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Histological Study of Gonad in the Female Knifefish, *Brachyhypopomus gauderio*

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ABSTRACT

Six stages of the female *Brachyhypopomus gauderio* ovary were described. Two of these belong to the primary growth phase: primary growth one nucleolus (PGon) and primary growth peri nucleolus (PGpn), during which the oocytes are small in diameter and free of cortical alveoli. The third, fourth, and fifth stages represent secondary growth, characterized by the presence of cortical alveoli, which increase in number as the stages progress. This phase is called vitellogenesis and is divided into three stages: primary, secondary, and tertiary vitellogenesis. The final stage, known as periovulatory (PO), is marked by the oocytes being fully filled with cortical alveoli and the disappearance of the nucleus, indicating that the eggs are ready to be laid.

INTRODUCTION

Brachyhypopomus gauderio is a species of benthic fish belonging to the Hypopmid family native to South America **(Triques & Khamis, 2003; Loureiro & Silva, 2006; Giora *et al*., 2008; Giora & Malabarba, 2009)**.

The knifefish, *B. gauderio* is a model species that has specific strategy in its offspring through protecting its eggs in deep holes away from the enemies. It was classified by **Giora and Malabarba (2009)** as a new species under the Hypopmid family. Moreover, it is widely distributed in South America, especially in Brazil, Uruguay, Paraguay and Argentina **(Correa *et al*., 2011)**.

B. gauderio livelihood is limited to fresh water bodies such as rivers, flood regions, lakes, with plenty floating plants and in slow run creeks **(Correa *et al*., 2011)**. According to morphological description of **Giora and Malabarba (2009)**, the color body of *B. gauderio* varies from brownish to yellowish with darker head than other parts of the body.

The male differs morphologically from female in respect to the tail; female has thin cylindrical tail; whereas, in adult male the caudal filament is vertically widened, and become more laterally flattened in males during the time of reproduction **(Giora & Malabarba, 2009; Alshami *et al*., 2020)** (Fig. 1).

Giora *et al*. (2014) studied the habits of feeding and reproduction of *B. gauderio* in Brazil. They reported that *Brachyhypopomus gauderio* is carnivores that feed on a wide range of small crustaceans and insects. Regarding the reproductive behavior of these individuals, they indicated that the species begins reproductive activity during spring and summer. The size of individuals involved in mating is approximately 108mm for males and around 104mm for females. The number of eggs laid by females ranges from 300 to 800.

Brachyhypopomus gauderio is known to be a gregarious species, and its reproductive strategy is classified as a polygynous system **(Perrone & Silva, 2016)**. Members of the order Gymnotiformes exhibit specific behaviors during spawning. They spawn and conceal their eggs exclusively at night, and the spawning process occurs in phases **(Kirschbaum & Schugardt, 2002; Giora *et al.*, 2014)**. *B. gauderio* is important for various studies, including genetics, development, and clinical research, making it necessary to study the developmental stages of ova to determine the characteristics of each stage.

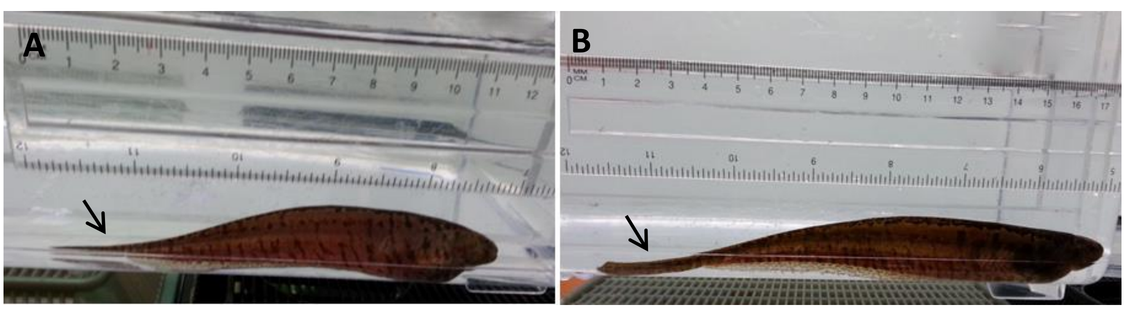

Fig. 1. The blunt nose knifefish *B. gauderio*; **(A)** Adult female 110mm; **(B)** Male 160mm **(Alshami *et al*., 2020)**

MATERIALS AND METHODS

Knifefish *B. gauderio* rearing

The knifefish *Brachyhypopomus gauderio* were cultivated in aquariums designed to mimic their natural conditions, as noted in a study by **Giora and Malabarba (2009)**, with a temperature of 26°C and a pH of 7.3. The water used in the aquariums was continuously aerated fresh water with a conductivity of 300µS/ cm, and it was changed daily. The photoperiod was set to 16 hours of light and 8 hours of darkness. Fish were fed once daily with frozen blood worms and twice daily with saline shrimp (*Artemia salina*).

To induce reproduction and spawning, the water conductivity was altered by adding 25% deionized water twice a week. Three females and one male were kept in the same container, and the nests for the eggs consisted of one to two plastic tubes with small pores attached to the side of the aquarium, where the fish could deposit their eggs. Eggs were collected from the tubes after the females laid the fertilized eggs. Mature females lay eggs every two to three weeks.

Harris hematoxylin/eosin staining

Eggs were fixed in 4% PFA/PBS for 4-5 days, then washed with distilled water 2-3 times and dehydrated through an increasing series of ethanol. They were then cleared with xylene in two stages (1:1 xylene, followed by paraffin only) and embedded in paraffin wax (Sigma-Aldrich) using a Shandon Citadel Tissue Processor 2000 (Thermo Scientific).

Sections of the blocks were cut at a thickness of 5µm using a rotary microtome (Leica Biosystems RM2125 RTS). Harris hematoxylin and eosin staining was performed using a Shandon Varistain 24-4 automatic slide stainer (Thermo Scientific), and the slides were coverslipped with Histomount (National Diagnostics). Photographs were taken using a Zeiss Axioskop 40 microscope.

RESULTS AND DISCUSSION

The histological sectioning of the *Brachyhypopomus gauderio* ovary revealed different stages of oocytes (Fig. 2), reflecting the reproductive strategy of *B. gauderio* as fractional spawners. This strategy allows the fish to reproduce multiple times throughout the year when food and environmental conditions are suitable. **Giora *et al.* (2014)** studied the reproductive biology of *B. gauderio* and found that the ovary contains various stages of oocytes, including mature oocytes, as well as some that have not yet begun the process of vitellogenesis. Their analysis indicates that the spawning strategy is sporadic and depends on the availability of mature oocytes.

Depending on the fish species, reproductive strategies can vary. Some fish lay a high number of eggs without parental care, while others lay a limited number but provide protective shelter and parental care. Additionally, some hermaphrodite species can change sex based on environmental conditions and reproductive needs. Ultimately, these strategies aim to prevent extinction **(Gavriilidis, 2022)**. *B. gauderio*, like other species in this family, follows a pattern of laying eggs in frequent batches throughout the year **(Perrone & Silva, 2016)**.

Based on the developmental stages of the *B. gauderio* ovary, five distinguishable stages were identified, starting with the primary growth of oocytes and culminating in mature ova before spawning (Table 1).

1. **Primary growth one nucleolus (PGon):** Characterized by small oocyte size and the expansion of the nucleus, with a single nucleolus as the most distinctive feature.

2. **Primary growth peri nucleolus (PGpn):** At this stage, the oocytes are slightly larger, with a greater area of cytoplasm stained darkly with eosin. Many nucleoli start to appear, and the oocyte membranes are not clearly visible.

3. **Vitellogenesis:** This stage includes three main sub-stages:

    - **Primary vitellogenesis (Vtg1):** The initial stage of vitellogenin formation, where cortical alveoli aggregate around the outer peripheral line of the oocyte.

    - **Secondary vitellogenesis (Vtg2):** The progression of vitellogenin formation, with cortical alveoli covering more of the ooplasm.

    - **Tertiary vitellogenesis (Vtg3):** At this stage, cortical alveoli occupy most of the ooplasm, and the oocyte membrane becomes thicker and more visible.

4. **Maturation stage (Periovulatory, PO):** The eggs are ready to be laid, characterized by a complete filling of cortical alveoli throughout the ooplasm (Fig. 3). The nucleus disappears, migrating to the animal pole, while the oocyte membrane thickens and is surrounded by perifollicular cells (Fig. 4). Perifollicular cells play a crucial role in oocyte maturation and are involved in regulating ovarian hormones. Follicular cells produce peptides that adjust the synthesis of ovarian hormones **(Robinson *et al*., 2009; Katz, 2012)**.

As oocytes develop, the number of nuclei increases, which is associated with oocyte maturation and the synthesis of rRNA important for protein synthesis **(Fu *et al.*, 2024)**. The nuclei within oocytes differ in morphology and become transcriptionally inactive as the oocyte matures, contrasting with the nuclei in somatic cells; they can be metaphorically referred to as nucleolar precursor bodies **(Kyogoku *et al.*, 2014; Amer & Ahmed, 2019)**.

With the onset of vitellogenin production, the oocyte enlarges to provide sufficient nutrients for the developing embryo. During oocyte maturation, the oocyte walls are formed and they increase in thickness. The walls of the oocyte are believed to play an important role in maturation. **Hart (1990)** noted that oocyte walls have physiological functions, including preventing polyspermy and providing protection for the embryo throughout its developmental process.

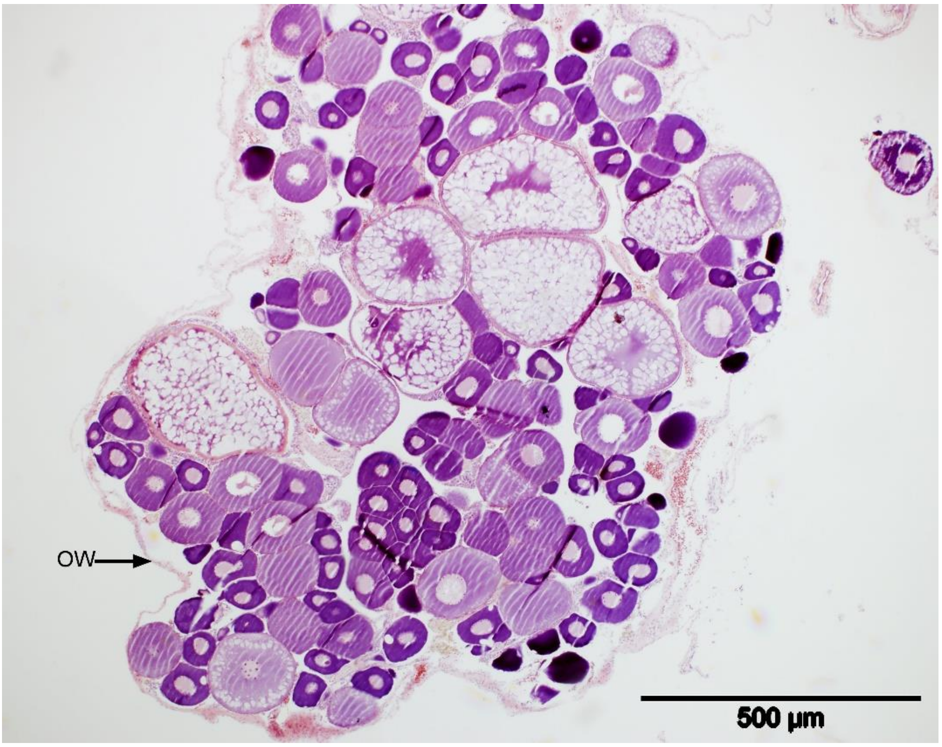

Fig. 2. Histological section of the ovary of *B. gauderio* contains the different stages of oocyte, OW: oocyte wall

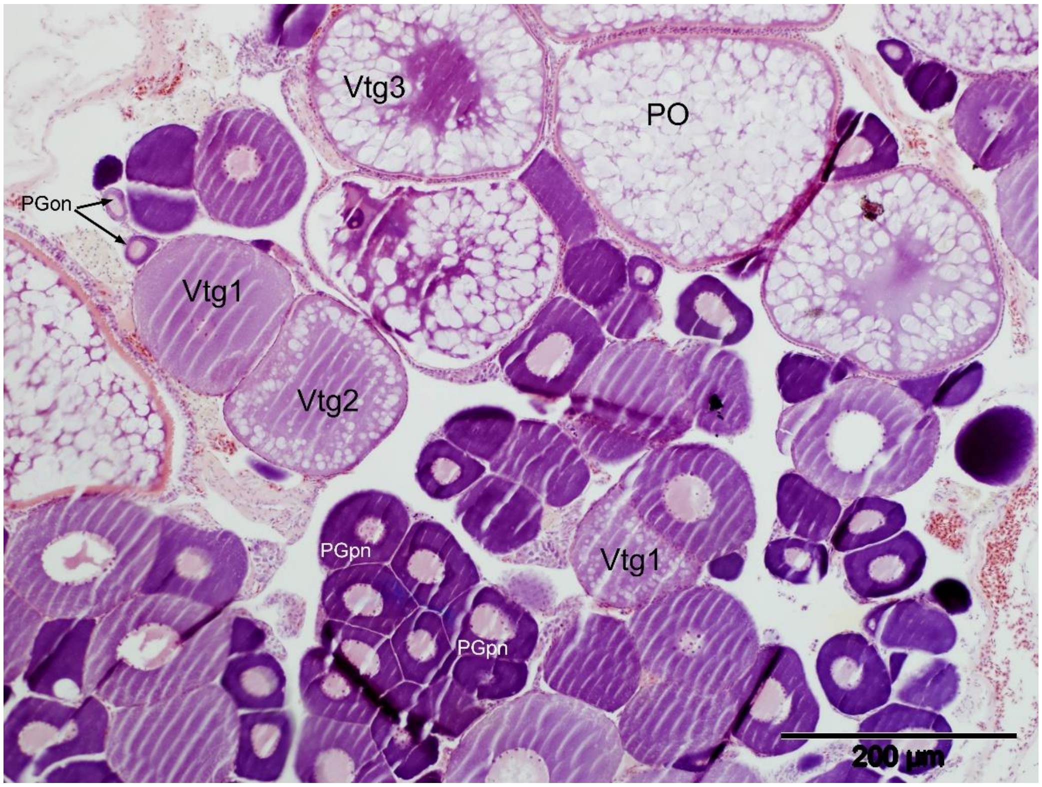

Fig. 3. The stages of oocyte development in *B. gauderio* , PGon: primary growth one nucleolus; primary peri-nucleolus; Vtg1: primary vitellogenesis; Vtg2: secondary vitellogenesis; Vtg3: tertiary vitellogenesis; PO: periovulatory

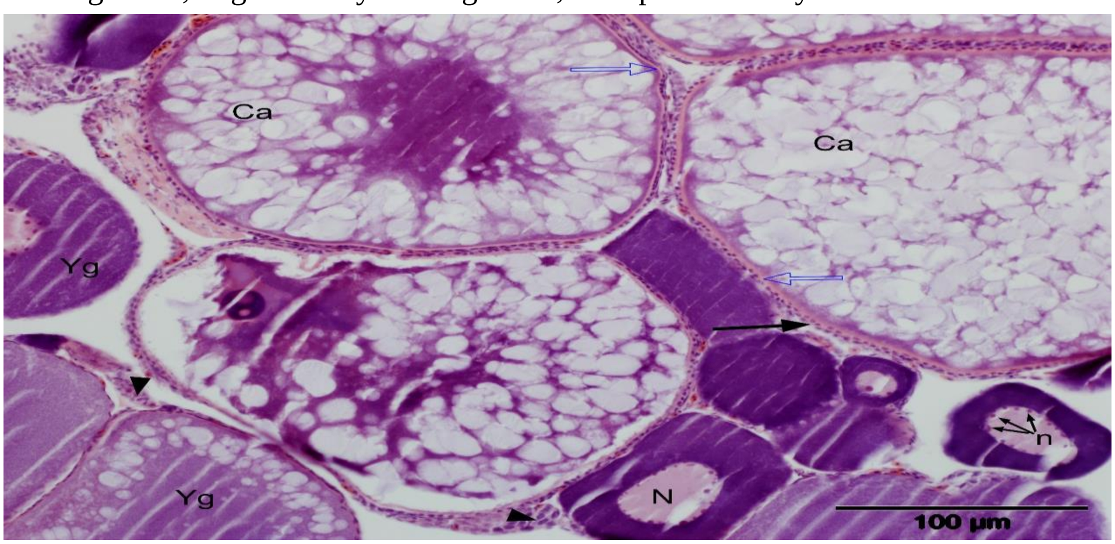

Fig. 4. The structure of oocyte stages, Ca: cortical alveoli; N: nucleus; n: nucleolus; Yg: yolk globules; bold arrows: granulosa; unfilled arrows: zona radiata; arrow head: the perifollicular cells

Table 1. Characterization of developmental stages of *B. gauderio* ovary. These stages of oocyte development division are based on details in the studies of **Brown-Peterson *et al*. (2011)** and **Grier *et al*. (2009)**

Growth phase	Stage of development	Description	Size of oocyte (µm)
Primary growth	Primary growth one nucleolus (PGon)	Oocyte are small in diameter; the nucleuses are larger than PGpn stage and it covers more area and surpasses the cytoplasmic size; the nucleolus is only one. The cytoplasm is stained deeply with eosin.	21-24
	Primary growth peri nucleolus (PGpn)	Oocyte are little larger, the nucleus to cytoplasm ratio is less than that in PGon stage, more than one nucleolus are present, the cytoplasm is still stained darkly with eosin.	45-71
Secondary growth	Primary vitellogenesis (Vtg1)	Cortical alveoli appear in this stage, but in less number, the yolk globules occupy the large area of cytoplasm and stain becomes lighter, the nucleus: still observed.	92-126
	Secondary vitellogenesis (Vtg2)	Cortical alveoli number becomes more and aggregates around the outer periphery of oocyte, Cortical alveoli block more area of yolk globules.	120-124
	Tertiary vitellogenesis (Vtg3)	Cortical alveoli occupy the most of ooplasm, oocyte membrane becomes thicker and visible.	150-190
Mature	Periovulatory (PO)	Cortical alveoli fill completely all the ooplasm, the nucleus disappears, the oocyte membrane is thicker and surrounded with perifollicular cells	198-250

CONCLUSION

To summarize, there are six developmental stages of the *Brachyhypopomus gauderio* ovary: primary growth one nucleolus (PGon) and primary growth peri nucleolus (PGpn) belong to the primary growth phase, while primary vitellogenesis (Vtg1), secondary vitellogenesis (Vtg2), and tertiary vitellogenesis (Vtg3) are part of the secondary growth phase, culminating in the mature periovulatory (PO) stage.

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