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Influence of water temperature on induced reproduction by hypophysation, sex steroids concentrations and final oocyte maturation of the "curimatã-pacu" *Prochilodus argenteus* (Pisces: Prochilodontidae)

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ABSTRACT

Most fishes with commercial importance from the São Francisco basin are migratory and do not complete the reproductive cycle in lentic environments, such as hydroelectric plant reservoirs, hence natural stocks are declining and there is an urgent need to reduce the pressure of fishing on those wild populations. Therefore, studies on reproductive biology and its relationship with endocrine and environmental factors are key to improving the cultivation techniques of Brazilian fish species. This study examined the influence of water temperature on sex steroid concentrations (testosterone, 17β-estradiol and 17α-hydroxyprogesterone), spawning efficiency, fecundity, fertilisation rate, larval abnormality rates and involvement of the cytoskeleton during the final oocyte maturation of *Prochilodus argenteus* under experimental conditions. The results of our study showed that in captivity, sex steroid plasma concentrations and spawning performance of *P. argenteus* were clearly different for fish kept in water with different temperature regimes. In lower water temperature (23 °C), it was observed that: 33% of females did not ovulate, fecundity was lower and vitellogenic oocytes after the spawning induction procedure exhibited a smaller diameter. Moreover, concentrations of 17β-estradiol and 17α-hydroxyprogesterone were lower and there was a delay in the final oocyte maturation and, consequently, ovulation and spawning. Our experiments showed direct influence of water temperature in the process of induced spawning of *P. argenteus*. Changes in water temperature also suggest the tubulin involvement in the nuclear dislocation process and the possible action of actin filaments in the release of polar bodies during final oocyte maturation of *P. argenteus*.

1. Introduction

The fish pituitary produces two types of gonadotropin (GTH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) [31]. The temporal pattern of secretion in several fishes suggests that FSH has a dominant role regulating vitellogenic growth of follicles, in part through stimulation of 17β-estradiol (E2) synthesis by ovarian follicles, and E2 regulates ovarian development through its control of vitellogenin synthesis. LH induced cascade of maturation-inducing hormone (MIH) synthesis followed by maturation or metaphase-promoting factor (MPF) production is well studied in fish [30,47]. The 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) which has 17α-hydroxyprogesteone as a precursor, has been identified as MIH in several fish and the action of this MIH in inducing the resumption of meiosis in teleost oocytes has also been reported [31].

Fish final oocyte maturation completes the process of oogenesis and results in the fertilisable female gamete or egg [14,16,31]. Final oocyte maturation is a remarkable transformation of the prophase I oocyte; a cell specialised for transcription, material uptake, nonexcitable, and unable to osmoregulate in fresh water, to the metaphase II egg, a cell different from the oocyte in many ways. In many teleosts, oocyte maturation is triggered by 17α, 20β-DP acting on a steroid–receptor in the cytoplasmic membrane [17,53,61]. This unconventional steroid–receptor interaction leads to the various non-genomic cellular changes that are the markers for oocyte maturation [31].

The fully-grown zebrafish oocyte residing in the ovary is covered by three cell layers, the ovarian epithelium, the theca and the follicle cell layer [24,48]. The oocyte and follicle cells have numerous gap junctions in common and, thus, are in chemical

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and electrical communication [20]. The oocyte has a high degree of endocytic activity and uses receptor-mediated endocytosis to take up vitellogenin transported from the liver. The fully-grown oocyte is in prophase I of meiosis with a central, large nucleus or germinal vesicle containing many nucleoli and a very high transcriptional activity [23].

Fecundity is an important variable for aquaculture, species management and conservation [54,57], and it estimates the reproductive potential of a fish species [4]. Fecundity depends on body size, oocyte diameter, spawning type and may vary as a result of different adaptations to environmental habitats and it provides important information for aquaculture, fisheries management, and conservation [52,56,58].

During the final oocyte maturation of the fish, the cytoskeleton participates in the ooplasm segregation [19]. Variations in water temperature can cause conformational changes as well as in the dynamics of the cytoskeleton [35,55], as aggregation of microtubules occurs when the temperature is high and its breakdown occurs when the temperature decreases [35], thus, interfering in the migration of the nucleus during final oocyte maturation [22]. Actin microfilaments, especially those present in the cortical region, also participate in the major events of final oocyte maturation in fish, such as cytoplasmic polarisation, formation of the first polar body, fertilisation and embryo morphogenesis [5,21,27]. During fertilisation, the cortical actin participates in the exocytosis of cortical granules, formation of the fertilisation cone, assisting the migration of the pronucleus and the release of the second polar body [5].

Efforts to control the reproduction of neotropical migratory fish began many years ago, but its success has been limited by intrinsic factors such as obtaining good breeders, and by extrinsic factors such as temperature, dissolved oxygen and other water properties which have prevented producers from achieving satisfactory results of artificial reproduction. The water temperature has great relevance in the reproductive process of many fish species, acting on gamete maturation, ovulation and spawning [2]. In culture conditions, neotropical migratory fish, when kept at temperatures below 25 °C, did not respond satisfactory to artificial reproduction procedures [40].

Prochilodus argenteus, popularly known as curimatã-pacu, is an endemic species in the São Francisco basin and is the most abundant migratory species in the Três Marias region, representing almost 50% of the total catch. It has an illiophagous feeding habit and is the largest member of the Prochilodontidae family, and is intensively used in hatcheries for restoring fishery stocks [42,43]. *P. argenteus* performs long-distance migrations upstream for spawning, and has high fecundity. It exhibits total spawning and its reproductive period extends from November to January in the rainy season, coinciding with the time of flooding, higher temperatures, and long photoperiods. In captivity, the curimatã-pacu prepares to reproduce and completing vitellogenesis, however, ovulation and spawning only occur after hormonal induction [40,42].

The application of hormonal therapies to induce spawning can be based on the administration of the gonadotropin-releasing hormone (GnRH), [9,29], by treatment with Ovaprim, a preparation containing a synthetic GnRH analogue with domperidone [51] or using the heteroplastic hypophysation method where commercial crude carp pituitary extract (CCPE) is injected into the coelomic cavity or intramuscularly [38,45,46,47,59]. However, in commercial aquaculture of Brazilian freshwater fish, spawning induction is usually performed with hypophysation, since this methodology, besides being economically beneficial, has high efficiency and produces eggs with high rates of fertilisation [44].

Considering the scarcity of studies on the influence of water temperature on final oocyte maturation and spawning of neotropical migratory teleosts, the objective of our study was to evaluate sex steroid concentrations, spawning, fecundity and the involvement of the cytoskeleton during induced spawning by hypophysation in females of *P. argenteus* utilising two regimes of water temperature: 23 and 26 °C. As *P. argenteus* females do not complete their reproductive cycle in the stretch of the river in the São Francisco near the Três Marias hydroelectric dam [3], and the water temperature in this environment has mean values near 23 °C during the reproductive period of this species, in our study, the fishes of first experimental group (group A) was kept in the tank with water temperature of 23 °C. According Sato et al. [42] in a water temperature of 26 °C, *P. argenteus* showed good results for induced spawning procedures, thus, in our work the second group (group B) was kept in water of 26 °C. The males were kept in a tank with water of 26 °C.

2. Materials and methods

2.1. Sampling

In order to perform the experiment, 150 specimens of *P. argenteus* were captured in the São Francisco River and kept for 2 years in a 200 m^3 tank (20 × 10 × 1 m) in the Hydrobiology and Fishculture Station of Três Marias, CODEVASF. The fish were feed with pelleted feed containing 36% of crude protein, at a level equivalent to 2% of their biomass per day during the storage period. The experiments were performed in duplicate, first in December 2005 and again in December 2006. In each experiment was used 30 females and one male, totalling 62 specimens of *P. argenteus.*

2.2. Induced spawning

For the experiments, only fish in advanced gonadal maturation stages were used. Females were selected by external morphological characteristics indicating that they were ready for spawning induction procedures, such as red urogenital papilla and bulging coelomic cavity. Males that released sperm with light pressure on the coelomic cavity were selected for the fertilisation procedures.

For each repetition, the specimens were divided into two groups of 15 females, which were transferred to 2.4 m^3 breeding tanks (3 × 1 × 0.8 m) with constant water circulation and the following characteristics: a dissolved oxygen concentration ranging from 5.5 to 6.5 mg L^{-1}, pH from 6.3 to 7.5 and an electrical conductivity of 58 to 87 μS cm^{-1}. The fish in group A were kept in a breeding tank with a water temperature of 23 °C, whereas fish in group B were kept in a tank with water of 26 °C.

The fishes of groups A and B were submitted to induced spawning by hypophysation according methodology established by [44]. Crude common carp pituitary extract (CCPE) was injected into the coelomic cavity. Males received a single dose of 5 mg CCPE/kg of body weight and females received two doses: a first dose of 1 mg of CCPE/kg and a second one of 5 mg of CCPE/kg, with an interval of 14 h in-between doses. Fertilisation was carried out by the dry method, using semen from a single male for each year of the experiment.

After the induced spawning procedures, the total length (TL), body weight (BW) and gonad weight (GW), ie weight of the spawned oocytes (the weight of free oocytes released plus the free oocytes retained in the ovaries was considered), were taken. We calculated the gonadosomatic index (GSI = GW × 100/BW) and Fulton condition factor (K = BW × 100/TL^3). The fish were killed by transversal section of the cervical medulla following the ethical principles established by the Brazilian College of Animal Experimentation (COBEA, http://www.cobea.org.br).