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Sex Differentiation and Gonadal Development of striped snakehead (*Channa striata* Bloch, 1793)

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Sex Differentiation and Gonadal Development of striped snakehead (*Channa striata* Bloch, 1793)

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Abstract. The striped snakehead *Channa striata* is a predatory fish whose life cycle still holds many mysteries. One of the obstacles to cultivating this fish is the difficulty of obtaining male broodstock. This study aimed to examine the sexual development of *C. striata*. Such information will be of benefit not only for the cultivation of *C. striata*, but also for sustainable management and exploitation of wild populations. Samples were collected from June to September 2017 in the inland waters (rivers and canals) of Barru and Bantaeng Districts, South Sulawesi, Indonesia. Gonads were observed both macroscopically and microscopically including gonad morphology, acetocarmine solution staining, and histology. We found that female *C. striata* were larger and more numerous compared to males in the same population (2 female:1 male). We identified five different oogenesis phases in *C. striata*: immature, maturing, mature, spawning (partial spawning), and post-spawning (spent). Some individuals of standard length 81-229 mm had an intersex (ovotestis) type gonad. In fish of standard length 110-241 mm, two types of gonads were found: a pair of ovaries suspected to be undergoing apoptosis, and a pair of developing testes. Two pairs of gonads were also found in an adult of length 381 mm, but both pairs were ovaries, of which one was in mature stage (ripe), the other undergoing apoptosis. This study suggests that *Channa striata* is at least potentially hermaphroditic, and its gonads can undergo a differentiation phase. We recommend further study on the genetic mechanisms and/or environmental influences which determine gonad development and sex differentiation in *C. striata*.

1. Introduction

Studies on fish reproduction require knowledge of gonad (oocyte and sperm) development. An improved knowledge and understanding of gonad development can lead to a deeper understanding of sex determination and differentiation, which in turn can serve as a basis for the application of biotechnological tools to increase aquaculture production. A review by Nelson et al. [1] found that, although over 32,500 species of fish have been described, very few sex-linked genes have been identified, most of which are associated with the male sex (e.g. genes dmy, gsdfy, sdY, etc.). In many fish sex is knows to be modulated by environmental factors affecting gonadogenesis, at critical points during early development (embryonic or juvenile phases) [2,3,4].

Unlike most vertebrates, in fish gonad development and sex differentiation are flexible processes, especially during early gonadal development, a labile or critical phase when fish tend to be extremely sensitive to environmental factors [2,5]. Fish sex can be changed during the early stages of development by factor such as changes in water temperature and pH [6,7], as well as the presence of endocrine disrupting chemicals (EDCs) in the water [8,9,10]. In many fishes, research has found that labile/critical phases can occur during early embryonic development, or during the juvenile phase before any differentiated cells (germ cells and additional cells) have been produced.

The striped snakehead *Channa striata* is a prized freshwater food fish, however processing technology is increasingly oriented towards medicinal uses. Striped snakehead culture is currently being developed in Indonesia to increase production, one reason being the decline in wild population abundance and distribution, due mainly to overfishing and habitat loss [11,12]. Overfishing of many wild striped snakehead populations was prompted by the discovery that this fish can accelerate would healing in humans due to its high albumin content. Striped snakehead habitat is increasingly being lost or degraded due to wetland conversion and other anthropogenic activities [11,12].

Challenges in striped snakehead culture include the paucity of data on reproductive characteristics, including gonad development, sex determination, and sex differentiation. Furthermore, there are no know morphological characters which can be used to reliably differentiate between male and female individuals during the maturing phase, and the sex ratio tends to be skewed towards females [12]. This background prompted the planning of a research programme with twin goals of increasing production and supporting conservation of the striped snakehead. This study focused specifically on striped snakehead gonad development and sex determination based on gonad morphology and histology, with an analysis of sex differentiation based on histological data. The results will provide a basis for the development and application of various technological means for increasing production and promoting conservation of the striped snakehead, as well as for predicting whether climate change is likely to affect the sex ratio of wild populations.

2. Materials and methods

2.1. *Striped snakehead sample collection*

Live *Channa striata* were collected from wetlands (creeks, swamps, canals, irrigation channels) in Barru and Bantaeng Districts South Sulawesi Province, Indonesia (Fig. 1) during June to September 2017 and brought to the Fish Biology Laboratory at Hasanuddin University, Makassar for study.

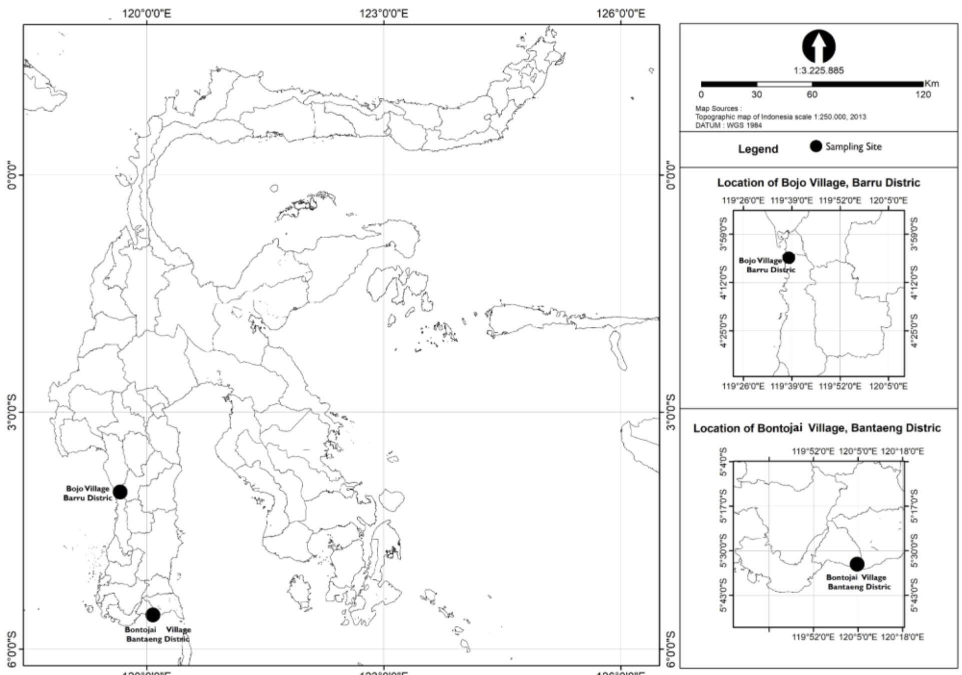

Figure 1. Map of striped snakehead sampling sites in Barru District (Bojo River) and Bantaeng District (irrigation canals) in South Sulawesi Province, Indonesia

Fish were caught using a traditional method, and random sampling produced a sample (N = 81) comprising 42 and 39 fish from Barru and Bantaeng district, respectively. The wet weight (w/w) and length (total length, TL and standard length, SL) of each fish were measured using standard procedures.

2.2. Sex determination

The sex of each striped snakehead was determined macroscopically (through dissection) and at the microscopic scale using an acetocarmine solution prepared according to [14] Guerrero & Shelton (1974) followed by histological examination [15]. The abdominal cavity was opened via a cut from the top of the pectoral fin to just behind the anus. Gonad maturity stage was determined macroscopically following a maturity key designed for fishery purposes. Gonad morphology was analysed descriptively following [16,17], with some modification. The gonads were then removed and a sample of around 0.5 cm was placed on a glass slide, 2-3 drops of acetocarmine were added; the sample was then finely chopped and observed under a binocular microscope at 4 x magnification.

2.3. Gonad histology

Following dissection, gonad morphology was observed using standard histological slide preparation methods. In small specimens the entire gonad was used, while for larger specimens segments were taken from the anterior, central and posterior region of both right and left gonads. The samples were fixed in 10% formalin solution for 24 hours. Each sample was sliced vertically to a thickness of 5 µm and stained with haematoxylin and eosin (H&E). Slide histology was observed and analysed using a MT-31 Binocular Entry-Level Advanced Compound Microscope and Euromex software, Microscope BS.1152-PLi, binocular. The maturity stage of ovaries was determined microscopically based on [18] (OECD, 2010) and a modified version of the gonad maturity scale in [19]. Based on morphological criteria, the method used [18] attributes a maturity value to ovaries and testes, that increases with gonad maturity (stage 0–5 for females; stage 0–4 for males). Completely undifferentiated or intersex individuals were not included in the maturity assessment.

3. Results and discussion

The striped snakehead is a predator whose life-cycle still retains many mysteries. Striped snakehead males and females are hard to distinguish without dissection, even in the mature phase. A secondary sexual character observed in both male and female individuals is a change in pigmentation, with black spots appearing on the ventral area and a reddish colouration of the urogenital area when an individual is in the mature phase.

The observed size at first maturity for *C. striata* from the Bojo River (Barru District was 115.60 mm TL/93.00 mm SL. In the Bantaeng wetlands, the size at first maturity was 230.00 mm TL/190.90 mm SL. The average size of adult *C. striata* was 292.10 ± 40.26 mm for females and 285.10 ± 85.71 mm for males.

Striped snakehead gonads observed were roughly tubular, situated below the intestines from the anterior abdominal cavity to the urogenital area. Gonad colour varied from off-white to brownish red and reddish white during immature and maturing phases, to yellowish-orange in the mature phase, Ovaries were longer than testes in fish of similar age/size. Early gonad development was seen in female striped snakehead of 113.6 ± 19.6 mm, while in males testes began to be detected at a length of 210.0 ± 42.4 mm.

Based on morphological, acetocarmine staining and histological traits, five ovarian developmental stages (Table 1) were determined for female striped snakeheads: immature (Figure 2), maturing (Figure 3), mature (Figure 4), spawning (Figure 5), and post spawning (spent) (Figure 6). This classification differs from the *Channa striata* ovarian maturity scale described by Al Mahmud et al. [20] which has four developmental stages: stage I (immature), stage II (maturing), stage III (mature), and stage IV (spent/recovering).

Table 1. Description of ovarian development in female striped snakeheads

Stage	Macroscopic appearance	Acetocarmine staining and Histology
I. *Pre-spawning*		
Immature Fig. 2	Small ovary, thin ribbon-like; transparent, yellowish white to reddish white in colour, oocytes not yet visible	Ova contains granules like sand; granule/oocyte diameter 0.022 to 0.528 mm
Maturing Fig. 3	Ovary straight, oocytes resemble white spots, reddish white in colour	Cytoplasm dominated by primary oocytes and cortical alveolar oocytes, oocyte diameter 0.033 to 0.550 mm
Mature Fig. 4	Ovary increases in size, oocytes clearly visible and yellowish-orange in colour	Dominated by late vitellogenic phase, yolk granules almost fill the ooplasm. The nucleus has not yet begun to migrate to the periphery, oocyte diameter 0.253 to 1.111 mm
II. *Spawning* Fig. 5	Oocytes yellowish in colour, ovary fully swelled and fills the abdominal cavity	Vitellogenesis has reached its peak; cells larger and more hydrated; nucleus has migrated toward the periphery of the cell and is in the process of dissolution. Ova dominated by oocytes in mature egg stage, oocyte diameter 0.033 to 1.484 mm
III. *Post-spawning* (*spent*) Fig. 6	Oocyte yellowish in colour, a few oocytes visible in ova that resemble membranes	Characterized by some oocytes undergoing atresia

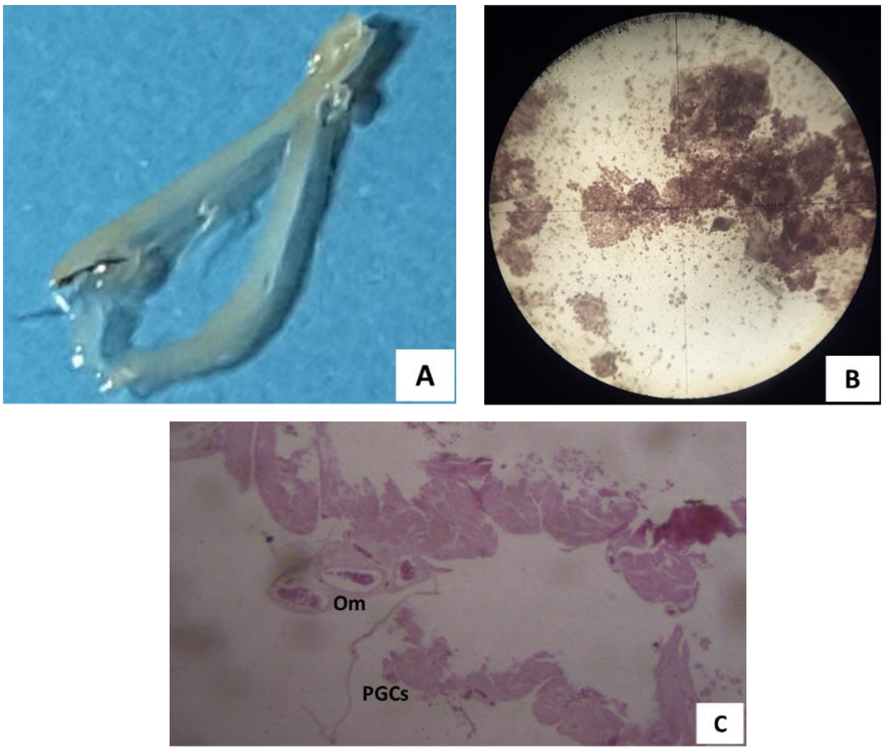

Figure 2. Immature stage, early differentiating *C. striata* gonad (80.0-136.0 mm TL, 65.0-112.0 mm SL) A: morphology B: acetocarmine staining C: histology: oocytes in meiosis (Om).

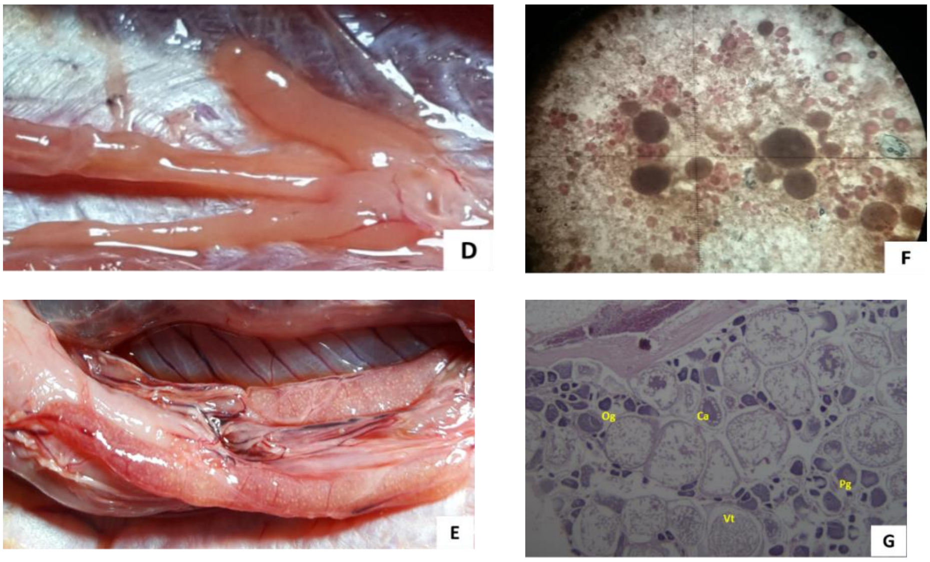

Figure 3. Maturing. Differentiating gonad of *C. striata* (230.0-287.0 mm TL, 190.9-233.0 SL) D,E. morphology F. acetocarmine staining G. histology: oogenia (Og), primary growth (Pg), cortical alveoli formation (Ca), vitellogenesis (Vt)

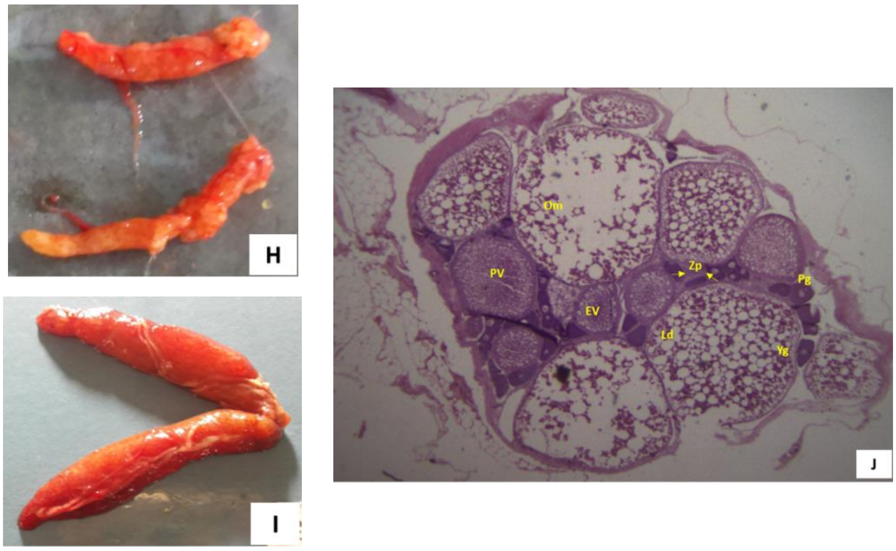

Figure 4. Mature differentiated *C. striata* gonad (240.0-336.0 mm TL, 200.0-280.0 SL). H and I. morphology J. histology: primary growth oocytes (Pg), yolk granules (Yg), zona pellucida (Zp), lipid droplets (Ld), early vitellogenic oocyte (EV) beginning to accumulate yolk granules peripherally, oocyte maturation (Om)

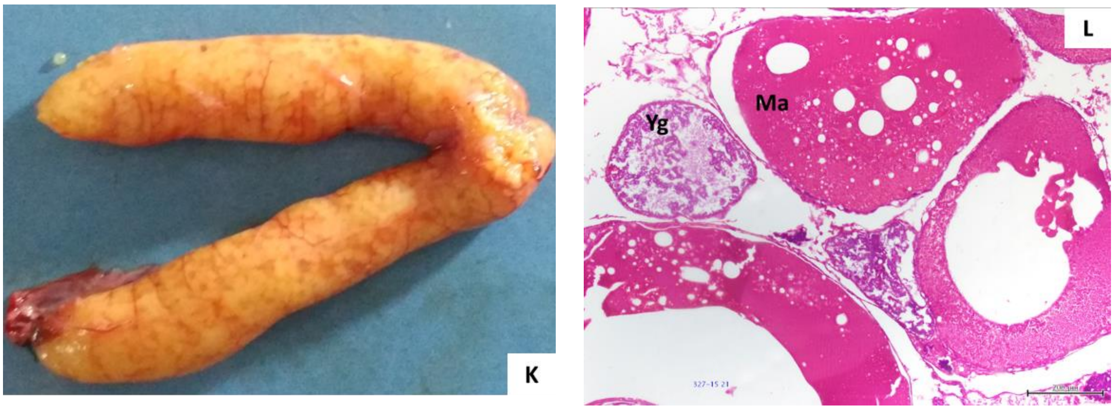

Figure 5. Spawning (311.45-465.0 mm TL, 258.73-395.0 mm SL). K: morphology, L. histology: yolk globule (Yg), mature egg (Ma).

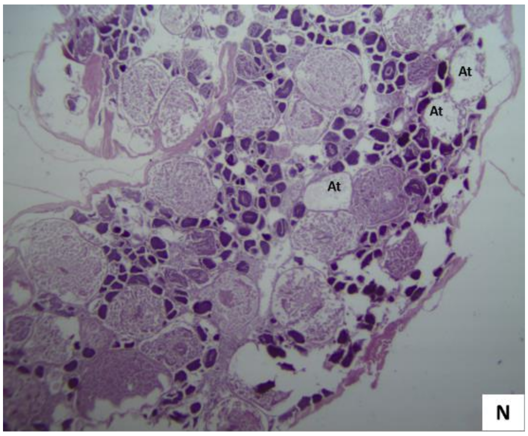

Figure 6. Post-spawning (spent) stage in *C. striata* (specimen 383.0 mm TL, 330.0 mm SL) with many atretic oocytes (At)

Microscopic examination and the oocytes size distribution of ripe ovaries indicated that *C. striata* exhibits an asynchronous ovarian growth pattern, with all oocyte types present during the spawning season, reflecting a long spawning period (Fig. 7). In asynchronous oocyte development, egg release is concomitant with oocyte recruitment, and therefore ovarian weight only changes slightly during the spawning season [24]. Similar asynchronous ovarian growth and fractional spawning patterns are reported in many Sparidae species, e.g. *Dentex dentex* [25] and *Pagrus pagrus*) [26].

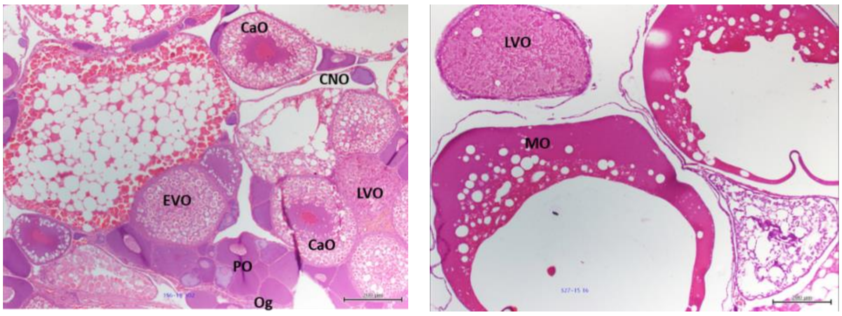

Figure 7. Mature oocytes reveal asynchronous ovarian development in *C. striata* (specimen 199.50 mm SL) Og: oogonia; CNO: chromatin nucleolar oocytes; PO: perinucleolar oocytes; CaO: Cortical alveolar oocytes; EVO: early vitellogenic oocytes; LVO: late vitellogenic oocytes; MO: mature oocytes

One of the challenges encountered during attempts to breed striped snakeheads is a difficulty in obtaining male broodstock. In this study the sex ratio (male;female) was around 1:2 in both the Bojo River, Barru District, and in Bantaeng District wetlands. A strong female bias (1:3) is also reported for the Bantimurung River *C. striata* population [21], while an even more extreme female bias (1;10.5) is reported from the Batangase area in Maros District, also in South Sulawesi Province, Indonesia [13].

The phenomenon of sex reversal appeared to be underway in the gonads of a number of male specimens of 110.00-241.00 mm SL (Fig. 8). Furthermore, the histological examination of several *C. striata* in the length rages 81.00-229.00 mm TL exhibited ovotestis (intersex) gonadal structures (Fig. 9). Macroscopic signs of ovotestis gonads include a milk white gonad colouration, overall slim and elongated thread-like shape with egg-filled protrusions standing proud of the gonad surface close to the anus (posterior end of the gonad). Microscopic examination of ovotestis samples showed a thick ovarian wall and thick lamellae, as well as testis tissue with spermatids present.

Hamlett [22] opined that the plasticity of sex determination is a hallmark of the bony fishes, including gonochoristic, protandrous and protogynous hermaphroditism, synchronised, sequential, and serial hermaphroditism. Sex reversal may occur once or several times during ontogeny; furthermore, sex reversal, sex determination and gonad development are often not fixed characters, and can be very sensitive to extrinsic/environmental factors such as temperature, pH, and contaminants [2,5,23]. This flexibility is atypical of vertebrates a whole, and means that temperature and pH during early gonad development can be applied to induce sex change in some fish species [6,7]. The observed instances of ovotestis and of the presence of eggs and spermatozoa in the same gonad lead the authors to suspect that the striped snakehead may be potentially hermaphroditic, either as a sequential or as a serial hermaphrodite. As far as we know, this is the first report of hermaphroditism in this species.

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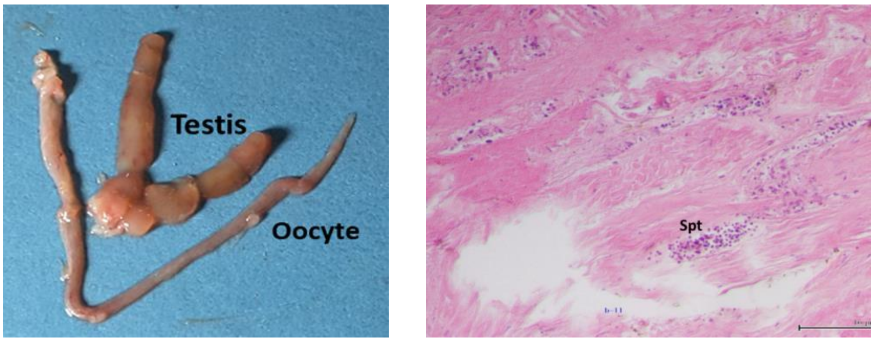

Figure 8. Ovotestis phenomenon (ovaries/ eggs and testes/sperm in the same individual) in a striped snakehead of 225.0 mm TL, 184.0 mm SL.
Spt: spermatid

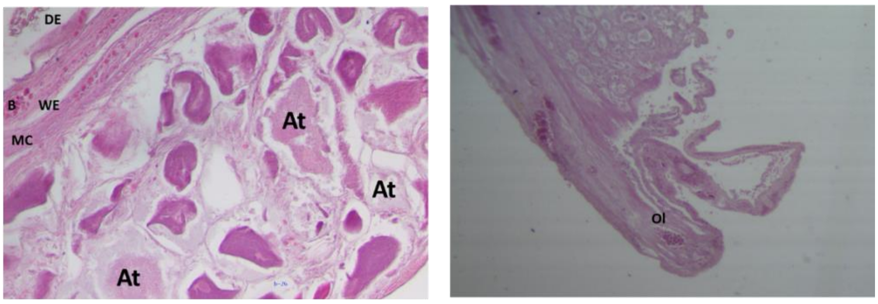

Figure 9. Hermaphroditism in *C. striata*. Immature ovary with the ovarian lamellae (Ol) containing spermatids.
WE: epididymis wall; DE: *ductus epididymis*; MC: smooth muscular cells; B: blood vessel; At: atretic follicles

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