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Changes in Plasma and Gonadal Steroid Hormones in Relation to the Reproductive Cycle and the Sex Inversion Process in the Protandrous Seabass, *Lates calcarifer*

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Plasma and gonadal levels of several gonadal steroids (testosterone, 11-ketotestosterone, androstenedione, 11β-hydroxyandrostenedione, 17β-estradiol, and estrone) were measured by RIA in the protandrous seabass, *Lates calcarifer*, throughout an annual reproductive cycle. Twenty to 25 fish were killed every month for gonadal and plasma sampling. Very low plasma levels of 11-ketotestosterone in females (monthly means always less than 75 pg/ml), and of 17β-estradiol (means always less than 68 pg/ml) and estrone (means always less than 42 pg/ml) in males did not fluctuate significantly during the cycle. Conversely, plasma concentrations of testosterone, estrone, and 17β-estradiol peaked during vitellogenesis in females (highest mean: 182 ± 121, 182 ± 32 and 598 ± 369 pg/ml, respectively) and testosterone and 11-ketotestosterone peaked during spermiation in males (highest mean: 189 ± 91 and 223 ± 94 pg/ml, respectively). When sex type are compared over the whole cycle, females displayed higher 17β-estradiol (172 ± 233.5 pg/ml) and estrone (79.5 ± 72 pg/ml) levels than males (57 ± 7.5 and 44 ± 62.5 pg/ml, respectively), while males had higher 11-ketotestosterone levels (153 ± 88 pg/ml) and, to a lesser extent, higher testosterone levels (128 ± 82 pg/ml) than females (51.5 ± 28 and 91.5 ± 60 pg/ml, respectively). Transitional fish always exhibit low plasma levels for these four steroids (testosterone 56.5 ± 12.5 pg/ml, 11-ketotestosterone 59 ± 23.5 pg/ml, 17β-estradiol 65.6 ± 36 pg/ml, and estrone 61 ± 47.5 pg/ml). Among gonadal androgens, 11β-hydroxyandrostenedione predominated in testes (3.95 ± 3 ng/g), except during spermiation (0.8 ± 0.5 ng/g), and remained low in ovaries (1.05 ± 1.4 ng/g). No differences were detected in gonads, for testosterone and 11-ketotestosterone whatever the sex type, but their concentrations were higher in vitellogenic and atretic ovaries. Androstenedione levels were slightly higher in testes (2.21 ± 2 ng/g) than in ovaries (1.53 ± 1.32 ng/g). Transitional gonads always showed low concentrations for these four androgens (testosterone 0.66 ± 1.77 ng/g, 11-ketotestosterone 0.14 ± 0.05 ng/g, androstenedione 0.3 ± 0.34 ng/g, and 11β-hydroxyandrostenedione 0.2 ± 0.23 ng/g). Gonadal 17β-estradiol was nearly undetectable in testes (0.06 ± 0.07 ng/g), low in ovaries (0.42 ± 0.46 ng/g), and strikingly high in transitional gonads (2.89 ± 1.64 ng/g) even at the very beginning of sex inversion. This suggests an important role for this estrogen in the protandrous sex inversion process in the seabass *L. calcarifer*.

The seabass, *Lates calcarifer*, is a protandrous hermaphroditic species (Moore, 1979; Davis, 1982) of great interest for both aquaculture and fisheries throughout the whole Indo-Pacific area. It has been introduced for breeding in French Polynesia (Fuchs, 1987; Aquacop *et al.*, 1990), where its reproductive cycle and sex inversion process have been described (Guiguen *et al.*, 1993). In these rearing stocks, sex inversion (March–April) occurs in postspawning males after the annual reproductive period (from October to February). In the seabass the sex inversion process is of particular interest as there are major morphological changes of the gonads, which lack preformed ovarian tissue (the gonad is not an ovotestis, and testicu-

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lar and ovarian tissues are found together only at one of the various transitional stages).

Yamamoto (1969) suggested that steroids are the natural inducers of gonadal sex differentiation in gonochorist fish, estrogens being the gyno-inducers and androgens the andro-inducers. So far, limited physiological data support this hypothesis, including, detection of steroids (Rothbard *et al.*, 1987; Baroiller, 1988; Nakamura and Nagahama, 1989; Feist *et al.*, 1990; Grace *et al.*, 1992) and of precocious steroidogenic activities in gonads around sexual differentiation in some gonochoristic fish (Van den hurk *et al.*, 1982; Baroiller, 1988; Baroiller *et al.*, 1988; Nakamura and Nagahama, 1985; Eckstein *et al.*, 1988). However, sexual inversion can be obtained by exogenous treatments with steroids (Hunter and Donaldson, 1983).

In hermaphroditic species, experiments have been performed to induce sex inversion by steroidal treatment (Chen *et al.*, 1977; Okada, 1964; Tang *et al.*, 1974; Reinboth, 1975; Roberts and Schlieder, 1983; Kuo *et al.*, 1988; Kramer *et al.*, 1988; Cardwell and Liley, 1991; Kime *et al.*, 1991, Chang and Lee, 1992), but results have been inconsistent in some species (Reinboth, 1970), and only a few of these studies were based on a knowledge of the physiology of that particular animal. In the present study, some of the endocrine events associated with the protandrous sex inversion process have been examined to identify appropriate treatment for inversing sex in the seabass, *L. calcarifer*. Thus, several common steroids known for their physiological actions in most of the fish and for their efficiency when used for experimental sex inversions (testosterone, 11-ketotestosterone, and 17β-estradiol) have been determined. Furthermore, estrone in plasma and androstenedione and 11β-hydroxyandrostenedione in gonads were measured to compare changes of the androgens to the estrogens during the protandrous sex-inversion process in *L. calcarifer*. The study of gonadal steroids was performed to assess the availability of steroids for the gonad, which is probably one of the main steroid target organs during sex differentiation and sex inversion process.

MATERIALS AND METHODS

Fish Sampling

Fish belonged to a 3-year-old cohort of *L. calcarifer* previously described (Guiguen, 1992; Guiguen *et al.*, 1993). Gonadal maturation and transitional stages were determinated histologically during the course of the study cited above and are described in Table 1.

Each fish was anaesthetized by immersion in sea water containing 2-phenoxyethanol (0.6 ml/liter). Blood was collected from the cardiac sinus into a heparinized syringe (1000 IU/ml lithium heparinate in 0.9% NaCl), kept on ice before centrifugation (3000*g*, 15 min, 4°), and was stored −20° until analysis. Subsequently, each fish was killed by a blow to the head, and a piece of gonad (a transverse section where possible) was removed and kept at −20° until analysis.

Extraction of Plasma Steroids

Before extraction, tritiated testosterone and 17β-estradiol (2500 dpm each) were added to each plasma for estimation of recovery. Plasmas were then extracted twice with 5 vol of cyclohexane/ethyl acetate 50/50. The pooled organic extracts were evaporated under air flow at 40°. Only this organic fraction (unconjugated steroids) was kept for RIA measurements.

Extraction of Gonadal Steroids

Before extraction, 0.8 to 1 g of gonad was homogenized (Polytron PTA 10TS) in 2 ml saline solution (2% NaCl), to which had been added tritiated testosterone and 17β-estradiol (2500 dpm each) for estimation of recovery. Each sample was then homogenized again after addition of 8 ml ethanol, submitted to sonication (Branson sonifier B-12, 2000 Hz), frozen at −20° overnight, and centrifuged (3000*g*, 15 min, 4°), and the supernatant recovered. A second extraction was made following the same protocol with 5 ml of aqueous ethanol (80%), and the two supernatants were pooled. This ethanolic solution was partially evaporated under air at 40° and extracted twice in 5 vol of cyclohexane/ethyl acetate 50/50, and the resultant organic extract was evaporated under air at 40°.

TABLE 1
GONADAL STAGES IN THE SEABASS, *Lates calcarifer*

Sex type	Index	Histological features (gonadal maturation stage)
Male	M1	Mostly gonia (testis gonia)
	M2	Mostly spermatocytes and spermatids (spermatogenesis)
	M3	Mostly spermatozoa (spermiation)
	M4	Testicular lobules devoid of spermatozoa (post-spawning)
Transitional	T1	Degeneration of male testicular tissue
	T2	Appearance of ovarian tissue with still degenerating testicular tissue
	T3	Ovarian tissue <50% within histological cross-section (no testicular tissue)
	T4	Ovarian tissue >50% within histological cross-section (no testicular tissue)
Female	F1	Gonia and previtellogenic oocytes (previtellogenesis)
	F2	Vitellogenic oocytes <50% within histological cross-section (early vitellogenesis)
	F3	Vitellogenic oocytes >50% within histological cross-section (vitellogenesis)
	F4	Oocytes atresia (atretic)

Separation of Steroids

Groups of steroids were separated on Sephadex LH20 (Pharmacia) columns. The dry residue was dissolved in 300 µl dichloromethane/methanol (95/5), then transferred to a Sephadex LH20 column (140 × 5 mm). Elution was performed in the same solvent system: the first 1.5-ml fraction contained androstenedione, the second 1-ml fraction contained testosterone, 11-ketotestosterone, and 11β-hydroxyandrostenedione, the third 2-ml fraction contained estrone, and the fourth 3.5-ml fraction contained 17β-estradiol. The first, second, and fourth fractions were evaporated under air at 40° and samples were kept in 200 µl ethanol at −20 until assay.

Recoveries for 17β-estradiol and testosterone after this column chromatographic step were respectively 61 ± 11.2% (N = 60) and 79.9 ± 11.8% (N = 60). Recoveries for the other steroids were estimated according to the 17β-estradiol recovery for estrone and to the testosterone recovery for 11-ketotestosterone, androstenedione, and 11β-hydroxyandrostenedione.

Assays

Before assays, ethanol was evaporated under air at 40°, and the dry residues were dissolved in phosphate buffer (0.01 M, pH 7.25) containing 0.1% gelatine. Estrone and 17β-estradiol were measured according to Fostier *et al.* (1978) except that bound steroids were precipitated with polyethylene glycol (Fostier *et al.*, 1982). The 17β-estradiol antibody was used at a final concentration of 1:3000 and its cross-reactivities with other steroids were respectively: 16-epiestriol 12%, estrone 9.5%, and estradiol-17d, 16-ketoestradiol-17β, 2-metoxyestrone, estriol, and testosterone less than 1%. The estrone antibody was used at a final concentration of 1:3000 and its cross-reactivities were less than 0.8% for 17β-estradiol, 16-epiestriol, and 16-ketoestradiol-17β and less than 0.1% for 2-metoxyestrone, testosterone, and 11-ketotestosterone. Testosterone, 11-ketotestosterone, androstenedione, and 11β-hydroxyandrostenedione were measured according to Fostier *et al.* (1982) and antibodies were used at final concentrations of 1:3000, 1:36,000, 1:3700, and 1:1200, respectively. Cross-reactivities are given in Fostier and Jalabert (1986) for testosterone and androstenedione antibodies and in Fostier *et al.* (1982) for 11-ketotestosterone antibody. 11β-Hydroxyandrostenedione antibody was a gift from Professor R. Reinboth (Zoological Institute, Mainz University, Germany). Sensitivity of the 11β-hydroxyandrostenedione assay was approximately 5 pg/tube and cross-reactivities of the antibody were 11β-hydroxy-5α-androstan 54.6%, androstenedione 4.8%, 11β-hydroxytestosterone 4.25%, and testosterone, 11-ketotestosterone, and androsterone less than 0.05%.

Expression of Results and Statistics

Because of the great variation in gonadal weights (means ± SD according to the stage: M1, 4.2 ± 1.5 g; M2, 8.5 ± 3 g; M3, 65.7 ± 23.6 g; M4, 6 ± 1.2 g; T1, 3.1 ± 0.2 g; T2, 5.2 g; T3, 4.1 ± 2.1 g; T4, 5.6 ± 3 g; F1, 40.8 ± 7 g; F2, 70.6 ± 29.5 g; F3, 473.1 ± 76.1 g; F4, 194.5 ± 111.5 g), data on gonadal steroid concentrations were expressed in ng/g of gonad. However, these data may not be quantitatively comparable because of high variation in the proportion of somatic tissue between sex types and during the sexual cycle.

Statistical analysis were performed using parametric one way analysis of variance (Statgraphic program), and multiple range comparisons were performed using a t test.

RESULTS

Plasma Steroid Levels

In general, the four plasma steroids assayed (estrone, 17β-estradiol, testosterone, and 11-ketotestosterone) were present at very low concentrations, i.e., less than 350 pg/ml except 17β-estradiol which reached 1 ng/ml in several females.

Steroid Level Fluctuations during the Reproductive Cycle

During the reproductive cycle (Fig. 1), females showed high levels of testosterone from November to January, during the period of vitellogenesis. After a rapid decline, these levels increased again slowly until September, but this rise was not statistically significant. In contrast, 11-ketotestosterone concentrations displayed small changes during the female cycle, with low levels all the year round, and monthly means were always below 75 pg/ml. 17β-Estradiol and estrone had very similar profiles. Concentrations were low during most of the female cycle and rose sharply to their highest levels in November at the beginning of the vitellogenesis.

In males, levels of estrone and 17β-estradiol were always very low and did not fluctuate significantly during the reproductive cycle. On the other hand, testosterone and 11-ketotestosterone fluctuated and showed similar changes in profiles. Concentrations increased slowly within 8 months from February to September–October, remained high from September to December, and then decreased.

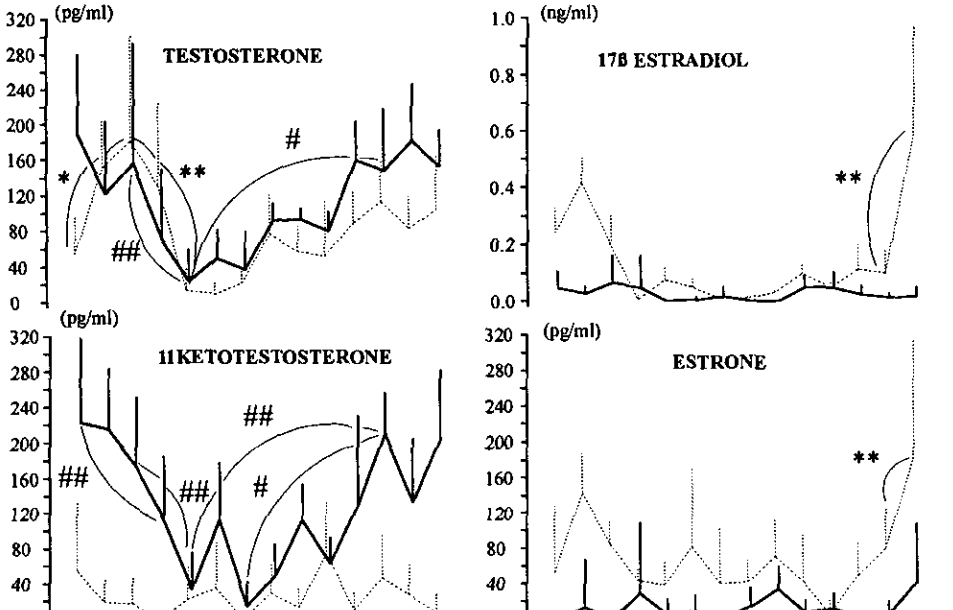

FIG. 1. Changes in certain plasma sex steroids in female (···) and male (—) seabass during an annual reproductive cycle. Data are expressed as means ± SD (vertical bars represent the standard deviation). The reproductive season (RS) in French Polynesia is indicated below the months scale. *, significant difference $P < 0.05$ for females. **, significant difference $P < 0.01$ for females. #, significant difference $P < 0.05$ for males. ##, significant difference $P < 0.01$ for males.

Comparison Based on the Sex Type and the Gonadal Maturation Stage

Estrone and 17β-estradiol concentrations in females (Fig. 2) were higher than in males ($P < 0.01$) and transitional fish ($P < 0.01$). These differences were particularly noticeable for vitellogenic females (F3) which exhibited the highest levels compared with the other ovarian maturation stages. Conversely, concentrations of testosterone and 11-ketotestosterone in males (Fig. 2) were higher than those of females and transitional fish ($P < 0.01$ 11-ketotestosterone; $P < 0.05$ testosterone). There were no significant differences in testosterone and 11-ketotestosterone concentrations at the various testicular maturation stages, except for testosterone between spermiation (M3) and postspawning stages (M4) (M4 < M3, $P < 0.01$).

Gonadal Steroid Levels

Comparison Based on the Sex Type and the Gonadal Maturation Stage

No difference was detected between the three sex types, male, transitional fish, and female (Fig. 3), for gonadal testosterone and 11-ketotestosterone. However, the two steroids were higher in vitellogenic (F3) and atretic (F4) ovaries compared with the other ovarian stages, or with male and transitional fish gonads. Gonadal androstenedione was higher in male than in transitional fish ($P < 0.01$) (Fig. 3). The levels were also significantly lower in testes at the spermiation stage (M3) compared with testes at the other maturational stages ($P < 0.01$). 11β-Hydroxyandrostenedione levels were higher in males than in transitional fish and females ($P < 0.01$) (Fig. 3) and the levels

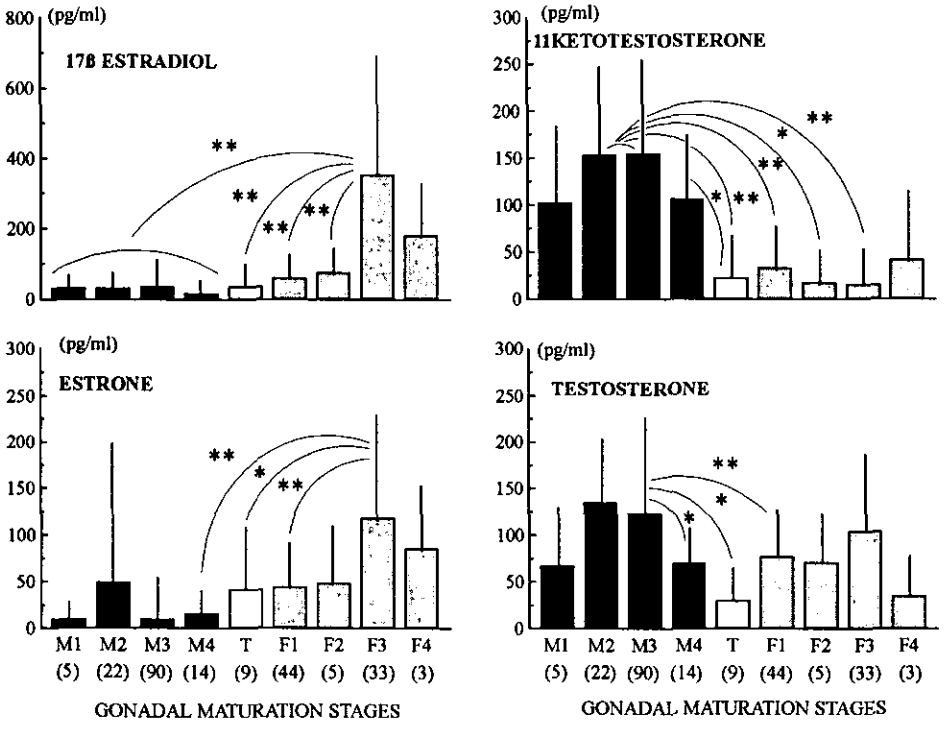

FIG. 2. Histograms showing plasma sex steroids for the various sex types and maturation stages in the seabass *Lates calcarifer*. Data are expressed as means ± SD (vertical bars represent the standard deviation). The number of fish is indicated in parentheses under each maturation stage and/or sex type. *, significant difference $P < 0.05$. **, significant difference $P < 0.01$. Measurements performed on transitional fish (T1 to T4) have been pooled (T).

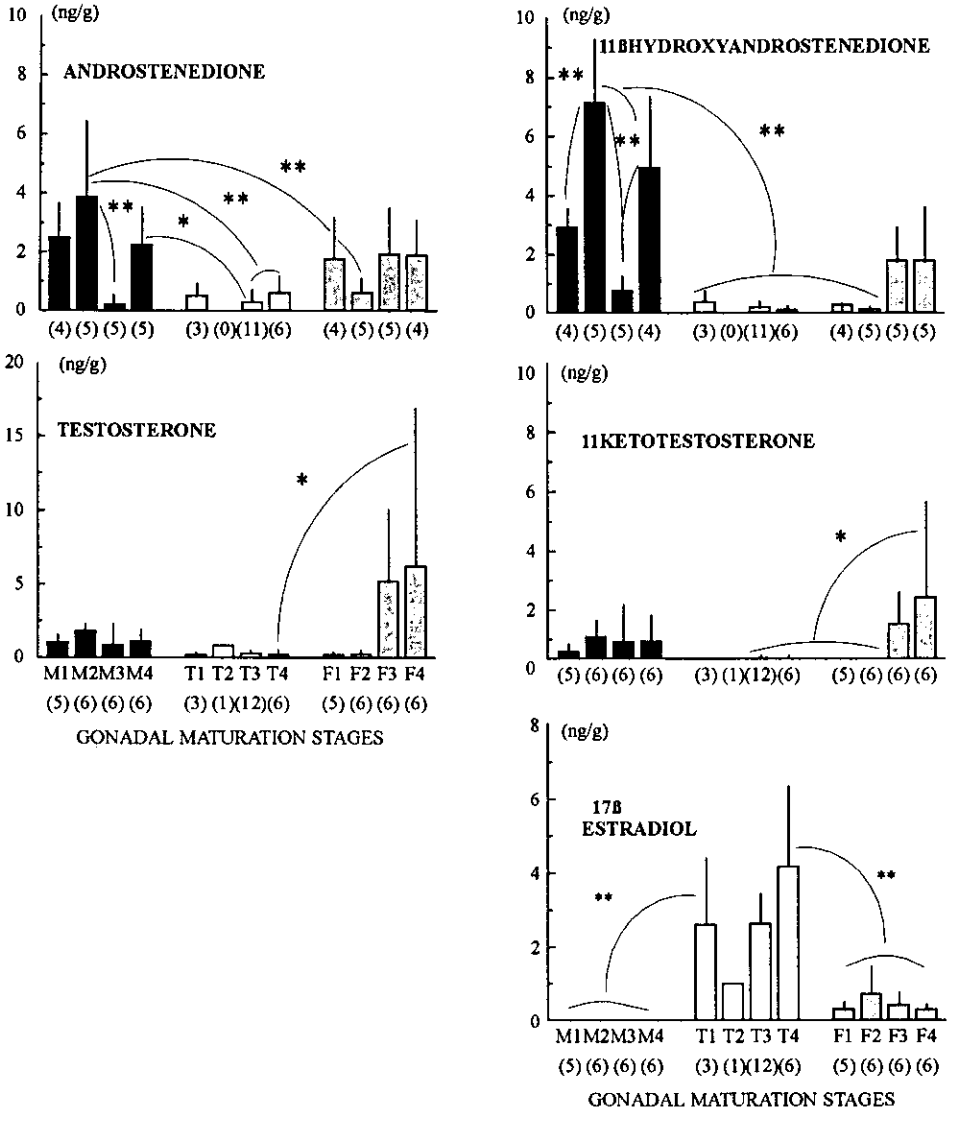

FIG. 3. Histograms showing the gonadal content of some sex steroids for the various sex types and maturation stages in the seabass *Lates calcarifer*. Data are expressed as means ± SD (vertical bars represent the standard deviation). The number of fish is indicated in parentheses under each maturation stage and/or sex type. *, significant difference $P < 0.05$. **, significant difference $P < 0.01$.

were higher during spermatogenesis (M2) and postspawning stage (M4) ($P < 0.01$). 17β-Estradiol was always below the detection level (5 to 10 pg/g) in testes (Fig. 3). In ovaries, 17β-estradiol levels were low. However, transitional fish showed very high 17β-estradiol gonadal concentrations that were significantly higher than in males and females ($P < 0.01$). These high levels of 17β-estradiol in transitional gonads were found even during the first stage of sex inversion (T1), which was characterized by degeneration of the testicular tissue, without discernible female germinal cells.

DISCUSSION

Depending on the species and on the sex type, plasma levels of testosterone seem to

be highly variable. In the protandrous *Sparidentex hasta*, levels were similar in males and females (Kime *et al.*, 1991). In *L. calcarifer*, testosterone was always present in plasma whatever the sex type, but was highest in males. Testosterone and 11-ketotestosterone in plasma were roughly similar in males whereas plasma testosterone levels in females were highest 1 month after the 17β-estradiol peak. These changes in plasma testosterone concentration during the reproductive cycle may be linked to its role as one of the possible precursors for 11-ketotestosterone in male and for 17β-estradiol in female (Scott *et al.*, 1980a,b; Prat *et al.*, 1990). Testosterone may also have a direct action on the maintenance of spermatogenesis (Billard *et al.*, 1982; Fostier *et al.*, 1987), in a similar way to that observed in testicular explants of goldfish, *Carassius auratus*, in which testosterone maintains spermatogonial divisions and meiosis (Remacle, 1976).

The possible role of testosterone in the sex inversion process seems to vary with species. In protogynous species, such as *Sparisoma viride*, testosterone did not appear to influence either the internal processes of sex inversion or the concomitant color changes (Cardwell and Liley, 1991). In the protogynous, *Thalassoma duperrey*, plasma testosterone did not vary whatever the sex type or the transitional stages (Nakamura *et al.*, 1989). On the other hand, plasma levels of testosterone increased during sex inversion in the protogynous, grouper, *Epinephelus microdon*, and decreased during sex reversion (Debas, 1989). In the protogynous, ricefield eel, *Monopterus albus*, and in the protandrous sparid, *Rhabdosargus sarba*, changes in plasma testosterone could be correlated with seasonal reproductive activities (Chan and Yeung, 1989). In fact, these and the present data do not support the hypothesis that testosterone is involved directly as an inducer of sex inversion in hermaphrodite species.

With regard to 11-oxygenated androgens, plasma 11-ketotestosterone and gonadal 11β-hydroxyandrostenedione levels were significantly higher in males than in transitional fish and females in *L. calcarifer*. Plasma 11-ketotestosterone fluctuated during the male reproductive cycle. Levels were elevated during the reproductive season and then decreased rapidly. Similarly, the highest levels of plasma 11-ketotestosterone were detected in males just before spawning in *Catostomus commersoni* (Scott *et al.*, 1984), and during spermiation in the halibut (Methven *et al.*, 1992) and *Dicentrarchus labrax* (Prat *et al.*, 1990). In fact, 11-ketotestosterone is a major androgen in plasma, but its specific role among other androgens in the control of spermiation remains to be demonstrated (Billard *et al.*, 1982). In the seabass, *L. calcarifer*, plasma 11-ketotestosterone fluctuated during the male reproductive cycle, but differences were not associated with different testicular maturation stages. This may be because of the permanent gametogenetic activity of male seabass (Guiguen, 1992; Guiguen *et al.*, 1993). Regarding the gonadal concentrations of steroids, 11β-hydroxyandrostenedione was the major steroid in the testes of *L. calcarifer*. Similarly, in *Gasterosteus aculeatus*, 11-ketotestosterone was the major plasma androgen during the reproductive season (Mayer *et al.*, 1990a), although testicular synthesis of adrenosterone and 11β-hydroxyandrostenedione was elevated (Borg *et al.*, 1989). Such a difference could result from the conversion of adrenosterone into 11-ketotestosterone by blood cells which may exhibit 17β-hydroxysteroid dehydrogenase activity (Mayer *et al.*, 1990b; Schulz and Blum, 1991). In a protogynous hermaphrodite, the ricefield eel *M. albus*, plasma 11-ketotestosterone was similar in the sex types (Chan and Yeung, 1989). In *T. duperrey*, plasma 11-ketotestosterone was highest in males and especially in secondary males (Hourigan *et al.*, 1991; Nakamura *et al.*, 1989). These high concentrations may be related to reproductive and aggres-

sive behavior rather than with the control of male gametogenesis. For example, in the protogynous *S. viride* the high plasma 11-ketotestosterone found in some males closely correlated with their aggressive behavior (Cardwell and Liley, 1990). However, a large increase in 11-ketotestosterone concentration was also observed in the latter species during the sex inversion process and the concomitant color change (Cardwell and Liley, 1987, 1991). In addition, a single injection of 11-ketotestosterone (5 μg/g) was sufficient to induce sex inversion, suggesting that this steroid may play an important role in the natural sex inversion process (Cardwell and Liley, 1991). In the grouper *E. microdon,* plasma 11-ketotestosterone concentrations were similar in the sex types, but levels increased during sex inversion and decreased during sex reversion (Debas *et al.,* 1990). Plasma 11-ketotestosterone concentrations were higher in male *Centropistes striatus,* a protogynous species, than in females (Cochran and Grier, 1991). A protandrous species, such as the sparid *Rhabdosargus sarba,* had undetectable plasma 11-ketotestosterone and 11β-hydroxytestosterone levels and did not show any difference between sex types (Yeung and Chan, 1987a). Conversely, concentrations of these two steroids were higher in males of another protandrous sparid, *S. hasta,* and increased both in males and in females during the reproductive season (Kime *et al.,* 1991).

In *L. calcarifer,* there are very low or undetectable concentrations of 11β-hydroxyandrostenedione and 11-ketotestosterone in transitional gonads, as well as low levels of 11-ketotestosterone in the plasma of transitional fish. However, the concentrations of these 11-oxygenated androgens exhibited a large difference between males and females.

An augmentation of estrogen plasma levels, and specially 17β-estradiol, during the period of growing vitellogenetic oocytes, occurs in many female teleosts (see Fostier *et al.,* 1983), revealing a clear relationship between high estrogen levels and vitellogenesis. In addition, the hepatic synthesis of a yolk protein precursor, vitellogenin, can be induced by 17β-estradiol in all the fish species investigated (Ho, 1987; Ng and Idler, 1983) and even in hermaphroditic species (Ng *et al.,* 1984). This relationship between estradiol and vitellogenesis seems also to be confirmed in *L. calcarifer* by the detection of high concentrations of estrogens in females at the beginning of the reproductive season. However, there is a sudden decrease in these plasma estrogens as early as December, although vitellogenesis continued until February (Guiguen, 1992; Guiguen *et al.,* 1993). This may indicate a transient very active phase of vitellogenin synthesis induction and hepatic release under the hormonal control of high estrogen levels at the beginning of vitellogenesis, followed by a steady phase which would not require such high estrogen levels. A similar evolution of 17β-estradiol levels was also observed in the plaice, *Pleuronectes platessa,* showing a return to basal levels, whereas the gonadosomatic index continued to increase (Wingfield and Grimm, 1977).

Estrogens may affect sex inversion in *L. calcarifer.* First, females had higher plasma levels of 17β-estradiol and estrone than males and transitional fish. Second, these plasma levels never fluctuated significantly during the reproductive cycle in males, and 17β-estradiol was never detected in the testis. Finally, 17β-estradiol concentrations in the gonads of transitional fish were very high, even during the first transitional stage characterized by the degeneration of the testicular tissue without any ovarian tissue.

Males of many gonochoristic species are characterized by low plasma estrogens (Fostier *et al.,* 1987). In protogynous hermaphrodites, higher levels were detected in females of *S. viride* (Cardwell and Liley, 1991), *T. duperrey* (Nakamura *et al.,* 1989), the ricefield eel (Yeung and Chan, 1987b),

and *C. striatus* (Cochran and Grier, 1991), but not in the grouper *E. microdon* (Debas *et al.*, 1990). In the sparid *R. sarba*, a protandrous species, there were higher concentrations of 17β-estradiol in plasma in females before the spawning season, but high levels of conjugated 17β-estradiol were also found in male plasma at the same time (Yeung and Chan, 1987a). In *Acanthopagrus schlegeli*, plasma 17β-estradiol peaked just before the reproductive season in males, whereas it increased later in females, at the beginning of the reproductive season (Chang and Yueh, 1990). In another sparid species, *S. hasta*, all the animals reached a peak of 17β-estradiol concentration before the reproductive season. Males that did not undergo sex inversion then exhibited decreasing levels of 17β-estradiol and increasing levels of 11-ketotestosterone. On the other hand, 17β-estradiol levels continued to rise in those fish that became or remained females, and no elevation in the concentration of 11-oxygenated androgens occurred in the plasma (Kime *et al.*, 1991). It was speculated that sex inversion could occur when the levels of ovarian 17β-estradiol secreted in the ovotestis reaches a threshold, dominant level, thus establishing a permanent female status (Kime *et al.*, 1991). Moreover, in these two species, 17β-estradiol treatments could trigger sex inversion (Chang and Lee, 1992; Kime *et al.*, 1991). All these data suggest that estrogens play an important role in the induction of the sex inversion process, at least in some protandrous fish. In *L. calcarifer*, the gonad is not an ovotestis as in protandrous sparids, and such an elevation of 17β-estradiol in male plasma during the reproductive cycle was not seen. However, gonadal concentrations of 17β-estradiol appeared very high as early as the very beginning of the inversion process. 17β-Estradiol treatment in males and transitional fish of the protogynous ricefield eel induced a complete degeneration of the testicular tissue (Tang *et al.*, 1974); Such also occurs in males of another protogynous fish, *Halichoeres poecilopterus* (Okada, 1964). High gonadal levels of 17β-estradiol observed in *L. calcarifer* in the early transitional stage could be responsible of the testicular tissue degeneration.

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