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Pituitary-gonadal Relationship in the *Catfish Clarias batrachus* (L): A Study Correlating Gonadotrophin-II and Sex Steroid Dynamics

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ABSTRACT—A heterologous radioimmunoassay was developed for measuring gonadotrophin-II (GTH-II) in the catfish *Clarias batrachus*. Serum and/or pituitary levels of GTH-II showed significant annual/seasonal variations in male and female catfish, which could be correlated with both gonadosomatic index and/or serum testosterone level. GTH-II was not detected in resting phase, increased during gonadal recrudescence to peak values in late prespawning /spawning phases, and declined to low values in postspawning phase. During gonadal recrudescence, the pituitary and serum levels of GTH-II maintained positive or inverse relationships implying differential rates of hormone release and synthesis/storage. Gonadectomy resulted in increased release of GTH-II; the release pattern varied in females and hemi-castrated or completely castrated males. In females, the GTH-II increase followed a distinct biphasic pattern with the peak rise at week 4 of ovariectomy. In males, castration resulted in significant rise of serum GTH-II levels at all duration except week 5, but the magnitude of the rise was higher in completely castrated fish (weeks 1, 2 and 3). Testosterone replacement in 3-week hemi-castrated fish restored the GTH-II level to that of the sham control vehicle group. In intact fish, administration of testosterone elicited an increase in serum GTH-II levels in the low dose (0.25 and 0.5 μg / g BW) groups and no change in the high dose (1.0 μg / g BW) group. Methallibure treatment inhibited GTH-II levels in a dose-dependent manner. The reduction was greater in males. Withdrawal of the drug treatment restored the GTH-II and testosterone levels after 15 days in the low dose group (2 μg / g BW). The results indicate that there exists a dynamic positive or negative feedback relationship between gonadal steroids and GTH-II, which is essential to control the release and availability of circulating GTH-II.

INTRODUCTION

The functional relationship between pituitary and gonads has often been studied qualitatively by correlating morphological/ histological changes in gonads and gonadotrophs during the natural reproductive cycle or following experimental approaches such as surgical or chemical hypophysectomy, gonadectomy or sex steroid treatment (Van Oordt and Peute, 1983). In recent years, introduction of radioimmunoassay of reproductive hormones has enabled quantitative analysis of hormonal changes in the pituitary-reproductive axis and defined the relationship and underlying regulatory mechanisms more precisely. Such quantitative analyses of gonadotrophin (GTH) and sex steroid levels are largely confined to a few species, notably salmonids, cyprinids and African catfish (Crim *et al*., 1981, Fostier *et al*., 1983, Swanson 1991, Tanaka *et al*., 1991, Schulz *et al*., 1994a, Breton *et al*., 1998). While sex steroid dynamics have been studied in a number of teleosts in relation to the annual reproductive cycle, gametogenesis and spawning, their correlation with GTH level have been studied only in a few cases due to non-availability of sensitive and specific GTH assays. Most fish species investigated have two GTHs, GTH-I and GTH-II, corresponding to the tetrapod FSH and LH (Suzuki *et al*., 1988; Querat 1994, Prat *et al*., 1996, Swanson and Dittman, 1997; Breton *et al*., 1998). African catfish (*Clarias gariepinus*) is a notable exception in which only GTH-II was reported (Koide *et al*., 1992; Schulz *et al*., 1995) although a recent report showed the existence of different molecular forms of varying glycosylation, but all belonging to the GTH-II type (communication from Dr. John Sumpter to Dr.Goos). According to Schulz *et al*., (1995), GTH-

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II performs all aspects of gonadal function viz., gametogenesis, steroidogenesis and spawning in the African catfish.

The catfish *Clarias batrachus* distributed in South-East Asian countries has been extensively used in endocrine research in reproduction. Being an air-breathing fish, it can tolerate low oxygen content in the aquatic environment and is used in waste water aquaculture as well. Different aspects of the reproductive physiology of the catfish have been described by various workers (Joy *et al*., 1989, Rai 1996). However, no attempt has been made to study GTH dynamics in this species apparently due to lack of an assay system. To this end, a heterologous radioimmunoassay using purified GTH-II and antiserum of the closely related species *Clarias gariepinus* was developed. The present study is an assessment of hormonal dynamics of the pituitary-gonadal-axis in relation to the annual reproductive cycle, gonadectomy, and testosterone and methallibure (a non-steroidal inhibitor of GTH secretion and also used as an agent of chemical hypophysectomy; Donaldson 1973, Senthilkumaran and Joy 1998) treatments.

MATERIAL AND METHODS

The reproductive cycle of *C. batrachus* can be divided into 5 phases : resting (November-January), preparatory (February-April), prespawning (May-June), spawning (July-August) and postspawning (September-October). Adult female and male catfish, weighing 70–80 g were collected from fish markets in and around Varanasi (latitude – 25°18'N and longitude(83°01'E). The following investigations were conducted.

Seasonal/annual study

Male catfish (80 ± 5 g) were sampled in the second week of each month for a year for the annual study. For seasonal study, both male and female catfish were sampled in the second week of January (resting), April (late preparatory), May and June (early and late prespawning), July (spawning), and September (postspawning) phases. In both studies, the fish were weighed, bled by caudal puncture (between 11–12 hr), sacrificed by decapitation and gonads were removed. Blood was allowed to clot at room temperature and serum was separated by centrifuging at 800 × g for 20 min at 4°C and stored at –20°C for hormone assays. In the seasonal study, pituitaries were collected for determination of GTH-II. The pituitary glands (numbers 5–8) were homogenized in 0.01 M sodium phosphate-buffer saline, pH 7.6. The homogenate was centrifuged at 5000 × g for 30 min at 4°C. The supernatant was stored at –20°C. The gonads were weighed for calculation of gonadosomatic index (GSI).

Gonadectomy

Fish were gonadectomised in preparatory (second week of April) or prespawning (first week of May) phases. For this, male and female fish were collected and acclimatized to laboratory conditions under natural photoperiod and temperature (13.20L:11.40D; 24±2°C) for two weeks. They were fed with minced goat liver during acclimation and experiments. The following experiments were conducted.

a. Castration : About 125 acclimated male fish each were used for castration and sham castration. The procedure was essentially the same adopted for ovariectomy of females (Senthilkumaran and Joy 1994). The testes alone (hemi-castration) or with the seminal vesicles (complete castration) were removed carefully. Completeness of castration was verified by examining the peritoneal cavity of the fish after killing. Mortality was 2% in hemi-castrated fish but varied from 15–20% in completely castrated group. The high mortality was due to accidental disruption of the intestine despite maximal care. The fish were sampled at 1, 2, 3, 4, and 5 weeks after the operation along with sham control. Blood was collected for separation of serum, as described above.

b. Testosterone replacement : Male fish were divided into 4 groups. Group 1 served as initial control and the fish were killed before the experiment. Group 2 fish were sham castrated, and groups 3 and 4 were hemi-castrated. After the first week of the operation, groups 2 and 3 fish were injected, ip, with propylene glycol (vehicle medium). Group 4 fish were injected with testosterone (Sigma, St. Louis, USA) in a dose of 1 μg/g BW, ip, daily for 21 days. Testosterone was dissolved in propylene glycol. The control fish received the same volume of the vehicle daily for 21 days. On termination of the experiment, fish were sacrificed and blood collected to separate serum which was stored at –20°C.

c. Ovariectomy : About 25 acclimatized female fish each were ovariectomized or sham ovariectomized in prespawning phase, as described by Senthilkumaran and Joy (1994). Completeness of ovariectomy was checked by examining the peritoneal cavity of the fish at the time of sampling. They were sacrificed at 2, 3, 4, 5, and 6 weeks of the operation. Mortality was negligible. Blood samples were collected and serum separated and stored at –20°C for hormone assay.

Testosterone administration

Acclimatized male fish in mid-preparatory phase (late March) were divided into 5 groups of 5 each. Group 1 (initial control) fish were sacrificed at the start of the experiment. They were weighed and blood was collected by caudal puncture. Serum was separated and stored at –20°C for measurement of hormones. The fish were sacrificed by decapitation immediately after blood collection. Group 2 fish were injected with vehicle (0.1 ml propylene glycol). Groups 3, 4 and 5 were injected with testosterone, ip, daily for 30 days in doses of 0.25, 0.5, and 1.0 μg/g BW, respectively. After termination of the treatment, the fish in all the groups were weighed and serum was collected and stored at –20°C.

Methallibure treatment

Two sets of experiments were conducted with low and high doses of methallibure (a generous gift of Mr. D.E. Riley, Zeneca Pharmaceuticals, England).

a. Low dose study : In early prespawning phase (May), acclimatized male fish were divided into 7 groups. Group I fish served as the initial control and sampled for serum collection before the experiment. Groups 2, 3 and 4 fish were daily injected, ip, with methallibure in a dose of 2 μg/g BW. Methallibure was dissolved in 2% polyethylene glycol. Group 5, 6 and 7 fish were injected with the vehicle (control). Group 2 and 5 fish were sacrificed after 15 days. In group 3 and 6 (methallibure groups), and 4 and 7 (control groups), the treatments were discontinued after 15 days and the fish were further maintained for 7 or 15 days, and sacrificed. Blood samples were withdrawn for serum collection.

b. High dose study : In late prespawning phase (June), acclimatized male and female fish each were divided into 5 groups. Group I served as initial control. Group 2 and 3 were injected with methallibure daily for 10 days in a dose of 20 μg/g BW of fish. Group 4 and 5 fish were injected with the vehicle. The group 2 and 4 fish were sampled after 10 days of the treatment. In group 3 and 5 the treatments were discontinued after 10 days and the fish were further maintained for 7 days and sacrificed. Blood samples were taken for separation of serum which was stored at –20°C.

Study parameters

a. Gonadosomatic index (GSI) : The weight of the testis was expressed in 100 g body weight of the fish.

b. Heterologous RIA for GTH-II : The method of Goos *et al.*, (1986), as described by Senthilkumaran and Joy (1994) was employed to measure GTH-II in the catfish using African catfish (*C. gariepinus*) GTH-II and antiserum. Displacement assays using serially diluted volumes (2.5–50 μl) of serum and pituitary extract (protein concentration 1 mg / 50 μl) of male and female *C. batrachus* in prespawning phase (June) were set up for comparisons with standard (homologous) displacement curves. The assay details were similar to that described earlier (Senthilkumaran and Joy, 1994).

c. RIA of testosterone : The RIA was carried out by the procedure of Abraham (1974), as described by Senthilkumaran and Joy (1994). [1, 2, 6, 7- ^{3}H (N)](testosterone (specific activity 92.1 Ci/mmol) was purchased from NEN, Boston, USA. Testosterone antiserum was obtained as a gift from Dr. Chandana Das, AIIMS, New Delhi through the courtesy of the ICMR, New Delhi.

Statistical analysis

All data were expressed as means ±SEM (standard error of mean). Parallelism of displacement curves of GTH-II in serum and pituitary extracts to standard curve of purified catfish GTH-II was tested by one-way analysis of variance (ANOVA). The data were analyzed by ANOVA, followed by Newman-Keuls' multiple range test ($P < 0.05$) for comparison of group means. Student's *t* test was applied to show statistical significance in some experiments.

RESULTS

Validation of heterologous RIA of GTH-II

The displacement curves (Fig 1) compiled from five different analyses showed parallelism (ANOVA) between the standard GTH-II and catfish serum or pituitary extract (June samples). The lower and the upper detection limits of the assay were 800 pg/ml and 12.5 ng/ml, respectively. Intra-and inter-assay coefficients of variation were 3.4% (±0.25 SD) and 7.6% (±0.63 SD), respectively for standard, and 6.1% (±0.49 SD) and 9.3% (±1.04 SD), respectively for catfish serum (50 μl).

Annual variations in GSI, and serum GTH-II and testosterone levels

In male fish, the GSI showed a significant annual variation (Fig 2; $F = 89.138$, $P < 0.001$, one-way ANOVA). The GSI was the lowest (33.40±6.84 mg/100 g BW) in January (resting phase), increased steadily during the preparatory and prespawning phases (February-June) to reach the peak value (278.28±10.2 mg/100 g BW) in July (early spawning phase). After spawning (August), the values decreased progressively during the postspawning phase. During October-December, the GSI values remained low. The values in May, June, and

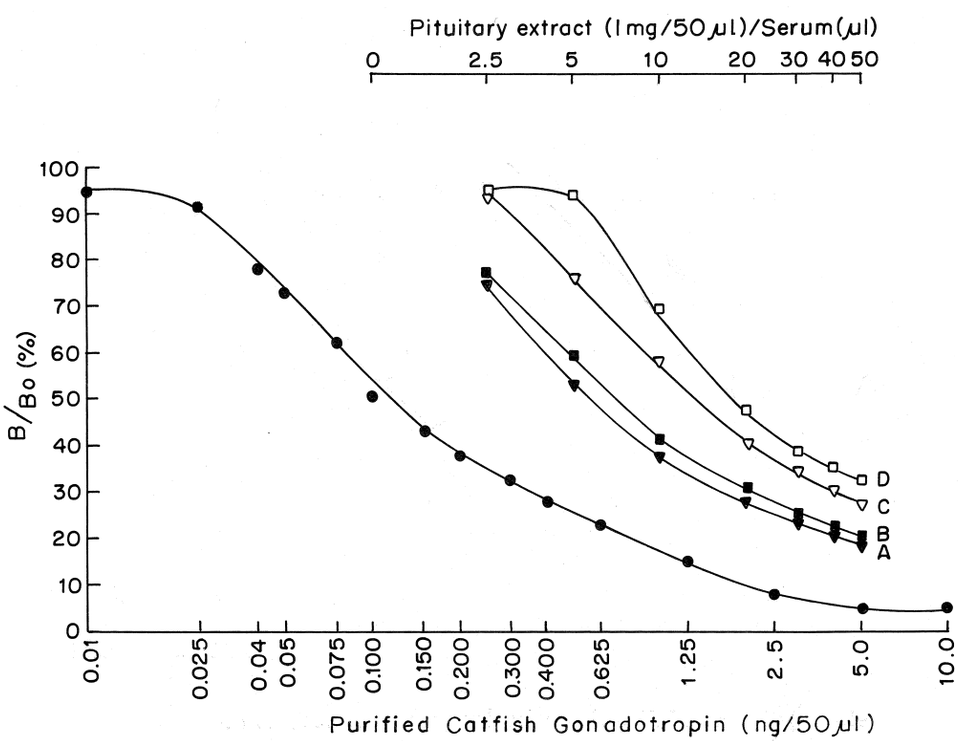

Fig. 1. Displacement curves of standard (*C. gariepinus*) gonadotropin-II (GTH-II;homologous) and serially diluted *C. batrachus* serum/pituitary extract (heterologous) using anti-catfish (*C. gariepinus*) gonadotropin-II serum. **A** and **C**, serum and pituitary extract of female fish, respectively. **B** and **D**, serum and pituitary extract of male fish, respectively. The serum and pituitary extracts (protein concentration 1 mg / 50 μl) were collected in June. Each point represents the average of five estimations. B/Bo = percentage of corrected average counts of standard or sample/ corrected average counts of zero standard (titre). Parallelism of displacement curves of serum and pituitary extracts to standard curve was tested by ANOVA.

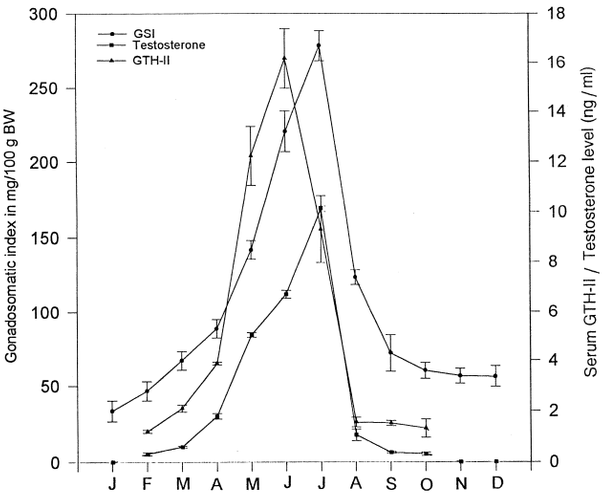

Fig. 2. Annual variations in gonadosomatic index (GSI) and serum levels of GTH-II and testosterone in male *C. batrachus* (mean ± SEM; n=5). One-way ANOVA - Newman-Keuls' test (see text for details).

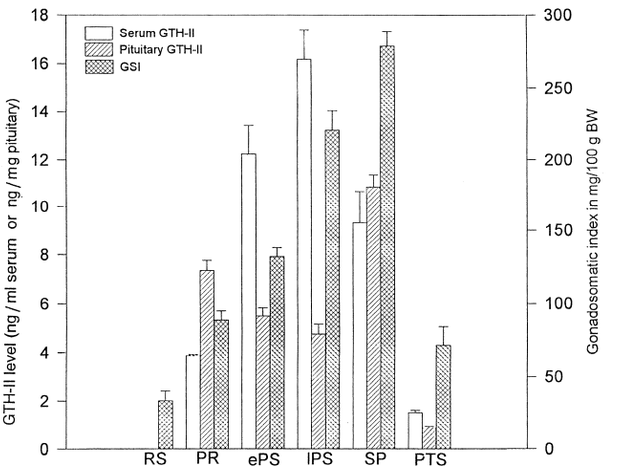

Fig. 3. Seasonal variations in serum and pituitary levels of GTH-II and gonadosomatic index (GSI) in male *C. batrachus* (mean ± SEM; n =5). Explanations to abbreviations: RS - resting phase; PR - preparatory phase; ePS - early prespawning phase; lPS - late prespawning phase; SP - spawning phase; PTS - postspawning phase. One-way ANOVA - Newman-Keuls' test (see text for details).

July were significantly different among them and also from all other values except those of May and August (Newman-Keuls' test, $P < 0.05$). Serum GTH-II showed a significant annual variation (F=72.17; $P < 0.001$, one-way ANOVA); the levels being not detected in November, December and January (resting phase). GTH-II appeared in the serum in February (1.2 ± 0.06 ng/ml) and increased gradually from the preparatory phase to attain the peak level (16.18±1.21 ng/ml) in June (prespawning phase). It declined in July (early spawning phase) with a further drastic drop in late spawning phase (August) and postspawning phases. Newman-Keuls' analysis showed that the GTH-II levels in the prespawning (May, June) and spawning (July) phases were significantly higher than all other values. Testosterone showed a significant annual variation (F=970.06; $P < 0.001$, one-way ANOVA) with the levels not detected in November, December and January (resting phase). It appeared in the serum in February (0.32 ± 0.06 ng/ml), increased gradually and attained the peak level (10.12 ± 0.12 ng/ml) in the early spawning phase (July). It declined in August (late spawning phase) to low levels in the postspawning phase. Newman-Keuls' analysis showed that the levels were significantly higher in July compared to those of preparatory (February-April), prespawning (May-June), late spawning (August), and postspawning (September-October) phases.

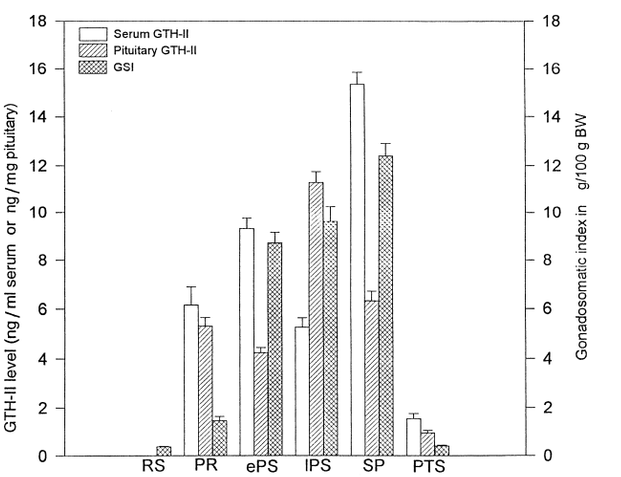

Fig. 4. Seasonal variations in serum and pituitary levels of GTH-II and gonadosomatic index (GSI) in female *C. batrachus* (mean ± SEM; n=5). One-way ANOVA - Newman-Keuls' test (see text for details).

Seasonal variations in pituitary and serum GTH-II levels and GSI in male and female catfish

In both males (Fig. 3) and females (Fig. 4), the serum levels of GTH-II showed significant seasonal variations (F=52.63, $P < 0.001$ for males and F=138.21, $P < 0.001$ for females, one-way ANOVA) with the peak levels in late prespawning phase (June) for males and in spawning phase (July) for females. The levels dropped subsequently to low values in the postspawning phase. On the other hand, the pituitary concentration of GTH-II which also showed significant seasonal variations (F=110.73, $P < 0.001$ for males and F=105.25, $P < 0.001$ for females, one-way ANOVA) displayed biphasic seasonal pattern with 2 peaks of activity. In both sexes, the minor peak was noticed in the preparatory phase. But the time of appearance of the major peak varied between sexes: in the prespawning phase (June) in females and spawning phase (July) in males. The GSI showed significant seasonal variations (F=93.32 for males and F=185.64 for females, one-way ANOVA, $P < 0.001$) with peak values in the spawning phase in both sexes.

Effects of castration on hormone levels

a. Hemi-castration : The removal of testis showed a significant variation in serum testosterone level (Fig 5; F =17.37, P

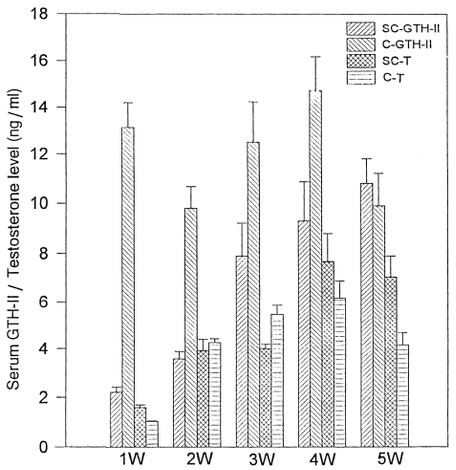

Fig. 5. Effects of hemi-castration (removal of testes) on serum levels of testosterone (T) and GTH-II (mean ± SEM, n = 5) in *C. batrachus*. Explanations to abbreviations: W : weeks; SC : sham castration group; C : castration group. One way ANOVA -Newman-Keuls' test (see text for details).

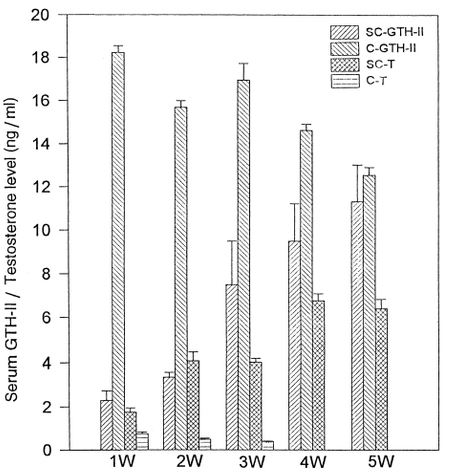

Fig. 6. Effects of castration (removal of testis and seminal vesicle) on serum levels of testosterone (T) and GTH-II (mean ± SEM, n = 5) in *C. batrachus*. Abbreviations as in Fig. 5. One way ANOVA - Newman-Keuls' test (see text for details).

< 0.001; one-way ANOVA) with a significant decrease in the first and fifth weeks, and a significant increase in the third week ($P < 0.05$; Newman-Keuls' test). Insignificant changes were only noticed in the second and fourth weeks. Newman-Keuls' analysis showed that in the hemi-castrated groups, the steroid level in week 1 was significantly lower than those at all other duration and at week 2, it was significantly lower than those of the third and fourth week levels. Serum GTH-II level showed an overall significant variation (F=15.19, $P < 0.001$; one-way ANOVA). The levels in the sham castrated groups increased compared to that of the initial control group apparently due to temporal variations. In the hemi-castrated groups, the levels were significantly high over the control group values at all duration except week 5. The levels varied over time; it declined at week 2, elevated at week 3 and 4 steadily, but again declined at week 5. The GTH-II level in the hemi-castrated group at week 4 was significantly high compared to the week 2 and 5 values.

b. Castration : The removal of both testis and seminal vesicle led to a significant decrease of serum testosterone level (Fig 6; F =131.30, $P < 0.001$, one-way ANOVA). The testosterone level was significantly lowered at week 1, 2, and 3 and was undetected at week 4 and 5. Serum GTH-II levels showed an overall significant variation (F=35.11, $P < 0.001$; one-way ANOVA). Newman-Keuls' analysis showed that the levels were significantly high over that of control groups at all duration except week 5. In the castrated groups, the mean values of GTH-II level also showed a gradual decline over time (3 to 5 weeks). The magnitude of the GTH-II increase was higher in the completely castrated groups, compared to corresponding values of hemi-castrated groups except at week 4 and 5.

Effect of testosterone replacement in 3-week hemi-castrated fish

Administration of 1 μg/g BW of testosterone for 21 days increased significantly (Fig 7; $P < 0.05$, Student's t test) the serum testosterone level in 3-week castrated fish when compared with that of castrated-vehicle group. Consequently, the increase in serum GTH-II level due to hemi-castration was restored to that of the sham vehicle group ($P < 0.01$, Student's t test).

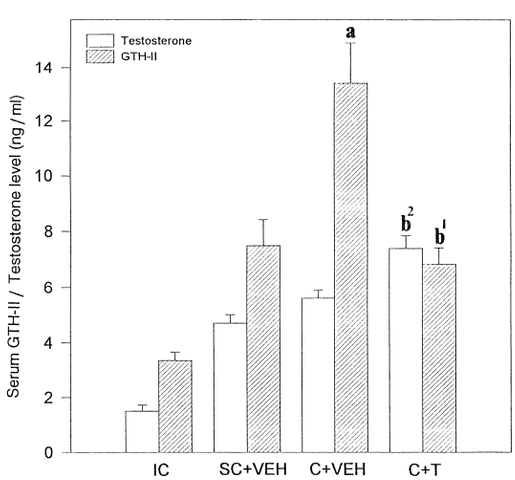

Fig. 7. Effects of testosterone administration on serum levels of testosterone and GTH-II in 3-week hemi-castrated *C. batrachus* (mean ± SEM, n = 5). **a** - comparison with sham control (SC + VEH) GTH-II level ($P < 0.05$); $\mathbf{b}^1$ - comparison with C + VEH group GTH-II level ($P < 0.01$); $\mathbf{b}^2$ - comparison with C + VEH group testosterone level ($P < 0.05$) (Student's t test).

Effect of different doses of testosterone on serum GTH-II level in intact males during preparatory phase

Administration of testosterone produced an overall significant variation on serum GTH-II level (Fig 8; $F = 23.14$; $P < 0.001$; one-way ANOVA). Serum GTH-II level registered significant increases in 0.25 and 0.5 μg dose groups, but did not vary significantly in the 1.0 μg group compared to that of the vehicle control group (Newman-Keuls' test, $P < 0.05$). The GTH-II level in the 1.0 μg group was significantly lower than that of the 0.25 and 0.5 μg groups.

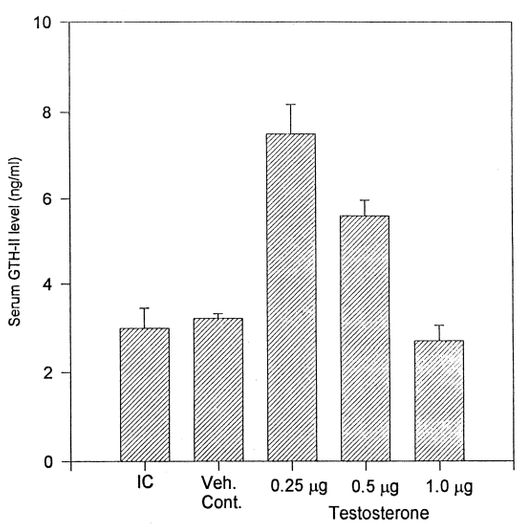

Fig. 8. Effect of different doses of testosterone on serum level of GTH-II in intact male. *C. batrachus* in preparatory phase (mean ±SEM, n=5). Explanations to abbreviations: IC -initial control; Veh. Cont-vehicle control. One-way ANOVA -Newman-Keuls' test (see text for details).

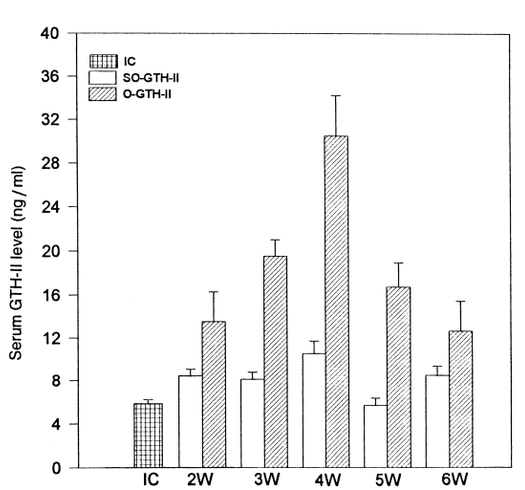

Fig. 9. Effect of ovariectomy on serum level of GTH-II in *C. batrachus* (mean±SEM, n=5). Explanations to abbreviations: IC - initial control; SO - sham ovariectomy; O - ovariectomy. One-way ANOVA - Newman-Keuls' test (see text for details).

Effect of ovariectomy on serum GTH-II level

Ovariectomy produced an overall significant variation on serum GTH-II level with a distinct biphasic pattern (Fig 9; $F = 14.68$, $P < 0.001$; one-way ANOVA). The GTH-II level increased to the peak in week 4 which is significantly higher than all other values (Newman-Keuls' test, $P < 0.05$).

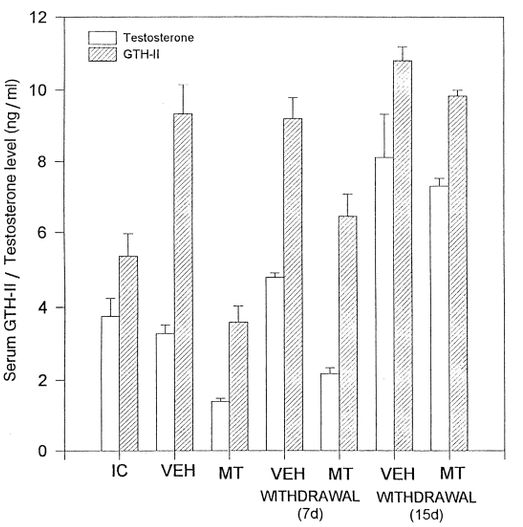

Fig. 10. Effects of injection of 2 μg/g BW of methallibure (15 days) and its withdrawal for 7 and 15 days on serum levels GTH-II and testosterone in male *C. batrachus* (mean ±SEM; n=5) in early prespawning phase (May). Explanations to abbreviations: IC - initial control; VEH -vehicle; MT - methallibure. One-way ANOVA - Newman-Keuls' test (see text for details).

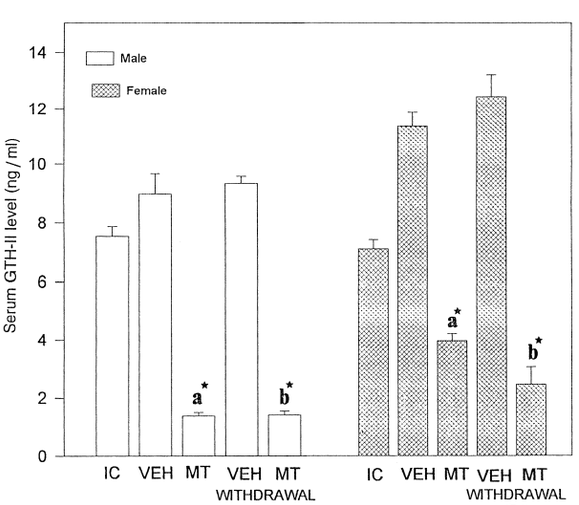

Fig. 11. Effect of injection of methallibure (20 μg/g BW for 10 days) and its withdrawal for 7 days on serum GTH-II level in late prespawning phase (June) in male and female *C. batrachus* (mean ± SEM; n=5). Explanations to abbreviations: IC - initial control; SC + VEH - Sham control +Vehicle; C +VEH-Castration +Vehicle; C +T–Castration +Vehicle. **a** - comparison with vehicle (VEH) groups; **b** - comparison with vehicle groups after withdrawal, $^{*}P < 0.001$; Students' t test.

Effects of methallibure treatment on serum GTH-II and/or testosterone levels

Administration of 2 μg/g BW of methallibure for 15 days produced overall significant effects on serum GTH-II ($F = 23.10$; $P < 0.001$, one-way ANOVA) and testosterone ($F = 23.70$, $P < 0.001$, one-way ANOVA) levels in males (Fig 10). The treatment decreased significantly both GTH-II and testosterone levels (Newman-Keuls' test, $P < 0.05$). On withdrawal of the treatment after 7 days, the hormone levels increased significantly compared to that of the methallibure group and on day 15 of the withdrawal, the levels were restored compared to corresponding control values. Administration of a high dose of methallibure (20 μg/g BW) for 10 days resulted in a marked reduction of GTH-II levels in both males and females (Fig 11; $P < 0.001$, Student's *t* test). The decrease was higher in males (84.63%) than females (65.20%). The GTH-II levels remained significantly low even after withdrawal of the treatment for 7 days in males and decreased even further in females.

DISCUSSION

The validation data of the heterologous RIA developed for GTH-II assay in the catfish (parallelism of displacement curves, coefficients of intra- and interassay variation and sensitivity limits) show that the assay is both sensitive and reliable to measure immunoreactive GTH-II in the serum and pituitary of the catfish. Since only one form of GTH, equivalent to GTH-II or maturational GTH was purified from the African catfish (Koide *et al.*,, 1992), the immunoreactive GTH measured in *C. batrachus* may correspond to GTH-II of other teleosts. Since the antiserum used in the assay was raised against the whole GTH molecule (Goos *et al*., 1986), the α-subunit of TSH might have also cross-reacted with it in the assay as evident from immunocytochemical staining of thyrotrophs in both *C. gariepinus* and *C. batrachus* (Goos *et al*., 1986, Joy *et al*., 1989). However, the TSH cross-reactivity might not have affected seriously the GTH-II assay as evident from the annual and seasonal variations. The changes in serum GTH-II level followed closely the GSI; the levels were not detected in November, December and January in the resting phase when the GSI was the lowest. If the TSH had cross-reacted substantially, some immunoreactivity could have been measured in serum samples collected in those months as well. Further, the pattern of GTH-II response to ovariectomy and castration also negate any major interference of TSH immunoreactivity.

Annual changes in serum levels of GTH-II and testosterone in male *C. batrachus* were correlated for the first time in this study which concur with similar patterns reported in *Heteropneustes fossilis* (Senthilkumaran, 1995) that co-inhabits tropical waters. The undetectable levels of serum GTH-II and testosterone, and a low GSI in November, December and January denote the functional quiescence of the pituitary-testis axis when the environmental photoperiod and temperature are in the lowest range in the annual scale in Varanasi. The detection of GTH-II and testosterone in blood from preparatory phase (February) onwards correlates very well with the increases of these variables that are known to activate the hypothalamo-hypophysial-gonadal (HHG) axis. The parallel rise in GTH-II, testosterone and GSI during the recrudescent phase indicates that GTH-II is involved in steroid synthesis vis-a-vis spermatogenesis, as has been reported in the African catfish (Schulz *et al*., 1994a,b). The early detection of the LH like GTH-II in circulation suggests that it may be involved in gametogenesis. In teleosts where two GTHs are described, this role is exercised by the FSH-like GTH-I (Swanson and Dittman, 1997; Breton *et al*., 1998). In the African catfish, LH-β immunoreactivity was detected in gonadotrophs 7 weeks after hatching corresponding to the time when FSH-β immunoreactivity was also detected in salmonids, the appearance of which coinciding with male gonadal sex differentiation (Saga *et al*., 1993; Schulz *et al*., 1997). The question whether GTH-I is present during the resting phase when GTH-II is not detectable needs future GTH characterisation studies. The undetection of GTH-II in circulation during the resting phase (November-January) may be due to the low level of the hormone (< 800 pg/ml). In *C. batrachus* the rise in serum testosterone indicates the elevated steroidogenic activity in the testis during the recrudescent phase although in many teleosts 11-oxygenated androgens are the major androgens (Fostier *et al*., 1983, Miura *et al*., 1994; Schulz *et al*., 1994a; Cavaco *et al*., 1995). The administration of testosterone stimulated spermatogenesis and Seminal vesicle (SV) secretory activity in this species (Singh and Joy, 1997). It is not clear whether the effect is direct due to testosterone or indirect due to its conversion. The parallel decline of serum GTH-II, testosterone and GSI after spawning indicates the onset of quiescent changes in the HHG axis in response to the decreasing photoperiod and temperature.

Both pituitary and serum GTH-II levels fluctuated considerably and the GTH release pattern may be parallel or inverse depending on gonadal maturity. This may reflect temporal changes in the secretory activity (synthesis, storage and release) of the gonadotrophs. In both sexes, the annual profile of serum GTH-II showed a single secretion peak (unimodal) with the peak level detected early in males (June) and later (July) in females. The sex difference may be due to the differential rate and tempo of gametogenesis, males maturing earlier than females, and the resultant gonadal feedback on the brain-pituitary axis. On the other hand, the pituitary GTH-II concentration showed two secretion peaks (bimodal); the minor peak in the preparatory phase and the major peak in late prespawning phase (June) in males and spawning phase (July) in females. Similarly, two peaks of pituitary GTH-II content were reported in female *H. fossilis*, the minor peak in early prespawning (May) and the major peak in early spawning phase (July) (Senthilkumaran, 1995). In the African catfish, the peak GTH content was observed in the beginning of breeding period (May) in males and two months later (July) in females (Van Oordt *et al*., 1987). Apparently, the variations in the annual cycles of hormone contents of the pituitary-reproductive axis are subjected to changes in external (photope-

riod and temperature) and steroid feedback influences, sex differences etc.). After spawning, both the pituitary and serum contents of GTH-II declined parallely indicating decreased and cessation of the hormone secretion in postspawning and resting phases.

It is well known that gonadal steroids exert positive or negative feedback on the brain and pituitary to regulate GTH secretion (Van Oordt and Peute, 1983, de Leeuw *et al*., 1987, Trudeau *et al*., 1993, Goos *et al*., 1999). A positive action of gonadal steroids on gonadotrophic activity has been demonstrated in a number of teleosts (Gielen *et al*., 1982, Dufour *et al*., 1983, de Leeuw *et al*., 1987). Cavaco *et al*., (1995) showed that testosterone, but not 11-ketotestosterone (11-KT), enhanced GTH-II storage and not its release in immature African catfish. In goldfish, testosterone stimulates GnRH-induced GTH-II release (Trudeau *et al*., 1993). In *C. batrachus*, the administration of testosterone resulted in dose-dependent effects on serum GTH-II levels in the preparatory phase. This can be interpreted in terms of differential effects of the steroid on gonadotrophic function. The low dose of 0.25 μg produced the maximal GTH-II build up in the serum implying enhanced hormone release (positive feedback). The median dose of 0.5 μg also elevated serum GTH-II level, but not to the same level as the low dose did, indicating a slowing down of hormone release. The high dose of 1.0 μg did not produce any significant effect on serum GTH-II release apparently due to negative feedback. But the effect of the high dose of the steroid on other aspects of secretion viz., synthesis and storage can not be ruled out (Goos *et al*., 1999).

Gonadectomy and steroid replacement are widely used to elucidate the nature of feedback of sex steroids in the control of GTH secretion. In *C. batrachus*, ovariectomy produced a significant elevation of serum GTH-II with a distinct biphasic release pattern, as has been reported in the catfish *H. fossilis* (Senthilkumaran and Joy 1994). The peak release was noticed at week 4, indicating an initial phase of increased release followed by a late phase of diminished release under consistent and progressive decline of circulating titre of E_2.

In male catfish, castration did not elicit any distinct biphasic pattern of GTH-II release during the period of the experiment, as in females. This was because GTH-II reached already the peak level within the first week unlike in ovariectomized females where the peak release occurred quite later (week 4). Furthermore, the quantum of GTH-II release in females is nearly two-fold of that in males. Thus, there is a distinct sex-related difference in the responsiveness and functioning of the pituitary-gonad axis, the females eliciting slow but high GTH-II release potential. The hemi-castration results revealed some new information on the feedback relationship of the pituitary-reproductive axis in males. This is attributed to the steroidogenic potential of the SV, a derivative of the sperm duct with exocrine secretory function (Schoonen and Lambert, 1986; Singh and Joy 1998). Removal of both testis and SV was, therefore, needed to completely eliminate testosterone from the serum (Fig. 6). The removal of the testis alone (hemi-castration) resulted in stimulation of testosterone synthesis and hypertrophic changes in the SV (present study). In hemi-castrated fish after an initial drop at week 1 the serum testosterone level was maintained or elevated up to 4 weeks because of this compensatory mechanism. However, the serum GTH-II level remained high up to 4 weeks. While the high titre of GTH-II in completely castrated fish can be explained on the basis of the negative feedback control by the decreasing steroid titre, it is difficult to explain the hormone interrelationships in hemi-castrates. In testis-extirpated *C. gariepinus*, the elevated GTH-II level was explained by the negative feedback exerted by decreased titres of testosterone and androstenedione (de Leeuw *et al*., 1986). The fact that replacement treatment with a high dose of testosterone (1 μg/g BW for 21 days) in 3-week hemi-castrated fish restored the GTH-II level to the sham control level indicate the existence of a negative feedback control by testosterone. Nevertheless, it might not have been controlled solely by testosterone, as we did not study the role of 11-KT or androstenedione in our experimental model. The decreased levels of both GTH-II and testosterone after long term castration may be due to the exhaustion of the pituitary - SV axis.

Methallibure is a non-steroidal inhibitor of GTH secretion and has been used as an agent of chemical hypophysectomy to inhibit gonadotrophic activity in teleosts (Donaldson, 1973, Murphy, 1980, Senthilkumaran and Joy, 1998). In the catfish, methallibure treatment inhibited serum GTH-II level in a dose and sex-dependent manner. The inhibition was more pronounced in males than females and in the high dose group (20 μg). Further, the inhibition was reversible depending on dose. The inhibition has been attributed to its differential actions on the hypothalamic monoamines that regulate GTH-II secretion (Senthilkumaran and Joy, 1998). The drug treatment stimulated dopamine that inhibits, and suppressed noradrenaline and serotonin that stimulate GTH-II secretion. Methallibure shows chemical similarity with diethyldithiocarbamate, a known inhibitor of dopamine-β-hydroxylase (Chang *et al*., 1995). The ability of the drug to inhibit GTH-II secretion vis-a-vis reproductive activity and the reversibility of the effects make it a suitable agent for population control of prolific breeders or weed fishes in fish farms.

In conclusion, the hormonal changes in the pituitary-gonadal axis in relation to the reproductive cycle, gonadectomy, and testosterone and methallibure treatments indicate a functional and dynamic relationship of GTH-II and sex steroids in the catfish.

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