MORPHOGENETIC MOVEMENTS DURING GASTRULATION OF *Rhamdia quelen* (TELEOSTEI, HEPTAPTERIDAE): EFFECTS OF TEMPERATURE INCUBATION

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Rhamdia quelen (jundiá) is a catfish native from Americas of high commercial value, which is newly introduced in aquaculture and has attracted wide interest of many scientists in respect to its biology. The study of teleost development is an increasing subject in recent years. Gastrulation is an early phase in the development of animal embryos, during which morphogenetic movements dramatically restructure the morphology of the embryo and direct the formation of germ layers. The main factor affecting the rate and the quality of embryonic development is temperature. Mature males and females of *R. quelen* were induced to reproduce through hypophysation. Incubation of fertilized eggs took place in 24 incubators of 1,3L. The incubators were divided in 4 groups kept in different temperatures: 24°C, 26°C, 29°C, and 32°C. Embryos samples were taken from incubators every 10-30 min and fixed in 4% Paraphormaldeyde in 0,1M PBS at 4°C for light microscopy. Samples were analyzed in a Zeiss Axiophot microscope. Embryos were measured using a computerized morphometry system (Sigma Scan, Jandel Scientific, v. 3.0). For scanning electron microscopy, samples are fixed in Karnowiski and analyzed in a JEOL-JSM 6360 LV. Photomicrographs of live embryos were taken with a Sony digital camera on a Quimis light microscope. At the time of mid-blastula transition, the embryo is composed of three distinct cell layers: the enveloping layer (EVL), deep cells, and the yolk syncytial layer (YSL) formed from the fusion of cells adjacent to the yolk cells [1]. The late blastula stage (Fig. 1A and B) precedes gastrulation. In this phase, the deep blastoderm cells move outwardly to intercalate with the more superficial cells [2], compacting the blastoderm. Gastrulation of *R. quelen* begins 3h20min (26°C) after fertilization with the morphogenetic movement named epiboly, characterized by the spread of EVL and the deep cells over the YSL. Active cell repacking by these so-called "radial intercalations" [4] may be a part of the driving force of early epiboly. After the blastoderm cells have covered about half the yolk cell (50% epiboly; Fig. 1C and D), a thickening occurs throughout the margin of the epibolizing blastoderm. This thickening occurs all around the margin and is called the germ ring (Fig. 1C and D), and it is composed of a superficial layer, the epiblast, and an inner layer, the hypoblast. The hypoblast is formed through involution and/or ingression superficial cells under the margin followed by their migration toward the animal pole [1]. Once formed, the cells of both the epiblast and hypoblast intercalate on the future dorsal side of the embryo to form a localized thickening, the embryonic shield [1]. In *R. quelen* the shield stage occurs 8h after fertlization (26°C) (Fig. 1E and F). This shield is functionally equivalent to the dorsal blastopore lip of amphibians, since it can organize a secondary embryonic axis when transplanted to a host embryo [5]. Concomitantly with the epiboly, the convergent extension makes one side of the blastoderm noticeably thicker than the other (Fig. 1G and H). Cell-labeling experiments indicate that the thicker side marks the site of the future dorsal surface of the embryo [3]. EVL and the deep cells move over the surface of the yolk to envelop it completely 11h after fertilization (26°C) (Fig. 1I and J). Tested temperatures (26 to 32°C) did not affect the occurrence of morphogenetic movements, gastrulas with similar morphological features, except to the size (Fig. 2) . Nevertheless, the temperature was direct proportional to development speed (Fig. 3).

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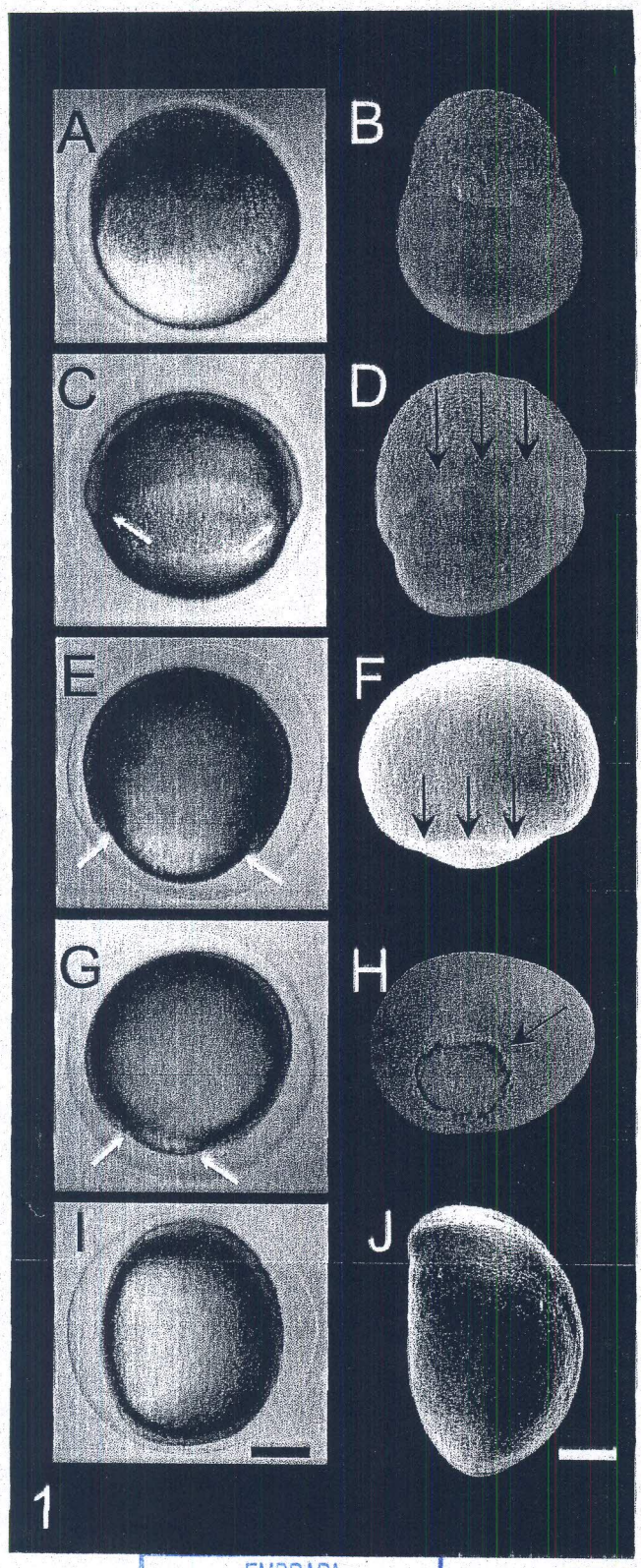

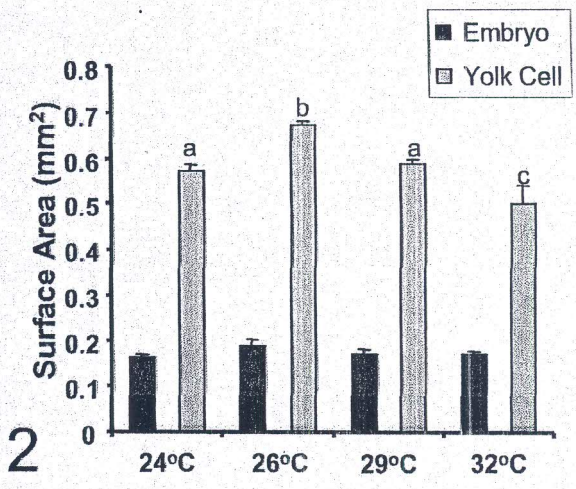

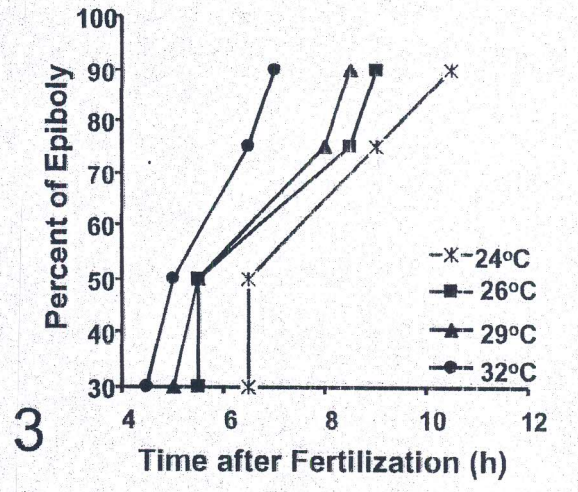

Figure 1: *Rhamdia quelen* embryo in (A and B) late-blastula stage (dome stage), (C and D) Germ-ring stage (50% epiboly), (E and F) 75% epiboly stage, (G and H) 90% epiboly stage, (I and J) Caudal Bud stage (100% epiboly). (A, C, E, G, and I) Light microscopy, Scale: 500 µm. (B, D, F, H, and J) Scanning electron microscopy, Scale: 500 µm. Lateral view, except to H (vegetal pole view). The arrows show the edges of blatoderm that suffer epiboly.

Figure 2: Embryo and yolk cell surface area of *Rhamdia quelen* at the end of gastrulation in four temperatures. Different letters correspond to significant differences (ANOVA, $P<0.05$).

Figure 3: Percent of epiboly in *Rhamdia quelen* embryo incubated in four different temperatures.