Embryonic Development of *Bryconamericus caucanus* (Characidae: Tetragonopterinae) under Laboratory Conditions

Desarrollo Embrionario de *Bryconamericus caucanus* (Characidae: Tetragonopterinae) en Condiciones de Laboratorio

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SUMMARY: In this study we describe the early stages of development of *Bryconamericus caucanus* under laboratory conditions. Mature females (mean weight 8.12 g) received two intraperitoneal doses of Carp Pituitary Extract (1st dose of 0.5 mg / kg at time 0 and 2nd dose of 5 mg / kg 12 h later), and mature males received a single dose of 0.5 mg / kg at the time of the 2nd dose to the females. Extrusion of eggs was performed at 152.25 Accumulated Thermal Units. Eggs of *B. caucanus* were yellow, round shaped, non-adhesive, and the perivitelline space after hydration was moderate. Hatching occurred 28 h 20 min after fertilization (21°C, 594.3 Accumulated Thermal Units). The morphological features of the egg and early embryo of *B. caucanus* were similar to previous reports in other members of the Tetragonopterinae, but the embryonic development was particularly long in this species.

KEY WORDS: Artificial breeding; Fish; Characiforms.

INTRODUCTION

The families Astroblepidae, Trichomycteridae and certain small characiforms such as *Bryconamericus sp.*, comprise the native ichthyofauna in small streams at medium and high elevations of the Andean region in Colombia. Because there is little information concerning the early stages of development of these species, contributions to this topic are important.

*Bryconamericus spp* occurs in a wide variety of freshwater ecosystems in the low and high elevations of South and Central America on both sides of the Andean cordillera (Vare & Siebert, 1990). Species such as *Bryconamericus caucanus* Eigenmann, 1913 are abundant in small streams and riverbanks (Roman-Valencia & Muñoz, 2001; Maldonado-Ocampo et al., 2005).

The embryonic development of fish is a complex process, and comprises the study of ontogeny, taxonomy, experimentation in biotechnology and bioindication of toxicity in aquatic environments (Botero et al., 2004). Although, detailed descriptions of early ontogeny of small characiforms under laboratory conditions are scarce, there is a vast amount of information about the embryonic development of characiform species (Romagosa et al., 2001; Nakatani et al., 2001; Börçato et al., 2004; Ninhaus-Silveira et al., 2006).

In this study, we describe the induced reproduction and the early stages of development of *B. caucanus* under laboratory conditions.

MATERIAL AND METHOD

Adult *B. caucanus* were captured with casting nets, in a ripe condition in “Alto de San Miguel” (6°2'43"N, 73°37'12"W) Antioquia, Colombia in December 2007. At this time of the year we expected to find maximum gonadal development. The animals were transported in plastic bags to the Biogenesis Laboratory (Facultad de Ciencias Agrarias, Universidad de Antioquia, 6°16'20"N, 75°35'18"W). The fish were kept in 15 gallon aquaria provided with biological filtration and a water reticulation system. One week after capture, females with swollen and soft abdomen and males with abundant milt obtained by gentle abdominal pressure were induced to reproduce.

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Six females (mean weight 8.12 g) received two intraperitoneal doses of Carp Pituitary Extract (Argent® chemical laboratories) dissolved in sterile physiological saline solution using a 1 ml syringe (1st dose of 0.5 mg/kg at time 0 and 2nd dose of 5 mg/kg 12 h later). Likewise, twelve males received a single dose of 0.5 mg/kg at the time of the 2nd dose to the females.

After the 2nd dose, the animals were observed regularly for signs of ovulation in females, and fertilization was performed using extrusion. Aquarium water was added to initiate sperm activation and fertilized eggs were incubated in a plastic container submerged in the aquarium. Furthermore, two females and two males were induced as was indicated above and left in an aquarium for natural spawning.

During the first 1 h 30 min, embryo samples were examined at 10 min intervals. Between 1 h 30 min post-fertilization until hatching, embryos were examined approximately every 60 min. Observations were made under a stereoscope and a light microscope (10x magnification) equipped with a digital camera Moticam 2300 (Motic®).

Embryonic periods, stages and terminology followed those of Kimmel et al. (1995).

RESULTS

The mean temperature throughout the experiment was 21°C. Eggs were obtained from all the induced females by extrusion and successful spawning in the “semi-natural method”. Extrusion was performed 7 h 15 min after the second dose, which corresponds to 152.25 Accumulated Thermal Units.

The embryonic development of B. caucanus is summarized in Table I, which is divided into seven periods. Eggs of B. caucanus were yellow, round shaped and non-adhesive, and the perivitelline space after hydration was moderate. Cleavage occurred during the first 2 h 30 min, embryo movements were noticed after 23 h and hatching occurred 28 h 20 min after fertilization (594.3 hours-grade). Larvae that had just hatched showed very little body pigmentation.

Fig. 1. Embryonic development of B. caucanus. a. Zygote; b. 2-cell; c. 4-cell; d. 8-cell; e. 16-cell; f. 32-cell; g. 64-cell; h. Blastula high; i. Blastula dome; j. Blastula 30% epiboly.
Table I. Description of the embryonic development of *Bryconamericus caucanus* (21 °C)

| Period        | Stage      | Time after fertilization | Description                                                                 | Figure |
|---------------|------------|--------------------------|-----------------------------------------------------------------------------|--------|
| Zygote        | 1-cell     | 0 – 20 min               | Formation of animal pole from fertilization to first cleavage               | 1 a    |
|               | 2-cell     | 40 min                   | First cleavage                                                              | 1 b    |
|               | 4-cell     | 50 min                   | 2x2 array of blastomeres                                                    | 1 c    |
|               | 8-cell     | 1 h                      | 2x4 array of blastomeres                                                    | 1 d    |
|               | 16-cell    | 1 h 30 min               | 4x4 array of blastomeres                                                    | 1 e    |
|               | 32-cell    | 2 h                      | Cycle 5 of cleavage                                                         | 1 f    |
|               | 64-cell    | 2 h 30 min               | Cycle 6 of cleavage                                                         | 1 g    |
|               | High       | 4 h 30 min               | Beginning of blastodisc flattening                                          | 1 h    |
| Blastula      | Dome       | 5 h 15 min               | Beginning of the epiboly                                                    | 1 i    |
|               | 30% Epiboly| 6 h                      | Blastoderm an inverted cup; margin reaches 30% of the distance between the animal and vegetal poles | 1 j    |
|               | Germ-ring  | 6 h 40 min               | Epiboly continues, and when it reaches 50% the germ ring appears (visible from animal pole) | 2 a    |
|               | Shield     | 7 h 25 min               | Embryonic shield visible from animal pole                                   | 2 b    |
| Gastrulation  | 75% epiboly| 9 h 30 min               | Dorsal side distinctly thicker                                              | 2 c    |
|               | 90-100% epiboly| 10 h 35 min         | Embryo elongation, blastoderm completely covers the yolk plug and closes    | 2 d    |
|               | Bud        | 15 h 35 min              | Prominent tail bud                                                          | 2 e    |
| Segmentation  | First somite| 16 h 30 min             | Formation of somite                                                         | 2 f    |
|               | 10-somite  | 17 h 40 min              | Optic vesicle and Kupffer’s vesicle                                         | 2 g    |
| Pharyngula    | 23 h       | 23 h                     | Movements by tail contractions                                              | 2 h    |
| Hatching      | 28 h 20 min| 28 h                     | Strong movements and hatching                                              | 2 i    |

Fig. 2. Gastrulation, segmentation, pharyngula and hatching periods in *B. caucanus*. a. Germ ring; b. Shield; c. 75% epiboly; d. 90-100% epiboly; e. Bud; f. First somite; g. 10-somite; h. Pharyngula; i. Hatching, free larvae.
Hatching of B. caucanus occurred at 594.3 Accumulated Thermal Units. This is higher than the hatching period previously reported for Astyanax altiparanae, Astyanax bimaculatus, and Tetragonopterus chalceus which varied between 410 to 624 Accumulated Thermal Units (Nakatani et al.; Sato et al., 2006).

The moderate perivitelline space of B. caucanus is similar to that reported by Nakatani et al. for A. altiparanae. These authors also showed that similarly to B. caucanus, the larvae of A. altiparanae and B. stramineus have little body pigmentation.

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