Reproductive Endocrinology of the Tropical Walking Catfish, *Clarias batrachus*.

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SUMMARY: Basic information on reproductive endocrinology is lacking in tropical fishes, although tropical areas harbour a wide variety of fish species. This paper provides information on plasma sex steroid levels in tropical walking catfish maintained under stable warm water conditions for two years. It was ascertained that males and females maintained mature gonads capable of undergoing ovulation at any time during the experimental period. Fish never underwent spontaneous spawning, but a single HCG injection could induce ovulation at any time of the year. Such fish also ovulated and spawned in response to water level and temperature manipulation. Changes in water temperature probably increase the sensitivity of fish to the change in water level. Endocrine changes following HCG injection were also monitored.

KEY WORDS: reproduction, endocrinology, maturation, spawning, walking catfish

INTRODUCTION

Most information on endocrinological control in fish reproduction has been derived from seasonally breeding fish such as salmon, rainbow trout1), carp2), bitterling3), channel catfish4), Indian catfish5), Thai catfish6), etc. On the other hand, little information is available relating to tropical fishes, which may breed repeatedly throughout the year. Tropical fish should receive more attention since tropical areas are rich in various fish species8) and many of these fish are economically important. This paper tries to provide information on endocrinological regulation of reproduction of these fishes using tropical walking catfish as a model.

FEMALE

Maturity under warm water conditions

To study the changes in ovarian maturity in the tropical walking catfish, females and males (7 months-old) were maintained under constant warm water temperature (23-25°C) and natural photoperiod until the age of 31 months-old. Histological observation of the oocytes revealed that 10-31 months-old females always possessed post-vitellogenic oocytes prior to final oocyte maturation (Fig.1.).

However, percentages of post-vitellogenic oocytes fluctuated and atretic oocytes were also observed. No spontaneous ovulation or spawning occurred during the experiment9). However, all females which had received a human chorionic gonadotropin (HCG) injection (0.8 IU/g BW) at the end of the experiment had succeeded in ovulation9). Plasma estradiol-17β and testosterone started to increase at 8 months-old, and thereafter fluctuated at
high levels. Furthermore, testosterone levels became higher in 2 years-old fish than did those in yearling females. 17α-Hydroxyprogesterone (17α - P) levels also fluctuated, but average levels were lower than those of estradiol-17β and testosterone. Plasma 17α, 20α-dihydroxy-4-pregnen-3-one (17α, 12α - P), 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-P) and progesterone were below detectable limits. These results indicate that female walking catfish can maintain conditions of maturity for an extended period under warm water and natural photoperiod.

**Induction of spawning by controlling water level and temperature.**

This experiment was carried out to confirm the role of water level and water temperature in inducing the spawning of tropical walking catfish. Mature males and post-vitellogenic females that reared under 23-25 °C, were paired and induced to spawn by controlling water level and water temperature\(^1\). Results are shown in Table 1. Decreasing water level and returning it to its original level resulted in a low spawning rate (less than 6.7 %) at 23 °C. Decreasing water level with simultaneous increase in water temperature, followed by returning the levels to their original, gave high spawning rates (41.7-50 %); whereas the same treatment but without any water temperature decrease when the water level was returned to the initial level, gave a low spawning rate (16.7 %). Increasing water level only, failed to induce spawning. A high spawning rate was obtained also when changes in water level were carried out under high temperature of 28 °C. No fish spawned in the absence of the environmental simulation.

From the results, it is confirmed that water level and temperature play important roles in inducing spawning of tropical walking catfish. Changes in water temperature probably increase the sensitivity of fish to the change in water level. Prolonged exposure to high water temperature could also improve the sensitivity of fish.

**Induction of ovulation by HCG injection**

A series of trials on induced ovulation were conducted using female walking catfish to examine conditions of maturity under warm water conditions. For this purpose, females were maintained at 23-25 °C for a long period, either under 12L:12D or natural photoperiod. Using 12-34 months old females, induction of ovulation was achieved with a single injection of HCG (0.2, 0.4, 0.8, 1.6 or 3.2 IU/g BW) (Data not shown). In all the trials, HCG treatment succeeded in inducing ovulation at dosages of 0.4 IU/g BW or higher. Oocytes larger than 770 μm responded to HCG and ovulated. These results indicate that female walking catfish can maintain condition of maturity for an extended time under warm water conditions, and can respond to HCG irrespective of photoperiod.

**Steroid hormone profiles during induced ovulation**

The present experiment was performed in order to investigate the response of walking catfish to HCG and the resultant hormonal changes during ovulation\(^1\). Post-vitellogenic female walking catfish were given a single intramuscular injection of 0.8 IU HCG/g BW. Of 14 fish treated with a single injection of HCG, 5 fish ovulated at 20 hr and 9 fish at 24 hr following treatment (Fig. 2.).

![Fig. 2. Changes in the seven sex steroid hormone levels during HCG-induced ovulation in walking catfish. Arrow indicates the detectable limit of the assay](image-url)

Germinal vesicle breakdown (GVBD) occurred after an elapse of 12-16 hr. After HCG injection, plasma testosterone peaked at 4 hr, and then gradually decreased to initial levels at 24 hr. Progesterone levels started to increase at 4 hr, and exhibited a small peak at 12 hr. Plasma 17α-P levels began to increase at 8 hr, peaked at 12 hr, and returned to basal levels at 20 hr. Plasma 17α, 20β-P levels suddenly increased and peaked at 12 hr following treatment. Plasma 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S) levels also increased at 12 hr and peaked at 16 hr. Meanwhile, 17α, 20α-P, stereoisomer of 17α, 20β-P, starting to increase at 4 hr, reaching a peak at 12 hr, with maximum levels lower than those of 17α, 20β-P or 20β-S. These peaks were concomitant to the first occurrence of GVBD. Plasma estradiol-17β levels in HCG-treated fish remained constant throughout the experiment, whereas levels in the control group were seen to decrease. These results indicate that HCG is effective in inducing ovulation in walking catfish and suggest that 17α, 20β-P and/or 20β-S are the maturation inducing steroid (s) in this species.
MALE

Maturity under warm water conditions
To investigate the changes in testicular maturity in the tropical walking catfish, seven months-old males were maintained under constant warm water temperature (23-25°C) and natural photoperiod until the age of 31 months-old (Fig. 3).

Fig 3. Changes in the average levels of plasma steroid hormones from males (mean ± sem, n = 15)

Plasma 11-ketotestosterone (11-KT) and testosterone started to increase at 8 months-old, and thereafter fluctuated at high levels. However, testosterone levels became higher in 2 years-old fish than did those in yearling fish. 17α-P levels also fluctuated, but average levels were lower than those of testosterone and 11-KT. Plasma 17α, 20β-P and 17α, 20β-P levels fluctuated at low levels. These results indicate that male walking catfish can maintain conditions of maturity for an extended period under warm water and natural photoperiod.

Steroid hormone profiles following HCG injection
The present experiment was performed in order to investigate the response of mature male walking catfish to HCG12.

Short-term experiment. Mature male walking catfish received a single intramuscular injection of HCG (0.2 and 1 IU/g BW) or saline. Blood samples were taken at 0, 6, 12, 18, 24, 36, 48, and 72 hr. Long-term experiment. Mature male walking catfish received a single intramuscular injection of HCG (1 IU/g BW) or saline. On both experiment, plasma testosterone, 11-KT, 17α-P, 17α, 20β-P and 17α, 20β-P levels were measured by radio immunoassay (RIA), and 20β-S levels were measured by enzyme immunoassay (EIA).

The results can be summarized as follows (Fig. 4, 5). 1) HCG firstly induces an increase in plasma testosterone and 11-KT levels. 2) Thereafter, 11-KT levels decrease and progestin levels increase. 3) Finally, progestin levels decrease, and 11-KT levels increase again. These results clearly indicate that a shift in the steroidogenic pathway is induced in males in response to HCG.

Fig 4. Plasma testosterone, 11-KT, and 17α-P levels following HCG injection in long-term experiment. Each point represented as mean ± sem (n are equal to 8 and 6 for HCG and control group, respectively).

Fig 5. Plasma 20β-S, 17α, 20β-P, and 17α,20β-P levels following HCG injection in long-term experiment. Each point represented as mean ± sem (n are equal to 8 and 6 for HCG and control group, respectively).
Table 1. Spawning success after water level and water temperature manipulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment duration (days)</th>
<th>Number of fish</th>
<th>Number of spawning fish</th>
<th>Spawning rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: a sudden decrease in water level from 70 cm to 20 cm.</td>
<td>13</td>
<td>15</td>
<td>1</td>
<td>6.67</td>
</tr>
<tr>
<td>A': a sudden decrease in water level from 70 cm to 35 cm</td>
<td>7-10</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B: a sudden drop in water level from 23 °C to 28°C</td>
<td>7-10</td>
<td>18</td>
<td>8</td>
<td>44.44</td>
</tr>
<tr>
<td>C: a sudden drop in water level from 35 cm to 70 cm &amp; an increase in</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>16.67</td>
</tr>
<tr>
<td>water temperature from 23 °C to 28°C. After treatment temperature was</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>maintained at 28 °C while water level was returned to its original.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D: increase in water temperature from 23 °C to 28°C.</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D': increase in water temperature from 23 °C to 28°C.</td>
<td>24</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E: a sudden drop in water level from 23 °C to 35 cm at 23 °C, then</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>50.00</td>
</tr>
<tr>
<td>retu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: water temperature &amp; water level kept at 23 °C &amp; 70 cm.</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CONCLUSION

It was ascertained that catfish can maintain gonadal maturity for an extended time, without spawning under constant warm water temperature. Females maintained post-vitellogenic conditions by continually producing new oocytes and absorbing the old oocytes. Plasma steroid profiles in males suggest that fish are continually producing sperm. Furthermore, after an extended period of rearing under constant warm water conditions, catfish can respond well to environmental water level and temperature changes regarding the induction of spawning. In addition, females can be induced to ovulate by injection of HCG at any time of the year, regardless of photoperiod.

REFERENCE