23 : D 1) in winter in combination with constant low temperature (18°C) enhances vitellogenesis in the ovaries and proliferation of testes in the pre-spawning period. But final spawning or ovarian maturation and spermatiation are not possible, only by maintaining long photoperiod. In Notemigonus also the effect of long photoperiod and low temperature regime never stimulates final gonadal maturation or spawning (De Vlaming, 1975).

Very little is known about the effect of short photoperiod on the fish gonads. Few recent observations are those of Henderson (1963), Sehgal and Sundararaj (1970), Urasaki (1972), De Vlaming (1975), Whitehead et al. (1978b) and Bromage et al. (1982b).

According to Yoshioka (1963) in Oryzias latipes with short photoperiods (L 11 : D 13) the growth of oocytes is markedly delayed. Sehgal and Sundararaj (1970) have reported that the short photoperiod (L 9 : D 15) brings about ovarian regression in Heteromeunastes fossilis. Under the influence of shortened photoperiod in the rainbow trout, spawning was advanced by 6
and 12 weeks in regimes where the normal yearly cycle was
compressed into 9 and 6 months respectively (Whitehead et al.,
1978b).

In case of Gerra when short photoperiods (L 1 : D 23)
were maintained in April-May and the temperature of water is
higher, i.e., 26°C, the results show an evident change in the
condition of the ovaries. In this condition (short photoperiod)
the oocytes cease to develop further and remain as such except
that the values of GSI are slightly low. In the natural
environment, summer has at long day length and in this fish
the spawning period begins in May and lasts till August. The
condition of the ovaries show that mature oocytes and corpora
ametastica are present and the gonosomatic values are slightly
higher than those of the experimental fishes. Similar results
have been reported in Notemigonus (De Vlaming, 1975). In this
fish short photoperiod in combination with warm temperature
results in gonadal regression. The fishes used in these
experiments were in preparatory and spawning periods.

Very little is known about the effect of short photoperiods
on the male reproductive organs of fishes. In Notemigonus
(De Vlaming, 1975) short photoperiod and warm temperature regime
promotes spermatocyte proliferation. In rainbow trout short
photoperiod and low temperature advanced spermatogenesis (Breton
and Billard, 1977).

In Gerra under short photoperiod (L 1 : D 23) and warm
temperature (26°C) histologically there is no change in the testicular structure when compared to controls but the values of gonosomatic index increase in the experimental fishes when compared to controls (Table 3). Similar results have been demonstrated by Urasaki (1972b).

The advancement or regression of the gonads either by long or short photoperiod may be indirectly controlled by endocrine mediation (Combs et al., 1959; Henderson, 1963; Sundararaj and Goswami, 1968; Gwinner, 1973; Breton et al., 1975; Crim et al., 1975; Whitehead et al., 1978; Baggerman, 1980; Bromage et al., 1984).

In white brook fish, Elliott et al. (1984) have shown that due to increased (long) photoperiods there is a change in endocrine serum. Along with these hormonal changes he observed an increase in GSI and also an incorporation of yolk in the ovary. Similar changes have been noted in other fish species also (Davis, 1977; Eliassen and Vahl, 1982). Possibly, such changes are present in all fish. In case of Gerra the values of gonosomatic index shows an increase in both the ovaries and testes during long photoperiod. Thus it can be concluded that the hormonal changes are responsible in increasing the gonosomatic values.

According to Whitehead et al. (1978) during increasing or decreasing photoperiod there is a change in gonadotropin levels. This supports the hypothesis of a photoperiod control
of reproduction by the pituitary probably via the hypothalamus with a subsequent induction by gonadotropin hormone and of release of steroids from the gonads. In the light of endocrine control during short photoperiod, Sehgal and Sundararaj (1970) investigated that short photoperiod probably inhibits the tonic production which, in turn, leads to oocyte atresia and eventually regression of the ovary. Similarly in the present study during short photoperiod the ovaries cease to develop further and this is probably due to inhibition of secretion of gonadotropin hormone.

In fishes, studies on the effects of photoperiods have shown that the pineal organ may have antigonadotropin or progonadotropin effects. Harasaki (1972b) investigated in Oryzias latipes that under 14 hrs of illumination condition, without pinealectomy, there was a little effect on ovarian development. Therefore, it seems probable that the pineal is concerned in ovarian maturation particularly during long photoperiod. The gonosomatic index of blinded female Oryzias kept under constant light was much higher than that of the fish exposed to constant darkness. Similarly in Garra during long photoperiod gonosomatic value is much higher than the fish exposed to short photoperiod.

However, it has been suggested that photoreceptive organs, such as eye and pineal, not only cease their stimulating action on gonadal development but also inhibit the growth of the gonad under short illumination conditions.
Recently, De Vlaming and Vodicnik (1977) have observed that in *Notemigonus crysoleucas* exposed to long day length, the pineal favours reproductive activity but it retards reproductive processes in fish maintained on short photoperiod. They have suggested that the effect of pinealectomy on reproduction vary with photoperiod but are mediated via the hypothalamus and pituitary. Similarly in case of *Garré* long photoperiods have a stimulatory effect on the reproductive organs and under short photoperiods the reproductive organs cease to develop.

Montiel and Peter (1980) investigated in goldfish that the pineal organ is important in the control of gonadotropin hormone secretion and gonadal development. During long photoperiod the pineal stimulates gonadal development by increasing serum gonadotropin hormone level and during short photoperiod the gonadal development is suppressed.

Very recently Jonathan and Taylor (1983) have reported that the pineal gland does not seem to play an essential role in *Fundulus heteroclitus* reproduction as an endocrine organ. But possibly, the pineal functions as a photoreceptor for reproduction under natural conditions.

In the present investigation the ascorbic acid contents in the gonads have been studied in both the experiments (long photoperiod and short photoperiod). The long photoperiod experiment was conducted between December to February and the
gonads were in an immature stage. At the end of the experiment, gonads reached the maturation stage. Therefore, the ascorbic contents decrease to lower level while the ascorbic acid content of control fishes remains higher. Similarly during short photoperiod the ascorbic acid contents are lower in experimental fishes but it is not too low than control fishes, because histologically there is not much change in experimental and control fishes.
(8) TEMPERATURE

INTRODUCTION

Environmental control of reproduction has been worked out in a few teleost fishes. The major factors controlling reproduction in teleost fishes are the photoperiods and temperature and onset of seasonal rains.

In the Northern hemisphere, cyprinid fish spawn in spring when the water temperature reaches its maximum. In some species of cyprinid fish like *Cottus gobio* (Ahsan, 1966) and *Carassius auratus* (Gillet et al., 1977), a sudden rise of temperature in winter can apparently block gametogenesis in laboratory conditions. In other species such as *Lepomis cyanellus* (Kay and Hasler, 1972) an increase of temperature associated with long photoperiod stimulates gonadal development in winter. These experiments suggest that temperature and photoperiod interact in the control of gametogenesis in fishes.

According to some investigators (Henderson, 1963; Jalabert, 1976) out of these two major environmental factors, temperature and photoperiod which regulate the annual reproductive cycles of teleost fish, the photoperiod factor is of greatest importance in Salmonid species. In northern temperate fishes, the photoperiod changes have been found to be particularly significant

Billard and Breton (1977) showed that a high temperature in winter, lowers sperm production than at normal spawning period. Other workers have shown that temperature dominates over photoperiod in the control of reproductive cycle (Matthews, 1939; Bullough, 1940; Merriman and Schadle, 1941; Medlen, 1951; Wiebe, 1968; Berton and Billard, 1977; Kime, 1982 and Kime and Hews, 1982).

In the present study, the effect of temperature has been seen on the reproductive organs of Garra and the results are compared to those seen with normal water temperatures in the natural environment from where the fish were collected.

OBSERVATIONS

The experiments were conducted between 31st December, 1983 to 15th February, 1984 (45 days) to determine the effect of temperature on gonads. This period was specially selected because the natural water temperature is very low (18°C) at this time of the year and also at a time when preproductive activity normally occurs.

Experiments were conducted with water temperature fixed between 30±1°C and with simultaneously maintained control fishes. Marked changes were seen in the histological structure.
of the gonads in the experimental fishes.

(a) **Effect of temperature on the ovaries:**

In control fishes the ovaries show the presence of immature oocytes as it is the post-spawning phase in the natural environment. The oocytes are mainly in the early and late perinucleolar stages but the other stages such as yolk vesicle, pre-mature and mature stages are absent. The interfollicular spaces and interlamellar cavity are prominent (Fig. 80).

In the experimental fishes with high temperature, marked and peculiar changes are visible in the structure of the ovaries. Besides the usual early and late perinucleolar stages few mature oocytes are also seen in the ovary. The interfollicular spaces and interlamellar cavities are very much reduced due to the increase in the number of mature oocytes. Another interesting change seen is that a few immature oocytes directly change into corpora atretica which are normally not seen in the regular cycle (Figs. 84 and 85).

The results show that in the ovaries higher temperature induced a rapid jump to the spawning period since mature oocytes and corpora atretica are formed (Figs. 86 and 87). Increased temperature also stimulated the rate of oogenesis in the experimental fishes which is evident from the presence of good number of only early and late perinucleolar oocytes
in the controls.

The gonosomatic index of the experimental fishes also increases when compared to the control fishes (Table 3). It shows that the rate of oogenesis is increased during this period.

(b) **Effect of temperature on the testis:**

In control experiments the testis of *Garra* shows the same histological structure which is seen at that time of the year in the normal cycle. In the control fishes the testis consists of primary germ cells, spermatogonia and primary spermatocytes but later spermatogenetic stages are absent (Fig. 88).

With high temperature mitotic activity becomes very rapid. The testes resemble those of spawning period in which the testis contains spermatids and spermatogonia besides primary germ cells, spermatogonia and spermatocytes (Fig. 90).

The results indicate that high temperature induces a rapid increase of the late spermatogenetic stages (Spermatids and sperms) with a marked stimulation of mitosis activity resulting in large numbers of dividing spermatocytes and spermatids (Fig. 91).

Thus higher temperature promoted stages from preparatory to maturation division. At normal temperatures in contrast, the early stages of spermatogenesis or mitotic activity are
well seen.

As regards the gonosomatic index there is significant
difference in gonosomatic index of control and experimental
fishes. The gonosomatic values of experimental fishes were
higher than controls (Table 3 and Histogram 1).

Some morphological changes are also seen both in the male
and female experimental fishes. The abdomen of both the sexes
becomes bulgy due to the increase in size of the gonads and
the tail portion becomes narrow.

The ascorbic acid content in the gonads of control and
experimental fishes do not show much difference, however, it
is lower in the experimental fishes but not significantly
low (Table 4 and Histogram 2). This can be explained due to
the fact that experimental and simultaneous control fishes are
mature fishes and the ascorbic acid contents in the mature
gonads of Garra is always lower when compared to immature ones.

These results show that temperature does have an effect
on the ovaries and testes as indicated by the rapid increase
in the formation of spermatozoa and spermatozoa in the testis
and mature oocytes and enhancement of vitellogenesis in the
ovaries.
TABLE 3

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>GONOSOMATIC INDEX (GSI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALE</td>
</tr>
<tr>
<td></td>
<td>EXPERIMENT</td>
</tr>
<tr>
<td>LONG PHOTOPERIOD</td>
<td></td>
</tr>
<tr>
<td>2.267</td>
<td>1.243</td>
</tr>
<tr>
<td>SHORT PHOTOPERIOD</td>
<td></td>
</tr>
<tr>
<td>3.320</td>
<td>3.365</td>
</tr>
<tr>
<td>TEMPERATURE</td>
<td></td>
</tr>
<tr>
<td>1.787</td>
<td>1.243</td>
</tr>
</tbody>
</table>

Explanation - Table showing the average gonosomatic index of female and male gonads of Perca during different experiments.
HISTOGRAM 1

EXPLANATION - GONOSOMATIC INDEX IN THE
EXPERIMENTAL AND CONTROL FISHES
UNDER THE INFLUENCE OF TEMPERATURE,
LONG AND SHORT PHOTOPERIODS.

ABBREVIATION
- ■ CONTROL FEMALE AND MALE
- □ EXPT. FEMALE
- □□ EXPT. MALE

GONO-SOMATIC INDEX

3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000

FEMALE MALE FEMALE MALE FEMALE MALE

TEMPERATURE PHOTOPERIOD PHOTOPERIOD
LONG SHORT
### TABLE 4

<table>
<thead>
<tr>
<th>Gonads</th>
<th>Ascorbic Acid Content µg/100 mg</th>
<th>Long Photoperiod</th>
<th>Short Photoperiod</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.271</td>
<td>0.235</td>
<td>1.271</td>
<td></td>
</tr>
<tr>
<td>Experim.</td>
<td>0.980</td>
<td>0.362</td>
<td>1.141</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(p &lt; .05 S)</em></td>
<td><em>(p &lt; .00 HS)</em></td>
<td><em>(p &lt; .05 S)</em></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.965</td>
<td>0.129</td>
<td>1.965</td>
<td></td>
</tr>
<tr>
<td>Experim.</td>
<td>0.723</td>
<td>0.235</td>
<td>0.796</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(p &lt; NS)</em></td>
<td><em>(p &lt; NS)</em></td>
<td><em>(p &lt; .001 HS)</em></td>
<td></td>
</tr>
</tbody>
</table>

Explanation - Table showing the ascorbic acid content in the gonads of *Garr* during different experiments.

Abbreviations: S - Significant; HS - Highly significant; NS - Not significant.
HISTOGRAM 2

EXPLANATION: ASCORBIC ACID CONTENT IN THE EXPERIMENTAL AND CONTROL FISHES UNDER THE INFLUENCE OF TEMPERATURE, LONG AND SHORT PHOTOPERIODS

ABBREVIATION

- □□□□ CONTROL FEMALE AND MALE
- ■■■ ■ EXPT. FEMALE
- □□□□ EXPT. MALE

ASCORBIC ACID CONTENTS (µg/100 mg)

TEMPERATURE

PHOTOPERIOD

LONG

PHOTOPERIOD

SHORT
Fig. 80. Photomicrograph of T.S. of ovary during long photoperiod and temperature (control) showing the early and late perinucleolus stages (Azan) 100 X.

Fig. 81. T.S. of ovary during long photoperiod (experimental) showing mature ovum and perinucleolus stage (Azan) 100 X.

Fig. 82. T.S. of ovary during short photoperiod (control) showing mature ovum and corpora atretica (Azan) 50 X.

Fig. 83. T.S. of ovary during short photoperiod (Experimental) showing early and late perinucleolus stages and yolk vesicle stages (Azan) 100 X.
Fig. 84. T.S. of ovary during temperature (Experimental) showing mature ovum and early and late perinucleolus stages (Azan) 100 X.

Fig. 85. T.S. of ovary during temperature (Experimental) showing mature ovum (Azan) 200 X.

Fig. 86. T.S. of ovary during temperature (Experimental) showing the corpora atretica and early and late perinucleolus stages (Azan) 100 X.

Fig. 87. T.S. of ovary during temperature (Experimental) showing the corpora atretica (Azan) 100 X.
Fig. 88. T.S. of testis during long photoperiod and temperature (control) showing primary germ cells and spermatogonia (Azan) 100 X.

Fig. 89. T.S. of testis during long photoperiod (Experimental) showing spermatocytes beside spermatogonia and distinct interstitial cells (Azan) 400 X.

Fig. 90. T.S. of testis during temperature (Experimental) showing mainly spermatids and spermatozoa besides spermatocytes (H.E.) 400 X.

Fig. 91. T.S. of testis during temperature (Experimental) showing spermatids and spermatozoa (H.E.) 400 X.
**Fig. 92.** T.S. of testis during short photoperiod (Control) showing only late spermatogenetic stages (H.E.) 50 X.

**Fig. 93.** T.S. of testis during short photoperiod (Control) showing spermatids and spermatozoa (H.E.) 200 X.

**Fig. 94.** T.S. of testis during short photoperiod (Experimental) showing late spermatogenetic stages (Azan) 50 X.

**Fig. 95.** T.S. of testis during short photoperiod (Experimental) showing spermatids and spermatozoa (Azan) 200 X.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
<td>Blood vessel</td>
</tr>
<tr>
<td>CA</td>
<td>Corpora atratica</td>
</tr>
<tr>
<td>EPN</td>
<td>Early perinucleolus</td>
</tr>
<tr>
<td>FL</td>
<td>Follicle layer</td>
</tr>
<tr>
<td>IC</td>
<td>Interstitial cell</td>
</tr>
<tr>
<td>LPN</td>
<td>Late perinucleolus</td>
</tr>
<tr>
<td>LYN</td>
<td>Late yolk nucleolus</td>
</tr>
<tr>
<td>MO</td>
<td>Mature ovum</td>
</tr>
<tr>
<td>OL</td>
<td>Ovarian lamellae</td>
</tr>
<tr>
<td>PGC</td>
<td>Primary germ cells</td>
</tr>
<tr>
<td>PN</td>
<td>Perinucleolus</td>
</tr>
<tr>
<td>PSC</td>
<td>Primary spermatocytes</td>
</tr>
<tr>
<td>S</td>
<td>Spermatids</td>
</tr>
<tr>
<td>SP</td>
<td>Spermatozoa</td>
</tr>
<tr>
<td>SPC</td>
<td>Spermatocytes</td>
</tr>
<tr>
<td>SPG</td>
<td>Spermatogonia</td>
</tr>
<tr>
<td>SSPC</td>
<td>Secondary spermatocytes</td>
</tr>
<tr>
<td>TA</td>
<td>Tunica albuginea</td>
</tr>
<tr>
<td>VM</td>
<td>Vitelline membrane</td>
</tr>
<tr>
<td>YN</td>
<td>Yolk nucleus</td>
</tr>
</tbody>
</table>
DISCUSSION

Numerous investigations have shown that photoperiod and (or) temperature are the most significant environmental factors involved in the regulation of reproductive cycles of fishes. According to Kime (1979) and Kime and Saksena (1980) the normal gonadal development and subsequent spawning may well be dependent upon the changing balance of gonadal enzyme activities which reflect seasonal changes of environmental temperature acting directly on the gonads. This differential effect of temperature on different enzymes may also be of relevance in many other systems not only in non-mammalian vertebrates, but also in invertebrates and in plants where biological activity shows a seasonal change in temperature.

Several other workers have linked the seasonal changes in teleost pituitary cytology with seasonal gonadal changes (Sokol, 1961; Robertson and Waxler, 1962a, b; Honma and Tumura, 1963; Lagios, 1965; De Vlaming, 1975; Gillet et al., 1978; Montela and Peter, 1978; Kime and Haws, 1982 and Montela and Peter, 1983). These pituitary changes are generally believed to be triggered by environmental factors mainly temperature and photoperiod.

The effect of temperature on gonadotropin hormone secretion seems to be very important. Under increased temperature the concentration of gonadotropin hormone also increases. A similar result has been found in Goldfish (Gillet et al., 1977).
In Gold fish, it has been shown that long exposure to high temperature stimulated gonadotropin secretion. According to Gillet et al. (1978) the increasing of gonadotropin hormone secretion is not always correlated with stimulation of gonadal development. Montala and Peter (1978) investigated that warm temperature alone appears as a stimulatory factor for gonadotropin secretion.

Ahsan (1968), in Cyprinus plumbens, found that high temperatures accelerate spermatogonial proliferation and hasten spermiation. Similarly, Lofts et al. (1968) in Fundulus heteroclitus postulated that an elevation of temperature accelerates the spermatogonial activity. In Cyprinus carpio, Gupta (1975) has shown that if the animals are acclimated to more than 20°C then soon after hatching they develop a total reproductive cycle in hot water after a precocious puberty and rapid growth.

The temperature may have a very pronounced effect on the testes of teleost fishes as observed by Kime (1979) and Kime and Saksena (1980). De Vlaming (1975) investigated in Notemigonus crysoleucas that during the preparatory period long photoperiod and warm temperature regime stimulates testicular development to the pre-spawning condition. According to De Vlaming (1975) long photoperiod in the absence of warm temperature does not promote final gonadal maturation in Notemigonus crysoleucas. On the other hand, Backiel (1964) suggested that warm temperature stimulated the last stages of spermatogenesis.
In the present study, the effect of temperature has been seen on the gonads of *Garra* during winter when normal water temperatures are very low 18°C and the gonads are in the late post-spawning period and early pre-spawning period. In this fish under natural conditions in the environment in the post-spawning fish low temperature promotes a rapid proliferation of primary spermatocytes from earlier stages in the spermatogenetic cycle. In the pre-spawning fish higher temperature in March and April hastens the late spermatogenetic activity.

The experimental fishes when kept at 30±1°C in the month of January when the controls were at 18°C showed marked changes in testicular histology. Mitotic activity becomes very rapid. There is a rapid increase in the late spermatogenetic stages, as a large number of spermatids and spermatozoa are seen. A marked stimulation of meiotic activity resulting in the formation of a large number of dividing spermatocytes and spermatids is noticed. Thus higher temperature stimulates testicular development from preparatory to the maturation phase.

In *Cymatogaster aggregata* (Wiebe, 1968) the temperatures at different times of the year appear to be more important than the day length in regulating oocyte formation and maturation. The production and early growth of oocytes was markedly enhanced by warm temperature. On the other hand, De Vlaming (1975) proposed that only warm temperature and long photoperiod stimulate spawning in the cyprinid *Notemigonus crysoleucas*. Further De Vlaming and Paquette (1977) showed that
high temperature induces gonadal regression in *Notemigonus crysoleucas*. Monteale and Peter (1978) have shown very peculiar changes in Gold fish. They noticed that when the female is exposed to warm temperature some oocytes developed atretic follicles and at the same time early stages of oocytes regressed.

Similar results have been observed in the ovaries of *Garra*. In the present study, the effect of temperature was seen on the ovaries during winter when the normal environmental water temperature, from where the fish were collected was very low (18°C) and the ovaries are in the late post-spawning and early pre-spawning periods. Under natural conditions the ovaries contain mostly immature oocytes particularly early and late perinucleolar stages but the other stages are absent.

The experimental fishes when kept at 30±1°C in this period showed marked and peculiar changes in the ovaries. In the ovaries besides the early stages the advance stages of oocytes are also seen particularly mature oocytes. The most peculiar and interesting change is that the oocytes developed atretic follicles while the early developing stages particularly yolk nucleolar stages of oocyte were seen to be regressed. Thus the higher temperatures promote stages from preparatory to late maturation stages.

According to Wiebe (1968) in *Cymatogaster aggregata*, the gonosomal values decreased due to an accelerated maturation
process in increased temperature (20°C) and release of spermatozoa. In Fundulus grandis (Wald and Meier, 1983), there is no significant changes in the GSI values in high temperature (28-30°C).

In the present study, the gonosomatic index is high in experimental fishes than the control ones.

As regards the ascorbic acid content in Garra, higher temperature brought about maturation of the gonads. Hence, the ascorbic acid content in the experimental fishes is lower when compared to controls. Normally, the ascorbic acid content in the ovaries of Garra is always higher than in the testes. Similarly, Seymour (1981) observed in Carassius carassius that the ascorbic acid content is always high in the ovaries when compared to the testes and that it is always less in a mature gonad. Immature gonads have a higher content of ascorbic acid.
CONCLUDING REMARK
Reproductive activity in Garra is a seasonal phenomenon. The reproductive cycle can be divided into three distinct phases, viz., pre-spawning, spawning and post-spawning. From a study of the morphology, histology, gonosomal index and ascorbic acid content of the gonads it can be concluded that the gonads show great development in the pre-spawning period and they present a spent condition in the post-spawning period.

Recent studies on reproductive biology of fishes has concentrated on the endocrine tissue of the gonads. The endocrinology of reproduction has been reviewed on the basis of steroid cells which control reproductive physiology of fishes.

The ascorbic acid contents in the gonads is important in the biosynthesis of sex steroids. From the present study on ascorbic acid level in the gonads of Garra it can be concluded that the content is always more in the female when compared with males. Also the ascorbic acid content is more in an immature gonad and less in a mature gonad. This suggests that ascorbic acid is necessary for the biosynthesis of steroid which is secreted by the mature gonad.

In Garra, raised levels of ascorbic acid in the ovary reflect the high rate of steroidogenesis associated in development of egg yolk. Also increased levels of ascorbic acid in both the gonads during the pre-spawning period also
suggests increased steroidogenesis in both the ovaries and testes.

The granulosa cells and the thecal cells are major sites of steroid synthesis in the teleost ovary while the interstitial cells are the cellular source of the testicular hormone. In \textit{Garra}, interstitial cells are observed in between the seminiferous tubules in the testis. The testis is of the tubular type without a lumen. Vacuolization in the interstitial cells occurs only during the spawning period, i.e., May to September. This behavior of the interstitial cells during the spawning period may probably be related to the activation of the other germ cells which are in the various stages of development and they may also suppress gonadotropin secretion from the pituitary.

The present study shows that the gonads mature under the influence of the pituitary gonadotropin and in turn secrete steroid for further processes of reproduction like ovulation and spermiation.

In \textit{Garra}, the data of GSI point out remarkable changes in their values during different periods of the gonadal cycle and may be correlated with seasonal changes in the gonads.

The yolk nucleus is present in \textit{Garra} and it has been shown to initiate vitellogenesis in this fish.

The hormones secreted by the fish pituitary regulates
reproduction, growth, colour change and reproductive behaviour. Hormones from the pituitary and gonads interact to regulate reproductive processes. Gonadotropin secretion in fish has been implicated in both inducing gametogenesis and vitellogenesis. The pituitary gonadotropins stimulate the gonadal tissues and gonadal steroids serve to check the activity of the pituitary gonadotropic cells through a feedback mechanism.

In the present study, after a detailed histological study of the pituitary with the classical staining techniques, six tinctorial cell types can be identified in the adenohypophysis. The rostral pars distalis consists of mainly deeply stained acidophils which are the prolactin cells. A few basophils are also found scattered in this region.

In the proximal pars distalis region mainly basophils are present which are intermingled with groups of acidophils. These acidophils are the growth hormone cells. Basophils in the proximal pars distalis are of two types. They are PAS positive cells which are shown to contain glycoproteins and Alcian blue positive cells shown to contain cysteine. The former have been regarded as the gonadotropin secreting cells and the latter as thyrotropin secreting cells. Very lightly stained acidophils bordering the neurohypophyseal processes are present in rostral pars distalis region and are regarded to be the corticotropes.

The pars intermedia contains mainly acidophils some of
which are darkly stained and the others lightly stained. This may be due to the variation in the activities of these cells. These are the melanotropes. Chromatophobic cells are scattered in the region of the rostral pars distalis and proximal pars distalis.

The neurohypophysis interdigitates with the adenohypophysis as in most teleosts but the main trunk enters the pars intermedia. Two types of nerve fibres are recognized. Deeply stained AF positive type A fibres and colourless type B fibres. Herring bodies and pituicytes are also present.

In Gerra, marked cytological changes are seen in the gonadotropes of proximal pars distalis during the different periods of the reproductive cycle. These changes have been correlated with the gonadal cycle of the fish.

In the post-spawning period of the fish, the gonadotropes appear vacuolated and depleted of secretion. At this stage, the secretory activity is very less and this can be correlated with the spent condition of the gonads in which the gametogenesis is yet to be accelerated. In the late post-spawning period, the basophils increase in size, the vacuoles disappear and the cytoplasm becomes granular showing the formation of secretory granules in the basophils. This secretory change in the gonadotropes is correlated with the formation of early stages of gametes in the gonads and absence of late stages.
In the pre-spawning period, the proximal pars distalis region of the pituitary is very much enlarged perhaps due to the increase in size of the basophil cells. The basophils become large in size and are full of secretion. There is a progressive trend in the process of granulation of basophils indicating an increased activity in these cells in the pre-spawning period. These changes can be correlated with the progressive development of the gonads during this period. In the later phase of spawning the basophils again show degranulation and appear vacuolated indicating a greater release of hormone.

Thus, it can be concluded that in Garra the granulation and degranulation of basophils show a definite correlation with the gonadal cycle indicating that the secretion of the gonadotropes are responsible for the process of spermatogenesis and vitellogenesis.

During the recent years the pineal of fishes has been extensively studied probably because there are contradictions regarding the role of this organ. There is now a general agreement that in fishes the pineal organ is a photoreceptive structure. It may also have a glandular function and there is substantial evidence to support the view that the pineal organ plays a reproductive role in bony fishes. Melatonin is shown shown to be synthesized in the pineal and the rate of synthesis is inhibited in continuous light.
Stimulatory effects of long photoperiods may be the result of reduced melatonin synthesis. More recent investigations have provided evidence for functional relationship between the pineal organ, photoperiod and seasonal reproductive strategies in teleost fishes. Environmental effects like photoperiods are mediated through the pineal organ.

In *Garra*, the pineal complex is a compact very well developed structure and consists of mainly sensory and supporting cells. Secretory granules are also visible in the lumen. The sensory cells consist of distinct outer and inner segments with ciliary processes projecting from the outer segment.

In *Garra*, exposure to long photoperiods (L 23 : D 1) in the pre-spawning phase indicate marked changes in the gonads. Oogenesis is enhanced in the ovaries and mature oocytes begin to develop. In males also spermatogenesis is enhanced. On exposure to short photoperiods (L 1 : D 23) in the spawning phase of the reproductive cycle, the ovaries regress and cease to develop further, in fact the process of oogenesis stops. In males short photoperiod shows no remarkable change in the testis when compared to controls.

These experiments were designed after the annual reproductive cycle under natural photoperiods and temperature was assessed. In the natural environment, this fish spawns in summer and with the onset of rains when there is an increased day length. Exposure of fish to short photoperiod at time of the year resulted in the regression of the ovaries, as
oogenesis is arrested. There are no remarkable effects on
the testes. With long photoperiod in winter when the natural
day length is decreased there is a stimulatory effect on both
the testes and ovaries because gametogenesis is enhanced and
later stages of oocytes, spermatids and sperms are seen in
the ovary and testis respectively.

Temperature also has a marked effect on the gonads of
Garra. In fish maintained under high temperature (30±1°C),
the ovaries show peculiar changes. Mature oocytes begin to
form rapidly and few immature oocytes directly change into
corpora atretica. In fact, the rate of oogenesis is stimulated
when compared to controls maintained under 18±1°C. In the
testes high temperature induces a rapid increase of the late
spermatogenetic stages, viz., spermatids and spermatozoa.
Some morphological changes are also seen in both males and
females maintained under high temperature. The abdomen of
both the sexes become bulgy due to the increase in size of
the gonads and the tail portion becomes narrow.

An overall assessment of the reproductive biology and
hormonal control in this fish reveals that environmental
factors such as photoperiods and temperature play an important
role to initiate and co-ordinate endogenous events leading to
reproduction. The endocrine system serves as a link between
these environmental stimuli and changes in the reproductive
system.