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BROODSTOCK DIETS AND SPAWNING OF *COLOSSOMA MACROPOMUM* AND/OR *PIARACTUS BRACHYPOMUS*

Tenth Work Plan, Feeds and Fertilizers Research 2 (10FFR2)
Final Report

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ABSTRACT

Hormonal treatments were used to induce final maturation, ovulation or spermation in male and female gamitana (*Colossoma macropomum)* and pacu (*Piaractus brachypomus)*. Plasma sex steroid hormone levels were also assessed prior to and after the hormonal treatments to better understand the process of induced spermiation or ovulation. The results of this study indicated that male gamitana and pacu responded positively to the hormonal treatments. In 2001, the concentrations of plasma sex steroids (estradiol-17β and testosterone) significantly increased after the hormonal injections whereas the levels of 11-ketotestoterone remained constant. In contrast, female gamitana (2001 and 2002) and pacu (2002) did not undergo final maturation/ovulation. The increase of estradiol-17β (E2), as well as the non-detection of 17,20β-dihydroxy-4-pregnen-3-one (17,20 βP) indicated that females were not ready to spawn when they were injected.

INTRODUCTION

Gamitana (*Colossoma macropomum*), pacu (*Piaractus brachypomus*) (Amazon drainage) and red belly pacu (*P. mesopotamicus*) (Parana River drainage) are becoming important species for aquaculture in South America (Saint-Paul, 1992; Roubach et al., 2003). Moreover, *C. macropomum* has been reported by the US Department of Agriculture (Situation and Outlook Report, USDA 1993) as a species of potential importance to US aquaculture.

Studies on artificial reproduction of pacu clearly indicated that this species has a synchronous type of oocyte development (Godinho and Gohindo, 1986; Romagosa et al., 1990). Spermiation and ovulation were induced with either human chorionic gonadotropin (hCG) or carp pituitary extracts (CPE). However, the success of final maturation and quality of the gametes obtained varied depend on treatment, and possibly of fish size. Females of 1.4 and 1.8 kg were used in the previously mentioned studies, however an increase of size to 3.5 ± 0.8 kg increased the rate of egg production and fertilization to 96% and 82%, respectively (Zaniboni Filho and Barbosa, 1996). Only recently the first data on steroid concentrations in blood plasma of pacu became available (Gazola et al., 1996; Gazola and Borella, 1997). This information in association with induced spawning can considerably improve the understanding of the final maturation and possibly allow the controlled reproduction of this species outside of their range of distribution.

Therefore, the objective of this study was to compare spawning performance and annual cycles of blood plasma steroid concentrations for *Colossoma* and/or *Piaractus* broodstock.

METHODS AND MATERIALS

On 13 November 2001, gamitana (*Colossoma macropomum)* were obtained from a stock of mature fish raised in a 1.2 ha pond (10C) at the Instituto de Investigaciones de la Amazonia Peruana (IIAP). Average weights (mean ± SD) of males and females were 6.82 ± 0.81 kg and 9.34 ± 0.89 kg, respectively. Four pairs of gamitana were moved into concrete indoor 0.75 m^3 tanks. In each tank, the male was separated from the female by a net. Both genders were injected with two doses of Conceptual® (luteinizing hormone releasing hormone analog, LHRHa). The concentration of preparation was 0.0042 mg of equivalents of active hormone per ml. Males and females were injected intraperitoneally with 1 ml kg^{-1} and 2.6 ml kg^{-1}, respectively. The priming dose (50% and 10% in males and females, respectively) was administered in the morning, whereas the resolving dose (50% and 90% in males and females, respectively) was injected at 20:00 h.

Blood was collected from the caudal vessel of unanaesthetized fish prior to the priming injection and two days after the injections using heparinized syringe. Blood samples were centrifuged at 1,500 g for 5 min and the resulting plasma was stored at –20°C until assays. The plasma concentrations of steroids (testosterone, estradiol-17β, 11-ketotestosterone and 17,20β-dihydroxy-4-pregnen-3-one) were measured by radioimmunoassay similar to those used previously (Ottobre et al., 1989) following ethyl-ether extraction. [1,2,6,7-^{3}H]testosterone (96.5 Ci/mmol) and [2,4,6,7,16,17-^{3}H]estradiol (141 Ci/mmol) were purchased from NEN Life Science Products (Boston, MA, USA). [^{3}H]11-ketotestosterone (11 kT 105 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Arlington Heights, IL, USA). [^{3}H]17,20β-dihydroxy-4-pregnen-3-one was a gift

Table 1. Radioimmunoassay characteristics of steroid hormones in *Colossoma macropomum*. T = testosterone, E2 = estradiol-17β, 11-kT = 11-ketotestosterone, and 17,20βP = 17,20β-dihydroxy-4-pregnen-3-one

Characteristics	T	E2	11-kT	17,20βP
Within-Assay Coefficient of Variation (%) (n = 6)	1.7	2.0	2.5	2
Between-Assay Coefficient of Variation (%) (n = 3)	9.2	3.9	6.1	5.9
Accuracy (Coefficient of Determination)	0.988	0.995	0.984	0.983
Sensitivity (pg ml^{-1})	2	1	2	1
Recovery of Extraction (%)	85.8	89.7	91.9	98.6

from Dr. A. Fostier (INRA, Rennes, France). Unlabelled steroids were purchased from ICN Pharmaceuticals (Costa Mesa, CA, USA), Sigma (St. Louis, MO, USA) and Steraloids (Wilton, NH, USA). The testosterone antiserum was provided by the Institute of Animal Physiology (University of Agriculture and Technology, Olsztyn, Poland), the estradiol-17β antiserum by Dr. R.L. Butcher (West Virginia University, WV, USA), 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; Fostier and Jalabert, 1986, respectively). The assay characteristics are shown in Table 1. Extraction blanks were below sensitivity of the assay for examined hormones and serial dilutions of plasma samples showed parallelism with the standard curve between 25 and 100 μL.

On 16 April 2002, ten *C. macropomum* were obtained from a broodstock raised in a 1.2 ha pond (10°C) at the IIAP. Average weights (mean ± SD) were 7.20 ± 2.15 kg. Blood samples were taken from the caudal vessel into a heparinized syringue, kept

Table 2. Plasma sex steroid hormones of male and female *Colossoma macropomum* before and after hormonal treatments. T = testosterone, E2 = estradiol-17β, 11-kT = 11-ketotestosterone, 17,20βP = 17,20β-dihydroxy-4-pregnen-3-one, and nd = not detected. For each sex, means within the same column with different letters are significantly different ($P < 0.01$).

	Plasma Sex Steroids			
	T (*pg ml^{-1}*)	*E2* (*pg ml^{-1}*)	*11-kT* (*pg ml^{-1}*)	*17,20βP* (*pg ml^{-1}*)
MALE (N = 4)				
Before Treatment	192 ± 74[a]	99 ± 75[a]	3,128 ± 1,132[a]	nd
After Treatment	815 ± 503[b]	618 ± 574[b]	3,574 ± 2,242[a]	nd
FEMALE (N = 4)				
Before Treatment	255 ± 137[a]	566 ± 183[a]	895 ± 1,111[a]	nd
After Treatment	296 ± 262[a]	2,235 ± 3,185[b]	782 ± 387[a]	nd

Table 3. Concentrations of Plasma sex steroid hormones of *Colossoma macropomum* collected on 16 April 2002. T = testosterone, E2 = estradiol-17β, 11-kT = 11-ketotestosterone, 17,20βP = 17,20β-dihydroxy-4-pregnen-3-one, and nd = not detected.

Fish	T (*pg ml^{-1}*)	E2 (*pg ml^{-1}*)	11-kT (*pg ml^{-1}*)	17,20βP (*pg ml^{-1}*)
1	119	130	274	nd
2	117	nd	538	nd
3	701	144	298	nd
4	118	163	75	nd
5	202	9	226	nd
6	677	242	651	nd
7	195	60	217	nd
8	241	57	1,938	nd
9	207	145	764	nd
10	58	nd	246	nd
	263 ± 231	119 ± 73	523 ± 542	nd

on the ice and then centrifuged at 1,500 g for 15 min and the resulting plasma was stored at –20ºC until assays. The plasma concentrations of steroids (testosterone, estradiol-17β, and 11-ketotestosterone) were measured by radioimmunoassay as described previously.

In October and November 2002 two experiments were carried out with breeders to address the problem of artificial reproduction of gamitana (four females and four males) and pacu (*P. brachypomus*) (three females and three males). Both species were injected with LHRH-analog and blood samples were collected for steroid hormone analysis prior to stimulation and 24–48 h after injection.

RESULTS

In November 2001, females did not respond to the hormonal treatment and no gametes were collected. Plasma sex steroid hormones prior to and after the injections are reported in Table 2. Following hormonal treatment, the concentrations of T and E2 in males significantly increased whereas only E2 significantly increased in females (Table 2). The concentrations of plasma sex steroid hormones including testosterone, estradiol-17β, 11-ketotestosterone, and 17,20β-dihydroxy-4-pregnen-3-one are summarized in Table 3.

In October through November 2002, males from both species provided copious amounts of sperm following injection (8–11 ml per fish) but no ovulation was recorded in females. Blood hormones will be analyzed and should provide an explanation of the status of maturation of gonads in comparison to our previous results from successful reproduction of pacu in 1999 (Dabrowski et al., unpubl.).

DISCUSSION

The results of this study indicated that male gamitana responded positively to the hormonal treatments. In 2001, the concentrations of plasma sex steroids (E2 and T) significantly increased after the hormonal injections whereas the levels of 11-kT remained constant. The levels of steroids measured in the present study were higher than the levels reported by Gazola and Borella (1997) in *P. mesopotamicus*, but similar to those reported by Dabrowski et al. (unpubl.) in *P. brachypomus*.

Although the LHRHa hormonal treatment was used previously to induce final maturation and ovulation in female *P. brachypomus* (Dabrowski et al., unpubl.), females of gamitana (2001 and 2002) and pacu (2002) did not undergo final maturation or ovulation. In females, the time of ovulation is characterized by an increase of plasma levels of T associated with the drop of E2 reflecting the decrease of the aromatase activity in the ovary (Fostier et al., 1983). In 2001, we observed a significant increase of E2 after the hormonal treatment indicated that the females were not ready to spawn and that they were still in the vitellogensis stage. The low levels of 17,20βP also confirmed this fact. As reported by Gazola et al. (1996), in *P. mesopotamicus*, a surge of 17,20βP was observed only at the time of ovulation. This steroid has been shown to be one of the most potent steroids for inducing final oocyte maturation and was found at high concentrations in the plasma of ovulating females of teleost fishes (Nagahama, 1987; Nagahama and Yamoshita, 1989).

ANTICIPATED BENEFITS

This project resulted in better understanding of reproductive physiology of important food fishes in the Peruvian Amazon, *Colossoma macropomum* and *Piaractus brachypomus*. Spawning performance remains a serious problem and reliable methods to improve fish maturation need to be further examined and researched. The further development of sustainable aquaculture of these two species will depend on improved environmental conditions and nutrition. In order for aquaculture of *Colossoma* and *Piaractus* to relieve some of the fishing pressure of these overharvested native species, stocking programs of fingerlings must be intensified. Consequently more research and data on physiology of maturation is needed (including steroidal patterns) that will result in successful completion of ovarian growth and final maturation, and production of high quality ova. The present project appeared to solve the problem of low sperm production since males of both species produced copious amounts of sperm.

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