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

Desarrollo Embrionario de *Bryconamericus caucanus* (Characidae: Tetragonpterinae) 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 hydratation was moderate. Hatching occurred 28 h 20 min after fertilization (21ºC, 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 (Vari & 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; Borçato *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" (6º2´43´´N, 73º37´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, 6º16´20´´N, 75º35´18´´W). The fish were kept in 15 gallon aquariums 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.

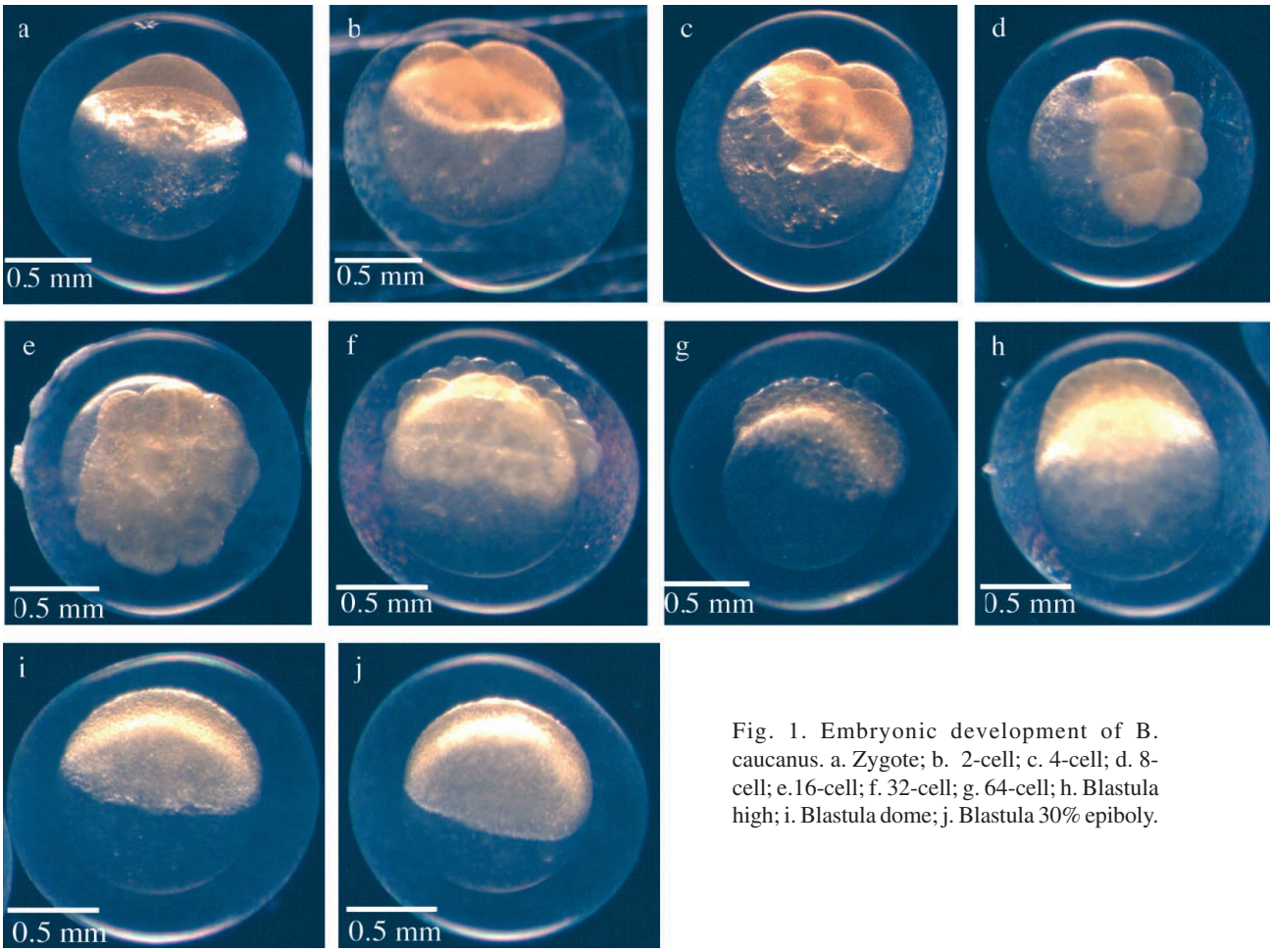

Fig. 1. Embryonic development of B. caucanus. a. Zygote; b. 2-cell; c. 4-cell; d. 8-cell; e.16-cell; f. 32-cell; g. 64-cell; h. Blastula high; i. Blastula dome; j. Blastula 30% epiboly.

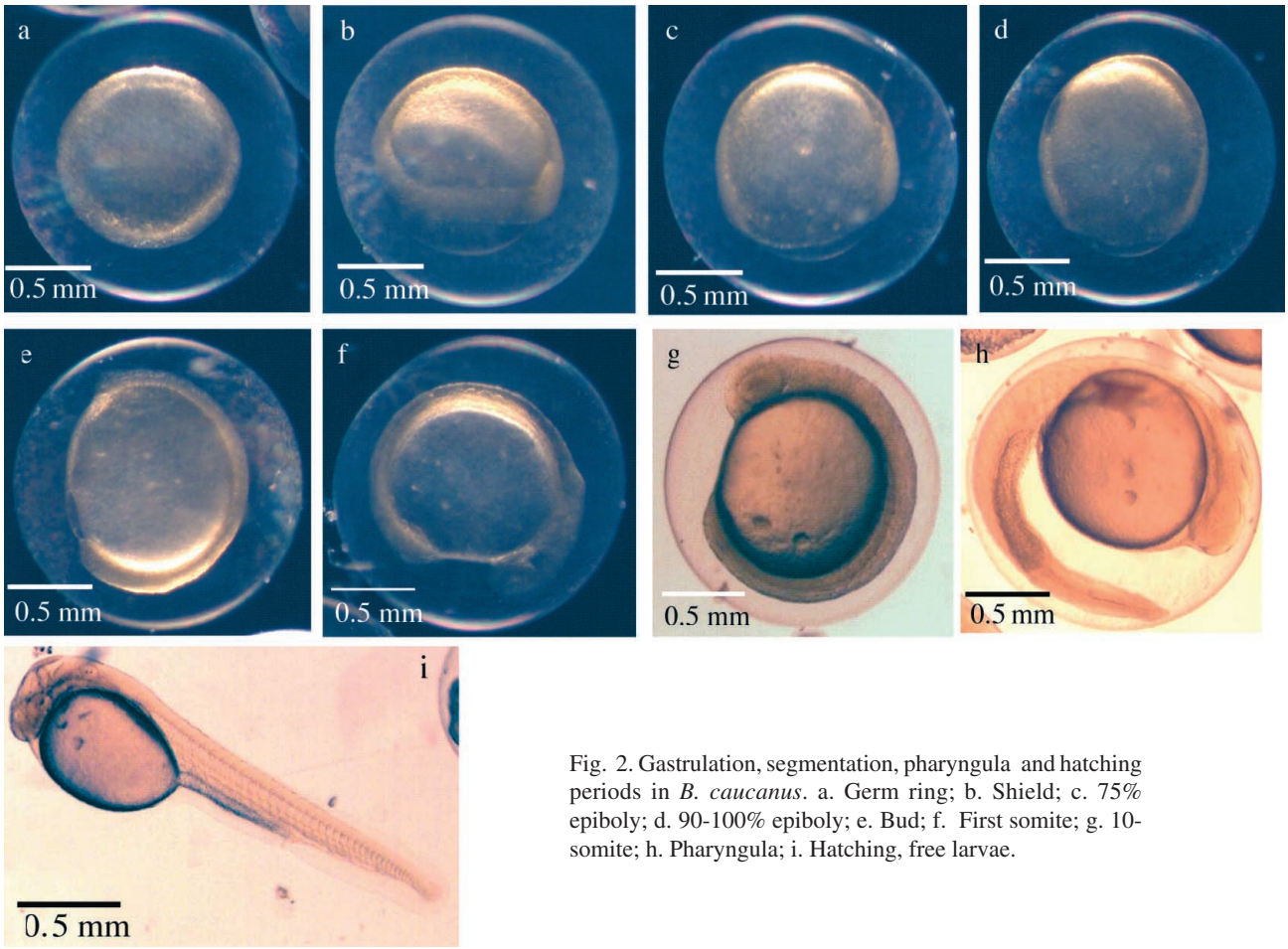

Fig. 2. Gastrulation, segmentation, pharyngula and hatching periods in *B. caucanus*. a. Germ ring; b. Shield; c. 75% epiboly; d. 90-100% epiboly; e. Bud; f. First somite; g. 10-somite; h. Pharyngula; i. Hatching, free larvae.

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
Cleavage	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	Cylcle 6 of cleavage	1 g
Blastula	High	4 h 30 min	Beginning of blastodisc flattening	1 h
	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
Gastrulation	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
	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	Movements by tail contractions	2 h
Hatching		28 h 20 min	Strong movements and hatching	2 i

DISCUSSION

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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RESUMEN: Se estudian, bajo condiciones de laboratorio, los estadios tempranos de desarrollo en *Bryconamericus caucanus*. Hembras maduras (peso promedio de 8,12g) recibieron dos dosis intraperitoneales de extracto de hipófisis de carpa (1a dosis de 0,5 mg/ Kg en la hora cero y 2ª dosis de 5 mg/Kg en la hora 12). Asímismo, los machos maduros recibieron una única dosis de 0.5 mg / kg en la 2ª dosis de las hembras. Se llevó a cabo desove en seco a 152,25 grados-hora luego de la aplicación de la 2ª dosis hormonal. Los huevos de *B. caucanus* eran amarillos, redondeados, no adhesivos y el espacio perivitelino luego de la hidratación fue moderado. La eclosión se registró 28 h 20 min después de la fertilización (594,3 grado-horas a 21ºC). La respuesta positiva a la hipofización y las características morfológicas del huevo y del embrión de *B. caucanus* fueron similares a los reportes previos en otros miembros de la subfamilia Tetragonopterinae, siendo el desarrollo embrionario de *B. caucanus* particularmente prolongado.

PALABRAS CLAVE: Characiformes; Peces; Propagacion artificial.

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