Oogenesis of the cardinal tetra *Paracheirodon axelrodi* Schultz (1956): a histological and histochemical study

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Abstract

A histological and histochemical study of *Paracheirodon axelrodi* oogenesis was conducted. Four types of oocytes were determined, presenting a thin zona pellucida and squamous follicle cells in all developmental stages. The yolk globules in vitellogenic oocytes are spherical and the micropyle possessed an ample vestibule and short micropylar canal. Atresic follicles were frequent, since ovulation did not occur. The histochemical reactions demonstrated the presence of neutral glycoproteins in the zona pellucida and follicle cells; glycoproteins and lipids in the yolk globules and carboxylated acid glycoconjugates in the cortical alveoli. The knowledge of the reproductive parameters becomes an important tool in captivity breeding programs, reducing the fishing effort on the native stock.

Keywords: *Paracheirodon axelrodi,* reproduction, oocyte, follicle atresia.

1 Introduction

Capture of small-sized ornamental fishes captured in the Negro River and tributaries present a great value in the national and international trade markets. Approximately 70% of the fish exported from the Amazon State comes from the Negro River (ANJOS, SIQUEIRA and AMORIM, 2007) with the municipalities of Barcelos and Santa Isabel do Rio Negro being the main sale locations (CHAO, 2001; FREITAS and RIVAS, 2006).

Although dozens of species are regularly exported from the Amazon as ornamental fish, the demand is centered on a small number of species. The main species is the cardinal tetra *Paracheirodon axelrodi* (SCHULTZ, 1956), representing almost 3/4 of the export trade in fish (ANJOS, SIQUEIRA and AMORIM, 2007). Its metallic blue color makes it one of the most popular species among Amazonian ornamental fishes traded. They are small-sized fish, reaching up to 5.1 cm in standard length (GEISLER and ANNIBAL, 1986). In the wild, most *P. axelrodi* species have longevity of little more than one year (GEISLER and ANNIBAL, 1986), but in captivity they are reported to live for more than five years (CHAO, 2001).

Recently, *P. axelrodi* has been the subject of studies in its natural environment (WALKER, 2004; MARSHALL, FORSBERG and THOMÉ-SOUZA, 2007). Recent experimental work provided a better understanding of its biology because this species is easy to maintain in captivity. Laboratory experiments conducted by Anjos and Anjos (2006) demonstrated batch spawning of the species and identified the fecundity to be between 154 and 562 adhesive eggs.

Like other ornamental species of economic importance and interest on the international market, aspects of the biology of *P. axelrodi* are still unknown (HARRIS and PETRY, 2001). Such information that can aid culturing techniques of this species on a larger scale is extremely necessary, thereby reducing the fishing pressures on native stocks. Thus, the present study contributes to a better understanding of the reproductive biology of *P. axelrodi* through histological and histochemical investigations of the oocytes of mature females.

2 Material and methods

Paracheirodon axelrodi specimens acquired from an aquarium store were maintained in a communal 100 L aquarium during nine months. Artificial lighting was controlled to achieve a 12:12 hours L:D photoperiod. The system was heated to maintain a temperature of 26 °C. During this time, the fishes were fed twice a day with regular commercial fish food (47% protein) and *Artemia salina* nauplii given 3 times a week as a food complement. Females (n = 8) were anesthetized and then sacrificed. Ovaries were removed and fixed in Bouin's fluid for 10 hours, embedded in paraffin inclusion, cut in 5 μm sections and stained by hematoxilin and eosin (HE). Histological sections derived from both ovaries were analyzed.

The oocyte development stages were established based on the morphological alterations of the nucleus, cytoplasm and coats (BAZZOLI, 2003). The presence of carbohydrates, proteins and lipids in the oocytes was determined using classical histochemical techniques according to Pearse (1985): Periodic acid-Schiff (PAS), salivary amylase + PAS, Alcian blue pH 2, 5, Alcian blue pH 0, 5, ninhidrina-Schiff and Sudan black B.

Oocyte morphometry was taken using a micrometric ocular piece in a light microscope. Mean diameter and standard deviation was established for each oocyte development stage.

3 Results

The oocyte development behaved in a progressive manner, beginning with smaller cells and ending with the largest cell of the lineage, the vitellogenic oocyte. Through histological analysis of the ovaries of *P. axelrodi* four stages of oocyte development were characterized:

- Initial perinucleolar oocyte (O1) (58.4 ± 11.6 μm): with a strongly basophilic ooplasm and nucleus with several nucleoli (Figure 1a);
- Advanced perinucleolar oocyte (O2) (114.4±18.0 μm): with a granular basophilic ooplasm and nucleus with nucleoli close to nuclear envelope (Figure 1a). There a conspicuous structure occurs in the ooplasma, the yolk nucleus;
- Previtellogenic oocyte (O3) (220.0 ± 8.2 μm): presenting discontinuities in the cortical alveoli in the peripheral ooplasm, thin zona pellucida and squamous follicle cells (Figure 1b); and
- Vitellogenic oocyte (O4) (474.9 ± 47.4 μm): with spherical and acidophyle yolk globules, thin zona pellucida and squamous follicle cells (Figure 1c, d). In the O4 a micropyle constituted of an ample vestibule and short micropyle canal was observed (Figure 1e).

The non-release of the vitellogenic oocytes caused them to process the resorption or atresia. This process was characterized by the hypertrophy of the follicle cells (Figure 1f), fragmentation of the zona pellucida and yolk degeneration (Figure 1g), and resorption of the oocyte content by the follicle cells (Figure 1h).

Through the histochemical reactions in the yolk globules, cortical alveoli, zona pellucida and follicle cells of *P. axelrodi*, it was possible to detect neutral glycoproteins in the zona pellucida and follicle cells; neutral glycoproteins and lipids in the yolk globules, and carboxylated acid glycoconjugates in the cortical alveoli (Table 1).

4 Conclusion

Determining the developmental characteristics of oocyte structures constitutes a primary step in understanding the reproductive guild of a species (SELMAN and WALLACE, 1989). The chemical content of these structures is also essential to understand their functional role in fertilization and in the initial development of the embryo (HART, 1990).

The reproduction in captivity of *P. axelrodi* has been obtained successfully by manipulating a series of events necessary to stimulate the posture process (ANJOS and ANJOS, 2006). Besides controlling confinement conditions, the food supply also plays an important role, acting directly on the process of vitellogenesis (BURTON, KAISER and HECHT, 1998). Although the cardinal females of this study completed their oocyte maturation, the absence of favorable conditions for spawning and their presence in a communal aquarium are pointed out as inhibiting factors for reproduction. There are cases in literature where individual *P. axelrodi* were kept in captivity for over four years without reproducing (CHAO, 2001).

Due to this unsuccessful reproduction, the vitellogenic oocytes were not released and resorption and atresia processes begun. In literature, follicle atresia shows as being caused by the administration of biocides, steroid hormones, temperature changes and feeding restrictions (GURAYA, 1986; BLAZER, 2002). The atresia process observed in the cardinal oocytes is similar to other studies (MIRANDA, BAZZOLI, RIZZO et al., 1999; DURKINA, 2006), characterized by the fragmentation of the zona pellucida, hypertrophy of follicle cells, yolk degeneration and resorption of oocyte content by the follicle cells.

The advanced perinuclear oocytes presented nucleoli close to nuclear envelope for the transference of ribonucleoproteins of the nucleus to the cytoplasm, destined to form the yolk nucleus, which takes part in the synthesis of oocyte organelles (BAZZOLI and GODINHO, 1995; TYLER and SUMPTER, 1996). The morpho-physiological events that occur during the formation of the yolk nucleus do not depend on gonadotrophins, taking place in the primary or pre-vitellogenic growth phase (PATIÑO and SULLIVAN, 2002).

There are vacuolar structures in the cytoplasm of the previtellogenic oocytes of teleosts, not stained or slightly basophile, the cortical alveoli, of a varied chemical composition depending on the species (BAZZOLI and GODINHO, 1994). The cortical alveoli are synthesized endogenously, involving the rough endoplasmic reticulum and the Golgi complex (TYLER and SUMPTER, 1996). They are analogous to the cortical granules of mammals, and their content is released in the perivitelline space at the moment of fertilization, constituting a blockage to polyspermy (YAMAMOTO, 1961). In *P. axelrodi*, the cortical alveoli contain carboxylated acid glycoconjugates, similar to other Neotropical Characiformes (BAZZOLI and GODINHO, 1994).

Glycoproteins and lipids were detected in the yolk globules of *P. axelrodi*. Carbohydrates, proteins and lipids are essential for the nutrition of fish embryos (KUNZ, 2004). In some cases they can represent more than 80% of the dry weight of the egg (CALLEN, DENNEBOUY, MOUNOLOU, 1980). These substances contained in the vitellogenin, which is a phosphoglycoprotein, are synthesized in the liver and incorporated to the yolk globules (SELMAN and WALLACE, 1989; KUNZ, 2004).

Fish oocytes, like other vertebrates, are surrounded by an acellular and acidophyle envoltory, the zona pellucida, which presents varied thickness and number of layers, depending on the species (BAZZOLI, 1992). In teleosts, the zona pellucida is more complex and elaborated, presenting a specialized area, the micropyle, which allows for the entrance of the fertilizing sperm directly without the occurrence of an acrossomal reaction, which occurs in most vertebrates (REDDING and PATIÑO, 1993). The micropyle morphology is also variable according to the diameter of the sperm head (RIZZO and GODINHO, 2003). Micropyles in freshwater fish have been divided into four types (KUNZ, 2004). In *P. axelrodi* the micropyle presented an ample vestibule and a short micropylar canal which opens to the inner surface of the envelope, like some Balitoridae, Callichthyidae, Cichlidae, Cyprinidae, Doradidae, Erythrinidae, Gasterosteidae, Loricariidae and Pimelodidae (RICARDO, AGUIAR, RIZZO et al., 1996; RIZZO, 2001; KUNZ, 2004).

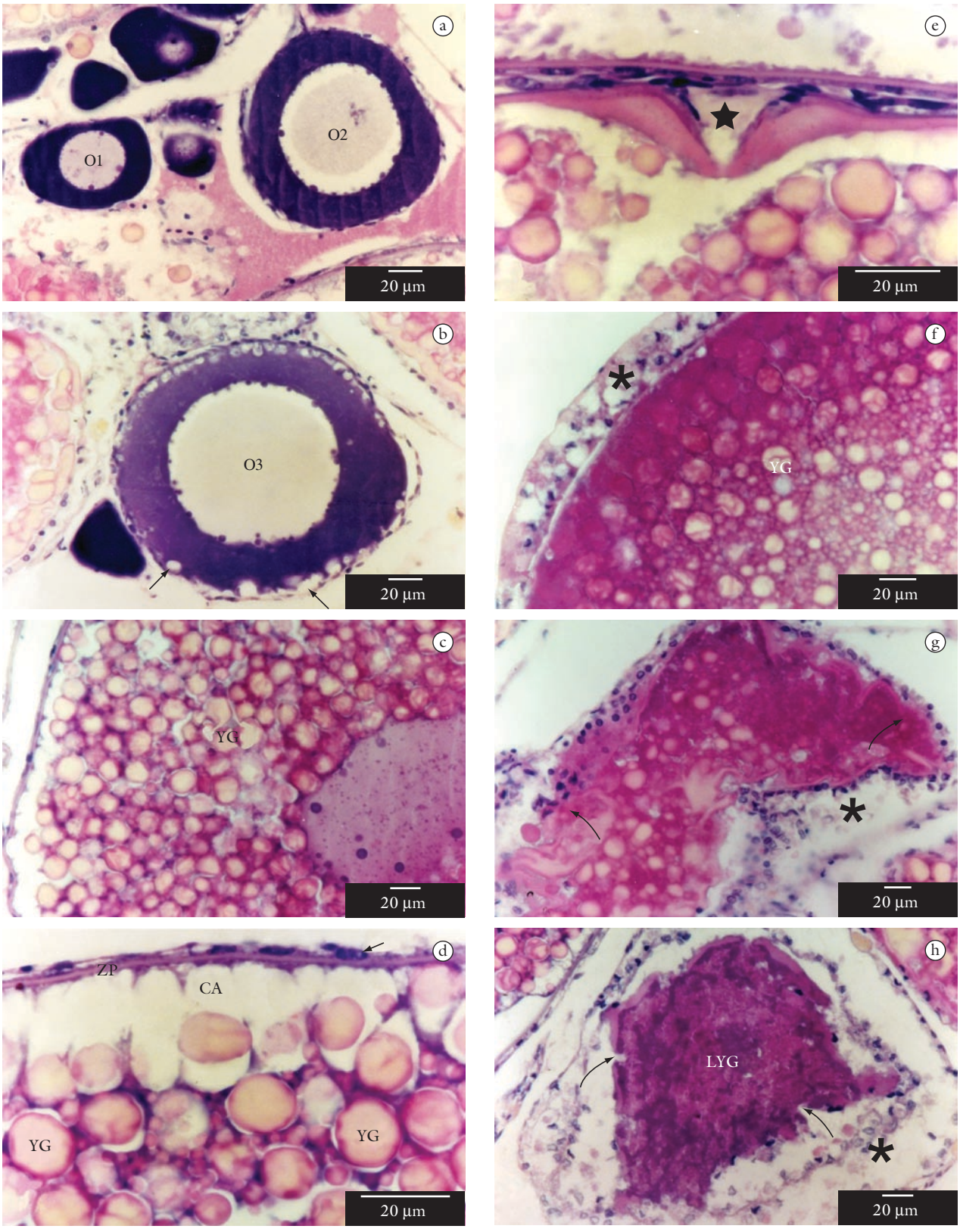

Figure 1. Transverse sections from ovaries of *P. axelrodi* stained with HE. a) initial perinucleolar oocyte (O1) and advanced perinucleolar oocyte (O2); b) cortical alveoli (arrows) in previtellogenic oocyte (O3); c) vitellogenic oocyte full of yolk globules (YG); d) vitellogenic oocyte with squamous follicle cell (arrow), thin zona pellucida (ZP), cortical alveoli (CA) and yolk globules (YG); e) micropyle (star) in vitellogenic oocytes; f) atresic follicle with hypertrophied follicle cells (asterisk); g) atresic follicle with hypertrophy of the follicle cells (asterisk), fragmented zona pellucida (arrow) and yolk degeneration; and h) atresic follicles with degenerated yolk globules (LYG), hypertrophy of follicle cells (asterisk) and slits in the zona pellucida (arrow). Scale bar = 20 µm.

Table 1. Histochemical reactions Periodic acid-Schiff (PAS), salivary amylase + PAS (AS), Alcian blue (AB), Ninhidrina-Schiff (NS) and Sudan black B (SB) in the yolk globules, cortical alveoli, zona pellucida and follicle cells of *P. axelrodi*.

Structure	Histochemical reactions						
	PAS	AS	AB 0.5	AB 2.5	NS	SB	Content
Yolk globules	+	+	–	–	+	+	Neutral glycoproteins and lipids
Cortical alveoli	–	–	–	+	+	–	Carboxylated acid glycoconjugates
Zona pellucida	+	+	–	–	+	–	Neutral glycoproteins
Follicle cells	+	+	–	–	+	–	Neutral glycoproteins

+ = positive reaction; and – = negative reaction.

The precursor proteins from the zona pellucida are synthesized by the oocyte and liver (PATIÑO and SULLIVAN, 2002). In most teleosts, when completely formed, the zona pellucida is constituted of two layers: an internal thin layer, rich in polysaccharides and an external striated one, composed of 3 to 4 sub-units derived from precursor proteins, named choriogenins (FUJITA, SHIMIZU, HIRAMATSU et al., 2002). Substances are exchanged in the developing oocyte through the existing channels in the zona pellucida (KUNZ, 2004). In a similar way to other Neotropical teleosts (BAZZOLI and RIZZO, 1990; RIZZO and GODINHO, 2003), *P. axelrodi* also presents a zona pellucida constituted of two layers of neutral glycoproteins.

The follicle cells originate from the germinal epithelium of the ovary when they become prefollicle cells and emit processes that surround the oocytes when in meiotic division (GRIER, 2000). In the follicle cells of *P. axelrodi,* neutral glycoproteins were detected, which can be related to follicle growth, to the production of the hormone that induces maturation and to the zona pellucida development (PATIÑO and SULLIVAN, 2002).

Considering that *P. axelrodi* populations in their natural habitat have been increasingly exploited, studies approaching oogenesis aspects are important, because they are basic for breeding, and this becomes an alternative to reduce pressure on the native stocks.

Acknowledgments: The authors are grateful to the Brazilian Research Foundation CNPq, FAPEMIG and Capes for financial support.

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Received October 1, 2008
Accepted April 14, 2009