Part V

Seasonal changes in the pituitary gland of *Clarias batrachus* and *Mastacembelus panchilus* in correlation with the reproductive cycle.

1. Introduction

Pituitary-gonadal relationship in fish has been now well established. Dodd (1955) and Roar (1955) have amply reviewed that the secretion of the meso-adenohypophysis (proximal pars distalis) controls the activity of the gonads. Gonadotrophs and thyrotrophs have been successfully demonstrated experimentally in *Astyanax mexicanus* (Atz, 1953), in African blind cave fish, *Caecobarbus geerstii* (Olivereau and Herlant, 1954), in *Phoxinus phoxinus* (Barrington and Matty, 1955) and in *Lebias reticulatus* (Sokol, 1955).

The correlation of the changes of the pituitary gland with the reproductive cycle has been described in *Fundulus heteroclitus* (Matthews, 1939), in *Cyprinus carpio* and *Carassius auratus* (Scruggs, 1951), in *Carassius auratus* (Beach, 1959), in *Heteropneustes fossilis* (Sundararaj, 1959 and 1960), in *Phoxinus phoxinus* (Bhargava, 1966), in *Tor* (*Barbus*) *tor* (Rai, 1966), in *Puntius ticto*, *Ambassis range* and *Rohtas cotio* (Kaur, 1968), in *Oxygastor bacaila*, *Gerre gotyla* *gotyla* and *Nandus mandus* (Raizada, 1969), in *Poecilia reticulata* (Sage and Bromage, 1970),
in *Tilapia nila* (Ryder, 1970) and in *Rasbora daniconius* (Raizada, 1972).

Little work has been done on the statistical assessment of the seasonal changes in the cell size of the pituitary gland. The seasonal changes in the size of the basophils and acidophils have been studied statistically in relation with the reproductive cycle in *Astyanax mexicanus* (Rasquin, 1949), in *Cyprinus carpio* (Scruggs, 1951), in *Salmo salar* (Olivereau, 1954), in *Phoxinus phoxinus* (Bhargava, 1966), in *Ambassia ranga*, *Puntius picta* and *Rohtas cotio* (Kaur, 1968) and in *Rasbora daniconius*, *Oxygastor baciai*, *Carassius gosyla gosyla* and *Nandus nandus* (Raizada, 1969).

The present study deals with the cytological changes of the pituitary gland (especially the proximal pars distalis) of *Clarias batrachus* and *Mastacembelus panchax* in correlation with the reproductive cycle. The cell size (mean maximum length) of the basophils and acidophils of the proximal pars distalis region of the pituitary gland of *Clarias batrachus* has also been studied statistically which further supports the pituitary-gonadal relationship of the fish.

2. Observations

There is enough evidence to show that the teleost pituitary undergoes certain changes in different seasons and these changes are associated, in some way or the other, with the reproductive cycle. The changes taking place in the cells
of rostral pars distalis and pars intermedia of *Clarias batrachus* and *Mastacembelus pancalus* are not so marked as to correlate them with the reproductive cycle. It is only the changes in the cells (basophils and acidophils) of proximal pars distalis which exhibit close correlation with the reproductive cycle. The basophils as well as the acidophils show variations in their cell size (maximum length) in different seasons of the year.

It has been further observed in this study that the basophils of the proximal pars distalis show more pronounced changes than those of the acidophils.

A. **Cytological changes in the basophils and acidophils of proximal pars distalis of the pituitary gland**

*Clarias batrachus*

In the proximal pars distalis of *Clarias batrachus* chief types of cells present are one type of acidophils and two types of basophils (refer page 25). It has been observed that the acidophils, with the staining methods used, do not exhibit any well marked cytological changes during the annual cycle. The basophils are differentiated into two types, i.e., deeply staining basophils or Basophil I and lightly staining basophils or Basophil II (Refer page 25). Of these two types of basophils, pronounced cytological changes are observed only in Basophil I during the reproductive cycle.
(a) **Post-spawning period** (mid October to January)

During this period the shape of the basophils (Basophil I) is polygonal with a big round nucleus. The cytoplasm of these basophils gives a smooth appearance as the production of AF+ve stainable secretory granules inside them is very scanty. Both the cell as well as the nuclear boundaries are distinct (Fig. 40 A, p. 209).

The acidophils have a well marked cell outline, a distinct nucleus and a nucleolus. The cytoplasm is sparsely granulated which would indicate that the production of their hormones, implicated in the growth or rebuilding processes of the body after spawning, has started.

During this period a large number of chromatin-nucleolus and perinucleolus stages of oocytes are present in the ovary. In the late post-spawning period (January) few early yolk-vesicle stages of oocytes also appear. The testis lobules contain a large number of early stages of spermatogenesis, especially the primary germ cells and spermatogonia (Table 7, p.177). The formation of large numbers of early cogenetic and spermatogenetic stages, during this period are, therefore, associated with the scanty secretions of the cells of the proximal pars distalis.

(b) **Pre-spawning period** (February to May)

There are no appreciable changes in the basophils of early pre-spawning period (i.e. February) from those of
Fig. 40 - Photomicrographs of the vertical longitudinal sections of the proximal pars distalis region of the pituitary gland of *Clarias batrachus* (AF) -

A.  - Showing the basophils with smooth cytoplasm in November fish
B.  - Showing most of the granulated basophils and few degranulated basophils in April fish
C.  - Showing highly degranulated basophils in June fish
D.  - Showing vacuole-like structures in August fish

Abbreviations:

A.  - Acidophils
B.  - Basophils
D.B. - Degranulated basophils
G.B. - Granulated basophils
V.  - Vacuole-like structures
post-spawning periods but in March and April the cytoplasm of the basophils no longer remain homogeneous but becomes somewhat more granulated. This is an indication that the process of hormone formation and its partial diffusion outside the cell is somewhat increasing than before. It can also be implicated that the rate of production of granules during these months is more than the rate of their release. In May, the basophils show intense granulation. However, the degranulation process also starts in a few basophils. The cytoplasm and the nucleus of the basophils do not show any other change during these months. Intracellular basophilic secretory droplets, as reported in some teleosts, are not seen. (Fig. 40 B, p. 209).

No appreciable change is observed in the acidophils of this period from those of the post-spawning period.

The oocytes present in the ovaries of these months are mostly of perinucleus and yolk-vesicle stages. From February to May there is almost a gradual decrease in the number of primary germ cells and spermatogonia. The primary and secondary spermatocytes show a considerable increase in their number. The spermatids appear in March while spermatozoa start differentiating in May.

It is, therefore, inferred that the secretions of the Basophil I are clearly involved in the maturation process of the oogenetic and spermatogenetic stages during this period. Also the secretion of the acidophils seems to be implicated in
the bodily metabolic processes during this period.

(c) **Spawning period** (early June to early October)

In June and July there is greater degranulation in the cytoplasm of a large number of basophils and consequent to this process there is an appearance of small vacuole-like structures in the cytoplasm of most of the basophils (Fig. 40 C, p. 209). In August and September the basophils are highly degranulated. There is an enormous increase in the number and size of the vacuole-like structures as a result of which the cell outline does not appear to be distinct though it can be easily distinguished (Fig. 40 D, p. 209). It is noteworthy that in October the cytoplasm of the basophils becomes smooth. The cell outline and the nuclear membrane are well defined which indicate that the secretory products have been released from the basophils by this time and the cytoplasm has been restored to its normal condition. During these months, therefore, there seems to be greater release of hormones than its production by these cells. The acidophils, during this period, show distinct cell outline and the nuclear membrane. The cytoplasm no longer remains granulated and becomes smooth.

The ovaries of this period show a large number of oocytes of yolk stage, prematuration stage and mature stage. In between these oocytes are also present some oocytes of early stages. The early stages of spermatogenesis, during
this period, decrease considerably in number. The number of spermatozoa increases and reaches its peak formation in August being 71.12% of different cell types of spermatogenesis (Table 7, p. 177). The secretions of Basophil I and their release, during this period, thus seem to be concerned with final maturation processes of the gonads as well as spawning behaviour and spawning of the fish.

In Clarias the granulation and degranulation of basophils shows a definite correlation with the reproductive cycle and spawning of the fish. The degranulation process takes place as a result of the release of the secretory granules from the cytoplasm of the basophils. This process is maximum in the month of August indicating that the rate of release of the hormones is greatest in this month. Since the spawning activity is greatest in August (which is evident from the fact that in the testes the number of spermatozoa is maximum in August (Table 7, p. 177) and in the ovaries the number of post-ovulatory follicles is maximum in August (Table 4, p. 143), the rate of release of the hormones (as indicated by the degranulation of basophils) in this month seems well justified.

Mastacembelus nanculos

In the proximal pars distalis of Mastacembelus two types of acidophils and two types of basophils are present (refer page 32). Out of the two types of basophils, the deeply staining
basophils designated as Basophil I (refer page 32) alone show marked changes during the different periods of the reproductive cycle. As in Carias, the acidophils of the proximal pars distalis of Mastacembelus do not exhibit well marked cytological changes during the annual cycle.

(a) Post-spawning period (mid November to January)

During this period the shape of the basophils (Basophil I) is more or less elongated. Their cytoplasm shows scanty granulation but still it is smooth and takes up a light stain. The nuclear wall and the cell outline are quite distinct (Fig. 41A, p. 215).

The acidophils (both Acidophil I and Acidophil II) show a clear cell outline, sparsely granulated cytoplasm and a clear nuclear membrane.

In November, oocytes of chromatin-nucleolus and peri-nucleolus stages are present in large number along with a few oocytes of prematuration and mature stage whereas the ovaries of December and January contain only the oocytes of chromatin nucleolus and peri-nucleolus stage. In January few oocytes of early yolk-vesicle stage also appear in some ovaries. Corpora atretica and post-ovulatatory follicles are very few in November and December while they are absent in January (Table 4, p. 143). Though all the stages of spermatogenesis are present in the
Fig. 41 - Photomicrographs of the vertical longitudinal sections of the proximal pars distalis region of the pituitary gland of *Mastacembelus bancanus* (AF) -

A. - Showing the basophils with smooth cytoplasm in December fish

B. - Showing the granulated basophils of March fish

C. - Showing highly degranulated basophils of July fish

D. - Showing the degranulated basophils of October fish

Abbreviations:

A. - Acidophils
B. - Basophils
D.B. - Degranulated basophils
G.B. - Granulated basophils
V. - Vacuole-like structures
testes of *Mastacembelus* round the year but during this period the primary germ cells and spermatogonia are abundantly present (Table 8, p. 181).

It is thus seen that the scanty production of hormones, during this period, by Basophil I and both types of acidophils seems to be implicated with the rebuilding processes of the gonads as well as the general metabolic processes after spawning.

(b) **Pre-spawning period** (early February to early May)

In February and March the secretory granules make their appearance in most of the basophils which become deeply stained. These AF+ve secretory granules lie in close aggregation (Fig. 41 B, p. 215). In April, the process of degranulation starts in few basophils while most of the basophils are highly granulated. The basophils show a clear cell outline and distinct nuclear membrane.

The granulation in the cytoplasm of both types of acidophils increases due to which they show a very clear cell outline.

During this period a number of oocytes of yolk-vesicle and early yolk stage appear in the ovaries along with the early stages of oocytes. Corpora atretica and post-ovulatory follicles are entirely absent (Table 4, p. 143). In the testes, the number of primary germ cells and spermatogonia decreases
gradually while the number of other stages of spermatogenesis increases considerably (Table 8, p.181). It shows that the maturation process in the spermatogenesis is accelerated during this period.

Thus there appears to be a complete correlation between the secretory activity of the cells of the proximal pars distalis and the maturation of the gonads as well as the general metabolic activities of the animal.

(c) **Spawning period** (late May to early November)

During the early part of the spawning period (i.e., in May) no appreciable change in the cytology of the basophils from the condition present in April is observed. The basophils in the months of July, August, September and October are highly degranulated showing vacuoles in the cytoplasm and the very sparse distribution of secretory granules in it (Fig. 41C, D, p.215).

Both types of acidophils have ill defined cell outline and the cytoplasm is devoid of secretory granules thus showing a smooth appearance. The nuclear membrane is well defined.

In the ovaries of May and June, the oocytes of yolk, prematuration and mature stage are predominantly present while the ovaries of July, August, September and October mostly contain mature oocytes. Other stages of developing oocytes are also present but are few in number. Both corpora atretica
and post-ovulatory follicles are present in good number (Table 4, p. 143). In the testes, the number of spermatozoa gradually increase and reaches the peak in July (63.86%) and August (62.13%), other stages of spermatogenesis are few in number.

Thus the changing events in the Basophil I and the gonads, during this period, indicate that the secretion of Basophil I is implicated with the spawning behaviour and spawning of this fish.

In *Mastacembelus pancalus* the process of degranulation starts in late pre-spawning period and continues throughout the spawning period. Pronounced degranulation showing the appearance of vacuole in the cytoplasm as seen in basophils of *Clarias batrachus* during the spawning period has not been observed in those of *Mastacembelus*. This may be due to the reason that in *Mastacembelus* sharp changes are not found as regards the maturation of the gonads in any particular month of the spawning period. This has been more particularly observed in case of testes where the spermatozoa are present throughout the year and there is no sharp variation in their number in any month except in the month of July, August and September when they are 63.80%, 62.13% and 60.47% respectively (Table 8, p. 181).
B. **Seasonal changes in the maximum length of acidophils and basophils of proximal pars distalis of the pituitary gland.**

It is now established that the proximal pars distalis is associated with the maturation of gonads and spawning behaviour of the fish. During the different periods of the reproductive cycle, the acidophils as well as the basophils show changes in their length. It is, therefore, in the fitness of things to study this variation in length statistically in case of *Clarias batrachus* and confirm the histological changes that take place in the pituitary in relation to different seasons.

This statistical study is based on similar work in the minnow, *Phoxinus phoxinus* (Bhargava, 1966) and the method used in the measurement of acidophils and basophils in the present study is the same as used in the above study. In order to measure the size, only those cells were considered which were found abutting on the process of neurohypophysis. The criterion for selection of such cells is based on the reason that these cells represent their maximum length in a particular period of the year. Other cells not abutting the processes of the neurohypophysis are those which are cut in various planes and hence do not represent their maximum length. In this method a monthly sample of eight specimens of *Clarias* were taken into consideration. After a general survey of the sections under high magnification of the microscope i.e. X 675, approximately,
five acidophils and five basophils (abutting on the processes of neurohypophysis) having the largest size were selected from the proximal pars distalis and measured from each one of the section in nine different planes (four on either side of the median sagittal section). In this way 360 acidophils and 360 basophils (45 acidophils and 45 basophils for each one of the eight fish) were measured from each monthly sample.

The eight monthly fish were compared with each other so as to see that they are not significantly different from each other at 5% level. This was done by applying the student 't' test formula for significance probability (Brownlee, 1949) which is as follows:

\[
t = \frac{\bar{x}_1 - \bar{x}_2 \text{ or } M_1 - M_2 \sqrt{\frac{N_1 N_2}{N_1 + N_2}}}{\sqrt{\frac{\sum (x_1^2) - (\sum x_1)^2}{N_1} + \frac{\sum (x_2^2) - (\sum x_2)^2}{N_2}} \sqrt{N_1 + N_2 - 2}}
\]

where \( \bar{x}_1 \) and \( \bar{x}_2 \) are figures in \( \mu \) giving the mean maximum length of the cells of two fish compared, \( N_1 \) and \( N_2 \) is the number of cells in each fish, i.e. 45 and \( N_1 + N_2 - 2 = 88 \) represents the degree of freedom allowed for determining the significance probability. In this way the number of remaining (not significantly different) fish in each monthly sample varied
from 6 to 8 fish.

The standard deviation for the mean maximum length of the cells (both acidophils and the basophils) in a monthly sample of fish (not significantly different) was calculated by the following formula (Brownlee, 1949):

$$ S.D = 6 = \sqrt{\frac{\bar{x}^2 - \left(\frac{\sum x}{N}\right)^2}{N}} $$

Where $N = 360$ (number of cells) or less depending upon the number of fish in a monthly sample and $x$ is the length of each one of the cells in $u$.

The standard error for the mean maximum length of each month was calculated by the following formula:

$$ S.E. = \frac{6}{\sqrt{N}} $$

where $6$ is the standard deviation and $N$ is the number of cells.

The values for the mean maximum length of basophils and acidophils, the standard error, and the standard deviation of the mean length for a population of cells in each month for *Clarias batrachus* is plotted in (Fig. 42, p. 224) and have been shown in the (Table 9, p. 222).

In order to ascertain that the curve showing mean maximum length in different months is really significant, a monthly
### Table 9

<table>
<thead>
<tr>
<th>Months</th>
<th>Acidophils</th>
<th></th>
<th>Basophils</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean maximum length in ( \mu )</td>
<td>S.E.</td>
<td>S.D.</td>
<td>Mean maximum length in ( \mu )</td>
</tr>
<tr>
<td>July</td>
<td>6.91</td>
<td>0.03</td>
<td>0.76</td>
<td>12.95</td>
</tr>
<tr>
<td>August</td>
<td>6.81</td>
<td>0.05</td>
<td>0.88</td>
<td>12.07</td>
</tr>
<tr>
<td>September</td>
<td>6.06</td>
<td>0.04</td>
<td>0.89</td>
<td>11.99</td>
</tr>
<tr>
<td>October</td>
<td>5.87</td>
<td>0.02</td>
<td>0.54</td>
<td>8.94</td>
</tr>
<tr>
<td>November</td>
<td>4.67</td>
<td>0.03</td>
<td>0.77</td>
<td>8.27</td>
</tr>
<tr>
<td>December</td>
<td>4.64</td>
<td>0.03</td>
<td>0.75</td>
<td>7.80</td>
</tr>
<tr>
<td>January</td>
<td>4.56</td>
<td>0.03</td>
<td>0.78</td>
<td>7.40</td>
</tr>
<tr>
<td>February</td>
<td>4.92</td>
<td>0.03</td>
<td>0.78</td>
<td>7.48</td>
</tr>
<tr>
<td>March</td>
<td>5.37</td>
<td>0.04</td>
<td>0.96</td>
<td>8.16</td>
</tr>
<tr>
<td>April</td>
<td>6.07</td>
<td>0.04</td>
<td>0.86</td>
<td>8.48</td>
</tr>
<tr>
<td>May</td>
<td>5.89</td>
<td>0.04</td>
<td>0.85</td>
<td>9.10</td>
</tr>
<tr>
<td>June</td>
<td>6.67</td>
<td>0.05</td>
<td>1.16</td>
<td>11.98</td>
</tr>
</tbody>
</table>

**Explanation:** Table showing the value of the mean maximum length (given in \( \mu \)), the standard error (S.E.) and the standard deviation (S.D.) of the acidophils and basophils in each month of *Clarias batrachus*.

**Note:**
- Post-spawning period: mid October to January
- Pre-spawning period: February to May
- Spawning period: early June to early October
Fig. 42 - Curves for basophils and acidophils of the proximal pars distalis of *Clarias batrachus*, to show the seasonal changes in their maximum length during different months. The standard deviation for the mean maximum length of each month is shown by vertical lines.
FIG. 42

MEAN MAXIMUM LENGTH OF BASOPHILS IN μm

MEAN MAXIMUM LENGTH OF ACIDOPHILS IN μm

MONTHS

MONTHS
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>7.55/$\infty$</td>
<td>15.30/$\infty$</td>
<td>29.48/$\infty$</td>
<td>24.42/$\infty$</td>
<td>34.95/$\infty$</td>
<td>42.51/$\infty$</td>
<td>40.15/$\infty$</td>
<td>29.13/$\infty$</td>
<td>32.08/$\infty$</td>
<td>2.33/$\infty$</td>
<td>1.71/$\infty$</td>
</tr>
<tr>
<td>February</td>
<td>-</td>
<td>8.45/$\infty$</td>
<td>24.14/$\infty$</td>
<td>27.82/$\infty$</td>
<td>27.27/$\infty$</td>
<td>42.13/$\infty$</td>
<td>30.54/$\infty$</td>
<td>20.96/$\infty$</td>
<td>23.08/$\infty$</td>
<td>5.23/$\infty$</td>
<td>5.96/$\infty$</td>
</tr>
<tr>
<td>March</td>
<td>-</td>
<td>-</td>
<td>13.04/$\infty$</td>
<td>9.15/$\infty$</td>
<td>19.93/$\infty$</td>
<td>23.45/$\infty$</td>
<td>23.08/$\infty$</td>
<td>12.16/$\infty$</td>
<td>10.52/$\infty$</td>
<td>13.34/$\infty$</td>
<td>13.86/$\infty$</td>
</tr>
<tr>
<td>April</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.29/$\infty$</td>
<td>9.30/$\infty$</td>
<td>16.55/$\infty$</td>
<td>12.27/$\infty$</td>
<td>0.18/$\infty$</td>
<td>4.49/$\infty$</td>
<td>29.13/$\infty$</td>
<td>28.36/$\infty$</td>
</tr>
<tr>
<td>May</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.17/$\infty$</td>
<td>20.20/$\infty$</td>
<td>15.33/$\infty$</td>
<td>1.82/$\infty$</td>
<td>0.59/$\infty$</td>
<td>24.14/$\infty$</td>
<td>24.75/$\infty$</td>
</tr>
<tr>
<td>June</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.00/$\infty$</td>
<td>3.46/$\infty$</td>
<td>9.64/$\infty$</td>
<td>14.46/$\infty$</td>
<td>33.30/$\infty$</td>
<td>33.86/$\infty$</td>
</tr>
<tr>
<td>July</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.60/$\infty$</td>
<td>16.71/$\infty$</td>
<td>44.68/$\infty$</td>
<td>47.93/$\infty$</td>
<td>49.06/$\infty$</td>
</tr>
<tr>
<td>August</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.50/$\infty$</td>
<td>13.99/$\infty$</td>
<td>38.44/$\infty$</td>
<td>40.31/$\infty$</td>
</tr>
<tr>
<td>September</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.19/$\infty$</td>
<td>27.29/$\infty$</td>
<td>28.08/$\infty$</td>
</tr>
<tr>
<td>October</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29.72/$\infty$</td>
<td>30.82/$\infty$</td>
</tr>
<tr>
<td>November</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.65/$\infty$</td>
</tr>
</tbody>
</table>

Explanation: Table showing the values of 't' between the samples of the acidophils (mean maximum length) of different months in *Clarias batrachus* at $\infty$ degrees of freedom. The figures underlined are not significantly different at 5% level.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>0.59&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>7.99&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>11.84&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>17.78&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>41.21&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>55.83&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>38.20&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>42.81&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>16.72&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>9.23&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>2.50&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>February</td>
<td>-</td>
<td>7.31&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>7.17&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>12.11&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>30.15&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>39.11&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>28.52&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>31.43&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>11.15&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>5.71&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>5.03&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>March</td>
<td>-</td>
<td>-</td>
<td>3.66&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>10.28&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>25.85&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>50.36&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>36.60&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>37.68&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>8.83&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>1.21&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>4.59&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>April</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.04&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>34.30&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>48.86&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>43.59&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>35.62&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>5.35&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>2.43&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>9.24&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>May</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27.22&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>40.27&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>25.74&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>28.38&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>1.87&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>9.08&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>16.44&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>June</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.94&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>9.76&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>29.57&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>34.66&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>38.35&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>July</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.41&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>4.93&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>43.32&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>50.55&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>62.69&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>August</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.79&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>31.07&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>36.61&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>45.90&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>September</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.72&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>35.91&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>45.07&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>October</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.65&lt;sup&gt;∞&lt;/sup&gt;</td>
<td>15.26&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
<tr>
<td>November</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.63&lt;sup&gt;∞&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Explanation: Table showing the values of 't' between the samples of the basophils (mean maximum length) of different months in *Clarias batrachus* at <sup>∞</sup> degrees of freedom. The figures underlined are not significantly different at 5% level.
sample of cells was compared with another monthly sample by applying the same student 't' test formula. The values obtained after comparison for both basophils and acidophils are given in Tables 10, p. 225; 11, p. 226).

The acidophils of proximal pars distalis of Clarias do not show any marked cytological changes (refer page 206) which can be correlated with different periods of the reproductive cycle. However, the acidophils exhibit a cyclic variation in their maximum length during the annual reproductive cycle.

The maximum length of acidophils is almost similar in the months of November and December (Table 9, p. 222) which is evident by the fact that the November sample is not significantly different on comparison with that of December (Table 10, p. 225). In January the acidophils show a little decrease in their size. The reduction of the length of the acidophils during this period seems to indicate a comparatively lower metabolic rate after spawning which, however, is implicated in the rebuilding process of the fish. With the advent of the pre-spawning period the acidophils begin to increase steadily in length till April while in May they again show a slight reduction in length in comparison to April. On a comparison of the monthly samples of this period with one another it is seen that the variation in the maximum length is significantly different (Table 10, p. 225). This increase in the length of the acidophils appears to be concerned with the high metabolic rate of the fish responsible in the body growth. The acidophils increase in length further until July when their maximum length is highest.
From August onwards there is a gradual decrease in their maximum length until the end of the spawning period. The values of 't' obtained after the comparison of the monthly samples of the spawning period with one another are significantly different (Table 10, p. 225). This increase in the length of the acidophils during spawning period till July appears to be concerned with the high metabolic rate of the fish resulting in the body growth and reproductive behaviour of the fish.

With the help of the staining techniques used in the present study, it is observed that there is no visible cytological activity in the acidophils and therefore, it seems likely that the hormones produced in the acidophils control some activities of the fish other than the maturation of gonads and spawning in the reproductive cycle.

The basophils show a sharp reduction in length in the post-spawning period from the condition present in November. This variation is significantly different (Table 9, p. 222). In the pre-spawning period the maximum length begins to increase steadily and continues to progress in the spawning period until July. Thereafter the maximum length of the basophils decreases gradually till the end of the spawning period. On a comparison of monthly samples of December, March and July (representing post-spawning, pre-spawning and spawning periods respectively) it is seen that the values of 't' are significantly different. A comparison of March and December sample shows that they are also significantly
different though the value of 't' is small being 4.59 (Table 11, p. 226). It is thus inferred that the basophils appear to become active right from the beginning of the pre-spawning period resulting in the production of secretory granules. This activity appears to be associated with the high metabolic rate of the fish and results in the maturation of the gonads. The increase in the length of the basophils during the early spawning period (Table 9, p. 222) and their highly degranulated condition during the spawning period (which has been observed cytologically) indicates that the secretory activity of the basophils during this period may be responsible for the final maturation process of the gonads and spawning of the fish.

The cyclic variations in the maximum length of the acidophils and basophils closely follow the variation present in the gonad volume (Table 3, p. 142) during the reproductive cycle.

3. Discussion

The rostral pars distalis in *Clarias* and *Hastacembalus* does not exhibit any marked cytological change which may be correlated with different periods of the reproductive cycle of the fish. A similar condition of the activity of this gland has been reported in *Gasterosteus aculeatus* (Bock, 1723), *Carassius auratus* and *Cyprinus carpio* (Scruggs, 1951), *Girrhina reba* (Sathyanesan, 1958), *Heteropneustes fossilis* (Sundararaj, 1959 and 1960), *Girrhina mrigala* (Bachan Lal, 1964),
Gobius giuris (Rajalakshmi, 1966), Phoxinus phoxinus (Bhargava, 1966), Ambassia ranga, Puntius ticto and Rohtee cotio (Kaur, 1968) and Rasbora daniconius, Oxygaster bacaila, Carra gotyla gotyla and Nandus nandus (Raizada, 1969).

Similarly cells of the pars intermedia in Clarias and Mastacembelus do not exhibit well marked cytological changes which could be correlated with their reproductive cycle.

However, prominent changes are observed in the Basophil I of proximal pars distalis which are concomitant with the cyclical changes in the reproductive activity in Clarias and Mastacembelus. Similar cytological changes in the basophils of the proximal pars-distalis have also been studied in correlation with the reproductive cycle in Fundulus heteroclitus (Matthews, 1936), in some teleost pituitaries (Kerr, 1940, 1948), in Cyprinus carpio and Carassius auratus (Scruggs, 1951), in Salmo salar (Olivereau, 1954), in Lebistes reticulatus and Xiphophorus helleri (Stock, 1953), in Cirrhina reba (Sathyanesan, 1958), in Heteropneustes fossilis (Sundararaj, 1959, 1960), in Fundulus heteroclitus and Lebistes reticulatus (Sokol, 1961), in Cirrhina mrigala (Bachan Lal, 1964), in Gobius giuris (Rajalakshmi, 1966), in Phoxinus phoxinus (Bhargava, 1966), in Ambassia ranga, Puntius ticto and Rohtee cotio (Kaur, 1968) and Rasbora daniconius, Oxygaster bacaila, Carra gotyla gotyla and Nandus nandus (Raizada, 1969).

The intra-cellular acidophilic spheres in Perca fluviatilis (Kerr, 1942), colloid bodies in Cirrhina mrigala (Bachan Lal, 1964),
acidophilic globules in *Gobius giuris* (Rajalakshmi, 1966), and
AF+ve droplets or globules in *Phoxinus phoxinus* (Bhargava, 1966)
as found in the cytoplasm of the basophils of proximal pars -
distalis are absent in case of *Clarias* and *Mastacembelus*. But
the secretory activities in the basophils at different times of
maturation and spawning periods of the fish, are clearly visible.

Bretschneider and Duyvene de Wit (1947) in *Rhodeus amarus*,
Kerr (1948) in *Leuciscus rutilus*, Scruggs (1951) in *Carassius*
auratus and *Cyprinus carpio* have suggested gonadotropic function
of the basophils of meso-adenohypophysis. Dodd (1955) and Hoar
(1955) have put forth ample evidence to prove that the secretions
from the pituitary gland control the activity of gonads. But
Sokol (1961) is of the opinion that the relation between the
reproductive cycle in both *Fundulus* and *Lebistes* and the pronounced
fluctuations in the secretory activity of the basophils cannot be
established with certainty by the morphological study of the normal
animals alone. Rajalakshmi (1966) in *Gobius giuris* and Bhargava
(1966) in *Phoxinus phoxinus* have suggested the gonadotropic
function of the basophils of the meso-adenohypophysis.

In the present study the degranulation of basophils has been
associated with the release of hormones for the maturation of gonads
and spawning behaviour of the fish. In *Clarias* vacuole-like
structures appear in the process of degranulation while in case of
*Mastacembelus* such vacuole-like structures do not appear. Instead,
the secretory granules (which are closely aggregated in the
granulated basophils) become sparsely distributed and as the
process of degranulation increases, the amount of these secretory granules becomes lesser. It has been observed that the rate of production of these secretory granules which represent the hormones during the spawning period is less than the rate of their release resulting in the degranulation of basophils.

By bioassay experiments Ramaswami and Sundararaj (1957, 1958a, 1958b) and Sundararaj (1959) have shown that the basophils secrete both the follicle stimulating and luteinising hormones in the female of *Heteropneustes fossilis*, Sundararaj (1960) has further assumed that the basophils in the male pituitary of this fish also secrete these hormones. In the present study, the degranulation process appears in the late pre-spawning period indicating that the hormones released due to the degranulation help in the maturation of gonads and at the height of breeding season, the degranulation is maximum in August in *Clarias* and in September in *Mastacembelus* which points out that the hormones released also seem to control the spawning behaviour of the fish.

Olivereau (1954) has shown that the fuchsinephils (acidophils) of the meso-adeno-hypophysis discharge their granules into the neuro-intermedia lobe of *Salmo salar* where they form after fusion brilliantly staining droplets which have been regarded as somatotropin. Sathyanesan (1958) has shown that the secretory acidophils are present only in the regression period of *Cirrhina reba* suggesting the possibility of their having some role in the repair of the gonads. In the present study it has been observed that the acidophils of proximal pars distalis in *Clarias* and
*Mastacembelus* do not show any marked secretory activity. This is in conformity of similar observations in *Phoxinus phoxinus* (Bhargava, 1966), *Ambassius ranga*, *Puntius ticto* and *Rohdea cotio* (Kaur, 1968) and *Rasbora daniconius*, *Oxygaster bacaila*, *Garra goltla goltla* and *Nandus nandus* (Raizada, 1969).

Scruggs (1951) used a method in which cell size was measured in four sagittal planes of the übergangsteil in alternate fields. The normal possibility is that in sections, the cells are cut in different planes and hence the length of the cells may not represent the maximum length for that period. Bhargava (1966) has taken only those cells of the meso-adenohypophysis into consideration which were found abutting on the processes of neurohypophysis in *Phoxinus phoxinus*. Kaur (1968) in *Ambassius ranga*, *Puntius ticto* and *Rohdea cotio* and Raizada (1969) in *Rasbora daniconius*, *Oxygaster bacaila*, *Garra goltla goltla* and *Nandus nandus* have used the method devised by Bhargava (1966) for similar studies. The criterion of selection of these cells which abutt on the processes of the neurohypophysis is justified in the way that these cells represent their maximum length, as during the process of development, the neural part interdigitates with the glandular component of the pituitary with a mesodermal stratum in between them.

In the present study on *Clarus betrachus* both the acidophils and basophils show cyclic changes in their maximum length. The body growth and reproductive behaviour such as search of food and mate involving long swimming movements appear to be related with the increase in the maximum length of the acidophils. Whereas
the increase in the maximum length of the basophils during the pre-spawning and spawning periods seems to be associated with the maturation of gonads and spawning in the fish. The changes in the gonad volume have also been found to be related with the changes in the maximum length of the basophils. In this way the present study of the seasonal changes in the maximum length of the acidophils and basophils of the proximal pars distalis confirms similar observations in the minnow, Phoxinus phoxinus (Bhargava, 1966), in Ambassis ranis, Puntius ticto and Rohtee cotto (Kaur, 1968) and in Rasbora daniconius, Oxystom bacaila, Garra golya golya and Nandus nandus (Raizada, 1969).