AQUACULTURE CRSP 22ND ANNUAL TECHNICAL REPORT

BROODSTOCK DEVELOPMENT AND LARVAL FEEDING OF AMAZONIAN FISHES

Eleventh Work Plan, Indigenous Species Development Research (11ISDR1B)
Final Report

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ABSTRACT

Growth and plasma sex steroid hormone (including, estradiol-17b, testosterone, and 11-ketotestosterone) levels were investigated in surubim *Pseudoplatystoma* sp. over a 1-year period. Growth of surubim in laboratory conditions was high. The gonadosomatic index (<1%) and the histological analysis (presence of only perinucleolar oocytes in female and spermatogonia in males) of the gonads indicated that the fish would not reach sexual maturity for at least a year. All three steroids measured (estradiol, testosterone, and 11-ketotestorone) did not show any significant variation throughout the year. Moreover, the concentrations were low in comparison to those reported in other catfish species and confirmed the results of the histological and morphological analysis. As a result, we postponed the induction of ovulation or spermiation until spring 2005.

INTRODUCTION

South American catfish (e.g., *Pseudoplatystoma coruscans, P. fasciatum,* and *P. tigrinum*) are potentially important species for commercial production in South America (Kossowski, 1996; Campos, 2004). In Peru, spawning of *P. fasciatum* and *P. tigrinum* occurs in February-March (Alcantara, personal communication). Final maturation and ovulation were achieved in several catfish species from South America using carp pituitary extracts or pituitary hormones (Cardoso et al., 1995; Kossowski, 1996). However, to the best of our knowledge, no information is available on the profiles of plasma sex steroids in both species and we could possibly use this information to synchronize ovulation/spermiation in these fish (Dabrowski et al., 1996). The annual changes in the blood plasma steroids as well as the surge of maturational hormones preceding the spermiation and ovulation can contribute to a better understanding of the dynamics of gonadal steroidogenesis. Moreover, such information will be useful in the development and standardization of breeding techniques through the use of natural and/or synthetic hormones. Our preliminary data indicated that the level of estradiol-17b and testosterone in females of *P. fasciatum* raised in a pond at the Instituto de Investigaciones de la Amazonia Peruana (IIAP) (Iquitos, Peru) in March averaged 0.35 ± 0.2 ng/ml and 3.18 ± 2.5 ng/ml (n=4).

The objectives of this study were (i) to determine gametogenesis and differentiation of ovary and testis in captive stock of South American catfish, *Pseudoplatystoma* sp., (ii) to determine changes in plasma sex steroid hormones during an annual cycle in *Pseudoplatystoma* sp., (iii) to induce reproduction of *Pseudoplatystoma* sp., and (iv) to assess blood plasma steroid response and gamete production (egg and sperm quality).

METHODS AND MATERIALS

On 7 March, 2003, 96 surubim (*Pseudoplatystoma* sp.) were transferred from the University of Wisconsin-Madison to the Aquaculture Laboratory (School of Natural Resources, The Ohio State University). Fish were approximately 1 year old. Fish were distributed according to their size into six 200-L tanks supplied with semi-recirculated water maintained at 25–30°C located in the greenhouse of the Department of Plant Biology 25–30°C. On 11 March, thirty fish were individually weighed. Blood samples were taken from the caudal vessel into a heparinized syringe, kept on ice and then centrifuged at 5,500 rpm at 4°C. The plasma was collected and stored at -20°C until radioimmunoassay. Fifteen fish were sacrificed and the gonads were removed and weighed. Gonadosomatic index (GSI) was calculated as GSI = (gonad weight x

100)/ total weight. The gonads were fixed in Bouin's solution for histological examination. Starting on 12 March, 2003, fish were fed a commercial diet (Biodiet Brood 5 mm, BioOregon, Inc., Warrenton, OR) 1% of their body weight. This diet is an extruded, non-frozen, semi-moist diet usually used for rearing pre-spawning adult salmon and trout. It contains 48% protein, 15% fat, 7% carbohydrate, 1% fiber, 9.5% ash, and 20.5% moisture. Fish were weighed every two-three months (Figure 1). Blood and gonads were sampled throughout the feeding period (Table 1).

The plasma concentrations of steroids (testosterone, estradiol-17b, and 11-ketotestosterone) were measured using radioimmunoassay methods similar to those used previously (Ottobre et al., 1989; Dabrowski et al., 2003) following ethyl-ether extraction. For the histological analysis, gonads fixed in Bouin's solution for 24h were transferred in 70% ethyl alcohol until use. The tissues were dehydrated in a series of ethyl alcohol and xylene baths and embedded in paraffin. Thin sections (5 mm) were cut, mounted on albumin-coated slides, stained in Mayer's haematoxylin and eosin, and examined by light microscopy.

RESULTS

On 11 March, mean GSI reached 0.4 ± 0.2% in females and 0.2 ± 0.1% in males. In females, ovaries were filled with oocytes at the perinucleolar stage, whereas lobules containing spermatogonia were observed in the testis of the males (Figure 2). Mean plasma testosterone was 208 ± 92 pg/ml and 234 ± 57 pg/ml in females and males, respectively. Plasma estradiol-17b levels were low in females and were similar to those observed in males. 11-ketotestosterone levels were significantly higher in males (Table 1). Because of the intent to save larger individuals, the fish weight is probably not representative for differences in sex-related dimorphism in growth (Table 1). On 13 June, the body weight of the small surubim averaged 151 ± 33 g (n=46), whereas the large ones averaged 339 ± 162 g (n=28). On September 16, 2003, the weight of the surubim reached 247 ± 78 g (n=46) and 490 ± 151 g (n=28) for the small and large surubim, respectively. The weight gains between June and September were 63% and 87% for the small and large surubim, respectively.

Growth characteristics of the surubim from February to April 2004 are reported in Table 3. All fish sampled on 19 February, 2004 for histological analysis were females and their ovaries were filled with oocytes at the perinucleolar stage. Gonadosomatic index (GSI) averaged 0.43 ± 0.13%. In February and April 2004, plasma sex steroid hormones were low and similar to those reported in March 2003 (Table 4). In April, one surubim presented a high level (>1 ng/ml) of 11-ketotestosterone (1185 ng/ml). Individual growth of surubim varied from fish to fish as reported in Figure 3.

DISCUSSION

Growth of surubim in laboratory conditions was high. Weight gain was higher in smaller fish (25-30%) than in larger fish (8 to 18%) (Table 3). On the basis of the present results, the commercial diet used (Biodiet Brood 5 mm, BioOregon) seems adequate to promote growth of surubim.

The gonadosomatic index (<1%) and the histological analysis (presence of only perinucleolar oocytes in female and spermatogonia in males) of the gonads in surubim indicated that the fish will not reach sexual maturity for at least a year. Brito and Bazzoli (2003) reported similar GSI (0.28 ± 0.15% and 0.07 ± 0.04% in females and males, respectively) in resting *P. coruscans* collected in the Sao Francisco River in Brazil. In contrast, these authors observed a significant increase of GSI when fish approached maturity (4.61% ± 2.10 and 1.97 ± 0.48% in female and male, respectively). Garcia et al. (2001) obtained the maximum value of GSI for *P. fasciatum* and *P. tigrinum* in February 2.8 and 1.1% (combined sexes), respectively. These fish were collected in the upper Amazon tributary, Rio Ucayali, Peru.

To the best of our knowledge, we report here for the first time the plasma sex steroid hormone levels in surubim. All three steroids measured (estradiol, testosterone, and 11-ketotestorone) did not show any significant variation throughout the year. Moreover, the concentrations were low in comparison to those reported in other catfish species (e.g., channel catfish, *Ictalurus punctatus*) in which estradiol and testosterone can reach up to 40 ng/ml in females prior to the spawning period (MacKenzie et al., 1989). The concentrations of steroids confirmed the results of the histological and morphological analysis indicating that the fish sampled during their second year of life made little advance toward their sexual maturity. As a result, we postponed the induction of ovulation or spermiation until spring 2005.

CONCLUSIONS

We have evidence that Amazonian catfish progressed toward maturity in our aquaculture indoor facility. It is essential to monitor fish growth and steroid status to better anticipate maturation and readiness for induced ovulation/spermiation in 2005.

ANTICIPATED BENEFITS

To our knowledge, the Aquaculture Laboratory is the only laboratory maintaining domesticated broodstock of *Pseudoplatystoma* sp. in the United States. Artificial breeding and propagation of this species will allow the optimization of larval and nursery rearing techniques that could accelerate acceptance of this catfish as a very

attractive aquaculture species.

Our attempts to induce maturation and potentially spawning of *Pseudoplatystoma* sp. may be considered as pioneering work in North America. Our studies on steroid profiles in blood of *Pseudoplatystoma* in captivity will serve as basis for comparisons with fish in natural or semi-natural (pond rearing) conditions in the Peruvian Amazon. Current studies are applicable to two species doncella (*P. fasciatum*) and tiger (*P. tigrinum*).

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Table 1. Schedule of surubim (*Pseudoplatystoma* sp.) sampling.

Date	Weighing	Blood sample	Gonad sample
3/11/03	30	30	15
6/13/03	74	0	0
9/15/03	74	0	0
12/12/03	74	0	0
2/1/04	8	0	8
2/19/04	66	13	6
12/4/04	59	18	0

Table 2. Reproductive characteristics of surubim *Pseudoplatystoma* sp. on March 11, 2003.

	n	Weight (g)	GSI (%)	E2 (pg/ml)	T (pg/ml)	11-kT (pg/ml)
Female	13	212 ± 74	0.41 ± 0.16	45 ± 22	208 ± 92	158± 113
Male	2	278 ± 61	0.20 ± 0.02	25 ± 1	234 ± 57	912 ± 491

Table 3. Growth characteristics of surubim *Pseudoplatystoma* sp. from February to April 2004.

Tank	n	February 19 Weight (g)	April 12 Weight (g)	Weight gain[1] (%)	Specific growth rate[2] (%/day)
1	11	445 ± 96	559 ± 139	25.6	0.19
2	10	498 ± 103	649 ± 146	30.3	0.22
3	16	317 ± 105	399 ± 159	25.8	0.19
4	12	710 ± 116	838 ± 163	18.1	0.14
5	5	1094 ± 293	1189 ± 335	8.7	0.07
6	5	1282 ± 309	1469 ± 445	14.6	0.11

WG = (final weight-initial weight) x 100 / initial weight

SGR= (ln (final weight) - ln (initial weight)) x 100 / time in days

Table 4. Plasma sex steroid hormones in surubim Pseudoplatystoma sp. sampled on February 19 and April 12, 2004.

Date	n	Weight (g)	E2 (pg/ml)	T (pg/ml)	11-kT (pg/ml)
February 19	13	430 ± 173	108 ± 65	239 ± 73	99 ± 60
April 12	18	823 ± 501	99 ± 72	234 ± 90	180 ± 280

Figure 1. Surubim *Pseudoplatystoma* sp. raised in 200-L tank at the Aquaculture Laboratory.

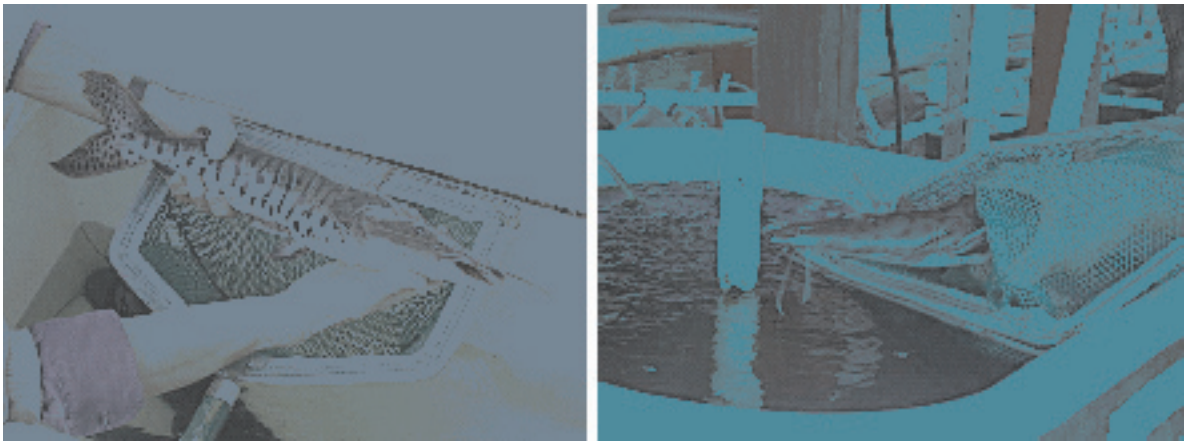


Figure 2. Cross-section of ovary (A) and testis (B) of catfish *Pseudoplatystoma* sp. sampled on March 11, 2003 (weights were 339 g and 321 g for the female and male, respectively). PO: peri-nucleolar oocyte, n: nucleus, c: cytoplasm, SP: spermatogonia. Black bar = 100 microns.

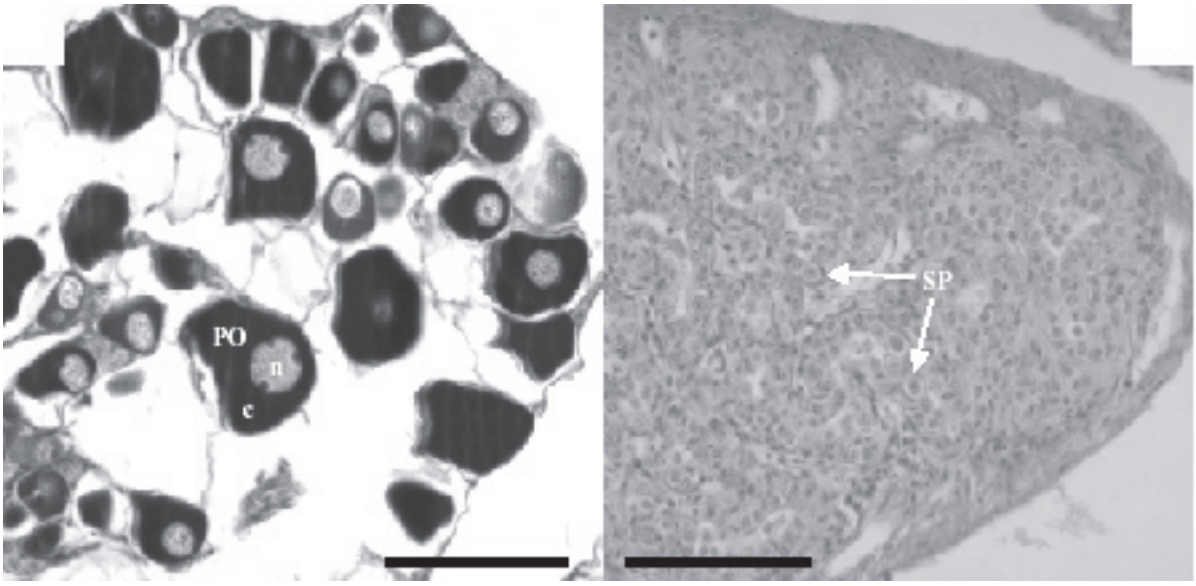
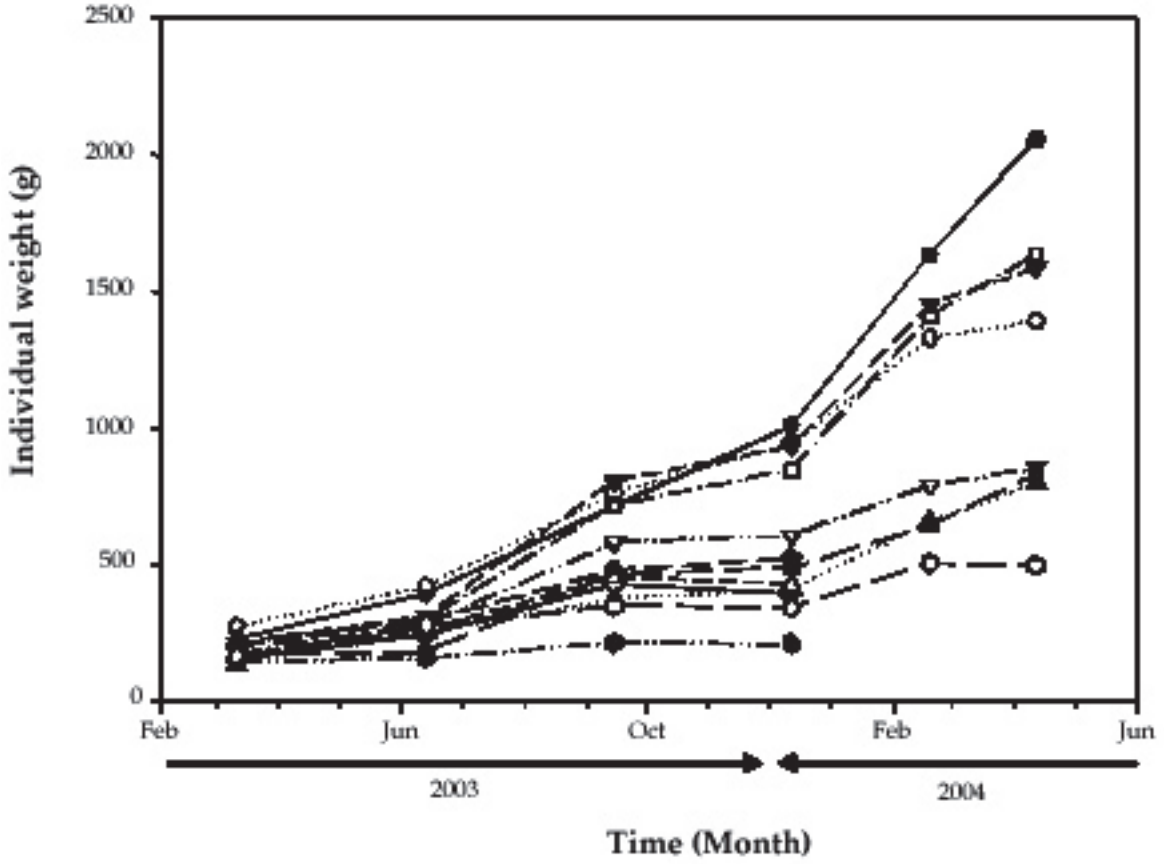


Figure 3. Examples of individual growth of *Pseudoplatystoma* in the Aquaculture Laboratory.



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