Aquaculture 452 (2016) 224–232

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

# Early puberty of farmed tambaqui (*Colossoma macropomum*): Possible influence of male sexual maturation on harvest weight

F.L. Almeida [a,*], J.S. Lopes [b], R. Crescencio [a], A.C.U. Izel [a], E.C. Chagas [a], C. Boijink [a]

[a] Brazilian Agricultural Research Corporation, Western Amazon; Rodovia AM 010-km 29, Cx postal 319, Cep 69010-970 Manaus, AM, Brazil
[b] Uninorte Laureate International Universities, Av. Joaquim Nabuco, 1469, Centro, Cep. 69020-010 Manaus, AM, Brazil

A R T I C L E  I N F O

*Article history:*
Received 2 April 2015
Received in revised form 21 October 2015
Accepted 24 October 2015
Available online 28 October 2015

*Keywords:*
Fish
Reproduction
Puberty
Aquaculture
Gametogenesis
Sex steroid

A B S T R A C T

Tambaqui is the main native fresh water fish in Brazilian aquaculture. Despite the increasing production of tambaqui (annual growth of approximately 8%), no study has addressed the effects of farming on tambaqui's reproductive physiology and growth performance. As part of an institutional portfolio that aims to increase the production of tambaqui per area of cultivation, the present work details the tambaqui's gametogenesis and steroidogenesis during 10 months of farming, until they reached the market size. Spermatogenesis is very precocious (750 g fish) and apparently is a rapid and constant process in tambaqui testes, with major concentration of mature fish in August. On the other hand, oogenesis is a much longer process that starts only in fish at around 1200 g and does not complete before harvesting (no mature female observed). The plasmatic concentration of sex steroids is very similar among immature male and female tambaqui. However, testosterone and estradiol rise in parallel with testis development while only the second rises with ovarian primary growth. Concentration of 17α-hydroxyprogesterone remains at basal levels during spermatogenesis of farmed tambaqui, but it slightly increases during oocyte primary growth. At harvest, the biometrical analysis reveals that female and male tambaqui yield different gutted weight in the order of 16% in favor of the former. Most probably due to the precocious gonad maturation that farmed males go through (5 m old), females are heavier at harvest (12 m old) and therefore stand out as the most profitable gender to be produced in the tambaqui farming industry.



## 1. Introduction

Puberty is the event that marks the beginning of the reproductive life of an individual. This process is very complex and involves a series of interdependent cascades that culminates in the production of gametes and sex hormones by the gonads, warranting the individual the capacity to reproduce. In females, the gamete production, called oogenesis, comprises several transformations that the oocytes undergo: the formation of the cortical alveoli, the accumulation of vitellogenin (vitellogenesis), the migration and breakdown of the germinal vesicle and the resumption of meiosis (arrested from ovary differentiation). Within this developmental process, the primary oocyte (located inside of an ovarian follicle) gives rise to a large secondary oocyte that is finally extruded from the follicular cells at the end of the maturational process. The development of the primary and secondary oocytes is marked by massive structural and functional changes that culminates with the release of the ovum on the ovarian lumen or abdominal cavity during spawning (Lubzens et al., 2010). On the other hand, the male puberty is characterized by the process of spermatogenesis, which begins with the high mitotic activity of the spermatogonia inside of the spermatogenic cysts (formed by their close relation with the specialized Sertoli cell). This proliferative phase results in the differentiation of spermatocytes that undergo the two meiotic divisions, originating the spermatids. These haploid cells enter the last spermatogenic phase, spermiogenesis, during which they pass through several important cellular transformations to form the highly specialized male gamete, the spermatozoa. Spermatogenesis completion is therefore the release of the flagellated spermatozoa into the seminiferous tubules by the rupture of the spermatogenic cysts (Schulz et al., 2010).

Oogenesis and spermatogenesis are fully coordinated by the endocrine system at different levels, namely brain, pituitary and gonads. In particular, the steroid hormones androgens, estrogens and progestins that are produced by and exert their effects on the gonads are crucial for both processes. Androgens are implicated in the transition of the oocyte into secondary growth, and are essential in all phases of spermatogenesis. For instance, testosterone increases in plasmatic levels as spermatogenesis progresses, then peaks during the final steps of spermiation and sperm release (Schulz et al., 2010). Estrogens are fundamental for oogonial proliferation and vitellogenesis in the females (Miura et al., 2007; King and Pankhurst, 2003; Feist et al., 1990) and play different roles during spermatogonial stem cell renewal

* Corresponding author.
*E-mail address:* fernanda.almeida@embrapa.br (F.L. Almeida).

http://dx.doi.org/10.1016/j.aquaculture.2015.10.031

(Miura et al., 1999; Amer et al., 2001) and Sertoli cell proliferation (Chaves-Pozo et al., 2007). Different progestins are essential for the final maturation of the oocytes and ovulation. In the males, progesterone is involved in the differentiation of spermatocytes and beginning of meiosis (Miura et al., 2006; Higuchi et al., 2013) but is mainly responsible for the last steps of spermiation, spermatozoa maturation and sperm motility (Amer et al., 2001; Ueda et al., 1985;; Nagahama, 1994; Miura et al., 1991; Miura et al., 1992; Scott and Sumpter, 1989). Moreover, a recent study showed a role of progesterone for the proliferation of early spermatogonia (Chen et al., 2012).

For fish farming, puberty and adult reproduction can be accompanied by reduction of growth, of flesh quality, incidence of aggression, reproductive behavior and increased susceptibility to diseases (Taranger et al., 2010). Meaning that, in general, puberty is a non-desirable event in aquaculture, unless when considering the broodstock management.

The negative effects of puberty specifically on fish growth are elucidated in different orders, mainly on economically relevant species. Different studies reveal the relationship between reproduction and body growth in tilapia *Oreochromis mossambicus* (Bhatta et al., 2012), salmonids (*Oncorhynchus tshawytscha*; Shearer and Swanson, 2000; *Oncorhynchus keta*; Onuma et al., 2010; *Oncorhynchus kisutch*; Yamamoto et al., 2011), sea bass *Dicentrarchus labrax* (Felip et al., 2008), Atlantic cod *Gadus morhua* (Taranger et al., 2006), yellowtail *Seriola quinqueradiata* (Miura et al., 2013), Atlantic halibut *Hippoglossus hippoglossus* (Weltzien et al., 2003) and others. Therefore, the knowledge about reproduction in teleosts and its effects on the final mass yield are a key point when a given aquaculture program is developing and moving from local to a large-scale production. Age at first maturation, spermatogenesis, oogenesis, reproductive behavior in captivity, sex hormones, the influence of puberty on growth and weight gain, etc. these data are all fundamental pieces of information when considering the development of a novel farming species. As well as optimizing fingerling production, this data can influence the development of techniques that enhance mass production per area.

Brazil is a country with enormous potential in the aquaculture industry. About 13% of the world's fresh water lies within Brazilian borders and the seacoast length is over 10,000 km. It is estimated that Brazil has a potential production of 20 million tons of fish per year (Sidonio et al., 2012). However, as the Brazilian aquaculture is still in its infancy, the actual production (544.5 kt in 2011) is not sufficient to cope with the national fish demand. Brazil still imports more than 180 kt of fish from different countries, which represents a large deficit in the food trade balance. Modernization of the aquaculture production line and development of technologies for each economically important fish species must therefore be a priority at this moment.

Tambaqui *Colossoma macropomum* is a scaled fish from the Characidae family, native to the Amazon basin, where it is traditionally the most appreciated fish for its meat. This characid has migration habits and can weigh more than 30 kg in the wild, while the average commercial size is 3 kg (Kubitza, 2004). In the scenario of the Brazilian aquaculture industry, this fish has been cultivated in all the five regions of Brazil representing the first native species with economic value (BRASIL, 2013). Due to the high quality and taste of its flesh, the tambaqui is also farmed in the neighboring countries Ecuador, Peru, Venezuela, Panama and Colombia (Chellapa et al., 1995; Roubach et al., 2003; Farias et al., 2010). With an estimated growth in production of 8% per year, tambaqui is the best native candidate to address the increasing internal demand for fish and thereafter to elevate the Brazilian aquaculture industry to international competitive levels.

Despite the importance and potential of tambaqui for the Brazilian aquaculture, no study has detailed the events that mark the puberty of the species under farming conditions, and the possible effects on the profitability of the activity. In this manuscript, we describe the pubertal spermatogenesis, beginning of oogenesis, sex steroid profiles and harvest indexes of farmed tambaqui. This is the first study detailing the early gonadal development of earth-pond-reared tambaqui, and the pioneer in comparing the biometry of males and females at the final harvest weight.

## 2. Material and methods

### 2.1. Animals and samples

Approximately 7000 fry tambaqui *C. macropomum* from one batch (obtained from two females and one male) were obtained from a commercial hatchery and kept in a 1 ha earth pond (initial density of 900 kg/ha) under farming conditions in the rural area of Rio Preto da Eva, Amazonas, Brazil (Farm Sagrada Família, Rodovia AM 010, km 64). Fish were fed twice a day ad libitum with commercial dry pellet containing 32% CP during the first two months, and thereafter pellets of 28% CP.

Every month, 12 fish were randomly sampled from November 2011 (when fish were 2 months old and average total weight 160 g) to September 2012 (average harvest size of 3000 g). For sampling, fish were first deeply anesthetized with Benzocaine 10%. Prior to sacrifice, blood samples were collected from the caudal vein and biometrical measurements were recorded individually (total weight, gutted weight and total length). The blood samples were immediately cooled in ice and centrifuged at 1300 g for 10 min for plasma separation. The plasma samples were stored at −80 °C until analysis. For the last sampling, when fish were ready for yielding, an additional 40 fish were collected for the final biometrical analysis. The higher number of samples (52 instead of 12) was more representative of the tambaqui harvest size and weight.

Gonads and liver were removed and weighed for the calculation of the gonadosomatic index (GSI) and hepatosomatic index (HSI) respectively (GSI = gonad weight × 100/total body weight and HSI = liver weight × 100/total body weight). The gonads were then cut in small fragments and fixed in Bouin solution for 12 h, rinsed in running tap water and stored in 70% ethanol at 4 °C. After dehydration, the fragments were embedded in paraffin according to conventional techniques. Sections of 5 μm thickness were mounted on histological slides and stained with hematoxylin-eosin for histological evaluation of the gonads. Representative images of each stage of the development of ovaries and testis were registered with an Axio Imager M2 Zen 2012 (Zeiss®).

For morphometric analyses of the oocytes in the last month of the study, the diameter of the largest 30 perinucleolar oocytes were measured individually in 5 females.

All the procedures during fish handling and sampling followed the ethical principles established by the Brazilian College of Animal Experimentation (COBEA).

### 2.2. Sex steroid assays

Blood was collected from all fish individually for measurement of sex steroid concentrations. Plasma sex-steroid levels were measured individually by enzyme linked immunosorbent assay (ELISA; DIA Source ImmunoAssays®, Nivelles, Belgium). Testosterone (T), 17β-estradiol ($E_2$), 17α-hydroxyprogesterone (OHP) were directly quantified in duplicates in each fish sample, according to the guidelines of the kit.

### 2.3. Statistics

Data are presented as mean ± SEM. Differences in mean GSI, HSI, T, $E_2$ and 17α-OHP of immature females and females in primary growth were analyzed by the Student T test. Differences in the monthly weights and lengths of males and females were also analyzed by Student T test. For the statistical analysis of the hormone levels in the different phases of male maturation, the one-way ANOVA was performed followed by the Newman–Keuls Multiple Comparison test.