Seasonal changes in serum testosterone, 11-ketotestosterone, and 17β-estradiol levels in the brown bullhead, *Ictalurus nebulosus* Lesueur

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The seasonal changes in gonadosomatic index (GSI) and serum testosterone, 11-ketotestosterone, and 17β-estradiol levels were measured in adult feral brown bullheads, *Ictalurus nebulosus* Lesueur. The maximum GSI of both male and female brown bullheads was considerably lower than that of most other teleostean species investigated. In males, the GSI began to increase in April concomitant with an increase in water temperature from 3 to 6°C. The maximum GSI levels were evident throughout May and June (during the prespawning and spawning periods). Peaks of serum testosterone and serum 11-ketotestosterone levels were evident in mid-April and late May to June, and in mid-April and mid-May, respectively. In females there was a rapid increase in GSI during May, when the ambient water temperature reached 16°C. The peak GSI was evident in mid- to late-May and had declined by early June. Peak serum testosterone and 11-ketotestosterone levels were evident in mid-April and again in late May, whereas peak 17β-estradiol levels were found in mid-May and mid-June. The peak serum testosterone levels in females were 4.5-fold higher than in the males, whereas the 11-ketotestosterone levels were similar in males and females.

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Les changements saisonniers de l'indice gonadosomatique (GSI) et des concentrations sériques de testostérone, de 11-cétotestostérone et de 17β-estradiol ont été mesurés chez des barbottes brunes *Ictalurus nebulosus* Lesueur en nature. L'indice GSI maximal des mâles et des femelles de cette espèce a atteint des valeurs de beaucoup inférieures aux valeurs obtenues chez la plupart des espèces de téléostéens. Chez les mâles, l'indice GSI a augmenté en avril alors que la température de l'eau est passée de 3 à 6°C. Les valeurs maximales de l'indice GSI ont persisté durant tout le mois de mai et tout le mois de juin (périodes pré-fraye et fraye). Les concentrations de testostérone sérique ont atteint un sommet à la mi-avril puis un autre vers la fin de mai et le début de juin, et les concentrations de 11-cétotestostérone maximales ont été enregistrées à la mi-avril et à la mi-mai. Chez les femelles, la valeur de l'indice GSI a augmenté rapidement en mai, alors que la température de l'eau a atteint une valeur de 16°C. La valeur de l'indice était maximale de la mi-mai à la fin de mai et a baissé au début de juin. Les concentrations maximales de testostérone et de 11-cétotestostérone ont été enregistrées à la mi-avril et de nouveau à la fin de mai, alors que les concentrations maximales de 17β-estradiol ont été enregistrées à la mi-mai et à la mi-juin. Les concentrations maximales de testostérone ont atteint des valeurs 4.5 fois plus élevées chez les femelles que chez les mâles, alors que les concentrations de 11-cétotestostérone étaient semblables chez les mâles et chez les femelles.

[Traduit par le journal]

Introduction

The recent development of relatively specific radioimmunoassays has enabled the quantification of serum levels of a number of gonadal steroid hormones in teleost fish. Studies of seasonal changes in serum gonadal steroid hormone levels have revealed the seasonal patterns of hormone levels in several species including goldfish (Schreck *et al.* 1974), flounder (Campbell *et al.* 1976), plaice (Wingfield and Grimm 1977), bluefish, mackerel (MacGregor *et al.* 1981), goby (Bonnin 1979), and several salmonid species (Sanchez-Rodrigues *et al.* 1978; Campbell *et al.* 1980; Stuart-Kregor *et al.* 1981; Fostier *et al.* 1982; Scott, Bye, and Baynes 1980; Scott, Bye, Baynes *et al.* 1980; Scott *et al.* 1983). To our knowledge, no such studies have been made in an ictalurid species despite the importance of these species in North American aquaculture (Lovell 1979; Bardach *et al.* 1972). Moreover, compared with many of the species studied so far, the ictalurids put less energy into gamete production (as evidenced by the extremely low gonadosomatic index (GSI) in these species) and more into parental care of the offspring (Blumer 1983). Consequently, the endocrine control of gametogenesis may differ markedly from other teleostean species.

The purpose of this study was to follow seasonal changes in the serum concentrations of several gonadal steroids including testosterone and 11-ketotestosterone in male brown bullheads and testosterone, 11-ketotestosterone, and 17β-estradiol in female brown bullheads, and to correlate the changes to the stages of gonadal maturation.

Methods and materials

The source and maintenance of the brown bullheads (*Ictalurus nebulosus*) used in this study was reported previously (Burke and Leatherland 1984).

All fish were necropsied between 1400 and 1800 the same day. The fish were weighed, killed by a blow to the head, and the blood was collected, after caudal severence, in plastic culture tubes. The blood was allowed to clot at 4°C, and the serum was separated after centrifugation and stored at −25°C in plastic serum storage vials. To evaluate the effect of transportation stress on blood hormones, fish were sampled immediately after their removal from the trap net or after transportation as follows: on 3 days (May 14, May 26, and April 3, 1982), approximately half the sampled fish (n = 14–22) were bled immediately after removal from the trap net, while the remainder were transported to Guelph for routine necropsy. There were no significant differences in serum steroid hormone levels (testosterone, 11-keto-

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testosterone, 17β-estradiol) between fish bled immediately after collection and those sampled after transportation to Guelph on any of the 3 test days.

The gonads were excised and weighed (±0.1 g) to determine the gonadosomatic index (GSI = gonad weight/body weight × 100); the mesenteric and urinary bladder tissues were carefully trimmed from the gonads prior to weighing. The gross appearance and color were noted for each gonad.

The serum samples were allowed to thaw at room temperature; 100-μL aliquots were extracted twice with 5 mL of methylene chloride, the extracts were dried at 37°C under nitrogen, and were reconstituted with ethanol. Aliquots of the ethanolic solutions were dried under nitrogen and reconstituted in phosphate buffer before being used in the three steroid radioimmunoassays. All assays were done in duplicate or triplicate.

Serum testosterone concentrations were determined by means of the radioimmunoassay (RIA) procedure described by Raeside and Middleton (1979) using sheep antiserum (P43-11). This antiserum cross-reacts significantly with dihydrotestosterone (61.5%), androstenedione (23.5%), and androstanediol (22.8%), but has little cross-reactivity with corticosterone (0.9%), progesterone (0.4%), 17β-estradiol (0.8%), or 11-ketotestosterone (1.3%). The assay sensitivity was 0.02 ng/mL (n = 14) in the samples. The intra- and inter-assay coefficients of variation were 7.0 (n = 14) and 6.1% (n = 14), respectively.

Serum 11-ketotestosterone concentrations were determined using a highly specific RIA procedure. Details of the assay and the antiserum characteristics have been published elsewhere (Leatherland *et al.* 1982). The assay sensitivity (calculated on the basis of serum concentration) of serum extracts was 0.15 ng/mL (n = 10). Intra- and inter-assay coefficients of variation were 8.0 (n = 6) and 8.5% (n = 6), respectively.

Serum 17β-estradiol concentrations were determined in females only by means of a RIA kit (Radioassay Systems Laboratories, Carson, CA, U.S.A. 90746). Intra- and inter-assay coefficients of variation were 8.0 (n = 6) and 10.0% (n = 6), respectively. The reported cross-reactivity of the antiserum with steroids other than 17β-estradiol was 6.5% with estriol, 5.2% with 17α-estradiol, 0.6% with estrone, and <0.01% with cortisol, corticosterone, progesterone, androstenedione, dihydrotestosterone, and testosterone.

The percentage recovery of testosterone, 11-ketotestosterone, and 17β-estradiol from methylene chloride extraction was 86, 90, and 92%, respectively.

Data of GSI and testosterone concentrations were subjected to a two-way analysis of variance with unequal sample size. Data of 11-ketotestosterone for males and females and of 17β-estradiol for females were separately subjected to a one-way analysis of variance with unequal sample size. Assumptions of constant variance and normality of errors were examined and appropriate data transformations were made if these assumptions were not satisfied. Where F-values indicated significance ($p < 0.05$), differences between means were compared with least significant means procedures (Kleinbaum and Kupper 1978). Student's t-test was used to test for significant differences in hormone levels between fish bled immediately after removal from the trap net and fish bled after transportation. Unless otherwise stated, the critical level of significance for testing hypotheses was $p = 0.05$. For clarity of presentation, arithmetic means with standard errors have been used in the table and the figures.

Results

Gonadosomatic index

The GSI of the males collected from September to mid-April ranged from 0.100 to 0.158, with no significant differences between any of the mean values (Fig. 1). In mid-April, mean GSI values rose significantly ($p < 0.01$) from the winter values to 0.218 in mid-May and retained this peak level with no significant differences until the end of June. At this time, the GSI fell rapidly to 0.108 in mid-July, which was significantly lower ($p < 0.01$) than in males sampled in June. A second smaller peak occurred when the GSI increased slightly throughout August to 0.158. This value was significantly ($p < 0.02$) larger than the values from males sampled in mid-July. By September, the GSI had decreased to the levels found in overwintering males.

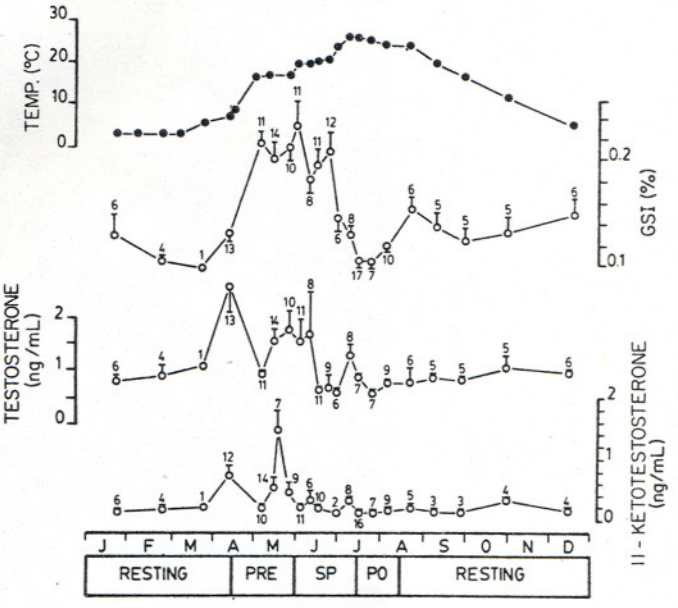

FIG. 1. Seasonal changes in serum testosterone and 11-ketotestosterone concentrations in relation to the seasonal pattern of gonadosomatic index (GSI) in male brown bullheads, *Ictalurus nebulosus* Lesueur. The stages of the reproductive cycle are based on GSI and histological data (Burke and Leatherland 1984). PRE, prespawning; SP, spawning; PO, postspawning.

The GSI in female fish showed no significant differences between any of the values from November to April (Fig. 2). The GSI rose rapidly from 1.65 in early May to a peak of 9.05 in late May, which was significantly different ($p = 0.01$) from the GSI in early May. The mean GSI subsequently declined rapidly and significantly ($p < 0.01$) to reach 0.48 at the end of July.

Between December and early March, the water temperature in Hamilton Harbour and in the holding pond was more or less constant at 3°C (Fig. 1). There was a progressive increase in water temperature from early March to 25°C in mid-July. The GSI values in males commenced to increase in mid-April when water temperatures were rapidly increasing (Fig. 1), whereas the GSIs in females started to increase later than those in males, when the ambient water temperature was in excess of 16°C (Fig. 2). The GSI in males declined from peak values in fish collected in late June, when water temperature was 20°C. The GSI in females commenced to drop 4 weeks earlier when the water temperature was 19°C.

Serum testosterone concentrations

With the exception of one collection, there were no statistically significant differences in serum testosterone concentrations between male and female brown bullheads during the latter part of the spawning, postspawning, and resting periods (Figs. 1 and 2). The exception was in early July during the spawning period, when the serum testosterone concentration of the females was significantly ($p < 0.01$) higher than that of the males. During the prespawning and early spawning periods, testosterone concentrations were significantly higher

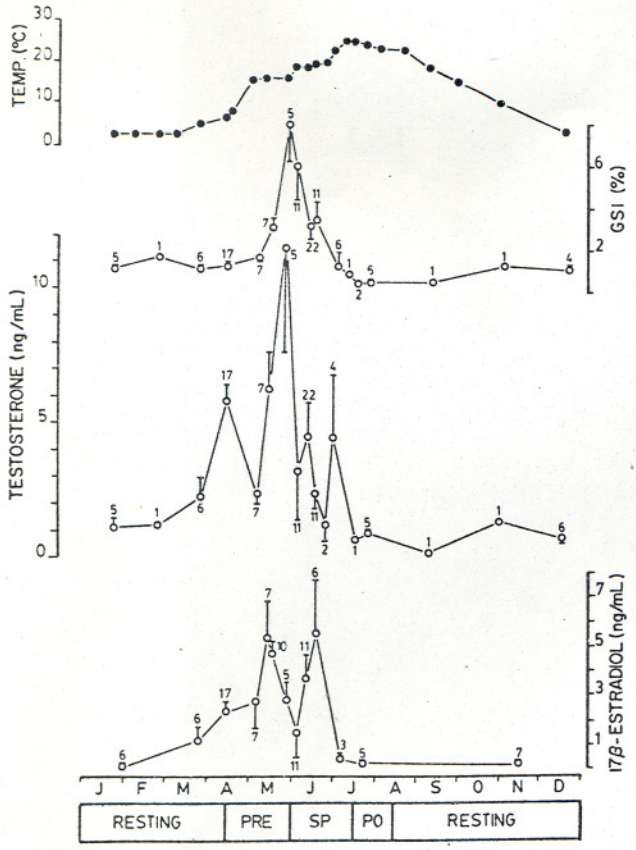

FIG. 2. Seasonal changes in serum testosterone and 17β-estradiol concentrations in relation to the seasonal pattern of gonadosomatic index (GSI) in female brown bullheads, *Ictalurus nebulosus* Lesueur. The stages of the reproductive cycle are based on GSI and histological data (Burke and Leatherland 1984). PRE, prespawning; SP, spawning; PO, postspawning.

($p < 0.01$) in females than in males.

Serum testosterone concentrations in males were low (<1 ng/mL) from mid-July until the end of March and did not alter significantly during this period. In mid-April, at the beginning of the prespawning period, the concentration increased significantly ($p = 0.01$) from the level found in late March but fell by early May to levels that were not significantly different from those of the overwintering males. There then appeared to be a secondary elevation of serum testosterone concentration lasting from mid-May until the 1st week in June (Fig. 1). By the middle of the spawning period in mid-June, the levels were significantly ($p = 0.02$) lower than those in early June. Female brown bullheads exhibited a pattern of serum testosterone levels which was similar to that of the males throughout most of the year (Fig. 2). Testosterone concentrations increased significantly ($p < 0.01$) in mid-April, at the start of the prespawning period, and then decreased significantly ($p < 0.01$) in early May. However, unlike the males, a second peak was evident at the end of May, during the early spawning period. Serum testosterone concentrations then decreased rapidly ($p < 0.01$) to overwintering levels at the end of June, during the middle of the spawning period.

Serum 11-ketotestosterone concentrations

In the males, serum 11-ketotestosterone concentration exhibited a bimodal seasonal pattern. A significant ($p < 0.01$) increase from the overwintering base line values was evident in mid-April (Fig. 1). Serum 11-ketotestosterone values then fell to base line levels by early May, and by mid-May began to increase again to peak at the end of May at a level which was significantly ($p < 0.01$) higher than at all other times. Thereafter, the hormone levels fell rapidly, and between early June and late February the values did not differ significantly.

TABLE 1. Seasonal changes in serum 11-ketotestosterone in female brown bullheads

Season	Serum 11-ketotestosterone concentration[a] (ng/mL)
August to February	0.18±0.02 (5)
April	0.48±0.10 (6)
Early May	0.25±0.05 (3)
Mid- to late-May	0.99±0.32 (8)
June	0.26±0.03 (18)

[a]Data are shown as means ± SE with n in parentheses.

An essentially similar pattern of 11-ketotestosterone cycles was evident in females (Table 1), although the levels in fish sampled in mid-April were not significantly different from those sampled during the overwintering period, early May, or June. The 11-ketotestosterone levels in females collected during mid- to late May were significantly higher than all other groups ($p < 0.01$, except when compared with the April sample where $p < 0.05$) (Table 1).

Serum 11-ketotestosterone levels were similar in males and females sampled at comparable times of the year (Fig. 1, Table 1).

Serum 17β-estradiol concentrations

Serum 17β-estradiol concentrations showed a bimodal seasonal pattern in female brown bullheads (Fig. 2). Base line values were evident between late July (postspawning period) and February. The levels rose progressively from February to peak in mid-May, after which there was a decline in serum 17β-estradiol values until early June when the values were not significantly different from those of the overwintering fish. During June, the 17β-estradiol values again increased progressively to peak again in late June, before falling to base line values by early July. The 17β-estradiol concentrations of the fish collected between early and late May (prespawning period) and early and late June (spawning period) were all significantly higher ($p < 0.05$) than those of fish collected during the postspawning and resting periods.

Discussion

Serum concentrations of the three steroid hormones measured in this study appeared to show a bimodal seasonal pattern (Figs. 1 and 2, Table 1), which thus differs from the single peaks of androgens or estrogens found in most other species (Wingfield and Grimm 1977; van Bohemen and Lambert 1981; Scott, Bye, and Baynes 1980; Scott, Bye, Baynes *et al.* 1980; Scott *et al.* 1982; Scott *et al.* 1983; Fostier *et al.* 1982; Kime and Manning 1982; Breton *et al.* 1983). However, in at least one other species, *Gobius niger*, there is evidence of a bimodal seasonal pattern of a plasma sex steroid (Bonnin 1979). Further studies are necessary to determine whether this apparent bimodality has physiological significance. The lack of correlation between the "peaks" of the three serum hormones measured supports the contention that there is considerable interplay between steroid hormones in the control of gamete production and maturation in teleosts.

The role of testosterone in the female reproductive cycle in teleosts is not clear, although it has been proposed as a precursor for 17β-estradiol in female teleosts (Lambert *et al.* 1971, cited in Campbell *et al.* 1976; Kagawa, Young, Adachi *et al.* 1982). In female brown bullheads, the two highest serum testosterone titres precede the two peak serum 17β-estradiol values by approximately 1 month. The highest serum testosterone values in female brown bullheads were 4.5-fold higher than the highest values in males. Similar findings were reported in winter flounder (Campbell *et al.* 1976), rainbow trout (Campbell *et al.* 1980; Scott, Bye, and Baynes 1980; Scott, Bye, Baynes *et al.* 1980), and bluefish (MacGregor *et al.* 1981), although this does not appear to be true for all teleostean species. For example, Wingfield and Grimm (1977) found that plasma testosterone levels in plaice were always higher in the males, and in goldfish (Schreck *et al.* 1974) and mackeral (MacGregor *et al.* 1981) androgen levels were similar in males and females.

Estrogen synthesis and release by the ovarian follicles in salmon appear to be under the influence of pituitary gonadotropins (Kagawa, Young, and Nagahama 1982), and estrogens are implicated in the stimulation of vitellogenesis by the liver (van Bohemen and Lambert 1981; Breton *et al.* 1983). In the brown bullhead, the 17β-estradiol peak evident in May was correlated with the period of rapid increase in gonadal weight (Fig. 2), possibly indicative of vitellogen incorporation into the oocytes during the prespawning period. However, a second 17β-estradiol peak was evident during the spawning period when vitellogen incorporation was presumably complete.

In several teleostean species examined to date, 11-ketotestosterone is considered to be the principal androgen, even though blood levels of the steroid are often lower than those of testosterone; 11-ketotestosterone levels are usually considerably lower in females than in males (Sangalang *et al.* 1978; Kime and Manning 1982; Stuart-Kregor *et al.* 1981; Leatherland *et al.* 1982; Scott, Bye, and Baynes 1980; Scott, Bye, Baynes *et al.* 1980). In the brown bullhead, the 11-ketotestosterone levels in males and females were essentially similar. It is not clear whether the 11-ketotestosterone levels in the females are physiologically meaningful or simply reflect the cross-reactivity of the antiserum with testosterone. However, there appeared to be a clear seasonal pattern of serum 11-ketotestosterone levels in female brown bullheads which was identical to that of the males.

In the males, the testosterone and 11-ketotestosterone exhibited bimodal seasonal patterns which were roughly correlated. The peaks of serum testosterone were evident in mid-April and from mid-May to early June, whereas those for 11-ketotestosterone were evident in mid-April and mid- to late May. The first increase in serum androgen titres was associated with testicular recrudescence. However, the middle of the prespawning period, when the GSI had reached its maximal value, was characterized by a decline in androgen levels (Fig. 1). The androgen titres subsequently increased again and the 11-ketotestosterone peaked early in the spawning period. These data support the concept proposed in other teleosts, namely that 11-ketotestosterone is related to spermiation. Peak levels of 11-ketotestosterone were coincident with spermiation in brook trout (Sangalang and Freeman 1974), brown trout (Kime and Manning 1982), and rainbow trout (Scott, Bye, and Baynes 1980; Fostier *et al.* 1982). Fostier *et al.* (1982) found a good correlation between the increase in plasma 11-ketotestosterone levels in rainbow trout and sperm release viability. Testosterone is probably one of the intermediate products in the synthesis of 11-ketotestosterone (Tamaoki *et al.* 1971; Ozon 1972) since in *in vitro* incubations of testicular tissues of Atlantic salmon (Idler and MacNab 1967), goldfish (Kime 1980), and yellow perch (Kime and Hews 1978) converted testosterone to 11-ketotestosterone.

In addition to being used as a precursor, testosterone possibly plays a role in the initiation and probable maintenance of spermatogensis, and appears to be important in the feedback processes of the hypothalmic–pituitary–gonad axis (Crim and Evans 1979; Crim *et al.* 1981).

In most freshwater temperate-zone teleosts studied, temperature and (or) photoperiod are proposed as the principal environmental factors regulating the timing of reproduction (Peter and Crim 1979; Peter 1981). In male brown bullheads, both GSIs and serum gonadal steroid titres rise concomitantly with the increase in water temperatures and photoperiod in the spring, possibly indicating that gonadal recrudescence in this species is also temperature and (or) photoperiod related.

The stress of transportation of the fish used in this study from the capture site to the laboratory did not appear to significantly affect plasma testosterone, 11-ketotestosterone, or 17β-estradiol levels. The effect of the capture on the hormone levels is harder to evaluate. The traps were set in late afternoon and emptied during the early morning, thus the fish could have been contained in the trap net for up to 20 h. In fish maintained at the University of Guelph during the winter, plasma steroid levels were comparable with those of fish sampled from the wild during the preceding and proceeding months, thus indicating that any stressor-induced effect did not elicit an increase in plasma hormone titres; the possibility of stressor-induced decreases in plasma hormone titres cannot be eliminated in this or any other study of feral teleosts.

The peak GSI values in both male and female brown bullheads were considerably lower than in other teleostean species, e.g., salmonids (Scott, Bye, and Baynes 1980; Scott, Bye, Baynes *et al.* 1980; Scott *et al.* 1983; Leatherland *et al.* 1982). This may explain why the ovary reaches its maximum size more rapidly than in other species, inasmuch as the investment in the ovary is considerably less than in other species. Moreover, the peak serum steroid hormone levels are also lower in the brown bullhead than in other teleosts (see references cited in Introduction). It is possible that the low hormone levels in the brown bullhead are related to the relatively small gonad size in this species.

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