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Toledo, OH 43604

Final Report

Project title: Changes in plasma levels of free and conjugated 17,20β-dihydroxy-4-pregnen-3-one during final maturation of yellow perch (*Perca flavescens*)

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1. Experiment 1: Determination of the plasma sex steroid hormones in Lake Erie yellow perch

1.1 Material and Methods

Yellow perch were caught on April 4, 1998 in commercial trap nets from the Western basin of Lake Erie. Fish spent up to 96 hours in the trap nets at approximately 3-

feet depth (7°C). Blood was collected from the caudal vessel of males (n=17) and females (n=15) using a heparinized needle and syringe. Blood samples were transported on crushed ice to the laboratory, centrifuged at 1,500 x g for 15 min. and the plasma was frozen at -20°C until radioimmunoassay. Fish were killed immediately after blood sampling, transported on crushed ice to the laboratory and measured for total weight (W_T ± 0.1 g), total length (L_T ± 0.1 cm), gonad weight (W_G ± 0.1 g). Sperm from ten individual males was collected and transported on crushed ice to the laboratory. Sperm density and motility (percentage and duration) after activation were measured 24h after sampling as previously described by Ciereszko and Dabrowski (1993). Following extraction with diethyl ether, plasma levels of free steroids testosterone (T), estradiol-17β (E2), 11-ketotestosterone (11-kT) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) were determined by radioimmunoassays (RIA) similar to those used previously (Ottobre et al., 1989).

A2. Results

All males sampled were spermiating. The levels of plasma T and 11-kT were high (Table 1) in comparison to the period prior to spermiation (Ciereszko et al., 1997). The concentrations of both androgens did not differ significantly ($P>0.05$). A positive correlation was found between those two androgens ($r = 0.79$, $n = 10$, $P<0.01$). Low levels of 17,20βP were determined in the plasma of spermiating males. Sperm concentrations (Table 2) were similar to those reported in captive yellow perch (Dabrowski et al., 1996). Duration of sperm motility was short (< 1 min) as reported in Iberian perch (Lahnsteiner et al., 1995).

Among the fifteen females sampled, three had already spawned. GSI was significantly higher ($P < 0.01$) in preovulating females than the ones in the postspawning stage (Table 3). The plasma sex steroid levels were also significantly higher in preovulating females than in postspawning yellow perch. In preovulating females, the high levels of T indicated the completion of oocyte growth. As reported in captive yellow perch, the levels of 17,20βP were low in comparison to those reported in some salmonid fish (Scott et al., 1983; Fitzpatrick et al., 1986), but in the same range as the levels reported in other percid species (Sulistyo et al., 1998; Malison et al., 1994; Barry et al., 1995).

1.3 Conclusion

Steroid concentrations in feral yellow perch do not indicate any disturbances in the maturation process in this species. However, additional studies are required to address possible correlation between blood steroids and quality (viability) of yellow perch gametes.

Table 1. Weight, gonadosomatic index (GSI) and plasma levels of free steroids testosterone (T), 11-ketotestosterone (11-kT) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) in wild male yellow perch.

	Weight (g)	GSI (%)	T (ng/ml)	11-kT (ng/ml)	17,20βP (ng/ml)
Males (n=17)	97.4 ± 11.[illegible]	3.4 ± 1.3	9.54 ± 1.34	[illegible].5 ± [illegible].73	[illegible].14 ± [illegible]

Table 2. Sperm characteristics in wild male yellow perch.

Sperm characteristics	Male (n=10)
Concentration (10^9 spz/ml)	62.2 ± 3.9
Motility (%)	54 ± 2.2
Duration of motility (s)	57.7 ± 6.5

Table 3. Weight, gonadosomatic index (GSI) and plasma levels of free steroids testosterone (T), estradiol-17β (E2) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) in wild female yellow perch.

	Females	
Characteristics	Preovulating (n=12)	Postspawning (n=5)
Weight (g)	151.8 ± 16.5*	267.0 ± 46.5**
GSI (%)	23.2 ± 2.0	3.5 ± 0.9
T (ng/ml)	24.8 ± 7.9	0.56 ± 0.24
E2 (ng/ml)	0.32 ± 0.09	0.03 ± 0.01
17,20βP (ng/ml)	0.45 ± 0.15	0.006 ± 0.003

* With ovaries ** without ovaries

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2. <u>Experiment 2: Determination of the plasma sex steroid hormones following spontaneous or induced ovulation in yellow perch</u>

The manuscript presented in Appendix 1 was submitted for publication to "Aquaculture" (March 2001).

Appendix 1

PLASMA STEROID LEVELS FOLLOWING SPONTANEOUS OR INDUCED OVULATION IN YELLOW PERCH *PERCA FLAVECENS*

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Abstract

This study examined the short-term changes in plasma sex steroids during natural (Experiment 1) and induced (Experiment 2) final maturation in out-of-season yellow perch *Perca flavescens*. In experiment 1, twenty female yellow perch were checked daily for ovulation. Ovulating yellow perch were stripped of eggs. Eggs from individual females were weighed and fertilized. Fertilization rate was determined at the embryonic eyed stage. Immediately after stripping, blood samples were collected. Plasma levels of testosterone (T), estradiol-17β (E2), 11-ketotestosterone (11-kT) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) were determined by radioimmunoassays (RIA). In experiment 2, eight female yellow perch per group were individually tagged and randomly assigned to one of the following treatment groups: (1) saline (0.7% NaCl), (2) des-Gly^{10}[D-Ala^{6}] LHRH-ethylamide (100 µg LHRHa/kg), and (3) LHRHa plus 17,20βP (1 µg LHRHa/kg + 2 mg 17,20βP/kg). Fish were injected intraperitoneally with two doses at a two-day interval. Blood samples were collected prior to injections and after ovulation/spawning. Plasma levels of T, E2, 11-kT and 17,20βP (free and conjugated) were determined by RIA. In experiment 1, low levels of 17,20βP, the maturational steroid inducing *in vitro* final maturation or ovulation in yellow perch, were recorded at spawning. In experiment 2, all fish injected with LHRHa released their eggs spontaneously during the week following injections. No control fish ovulated during this period, whereas only one injected with LHRHa + 17,20βP released eggs. In the control group, concentrations of E2 and 17,20βP did not show significant differences over the experimental period, whereas plasma T levels increased significantly indicating the completion of oocyte growth. In fish injected either with LHRHa or LHRHa + 17,20βP, the concentration of T or 17,20βP increased significantly after the first injection. Both T and free17,20βP significantly

declined in circulation after ovulation/spawning. Thus, it appears that LHRHa alone is highly effective in inducing gonadotropin II release and ovulation in yellow perch. Administration of 17,20β-P with LHRHa interfered with the action of LHRHa. Moreover, it appears that 17,20β-P was rapidly conjugated to its sulfated form.

Key words: reproduction, yellow perch, *Perca flavescens*, steroid

Introduction

In teleost fish, oocyte maturation is regulated by a maturation-inducing hormone (MIH) that is under control of the pituitary gonadotropins (specifically GtH_2). Two steroids, the 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and the 17,20β,21-trihydroxy-4-pregnen-3-one (20βS) have been identified as MIH (for review see Nagahama, 1987, Thomas, 1994). However, in some species such as the Indian catfish *Heteropneustes fossilis* (Goswami and Sundararaj, 1974) and the zebrafish *Brachydanio rerio* (Van Ree et al., 1977), it appears that the corticosteroids are the most effective steroids to induce *in vitro* the germinal vesicle breakdown (GVBD).

In yellow perch, *Perca flavescens*, *in vitro* incubation studies have demonstrated that 17,20βP was the most potent steroid inducing final oocyte maturation (Goetz and Theofan, 1979). There is also evidence that yellow perch ovarian tissue is able to convert progesterone or pregnenolone to 17,20βP (Theofan and Goetz, 1983). Ciereszko et al. (1997a) measured the levels of plasma estradiol-17β (E2) and testosterone (T) to describe the reproductive cycle of female yellow perch. This work did not include the pre-ovulatory period when the major changes in the level of MIH take place. To the best of our knowledge, there are no published data on the 17,20βP plasma concentration in this species. In two closely related percid species, the Eurasian perch *Perca fluviatilis* (Sulistyo et al., 1998) and the walleye *Stizostedion vitreum* (Malison et al., 1984; Barry et al., 1985), the levels of 17,20βP were low during the spawning season in comparison to those reported in salmonid species (< 1 ng/ml vs. 100-800 ng/ml). The authors of these studies suggested that percids require low levels of 17,20βP to induce final maturation and/or they rapidly metabolized this steroid. Therefore, the first objective of the present study (Experiment 1) was to

determine the short-term changes of plasma free 17,20βP during the spontaneous final maturation and ovulation of yellow perch. Levels of free E2 and testosterone T also were measured.

The second objective of this study (Experiment 2) was to induce the final maturation and ovulation in female yellow perch using des-Gly^{10},[D-Ala^{6}]-luteinizing hormone releasing hormone analogue (LHRHa) and LHRHa in combination with 17,20βP and to evaluate the effects of these hormonal preparations on the concentration of plasma sex steroids. Such information is critical for understanding the physiological mechanisms regulating these hormonal events and successful spawning of this species in controlled conditions. LHRHa was previously successfully used to induce ovulation in yellow perch (Dabrowski et al., 1994, Ciereszko et al., 1997a). Therefore, we hypothesized that, if 17,20βP is the MIH in this species, its addition may improve the effectiveness of the treatment. In this experiment, we also measured the level of conjugated (sulfated and glucuronidated) 17,20βP to determine if this steroid is rapidly metabolized in yellow perch.

Materials and Methods

Experimental fish

Three year-old yellow perch were spawned out-of-season for both experiments. Induction of out-of-season yellow perch by photothermal manipulations was described by Kolkovski and Dabrowski (1998). Briefly, yellow perch broodstock was exposed to high photoperiod (15 h light) and temperature (20°C) until January 1998. Then, the photothermal conditions were decreased gradually until April to reach 10 h light and 10°C, respectively. The chill period (occurring naturally from December to February) was maintained from April to June and was followed by a

gradual increase of photoperiod and temperature. During the photothermal manipulations, fish were fed with a commercial diet (Biodry 4000, Bioproducts Inc., Warrenton, OR). The maturity of fish was checked prior to both experiments. Four male (average body weight: 87.5 ± 10.3 g) and ten female (average body weight: 160.9 ± 5.8 g) yellow perch were anesthetized in a MS-222 solution (0.5 g/10 L) and weighed. Blood, collected from the caudal vessel with a heparinized syringe, was centrifuged at 1,500 g for 15 min at 4°C, and plasma was stored at -20°C until radioimmunoassays. After blood sampling, a number of females were killed and dissected. Ovaries were removed, weighed and gonadosomatic index (GSI) was determined as follows: GSI (%) = (GW x 100)/BW, where GW = gonad weight (g) and BW = total body weight (g).

Experiment 1: Levels of plasma sex steroids and fertilization rate after spontaneous ovulation

In September 1998, twenty females (average body weight: 134.0 ± 28.2 g) with round abdomens and ten spermiating males (average body weight: 89.0 ± 5.2 g) of out-of-season yellow perch were transferred from the Piketon Research and Extension Center (Piketon, OH) to the aquaculture laboratory at The Ohio State University (Columbus, OH). Fish were maintained in a recirculating water system at 10-12°C and exposed to a photoperiod of 14 h light/10 h dark. Females were checked daily for ripeness by examining the genital papilla and pressing the abdomen. Ovulating yellow perch were stripped of eggs. Ovulation occurred between September 21 and December 8, 1998, seven to four months before natural spawning. Eggs from an individual female were weighed, fertilized with pooled semen from three to four males and incubated in separate 5L-McDonald jars provided with a continuous water flow at 10°C. Fertilization rate was determined at the embryonic eyed stage (approximately 10 days) immediately after stripping the

females blood was sampled as previously described. Pseudo-gonadosomatic index (Pseudo-GSI) was estimated by dividing the weight of stripped eggs by the total body weight.

Experiment 2: Levels of plasma sex steroids after induced ovulation

This experiment was performed at the Piketon Research and Extension Center. Out-of-season yellow perch (average body weight: 181.3 ± 36.2 g) were maintained in a recirculating water system at 10-12°C and submitted to 14 h light. Twenty-four females were anesthetized in a MS-222 solution (0.5 g/10 L), weighed, and individually tagged for identification (Floy® Tag & Mfg. Inc., Seattle, WA). Eight females were randomly assigned to each of the following treatments groups: (1) saline (0.7% NaCl), (2) des-Gly^{10} [D-Ala^{6}] LHRH-ethylamide (100 µg LHRHa/kg), and (3) LHRHa plus 17,20β-dihydroxy-4-pregnen-3-one (100 µg LHRHa/kg + 2 mg/17,20β(P)/kg). LHRHa (Sigma, St. Louis, MO) was dissolved in 0.7% of NaCl whereas 17,20β(P) (Steroloids, Wilton, NH) was dissolved in ethanol and diluted 10 times in physiological saline. Fish were injected intraperitoneally with two doses at a two-day interval (August 29 and 31, 1999). Control fish were injected with the same volume of saline (0.2 ml per fish). Blood samples were collected prior to each injection and after ovulation or spawning as described previously.

Steroid analysis

[11,2,6,7-^{3}H]testosterone (96.5 Ci/mmol) and [2,4,6,7,16,17-^{3}H]estradiol (141 Ci/mmol) were purchased from New England Nuclear Life Science Products (Boston, MA). [2,4,6,7-^{3}H]-11-keto-testosterone (105 Ci/mmol) was purchased from Amersham Pharmacia Biotech

(Arlington Heights, IL). [^{3}H]17,20β-dihydroxy-4-pregnen-3-one was a gift from Dr. A. Fostier (INRA, Rennes, France). Unlabelled steroids were purchased from ICN Pharmaceuticals (Costa Mesa, CA), Sigma and Steraloids. The testosterone antiserum was provided by Dr. R.E. Ciereszko (University of Agriculture and Technology, Olsztyn, Poland), the estradiol-17β antiserum by Dr. R.L. Butcher (West Virginia University, WV), the 11-ketotestosterone antiserum by Dr. D.E. Kime (University of Sheffield, United Kingdom), and the 17,20β-dihydroxy-4-pregnen-3-one antiserum by Dr. A. Fostier. The characteristics of these antisera have been reported previously (Dabrowski et al., 1995, Butcher et al., 1974, Kime and Manning, 1982 and Fostier and Jalabert, 1986, respectively).

Following extraction with diethyl ether, plasma levels of free steroids testosterone (T), estradiol-17β (E2), 11-ketotestosterone (11-kT) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) were determined by radioimmunoassays (RIA) similar to those used previously (Ottobre et al., 1989). In experiment 2, levels of conjugated (sulfated and glucuronidated) 17,20βP were extracted from the plasma according to the procedure described by Scott and Canario (1992). Briefly, a 50 µl aliquot of plasma was vortexed vigorously with 4 ml diethyl ether. The bottom (plasma) layer was then frozen and the diethyl ether layer containing the free steroids was poured off and evaporated under a stream of nitrogen at 45°C. The plasma was dried under a stream of nitrogen at 45°C, redissolved in 25 µl of distilled water, incubated overnight at 45°C with 4 ml of trifluoroacetic acid/ethyl acetate (1:4 100, v/v) for acid solvolysis of sulfates, and the solvents evaporated under a stream of nitrogen at 45°C. The residue was redissolved in 500 µl sodium acetate buffer (pH 5.5) and the free steroids extracted with 4 ml diethyl ether. The residues were treated overnight with 2[illegible] µl of snail juice at 37°C for enzymatic hydrolysis of glucuronides and the free steroids extracted with 4 ml of diethyl ether as described above.

Assay characteristics for T, E2 and 11-kT were described previously (Ciereszko et al., 1997a and b). The within- and between-assay coefficients of variation were 1.8% and 5% for 17,20ºP, respectively. Sensitivity of the assay was 2 pg per tube. The coefficient of correlation between added and determined amounts of cold hormone was 0.996. The percentage of recovery of extraction was 73 ± 5%. Serial dilution of plasma samples showed parallelism with the standard curves.

Results

Control of the fish maturity prior to both experiments

Fifteen days before the beginning of both experiments, mean GSI of female yellow perch (n = 8) was 14.4 ± 2.9%, whereas the concentrations of plasma E2 and T reached 1.3 ± 0.2 ng/ml and 2.3 ± 0.6 ng/ml, respectively. Plasma levels of E2 were positively correlated with plasma levels of T (r = 0.71, n = 8, $P < 0.01$).

All males were spermiating. The levels of plasma 11-kT were significantly ($P < 0.05$) higher than T (8.1 ± 2.9 ng/ml vs. 2.9 ± 0.8 ng/ml, n = 4) and a positive correlation was found between those two androgens (r = 0.94, n = 4, $P < 0.01$). The levels of free 17,20ºP were significantly higher ($P < 0.01$) in males than in females (596 ± 2.7 pg/ml vs. 7 ± 4 pg/ml).

Experiment 1: Levels of plasma sex steroids and fertilization rate after spontaneous ovulation

Pseudo-GSI in spawning yellow perch varied from 13.1 to 31.8%. We were able to classify females into 4 categories according to the pseudo-GSI: <20%, 20 to 25%, 25 to 30% and >30%. Although the fertilization rate increased with the increase of the pseudo-GSI (Figure 1), no significant differences ($P > 0.05$) were found among groups. The concentrations of plasma sex steroids were low at spawning regardless of the pseudo-GSI category. Moreover, no significant differences ($P > 0.05$) were found among pseudo-GSI categories (Figure 1). At spawning, the levels of E2 and T dropped significantly ($P < 0.01$) to 0.12 ± 0.03 ng/ml and 0.35 ± 0.09 ng/ml, respectively, in comparison to those reported prior to the experiment. Although 17,20βP is the most important steroid to induce *in vitro* final maturation and ovulation in yellow perch (Goetz and Theofan, 1979), the concentrations of this steroid remained low (27 ± 7 pg/ml) at spawning relative to those reported in some salmonid fish.

Experiment 2: Levels of plasma sex steroids after induced ovulation

Mortality reached 62.5% in the LHRHa + 17,20βP group, whereas it amounted to 37.5% in the control and LHRHa groups. No mortality was recorded between the two injections. All fish injected with LHRHa released their eggs spontaneously during the week following injections. No control fish ovulated during this period, whereas only one injected with LHRHa + 17,20βP released eggs. The first ovulation occurred 4 days following injections in the LHRHa + 17,20βP group and in 5 days in the LHRHa group. In the control group, concentrations of E2 and free 17,20βP did not show significant differences over the experimental period, whereas plasma T

levels increased significantly ($P<0.01$) (Figure 2). In fish injected either with LHRHa or LHRHa + 17,20β-P, the concentration of T and free 17,20β-P increased significantly ($P<0.01$) after the first injection. However, the levels of free 17,20β-P were significantly higher ($P<0.01$) in fish injected with LHRHa + 17,20β-P than with LHRHa. Both T and free 17,20β-P were almost completely removed from circulation at the time of ovulation or spawning (Figure 2). The concentration of E2 increased after the first injection of LHRHa whereas it significantly ($P<0.01$) decreased in fish injected with LHRHa + 17,20β-P. In the LHRHa group, the levels of E2 significantly decreased ($P<0.01$) after the ovulation or spawning.

The levels of free and conjugated (sulfated and glucuronidated) 17,20β-P are presented in Figure 3. Over the experimental period, the sulfated 17,20β-P was the major form in the control and LHRHa group, ranging from 77 and 83% of the total 17,20β-P in control group and from 81 to 92% of the total 17,20β-P in the LHRHa group. In the LHRHa + 17,20β-P group, the sulfated form dominated prior to the first injection, representing 81% of the total. Prior to the second injection, the free form accounted for 58%, whereas the sulfated form to 37%. After the ovulation or spawning the percentage of free and sulfated of 17,20β-P were similar (51 and 46%, respectively). In the three groups, the concentrations of glucuronidated 17,20β-P were low, ranging from 1 to 5.5% of the total. In the control group, concentrations of free, sulfated and glucuronidated 17,20β-P did not show significant ($P>0.05$) differences over the experimental period. In the LHRHa and LHRHa + 17,20β-P groups, the free and sulfated 17,20β-P increased significantly ($P<0.01$) after the first injection. Levels in both groups dropped significantly ($P<0.01$) once the ovulation or spawning occurred.

Discussion

This study describes the endocrine changes associated with spontaneous or induced ovulation in female yellow perch. In experiment 1, the levels of both T and E2 significantly decreased at the time of the spawning as reported in previous studies on yellow perch (Ciereszko et al., 1997a) and other teleost fish (Wingfield and Grimm, 1977; Scott et al., 1984; Pankhurst and Conroy, 1987; Berlinsky and Specker, 1991; King et al., 1994). Treatment with LHRHa (Experiment 2) induced a significant rise of plasma T and 17,20βP. Patterns were similar to those reported in naturally or induced ovulating fish (Scott et al., 1980; Van der Kraak et al., 1984). As in experiment 1, the plasma sex steroid levels in fish injected with LHRHa significantly decreased once the eggs were released. The concentrations of T and E2 reached during spontaneous or induced ovulation were similar to the ones reported previously by Ciereszko *et al.* (1997a). In teleost fish, the drop of plasma E2 and T is associated with the ovulatory gonadotropin (GtH) surge and the shift in the steroidogenic pathway from C_{18} and C_{19} to C_{21} steroids (Nagahama et al., 1994).

Interestingly, the concentrations of free 17,20βP (C_{21} steroid) measured for the first time in the plasma of yellow perch ovulating spontaneously remained low in comparison to those reported prior to the spawning. In contrast, injections of LHRHa or LHRHa + 17,20βP induced a significant increase of the plasma 17,20βP, in both free and sulfated forms. Nonetheless, the levels reached were low in comparison to those reported in salmonids (Scott et al., 1983; Fitzpatrick et al., 1986), but were similar to those reported in two other percid species, the Eurasian perch (Salisbury et al., 1998) and the walleye *Stizostedion vitreum* (Malison et al., 1994; Barry et al., 1995). Previous investigations have shown that *in vitro* 17,20βP induced ovulation through the

production of prostagiandin in yellow perch (Goetz, 1979; Goetz and Garczynski, 1997). These processes took place only if the mature follicles were not isolated from the extrafollicular tissue prior to the incubation (Berndston et al., 1980; Goetz, 1997). Therefore, low concentrations of free 17,20β-P may be attributed to the paracrin character of this steroid. Moreover, the presence of high levels of sulfated 17,20β-P prior to and after the hormonal treatment indicated that 17,20β-P is rapidly metabolized to conjugated esters in yellow perch. The sulphated steroid may act as a spawning pheromone as reported in the goldfish (*Carassius auratus*) (Sorensen et al., 1995).

Among perciformes order, the final maturation and ovulation of studied species are mediated either by 20β-S or both 20β-S and 17,20β-P. The 20β-S was identified as the MIH in two species of the family Sciaenidae, the spotted seatrout (*Cynoscion nebulosus*) (Thomas and Trant, 1989) and the Atlantic croaker *Micropogonias undulatus* (Trant et al., 1986). In contrast, both 20β-S and 17,20β-P were detected in the plasma of two Perchichthyidae species, the striped bass *Morone saxatilis* and the white bass *M. chrysops* and the levels of both steroids significantly increased during GVBD (Berlinsky and Specker, 1996; King et al., 1994; Mylonas et al., 1994). To our knowledge, no attempts were made to test whether 20β-S is equipotent to 17,20β-P for inducing *in vitro* GVBD and ovulation of yellow perch oocytes. However, even though 20β-S can induce *in vitro* final maturation, it does not mean that this steroid is the MIH in this species. Thus, although 20β-S induced *in vitro* GVBD in plaice *Pleuronectes platessa* and dab *Limanda limanda* oocytes, this steroid was not detected *in vivo* in the plasma of these species during final maturation (Canario and Scott, 1987; Scott and Canario, 1987). The identification of two receptors, one for 20β-S and one for 17,20β-P in the ovary of spotted seatrout (Parino and Thomas, 1996; Pinter and Thomas, 1995) suggested that both steroids may play distinct roles in the final maturation and ovulation processes in these species. Therefore, we cannot reject the possibility that in yellow

perch the final oocyte maturation may be regulated by both 17,20βP and 20βS as suggested in striped bass (King et al., 1994), bamboolear wrasse *Pseudolabrus japonicus* (Matsuyama et al., 1995), and white bass (Mylonas et al., 1999). In this case, the failure to detect high levels of 17,20βP in plasma of yellow perch during ovulation will not only be related to its parasitic character and its conjugation but also to its utilization as substrate in the production of 20βS. Nonetheless, further studies are required (1) to assess the presence of 20βS in the yellow perch plasma, (2) to investigate the specific role of this steroid if detected.

In this study, we also investigated the effect of different hormonal treatments on the ovulation of yellow perch. LHRHa was successfully used to induce ovulation in numerous teleost fish (for review see Crim et al., 1987; Redding and Patino, 1993). After the first injection of LHRHa (at a dose of 100 µg/kg) or LHRHa (100 µg/kg) + 17,20βP (2 mg/kg), the plasma levels of T and 17,20βP significantly increased indicating that the eggs were ready to ovulate. Thus, it appears that LHRHa is highly effective in inducing GtH_2 release. However, only one female ovulated among the fish injected with LHRHa + 17,20βP. Administration of 17,20βP with LHRHa interfered with the action of LHRHa, probably by negative feedback on the GtH_2 production.

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Figures

Figure 1 Plasma levels of estradiol-17β (E2), testosterone (T) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and fertilization rate regardless of pseudogonadosomatic index (Pseudo-GSI) in female yellow perch after spontaneous ovulation.

Figure 2 Effects of NaCl, LHRHa and LHRHa+17,20βP injections on plasma sex steroids of female yellow perch prior to injections and after ovulation/spawning. Bars within treatment indicated by the same letter are not significantly different from each other at P<0.05.

Figure 3 Plasma levels of free and conjugated (sulfated and glucuronidated) 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) in female yellow perch prior to injections and after ovulation/spawning. Bars within treatment indicated by the same letter are not significantly different from each other at P<0.05.

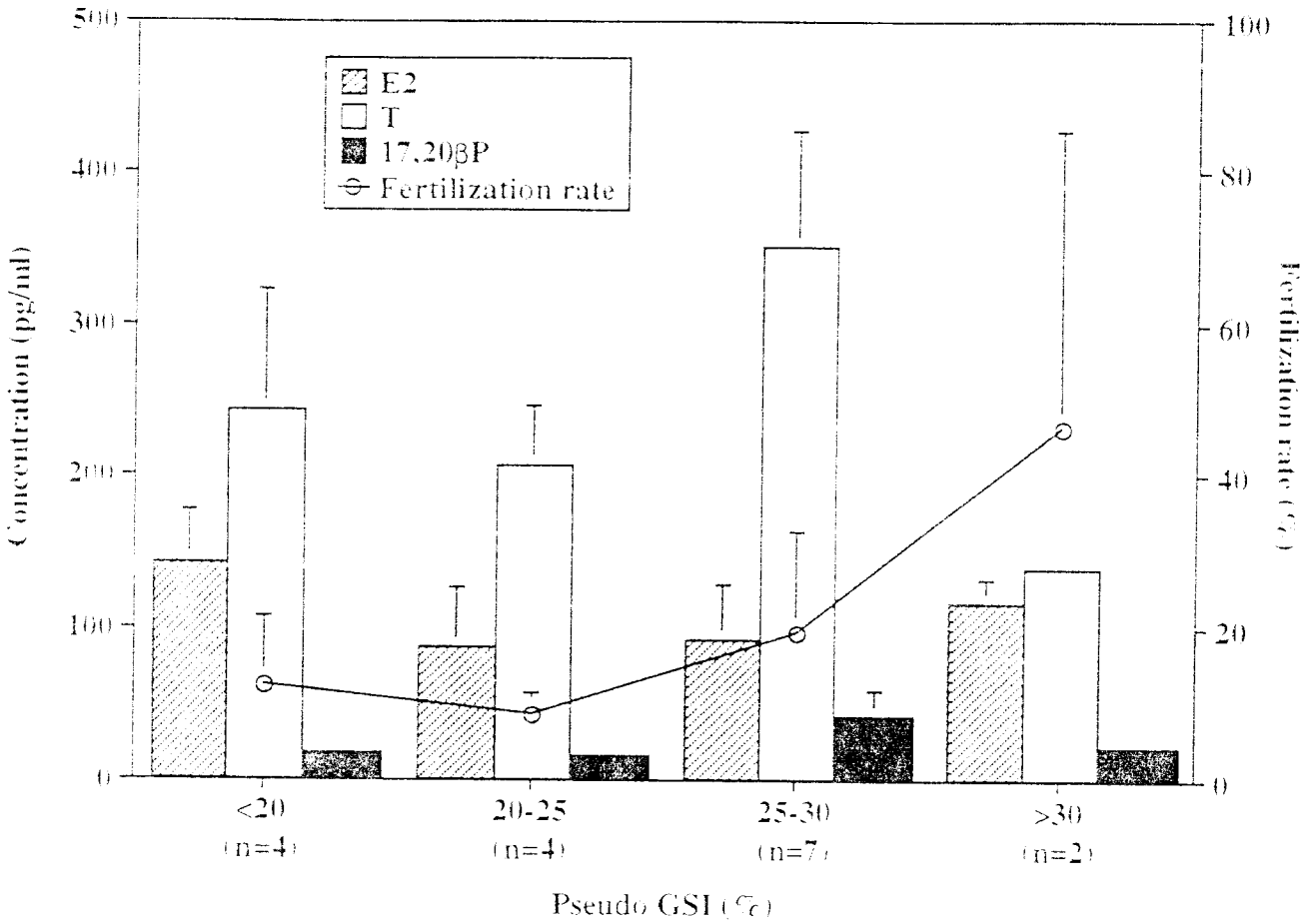
E2
T
17,20βP
Fertilization rate
Concentration (pg/ml)
Fertilization rate (%)
500
400
300
200
100
0
100
80
60
40
20
0
<20
(n=4)
20-25
(n=4)
25-30
(n=7)
>30
(n=2)
Pseudo GSI (%)

Figure 1

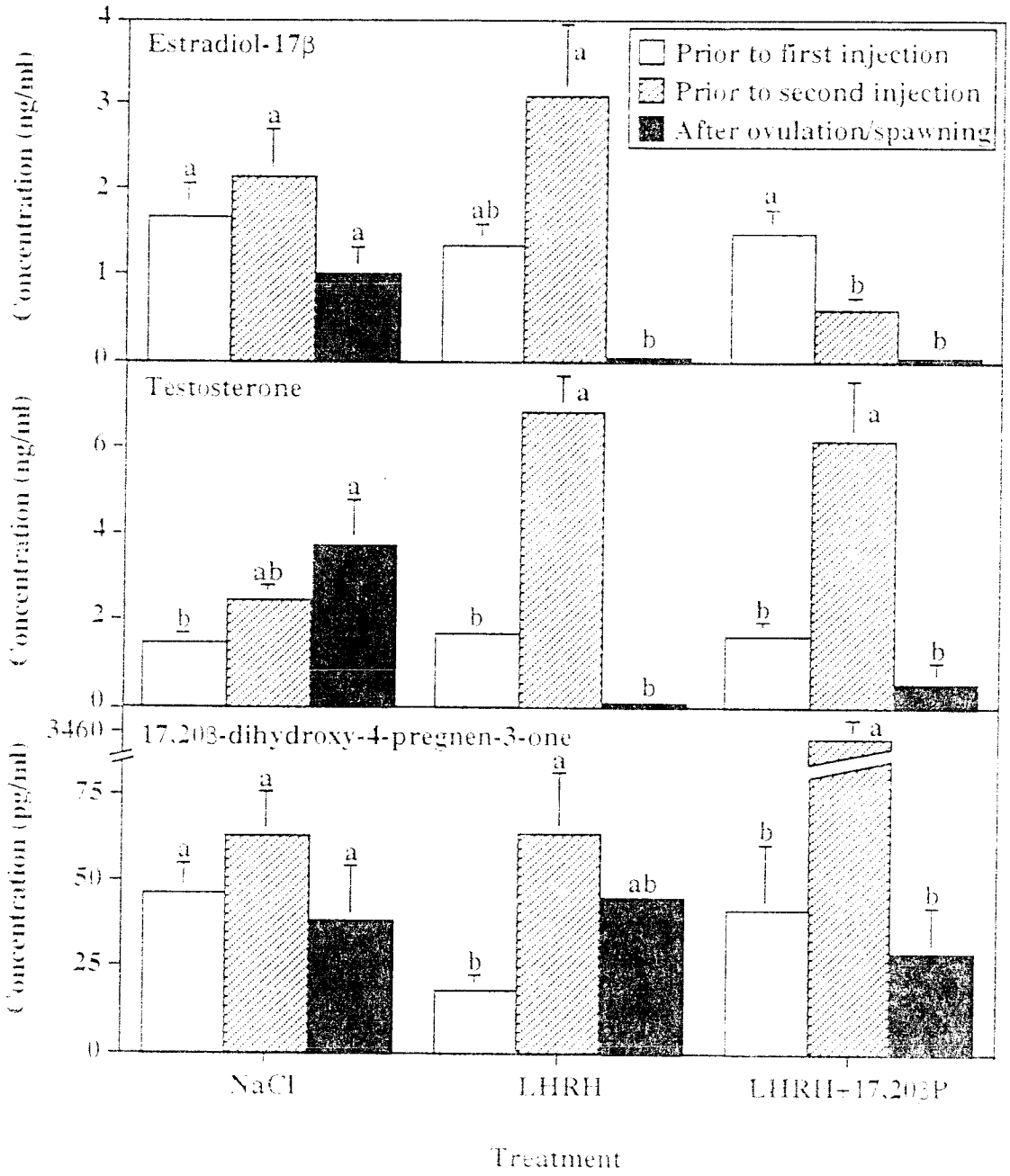
Estradiol-17β
Prior to first injection
Prior to second injection
After ovulation/spawning
Concentration (ng/ml)
Testosterone
Concentration (ng/ml)
17,20β-dihydroxy-4-pregnen-3-one
Concentration (pg/ml)
NaCl
LHRH
LHRH+17,20βP
Treatment

Figure 2

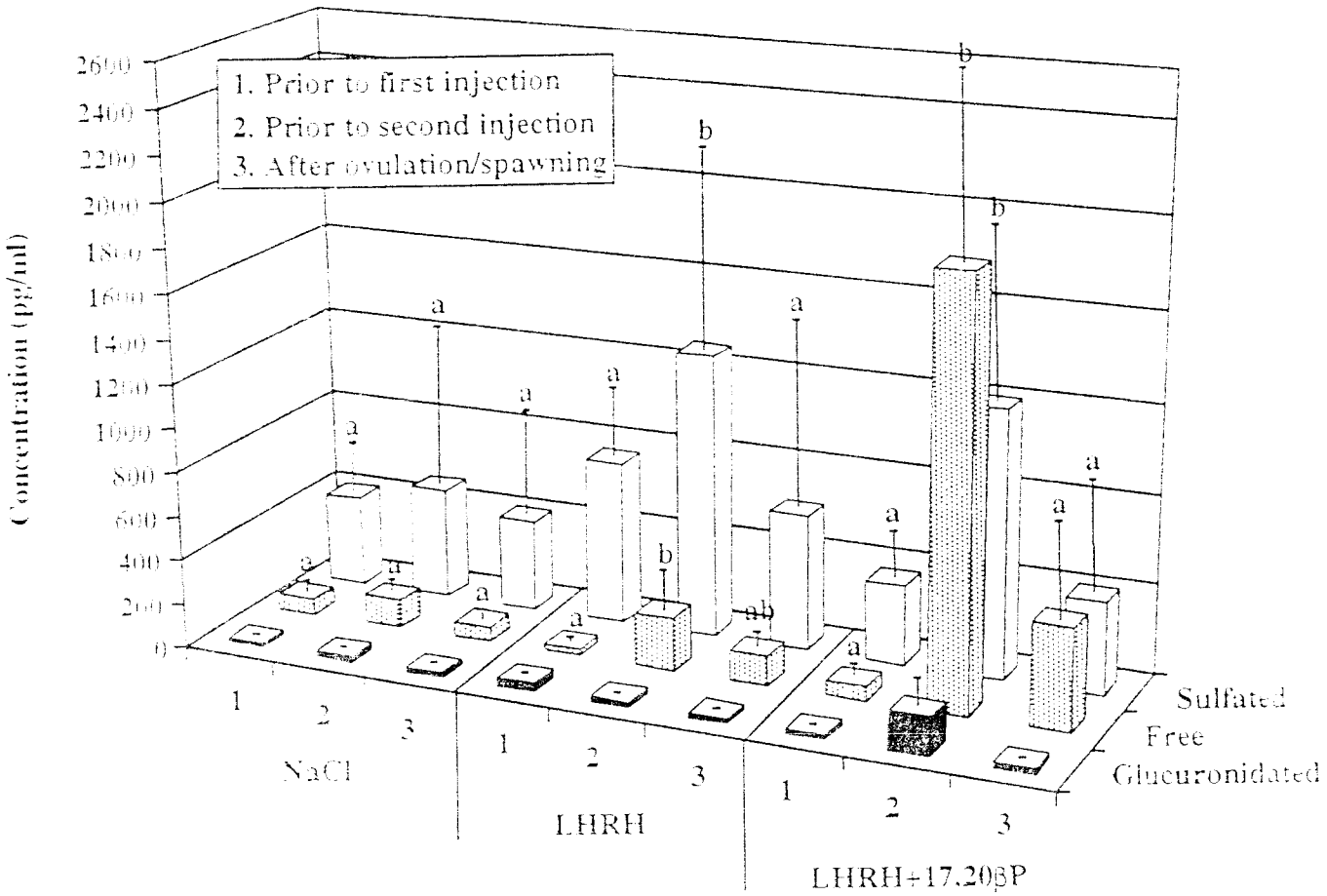
1. Prior to first injection
2. Prior to second injection
3. After ovulation/spawning
Concentration (pg/ml)
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
NaCl
LHRH
LHRH+17,20βP
Sulfated
Free
Glucuronidated

Figure 3