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E Montchowui a, P Compère b, M Thiry c, P Lalèyè d, J-C Philippart e & P Poncin e

a École Nationale Supérieure des Sciences et Techniques Agronomiques de Kétou, Université d'Abomey-Calavi, Kétou, BP95, République du Bénin
b Functional and Evolutive Morphology Laboratory, University of Liège, Bât. B6, Allée de la Chimie, 3, B-4000, Liège, Belgium
c Laboratory of Cell and Tissue Biology, University of Liège, 20 Rue de Pitteurs, 4020, Liège, Belgium
d Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01BP526, Cotonou, Republic of Benin
e Behavioural Biology Unit, University of Liège, 22 quai Van Beneden, 4020, Liège, Belgium

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Histological assessment of gonad maturation in *Labeo parvus* (Teleostei: Cyprinidae) in Benin

E Montchowui*, P Compère², M Thiry³, P Laléyé⁴, J-C Philippart⁶ and P Poncin⁵

1 Ecole Nationale Supérieure des Sciences et Techniques Agronomiques de Kétéu, Université d’Abomey-Calavi, BP95 Kétéu, République du Bénin; current address: Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01BP526 Cotonou, Republic of Benin
2 Functional and Evolutionary Morphology Laboratory, University of Liège, Bât. B6, Allée de la Chimie, 3, B-4000 Liège, Belgium
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5 Behavioural Biology Unit, University of Liège, 22 qual Van Beneden 4020 Liège, Belgium
* Corresponding author, e-mail: e.montchowui@yahoo.fr

A histological analysis was undertaken on the gonad development of *Labeo parvus* Boulenger 1902, a medium-sized cyprinid from the Ouémé River, Benin. Samples were collected monthly between April 2006 and March 2007 and were examined using routine macroscopic and histological techniques. Five macroscopic stages of gonad maturation were identified in the ovaries and testes and described histologically. Oocyte development was subdivided into five stages, based on the presence of chromatin nucleolus oocytes, perinucleolus oocytes, yolk vesicle oocytes, vitellogenic oocytes and mature oocytes. Four stages of spermatogenesis (spermatogonia, spermatocytes, spermatids and spermatooza) were observed. Comparisons of macroscopic and histological observations revealed that the temporal patterns of maturation activity obtained from visual examination closely reflected the seasonal histological trend in gonad development. Histological characteristics of spent ovaries and testes were similar to those of immature fish, indicating that *L. parvus* were synchronous iteroparous spawners.

**Keywords:** gametogenesis, maturation rhythm, oocyte development, ovaries, reproduction, testes

**Introduction**

Fishes of the cyprinid genus *Labeo* contribute significantly to subsistence fisheries in Africa (Skelton et al. 1991). *Labeo parvus* Boulenger 1902, a rheophilous fish which favours running water in rocky habitats, is widely distributed throughout West Africa (Lévêque 2003). In Benin, *L. parvus* is important in capture fisheries and is a popular food fish.

Little is known about the annual cycle of gonad development in *L. parvus*. Previous assessments were based exclusively on macroscopic observations, while seasonal variations were analysed only in terms of changes in the gonadosomatic index (Albaret 1982, Montchowui et al. 2007). Gonad maturation in *L. parvus* has not been studied at a histological level, and their sexual maturation is still poorly understood.

One of the most important questions in fishery biology is to determine the annual reproductive cycle of fishes (Ünver and Ünver Saraydin 2004). The identification of gonad development stages is considered an essential element in reproductive studies (West 1990). The methods used for evaluating ovary development stages in fish include measurements of oocyte size, histological examination, determination of gonadic indices, and external appearance of ovaries and oocytes. Although time-consuming and expensive, histology is considered the most precise technique, and is a powerful tool for the evaluation of fish maturity stages (West 1990, Paugy and Lévêque 1999).

The aim of this study was to describe, by means of histological techniques, ovarian and testicular development stages of *L. parvus* in the Ouémé Basin, Benin.

**Materials and methods**

**Study site and fish sampling**

The Ouémé is the largest river basin in Benin, comprising a 50 000 km² catchment area around the 510 km long river, stretching from the Tanéka mountains in the north to the eastern side of Lake Nokoué (Colombani et al. 1972). Peak discharge occurs in August and September. Due to its geographic location, this river is influenced by two distinct climates. In the north the climate is characterised by a tropical trend of alternate dry (November–March) and rainy (April–October) seasons, with wide-ranging temperatures (10–40 °C). Furthermore, the *harmattan*, a dry, hot wind which blows from north to south from November to April, accentuates the thermal and hygrometric amplitudes. The rainy season extends from May to September. The southern part of the basin is influenced by a sub-equatorial climate, with two rainy and two dry seasons. The long rainy season,
from April to July, has its highest rainfall in June. The short rainy season starts in September and ends in October. Temperatures range from 18 to 35 °C.

Fish were sampled at four stations along the river (Figure 1). Station 1, at Bétérou (09°11' N, 02°16' E), was in a rocky zone with swift currents. Station 2, at Atchakpa (08°04' N, 02°22' E), was also in a rocky zone. Station 3, at Toué (07°12' N, 02°17' E) on the Zou tributary fairly near its confluence with the Ouémé, marked the transition between the zones of swift-flowing water and the delta. Station 4, at Agonlin Lowé (06°39' N, 02°28' E), was within the Ouémé Delta.

Fish were captured monthly between April 2005 and March 2006 using gillnets set in the late afternoon and retrieved the following morning. Fish weights (body weight) were recorded to the nearest 0.01 g, using an electronic balance (Kern, Germany), and total length (TL) and standard length (SL) were measured to the nearest centimetre. The specimens were dissected, the gonads were removed and weighed to the nearest 0.01 g, and sex was determined by macroscopic examination of the gonads. Gonad maturity stages, as described by Weyl and Booth (1999), were determined macroscopically: (I) juvenile/resting, (II) initial maturation, (III) advanced maturation, (IV) ripe, and (V) spent.

**Histological analysis**

For histological analyses, tissue samples excised from the rostral, middle and caudal regions of ovaries and testes were fixed for 24–48 h in Bouin’s solution, then dehydrated in a graded series of alcohol baths, embedded in paraffin wax, and sectioned to 8 μm using a Reichert microtome. The sections were stained with haematoxylin and eosin (H&E) or Masson trichrome and examined with an Olympus Provis AX70 microscope equipped with an Olympus Camedia C70/70 camera. Determination of germ cell development stages on histological preparations was based on the terminology proposed by West (1990). The histological characteristics of germ cells were used to describe the stages of gonad maturation. Structural measurements for the early stage of oocytes were made on selected sections of photographs using VistaMetrix software to analyse photographs of the sections.

Oocyte diameter frequency distributions for females in all stages were determined by random selection of three sub-samples of oocytes from each ovary, and oocytes from each subsample were measured under a Nikon 6C-2 projecting microscope at 10× and 20× magnification. The percentages of the frequency of oocyte diameters in different size classes were then determined.

Quantitative analysis of ovarian development was made using the gonadosomatic index (GSI): GSI = (Pgo / (Pt − Pgo)) × 100, where Pgo is total gonad weight and Pt is total body weight. Means are given with standard deviation (mean ± SD).

**Results**

A total of 405 male and 461 female *L. parvus* were sampled. Males ranged from 10.4 to 25.1 cm TL (average: 18.8 cm), and females ranged from 11.7 to 28.8 cm TL (average 19.8 cm).

**Testicular germ cell development**

Testes were paired structures suspended in the body cavity by mesorchia. Each testis was surrounded by a tunica albuginea. A number of seminiferous tubules led into
secondary sperm ducts, joining posteriorly to form the main sperm duct. Four stages of spermatogenesis (spermatogonia, spermatocytes, spermatids, spermatozoa) were observed on histological preparations.

**Stage I: spermatogonia**
Spermatogonia constituted the primary germ cells. They appeared as small nests associated with the edges of seminiferous tubules. Spermatogonia were characterised by their large size, prominent cytoplasm and lightly basophilic nuclear chromatin (Figure 2a).

**Stage II: spermatocytes**
Primary and secondary spermatocytes, which had smaller...
nuclei than spermatogonia, formed islets on the edges of the seminiferous tubules (Figure 2b). Secondary spermatocytes were smaller than primary spermatocytes.

**Stage III: spermatids**
Spermatids, which were smaller than spermatocytes, also formed islets in contact with the edges of the seminiferous tubules. These cells were characterised by a compact chromatin mass (Figure 2c, d).

**Stage IV: spermatozoa**
Spermatozoa, characterised by their small size and intensely basophilic heads, appeared as the dominant cells in the lumen of seminiferous tubules before moving towards and accumulating within the sperm ducts (Figure 2e).

**Oocyte development**
Ovaries of *L. parvus* were paired organs located in the abdominal cavity against the dorsal wall, adhering to the swim bladder. The two lobes are fused in their posterior parts, forming the oviduct connected to the urogenital opening. Histologically, maturity stage was characterised in five developmental stages, using oocyte size and location, staining characteristics, number of nucleoli, presence of a follicular layer and the distribution of cytoplasmic inclusions.

**Stage I: chromatin nucleolus oocyte stage**
The oocytes were characterised by the presence of a centrally located nucleus (mean diameter 7 ± 2.23 μm, n = 15) containing 2–11 large nucleoli per section (Figure 3a). Chromatin appeared in the form of a few thick filaments within the nucleus. The oocyte cytoplasm, although not very abundant, was homogeneous and was slightly basophilic.

**Stage II: perinucleolus oocyte stage**
The oocyte nucleus (mean diameter 38 ± 15.73 μm, n = 15) contained multiple smaller nucleoli (12–64 per section) arranged on the periphery of the nucleus. In early perinucleolus oocytes (Figure 3b), a few large, polygonal shaped nucleoli could still be seen. Late perinucleolus oocytes became more spherical in shape (Figure 3c). The oocyte cytoplasm also became less basophilic in late perinucleolus oocytes. In the cytoplasm periphery of some perinucleolus oocytes a very basophilic area, corresponding to the Balbiani body, was also clearly seen. A few yolk vesicles, or cortical alveoli, were visible in the late perinucleolus oocytes (Figure 3c), marking the end of the primary growth phase. The perinucleolus oocytes were surrounded by a flattened follicular layer.

**Stage III: yolk vesicle oocyte stage**
During this phase many cortical alveoli had accumulated in the cytoplasm periphery (Figure 3d). The oocytes (mean diameter 85.87 ± 10.42 μm, n = 15) had a spherical appearance, but their nuclei were starting to become irregular in shape. Many nucleoli (more than 64 nucleoli per section) were clearly seen in the nuclear periphery. The vitelline membrane was observed between the oocyte and the follicular cells.

**Stage IV: vitellogenic oocyte stage**
The nucleus was centrally positioned and rather irregular in shape (Figure 3e). The number of nucleoli contained in the nuclear periphery was greater than 64 nucleoli per oocyte (mean diameter 131.87 ± 14.41 μm, n = 15). The yolk granules, which were at the cytoplasm periphery at the beginning of the vitellogenic phase, increased in both, size and number to form globules that occupied the entire central area of the cytoplasm (Figure 3e). Cortical alveoli were only seen at the cytoplasm’s periphery. The vitelline membrane was more conspicuous and increased in thickness.

**Stage V: mature oocyte stage**
The mature oocyte phase was the final stage of the process of oogenesis (Figure 3f, g). The nucleus migrated from the centre to the cell’s periphery. At this stage the oocyte was enriched with yolk proteins, as shown by its red colouration after H&E staining (Figure 3g). The cytoplasm (mean diameter 205.73 ± 27.19 μm, n = 15) was more voluminous and had a grainy appearance.

**Gonad maturation stages**
Depending on their maturity stage, the macroscopic appearance of the gonads changed in size, colour and turgescence. Five macroscopic stages of gonad maturation were revealed within the ovaries and testes by visual inspection. Figure 4 illustrates the histological appearance of the five macroscopic stages of the ovaries. The equivalent histological descriptions of the macroscopic appearance of various stages are summarised in Table 1.

**Gonadal maturation cycle**
To determine the spawning rhythm we examined the oocyte diameter distribution in the ovaries of immature to adult females as well as in the ovaries of females captured between two mature/spawning periods (February–October) (Figure 5). There were two categories of ovarian structure. The first (G1) comprised chromatin nucleolus oocytes and perinucleolus oocytes, found in immature females (Figure 5a). In the second category (G2), the distribution of oocyte diameters was wider, and this category was detected in both advanced mature females (Figure 5b) and mature females (Figure 5c). Between two successive spawning periods the oocytes in the initial developmental stages (in previtellogenesis) evolved into mature oocytes, but ovaries always contained chromatin nucleolus and perinucleolus oocytes. Histological observations made on the ovaries of *L. parvus* during the mature/spawning period also revealed the predominance of mature oocytes. In contrast, just after ovulation (Figure 5d), the appearance of the ovaries was similar to that observed in immature fish. They contained essentially oocytes in stages of initial development. Contrary to the ovaries of immature fish, a few postovulatory and atresic follicles were also present in spent ovaries.

**Discussion**
The present study provides the first histological description of gonad development stages and cycles in *L. parvus*. 
Figure 3: Transverse sections through ovaries of *L. parvus* illustrating oogenesis: (a) chromatin nucleolus oocytes; (b) early perinucleolar oocytes; (c) late perinucleolar oocytes and nucleus; (d) yolk vesicle oocyte with cortical alveoli forming in the cytoplasm periphery. The vitelline membrane is developed; (e) vitellogenic oocyte with central nucleus with the sequestration of yolk globules; (f–g) mature oocyte with migrating nucleus, cytoplasm filled with yolk globules. Abbreviations: ca = cortical alveoli, cno = chromatin nucleolus oocytes, epo = early perinucleolar oocytes, lpo = late perinucleolar oocytes, mn = migrating nucleus, mo = mature oocyte, n = nucleus, vm = vitelline membrane, vo = vitellogenic oocyte, yg = yolk globules, yvo = yolk vesicle oocyte. Staining: (a–g) haematoxylin and eosin, (f) Masson trichrome. Scale bars: (a–c), (g) = 5 μm; (d–e) = 14 μm; (f) = 49 μm
Five macroscopic stages of gonadal recrudescence could be identified. A similar number of stages was described for *Labeo cylindricus* in the Lake Chicamba in central Mozambique (Booth and Weyl 2000) and *Labeo victorianus* in the Kagera and Sio rivers in Uganda (Rutaisire and Booth 2004). Comparisons of macroscopic and histological observations revealed that the temporal patterns of maturation activity obtained from visual examination closely reflected the seasonal trend in gonadal development in *L. parvus* from the Ouémé River. The histological results obtained in this study therefore support the macroscopic observations previously used to stage *L. parvus* maturity, and suggest that the macroscopic criteria previously used for assessing gonadal recrudescence were adequate.

This histological study showed that, during oogenesis, the size of the oocytes increased considerably due to a progressive accumulation of lipid- and protein-yolk within the cytoplasm. Five stages of oocyte development were identified in *L. parvus*. Most authors divide the oogenesis process in teleost fishes into four to eight stages of oocyte development, depending on the species and the choice of criteria used (Nagahama 1983, Kestemont 1987, West 1990, Fishelson et al. 1996, Ünver and Ünver Saraydin 2004). Consequently, as in other studies (van der Merwe et al. 1988, Ünver and Ünver Saraydin 2004, Smith and Walker 2004), the present study used chromatin nucleolus oocytes and perinucleolus oocytes as indicators of the first stages of oocyte development in *L. parvus*. In certain studies, oogonia were used as indicators of the first stage of oocyte development in Cyprinidae (Booth and Weyl 2000, Rutaisire and Booth 2004). Likewise, we did not consider atresic follicles as a stage of oocyte development and our histological classification of oocyte development in *L. parvus* corresponded for the most part to that previously described for *Labeo capensis* (van der Merwe et al. 1988).

The development of oocytes as described in *L. parvus* is similar to that observed in many fish species. In particular, the multiplication of nucleoli during previtellogenesis is an event commonly reported in most species studied (Garcia et al. 2001, Santos et al. 2005, Thiry and Poncin).

**Figure 4:** Transverse sections through ovaries of *L. parvus* in different stages of maturation: (a) immature or initial maturation fish, ovary filled with previtellogenic oocytes; (b) advanced maturation fish; (c) ripe fish, ovary filled with mature oocytes; (d) spent fish. Abbreviations: ao = atresic oocyte, cno = chromatin nucleolus oocyte, epi = early perinucleolus oocyte, mo = mature oocyte, vo = vitellogenic oocyte, yvo = yolk vesicle oocyte. Staining: (a–b), (d) haematoxylin and eosin; (c) Masson trichrome. Scale bars: (a–b), (d) = 14 μm; (c) = 0.05 mm.
Table 1: Macroscopic and histological appearance of *L. parvus* gonads at various stages during maturation

<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic appearance</th>
<th>Histological appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage I:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>immature</td>
<td>A translucent gelatinous strip. Not possible to identify sex. GSI = 0.18 ± 0.09%.</td>
<td>Lumen of seminiferous tubules almost devoid of material, with only small clusters of a few spermatogonia (Figure 2a).</td>
</tr>
<tr>
<td><strong>Stage II:</strong></td>
<td></td>
<td></td>
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<tr>
<td>initial</td>
<td>Testes discernible as a small, thin, partially white band. GSI = 0.23 ± 0.12%.</td>
<td>Lumen of seminiferous tubules partially filled with primary and secondary spermatocytes (Figure 2b). A few small islets of spermatids and spermatozoa visible.</td>
</tr>
<tr>
<td>maturation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stage III:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>advanced</td>
<td>Testes increased in size. Sperm not extrudable. GSI = 0.63 ± 0.33%.</td>
<td>Seminiferous tubules almost completely filled with primary and secondary spermatocytes, spermatids and spermatozoa (Figure 2c and d). Spermatozoa islets larger and more frequent than in Stage II.</td>
</tr>
<tr>
<td><strong>Stage IV:</strong></td>
<td></td>
<td></td>
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<tr>
<td>ripe</td>
<td>Testes creamy white and showing constrictions. Sperm can be extruded. GSI = 1.61 ± 0.91%.</td>
<td>Testes filled with spermatozoa (Figure 2e). Few small areas of spermatids, and primary and secondary spermatocytes present.</td>
</tr>
<tr>
<td><strong>Stage V:</strong></td>
<td></td>
<td></td>
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<tr>
<td>spent</td>
<td>Testes reduced in size, dirty brown in colour. GSI = 0.26 ± 0.24%.</td>
<td>Similar to immature fish (Figure 2f). Seminiferous tubules do not contain gamete cells. Only a few clusters of spermatogonia and a few rare spermatids visible.</td>
</tr>
<tr>
<td><strong>Stage VI:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spent</td>
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</table>

2005). It is well established that oogenesis is characterised by the progressive storage of reserve materials, such as ribosomes, used later in embryonic development. The accumulation of vitelline vesicles in the ooplasm periphery during the first part of vitellogenesis and the appearance of many small vitelline globules under the layer of vitelline vesicles in a deeper layer of the ooplasm during the second part of vitellogenesis are events commonly reported in fish species in general (Nagahama 1983, Mutambue et al. 1991, Goubier et al. 1997). However, the sequential appearance of these reserves varies with fish species (Nagahama 1983, Sarasquete et al. 2002).

Notably, our study also demonstrated that the annual cycle of gametogenesis in *L. parvus* is synchronised. Spermatogonia in nests associated with the edges of seminiferous tubules divided synchronously and therefore were at the same developmental stage. Contrary to oogenesis, four stages of testicular germ cell were identified during spermatogenesis. The number of histological stages described here for *L. parvus* is similar to that described by Booth and Weyl (2000) for *L. cylindricus* in the Lake Chicamba, Mozambique, but differs from the six stages described by Rutaside et al. (2003) for *Labeo victorianus* in the Kagera and Sio rivers, Uganda. This difference can be attributed to the testicular germ cell development of each species and also to each author’s particular criteria.

*Labeo parvus* spermatogenesis occurred synchronously with the release of spermatozoa into the lumen of the seminiferous tubules. This is a common pattern in fish of the genus *Labeo* (Booth and Weyl 2000, Rutaisyre et al. 2003). The histology of ovaries further supports the hypothesis that *L. parvus* are synchronous spawners. At the beginning of gonadal recrudescence the ovaries contained only primary oocytes (chromatin nucleolus oocytes and perinucleolus oocytes). In the Ouémé River the first signs of gonad development were only apparent in March. These primary oocytes grew to reach the vitellogenic stage (Figure 4c) during reproduction in July–October (Montchowui et al. 2007). All ovulated oocytes were spawned during the annual fish migration into the adjacent floodplains. Histological analysis of spent ovaries revealed that they contained predominantly oocytes in initial developmental stages and a few atresic follicles (Figure 4d). This could explain the rapid drop in GSI from 21.5% to 0.4% observed...
in *L. parvus* between August and October. It also argues strongly in favour of a total spawning pattern. The remaining primary oocytes thus develop and mature during the next season, providing another batch of vitellogenic oocytes. The mitotic division of oogonia is in turn replaced by these oocytes. A full resumption of endogenous and exogenous vitellogenesis is necessary for the completion of a new spawning. As this resumption is conditioned by a new rainy season, in the Ouémé River the ovarian resting stage lasts a minimum of six months, from October to March.

This pattern of oocyte development is typical of iteroparous spawning fish species (West 1990). This occurrence has been described in different species such as *L. cylindricus* (Booth and Weyl 2000), *Chalcalburnus chalcoideus* (Ünver and Ünver Saraydin 2004) and *Brycon orthotaenia* (Gonçalves et al. 2006). *Labeo parvus* is thus a synchronous iteroparous spawner, annually spawning almost all its matured gametes over a short period throughout its lifespan.

Synchronous and total spawning by *L. parvus* in the Ouémé River can be considered a reproductive tactic favouring higher survival among juveniles in given environmental conditions. The gonad morphology and maturity development classes of *L. parvus* in the Ouémé River did not differ significantly from those of African *Labeo* species in other environments. However, in *L. victorianus* in the Sio and Kagera rivers, Uganda, spawning occurred at two periods, corresponding to the annual bimodal rainfall maxima (Rutaisire and Booth 2004). In *L. parvus*, spawning occurred only during the second rainfall maxima in July–October (Montchowui et al. 2007). Additional research is required to understand differences in reproductive strategies in different *Labeo* species.

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