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Effect of timing of hormonal induction on reproductive activity in lambari (*Astyanax bimaculatus*)

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Abstract

The objective was to evaluate the influence of the timing of hormonal induction, using gonadorelin or common carp pituitary extract (CPE), on the reproductive activity of female *Astyanax bimaculatus*. Fish (N = 44) were weighed, measured, and acclimatized to experimental conditions with a photoperiod of 12 h:12 h light:dark (L:D) for 10 days. Ovulation was induced with a single dose of CPE (6 mg/kg) or gonadorelin (80 μg/kg), given at 12:00 (halfway through the light phase (LP) or 24:00 (halfway through the dark phase (DP), in a 2 × 2 factorial design. The time of ovulation was calculated in degree hours and daily motor activity was recorded using a photocell. The fish were killed and the liver and gonads were weighed for calculation of gonadosomatic (GSI) and hepatosomatic (HSI) indexes, respectively. Absolute fecundity (AF), absolute fecundity relative to weight (AFRW) and length (AFRL), diameter of oocytes (mM), and percentage of oocytes with the germinal vesicle in a peripheral position (PPGV) were recorded. All females responded (ovulated). The female *Astyanax bimaculatus* had twilight motor activity rhythm. Females given CPE at 12:00 had a higher ($P < 0.05$) percentage of oocytes with the germinal vesicle in a peripheral position compared with the group that received gonadorelin in the same period (95 ± 6 vs. 79 ± 21%, mean ± SD). The absolute fecundity relative to weight was higher in groups induced at 12:00, regardless of the hormone used (LP: 805 ± 448 and 700 ± 214, for CPE and gonadorelin, respectively; dark phase: 580 ± 396 and 529 ± 105, $P < 0.05$). Both times used for hormonal induction with CPE and gonadorelin were suitable for inducing reproduction in lambari, although induction with CPE in LP had the best results.

Keywords: CPE; Daily rhythm; Gonadorelin; Oocytes; Induction of ovulation; Fish

1. Introduction

Daily biological rhythms are associated with environmental fluctuations that have a periodicity of 24 h (circadian rhythm), entrained to the cycle of light and dark. Light and temperature cycles play a crucial role in seasonal rhythms, such as those involved in fish reproduction [1,2]. For example, in teleost fish, ovulation and spawning usually occur at certain times of day, depending on the photoperiod [3]. Peaks of plasma GnRH concentrations vary among species of fish according to the time of day. This hormone is responsible for final maturation of oocytes [4]; therefore, time of day is one of the most important environmental variables affecting induced spawning. Muniz et al. [5] dem-

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onstrated the influence of photoperiod on the ovulation process in tambaqui (*Colossoma macropomum*) induced with luteinizing hormone releasing hormone (LHRH-gonadorelin). This was linked to plasma concentrations of sex steroids present during early stages of hormonal induction.

Common carp pituitary extract (CPE) is widely used to induce final maturation in Brazilian migratory fish, achieving acceptable results for several species [6] including *Prochilodus lineatus* [7] and *Brycon orbignyanus* [8]. However, the high cost of the CPE has encouraged studies on alternative hormones, e.g., ovaprim, hCG, GnRH, and GnRH analogues [9,10]. Gonadorelin (GnRH) in the form of lyophilized diacetate tetrahydrate has been used [5], but there are apparently no reports of using this hormone to induce ovulation in fish that have daily patterns of activity.

Astyanax bimaculatus (lambari) is widely distributed across South America [11], and is popularly referred to as lambari, yellow tail piaba, or yellow tail lambari. Reproductive activity begins once individuals reach 5 cm in length [12], and total or split spawning may occur, depending on environmental conditions [13]. Due to its small size, ease of manipulation in the laboratory and dependence on environmental conditions for reproduction, *A. bimaculatus* can be used as a model for other large tropical species. This species was used in tests of reproductive induction with CPE by Sato et al. [14]. However, there is little if any knowledge of the daily rhythm of motor activity and the influence of the timing of hormone application on induced breeding of lambari. Thus, the objectives of this study were to determine reproductive parameters of female lambari (*A. bimaculatus*) induced with gonadorelin or CPE at two times of the day, and to determine the daily rhythm of motor activity of this species.

2. Materials and methods

The experiments were performed in the laboratory of Physiology and Pharmacology in the Department of Veterinary Medicine, Federal University of Lavras (UFLA), during December 2009. A total of 44 adult female lambari, weighing 7.9 ± 2.4 g and measuring 8.4 ± 0.8 cm in length, were obtained from the aquaculture station of UFLA. Females suitable for hormonal induction were selected according to external features, e.g., swollen abdomen and a swollen and reddish genital pore, as described [14].

After selection, the fish were kept in 20-L glass aquaria (11 fish per aquarium) with a system of water recirculation, in which water from the aquaria was captured and passed through a mechanical and biological filter of 250 L for later use; this system maintains water quality. A photoperiod of 12 h:12 h light:dark (LD) was maintained using fluorescent lamps. The light intensity was 1173 lux, measured by a digital light meter Model LDR-208 (Instrutherm, São Paulo, São Paulo, Brazil). During the experimental period, the water temperature, dissolved oxygen concentration, and pH were maintained at 27 ± 1 °C, 6 ± 0.5 mg/L, and 6.5 ± 0.5, respectively. Throughout the experimental period, the fish were fed commercial extruded feed (40% crude protein) pellets (2 mm in diameter), at a rate of 2% of body weight, in two daily portions, provided during the light period.

During Experiment 1, daily motor activity of female lambari was determined, whereas in Experiment 2, reproductive end points were determined in females induced with gonadorelin or CPE given during the light or dark phase (2 × 2 factorial).

2.1. Experiment 1

Motor activity was recorded daily for 10 days, before performing the hormonal induction, using an infrared photocell (Model E3S-[SCAP]AD[R]62, Omron, Tokyo, Japan) installed on the exterior of each aquarium [15], each containing 11 fish. The photocell was activated by movement as fish passed in front of the photocell and records were sent to and stored in a computer connected to the photocell. A chart was created from the numerical data, showing the average daily motor activity over 10 days, with a trend line every 10 min. The type of motor activity (diurnal, nocturnal, or crepuscular) was determined by observing the time of maximum movement of fish within the photoperiod and performing a descriptive analysis of the data, according to methodology previously described [15,16].

2.2. Experiment 2

Fish were acclimatized to experimental conditions for 10 days. After 24 h of fasting, hormonal induction was induced with a single, im injection at the base of the pectoral fin, at two times, as follows: groups 1 and 2 received 80 μg/kg of gonadorelin at 12:00 (mid light [ML]) and 24:00 (mid dark [MD]), respectively, whereas groups 3 and 4 received CPE at a dose of 6 mg/kg, at the same times as groups 1 and 2, respectively. The experiment was conducted in a randomized design in a 2 × 2 factorial design (two hormones and two times of application), with 11 replicates, where