

Ovary Morphology and Reproductive Features of the Female Suckermouth Sailfin Catfish, *Pterygoplichthys disjunctivus* (Weber 1991) from Marikina River, Philippines

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Abstract

The ovarian histomorphology during the annual reproductive cycle of the non-native suckermouth sailfin catfish, *Pterygoplichthys disjunctivus* (Weber 1991), a highly invasive and dominant fish population in Marikina River, Philippines was described from July 2010 to June 2011. Six ovary development stages were described with defined oocyte diameters in immature, maturing and spawning stages. Minimum length of maturity for females was at 26 cm standard length (SL), although incidences of precocious sexual maturity in several females as small as 19 cm SL were seen during the peak spawning months. Female *P. disjunctivus* have a relatively short spawning period (June to September) coinciding with the rainy season, followed by a short regression stage (October to December) which overlapped with the long recrudescence stage (October to early June). The three oocyte diameters in vitellogenic ovaries and the short spawning season suggest that the females were iteroparous batch spawners.

Introduction

Invasive alien species (IAS) are one of the major causes of biodiversity loss in freshwater systems (Clavero and Garcia-Berthou 2005). Studies on the reproductive features of invasive fish species have explained how some exotic species rapidly proliferate in non-native environments (Garcia-Berthou 2007). Gonad characteristics provide insights on reproductive strategies and monitoring gonadal changes aid in determining patterns of reproductive phenology and spawning periodicity (Brown-Peterson et al. 2011). This information is essential in creating population models and management measures such as mechanical control to prevent their further spread in novel environments.

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Pterygoplichthys disjunctivus (Weber 1991) is an invasive loricariid catfish that originally inhabited the Amazon River basin of Brazil and Peru (Weber 2003) and Rio Madeira drainage of Brazil and Bolivia along with another species, *Pterygoplichthys pardalis* (Castelnau 1855) (Page and Robins 2006). The two species were introduced to the Philippines as “janitor fishes” through the aquarium fish trade (Hubilla et al. 2007). Individuals with intermediate abdominal pattern characteristics between the two species were observed in the river with no defined species-specific DNA barcodes, suggesting possible introgressive hybridisation between the two species (Jumawan et al. 2011; Quilang and Yu 2013).

With no known natural predators in the Philippines, janitor fishes have easily invaded inland waters, seriously threatening the livelihood of local fishermen by damaging aquaculture structures and gill nets through their bony external armour and contributing to water turbidity and erosion of riverbanks through its nest-building behaviour.

Pterygoplichthys disjunctivus females have been reported to spawn at small body sizes (Gibbs et al. 2008). Studies on the gonadosomatic index (GSI) and fecundity of *P. disjunctivus* in Volusia Blue Springs, Florida (Gibbs et al. 2008) and size structure indices of *Liposarcus multiradiatus* (Hancock 1828) in Southern Taiwan (Liang et al. 2005) were essential in explaining some reproductive features and the propensity of this fish for invasion. The gonad maturity stages, histochemistry of oocytes and reproductive phenology reported in this study should provide a better understanding on the dynamics of reproduction in this species.

Materials and Methods

Pterygoplichthys disjunctivus specimens were collected from five sites along the stretch of Marikina River, Philippines—Barangay San Jose, Rodriguez Rizal; Barangay Banaba, San Mateo; Barangay Tumana; Barangay Malanday and River Park (Fig.1) from July 2010 to June 2011. Fish were captured with a cast net (5 m long; mesh size of 3.8 cm). Due to absence of defined sexual dimorphism, *P. disjunctivus* males were inevitably collected and were excluded after sorting in the laboratory. Segregated females were sacrificed by immersing fish in ice 4 h prior to dissection. Standard length (SL) was measured to the nearest 0.1 cm using a measuring board. Body weight was measured with an electronic scale to the nearest 0.1 g and ovaries were weighed using an analytical balance of 0.1 to 0.01 g accuracy.

Ovaries were examined and assigned ovarian maturity stages by two methods: (1) visual-based macro-structural scale by which a micro-structural scale of oocyte diameter distribution was validated, and, (2) histological descriptions of the ovary to characterise the stages of ovarian maturity and the sizes of developing oocytes for each stage (Lowerre-Barbieri et al. 2011; Brown-Peterson et al. 2011). Ovaries were classified into maturity stages based on (1) colour (opalescent to yellow), (2) gross surface texture (grainy to smooth), and (3) eye estimate of the abdominal cavity occupied by the ovary (Mazzoni and Caramaschi 1997).

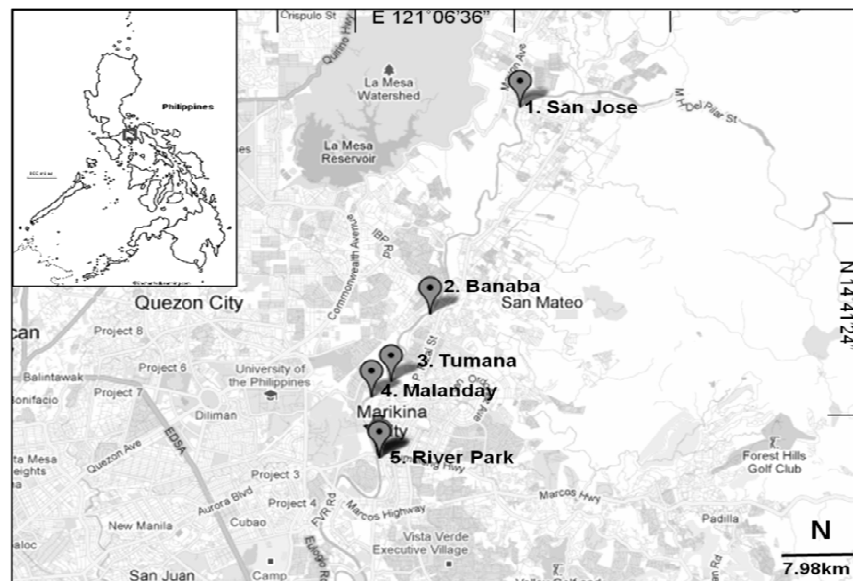


Fig. 1. Janitor fish *P. disjunctivus* collection stations (*) in Marikina River, Philippines.

A portion of each ovary was preserved in Bouin's solution for 12-24 h and then stored in 70% ethanol. Ovary fragments were dehydrated through a 75-90% ethanol series, embedded in paraffin and sectioned on a microtome. The 5-6 μm thin sections were stained with haematoxylin-eosin (HE). The occurrence of partially spent ovaries, identified by the simultaneous presence of post-ovulation follicles (POFs) and vitellogenic oocytes were used to determine spawning mode. To measure oocyte size ranges and their frequency at different stages of development, at least 30 oocytes were extracted from the apical, central and caudal sections of ovaries of five freshly dissected females for each maturity stage which were initially sorted through visual-based classification. To detect carbohydrates and proteins in vitellogenic and non-vitellogenic oocytes, a combination of Periodic Acid Schiff (PAS)-Alcian blue (AB) pH 2.5 (ArtisanTM) was used. For all measured structures, the mean and standard deviation were calculated.

Results

A total of 607 females ranging from 5-40 cm SL were sampled in this study. *Pterygoplichthys disjunctivus* is monomorphic externally, where no distinguishable external difference can be observed between sexes. The ovaries were cystovarian wherein the ovary lumen has continuity with the oviduct. Ovaries are largely asymmetric in weights in very mature samples (Fig. 2). Small ovaries from sexually immature females do not exhibit this difference in ovarian weights. The left and right ovaries are joined together along the medial-caudal portion by a thin connective tissue at the dorsal most end of the coelomic cavity, and are fused as a common duct. The flow of oocytes from this sinus is regulated by a sphincter such that a single aperture serves as a common gonadal/anal pore.

Oogenesis in *P. disjunctivus* was classified with respect to oocyte size, appearance of the nucleus, distribution of cytoplasmic inclusions and thickness of the ovarian wall. The progression of oocyte growth and development were as follows:

Primary growth phase

Perinucleolus (PN) stage (Fig. 3): This is the prominent stage of oocyte growth since they are present throughout the year, notably at the end of the reproductive period. This stage is characterised by the presence of perinucleolar oocytes with strongly basophilic cytoplasm surrounding a paler nucleus with several nucleoli found attached, often closely associated with oogonia and chromatin nucleolar cells (Fig. 3 A-D). During early PN stage, a small basophilic mass known as Balbiani's body (Fig. 3 C) accumulates and is often closely associated with the nucleus. During this time, oocytes are larger ($58.61 \mu\text{m} \pm 2.83$; range: $41.39\text{-}85.70 \mu\text{m}$) and a single thin layer of thecal cells appear to cover the oocytes (Fig. 3 D-E). Cytoplasm of PN is PAS non-reactive and AB reactive (Fig. 3 E).

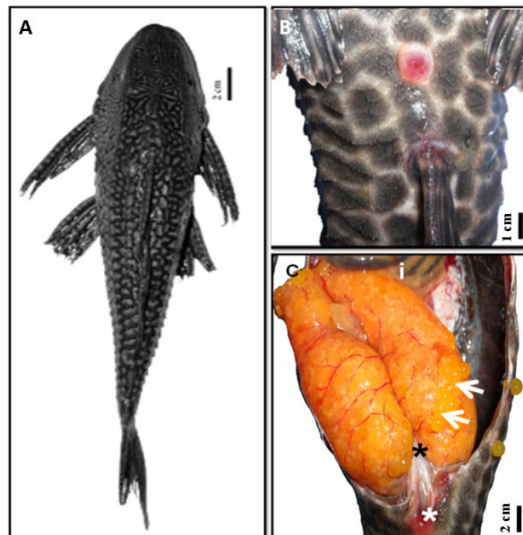


Fig. 2. Female janitor fish *P. disjunctivus* collected from the Marikina River system, Philippines. A: Dorsal view. B: Ventral view of the abdomen showing a single anal and urogenital orifice. C: Overview of a mature cystovarian ovary. *i*: intestine, white arrows: mature oocytes, black asterisk: rectum, white asterisk: common anal/gonadal orifice.

Secondary Growth (SG) Phase

Lipid vesicle (cortical alveoli) stage (Fig. 4): Lipid vesicles are the first structures to appear in the cytoplasm of SG oocytes, often in the midcortical zones (Fig. 4 A) of the early SG phase ($139.69 \pm 19.44 \mu\text{m}$, range: $130.22\text{-}154.21 \mu\text{m}$). Subsequent yolk deposition result in an increase in oocyte size in the late stage (144.56 ± 28.64 ; range: $141.15\text{-}148.22 \mu\text{m}$) wherein lipid vesicles eventually give rise to cortical alveoli (Fig. 4 B,D,E). A thin acellular zona radiata exhibits distinct banding and transverse striations covered by a single layer of follicular cells (Fig. 4 C-E).

Developing stage ovaries are dominated by oocytes with lipid vesicles and cortical alveoli. Lipid vesicles are PAS-negative surrounded by AB-positive cytoplasm (Fig. 4 E).

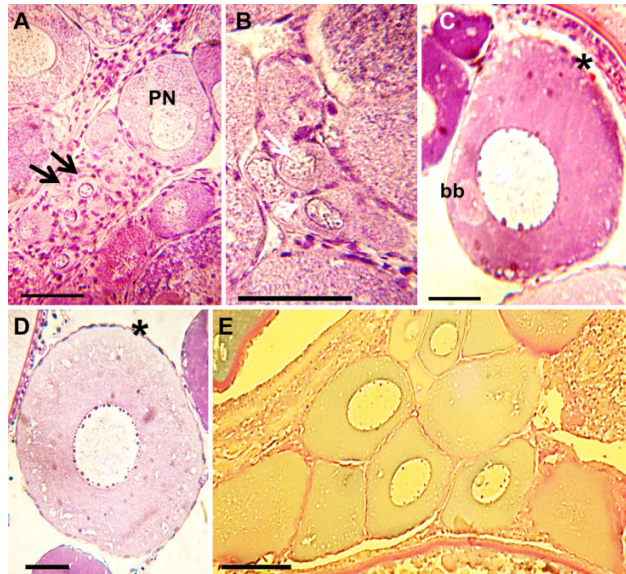


Fig. 3. Primary growth (PG) stage oocytes in *P. disjunctivus*. A: Cluster of oogonia alongside PN cells. B: Chromatin nucleolar cells. C: Early stage PN oocyte with defined bb. D: Late PN stage. E: AB weakly positive reaction in the cytoplasm of PG oocytes. Scale bar: 10 μ m. Black arrows: oogonia; white arrows: chromatin nucleolar; white asterisk: stromal cells; black asterisk: thecal cells; bb: balbiani body; PN: perinucleolar oocyte.

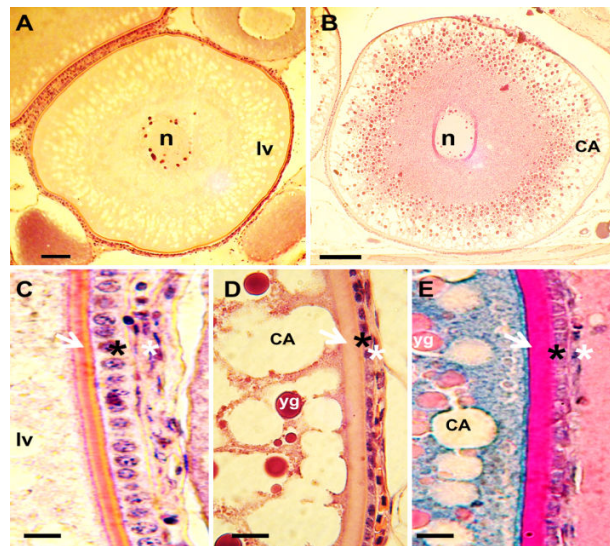


Fig. 4. Secondary growth (SG) oocytes in *P. disjunctivus*. A: Early lipid vesicle stage. B: Late CA stage. C: Peripheral cytoplasm of A showing zona radiata, a single layer of follicular cells and a loose layer of thecal cells. D: Peripheral cytoplasm of B showing CA and yolk globules. E: AB (-) CA and PAS (+) yolk globules and zona radiata in CA oocyte. Scale bar: 10 μ m. N: nucleus; yg: yolk globule; CA: cortical alveoli; lv: lipid vesicles; white arrow: zona radiata; black asterisk: follicular cells; white asterisk: thecal cells.

Maturation and Hydration stage

Yolk globule stage (vtg1; Fig. 5): The early maturation stage is characterized by the formation of yolk globules from the fusion of small coated yolk vesicles in the oocyte periphery (Fig. 5 B,C). Yolk globules tend to be concentrated along the outer margin of the yolk vesicle layer. Late yolk globule stage is characterized by an increase in oocyte size ($196.44 \pm 25.34 \mu\text{m}$; range: $181.45\text{--}212.56 \mu\text{m}$) and by the migration of the fused/coalesced globules to the center, forming a continuous border near the zona radiata (Fig. 5 B,D,E). Yolk globules are PAS-positive while the cytoplasm in both early and late stages are AB-negative (Fig. 5 E).

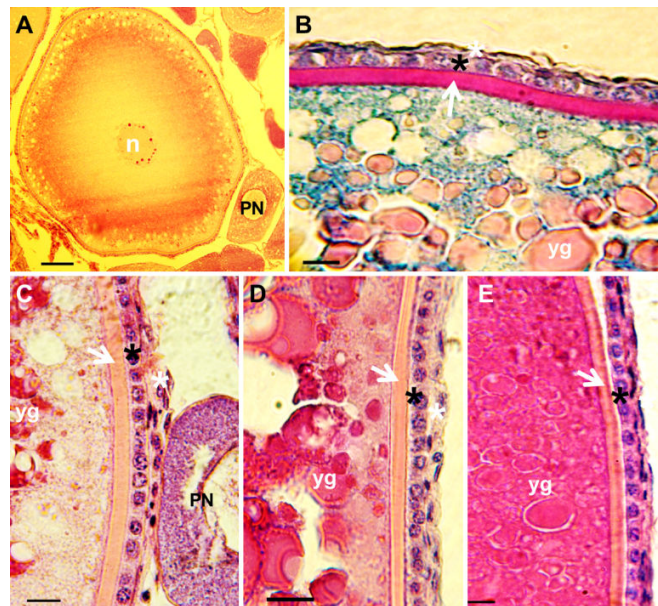


Fig. 5. Vitellogenesis in *P. disjunctivus*. A: Early yolk globule stage (vtg1). B: AB (+) non-globule cytoplasm; PAS (+) yolk globules and zona radiata. C: Peripheral cytoplasm in early stage (HE). D: Yolk globules start to fuse (mid-stage). E: Yolk globules coalesce with cytoplasm compacted (late stage; HE). Scale bar: $10\mu\text{m}$. *n*: nucleus, *PN*: perinucleolar oocyte, *yg*: yolk globules, white arrow: zona radiata, black asterisk: follicular cells, white asterisk: thecal cells.

Migratory nucleus stage (vtg2; Fig. 6 A): This stage is characterized by the migration of the nucleus to the animal pole, the disintegration of the nuclear membrane, and germinal vesicle breakdown (GVBD). Oil droplets appear in the cell periphery, often surrounding the nuclear membrane after the formation of yolk vesicles and globules (Fig. 6 A, inset). Follicular cells are now composed of two defined layers—a thick ($12.44 \pm 4.62 \mu\text{m}$, range: $10.32\text{--}18.62 \mu\text{m}$), brush-like single layer of granulosa cells and a thin theca externa. The zona radiata is conspicuously thinner ($4.521 \pm 1.01 \mu\text{m}$, range: $3.02\text{--}6.82 \mu\text{m}$) and reacts strongly to PAS (Fig. 6 D). The follicular layer is AB-positive (Fig. 6 E).

Ripe Stage (vtg3; Fig. 6 B): Vtg3 oocytes had the largest average diameter ($3243 \pm 144.32 \mu\text{m}$; range: $3115\text{--}3671 \mu\text{m}$) among the oocytes. The yolk globules in the cortical ooplasm are abundant. PAS-positive globules fuse to form a single mass of yolk.

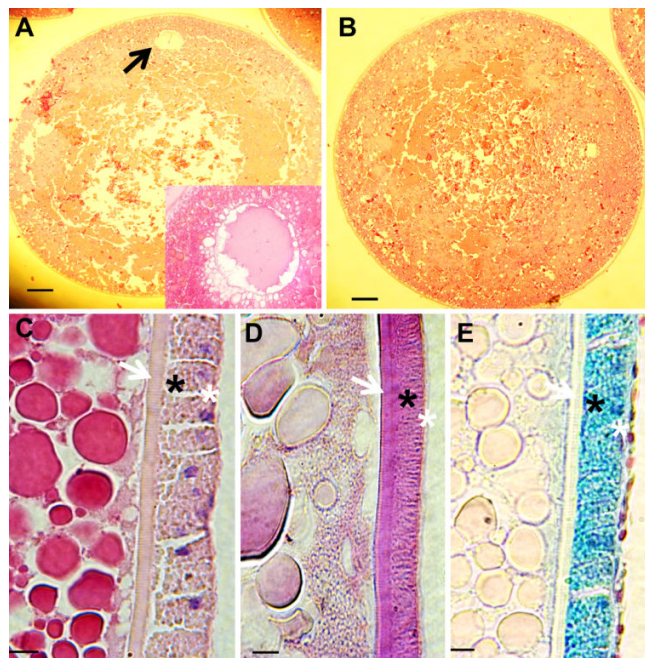


Fig. 6. Germinal vesicle breakdown (vtg2) and ripe oocyte (vtg3) stage in *P. disjunctivus*. A: Oocyte undergoing late stage vtg2. Inset: detail of (A). B: Ripe vtg3 oocyte. C: Peripheral cytoplasm of the ripe oocyte (HE). D-E: AB-PAS of peripheral cytoplasm: PAS (+) yolk globules and zona radiata; Granulosa layer (+) for AB test. Scale bar: A-B: 200 μ m; B-E: 10 μ m. White arrow: zona radiata, black asterisk: granulosa layer, white asterisk: thecal cells.

No positive material for either PAS or AB was ever observed in early PG oocytes. Cortical alveoli and yolk granules exhibit weak PAS reactions during the onset of vitellogenesis, but their reactions subsequently increase during advanced exogenous vitellogenesis. PG oocytes do not positively react with PAS; however, mucosubstances were detected in the cytoplasm through AB. The zona radiata is mainly proteinaceous in nature and reacts strongly with PAS from the onset to late vitellogenesis, while AB test for acid mucosubstances reacts weakly in the intergranular cytoplasm only of vitellogenic oocytes (Table 1).

Table 1. Alcian blue (AB) - Periodic Acid Schiff (PAS) histochemistry in *P. disjunctivus* oocytes

	Cortical alveoli	Oil globules	Yolk granules	Intergranular cytoplasm	Follicular envelope	Zona radiata
PAS	+	-	+	-	+/-	++
Alcian Blue	-	-	-	+	-	-

Intensity of reaction: -: none; +/-: weak; +: moderate; ++: strong

Table 2. Macroscopic and microscopic characteristics used to assign stages of ovarian maturity in *P. disjunctivus* from Marikina River, Philippines.

Stage	Macroscopic Features	Histologic features
Immature (Stage 1)	Small ovaries (0.25 ± 0.27 g; range: 0.1-1.66 g) in small females (≤ 16.68 cm SL ± 5.45 cm; range: 5-27 cm). Ovaries occupy $< 15\%$ of body cavity. Ovary surface thin, pink to transparent without visible opaque oocytes.	Ovaries contain mainly PG oocytes. No evidence of prior spawning activity. Cells densely packed, in close association with the ovarian wall (Fig. 7 A).
Maturing/ Developing (Stage 2)	Ovaries (10.47 ± 1.45 g; range: 1.28-14 g) occupy 20-40% of the body cavity in small females ($\leq 25.77 \pm 2.86$; range: 19-31 cm SL). Pale yellow to opaque oocytes of different sizes (1-2.5 mm).	Majority of oocytes yolked (mostly CA oocytes) but not hydrated (Fig. 7 B).
Mature/ Spawning capable (Stage 3)	Ovaries large (55.41 ± 9.28 g; range: 15-145 g), occupying up to 50-70% of the body cavity. Thin ovarian walls, highly vascularised, strong asymmetry between the left and right ovaries. Bright yellow oocytes (2.5-3 mm).	Vtg2-vtg3 oocytes with no visible POFs. Early stages of OM present. Fully yolked oocytes (migratory nucleus stage and hydrated oocytes) (Fig. 7 C).
Actively spawning (Stage 4)	Very recently spawned ovaries (71.7 ± 31.13 g; range: 22-165.1 g) slightly flaccid, occupying up to 85% of body cavity in mature (≤ 30.44 cm ± 4.62 cm SL; range: 19-38 cm SL) females. Oocytes large, bright yellow; hydrated.	Fully yolked oocytes with eminent release of gametes defined by late stage GVM, GVBD, hydration, and newly collapsed POFs (Fig. 7 D).
Spent/ regressing (Stage 5)	Ovaries occupy $< 20-40\%$ of body cavity in sexually mature females (29 ± 3.59 cm; range: 24-38 cm SL). Ovaries vary in weight (0.1-51.2 g) and color depending on time since spawning. <i>Newly spent</i> : Ovaries large but flaccid. <i>Late stage</i> : Ovaries appear bruised, dark purple, like deflated sacs with few or no leftover vitellogenic oocytes. Ovarian wall thick.	No fully yolked oocytes present (Fig. 7E); occasional observations of atretic vtg2 oocytes clustered along blood vessels. 90 % atresia of fully yolked oocytes and considerable occurrence of POFs. Brush-like fimbriae from the ovarian wall extend into the empty ovary lumen.
Recovering spent / regenerating (Stage 6)	Ovaries occupy $< 20-40\%$ of body cavity in sexually mature females. <i>Early stage</i> : Ovaries appear bruised; purple to dark pink; very similar to stage 1 but larger. <i>Mid-regenerating stage</i> : Light pink ovary with subtle vascularisation; no visible vitellogenic oocytes. <i>Late regenerating stage</i> : oocytes regain bright yellow color and increase in diameter.	<i>Early stage</i> : No fully yolked oocytes present with degenerating POFs. <i>Mid-regenerating</i> : Similar to Stage 1 except for the thick ovarian lining. <i>Late stage</i> : Oocytes mostly vitellogenic but not hydrated (Fig. 7 F).

CA-cortical alveolar stage; GVBD-germinal vesicle breakdown stage, GVM- germinal vesicle migration stage; OM-oocyte maturation stage, PG-primary growth phase, POF-post-ovulation follicle, Vtg1-primary vitellogenic stage, vtg2-secondary vitellogenic stage, vtg3-tertiary vitellogenic stage

Six arbitrary stages of oocyte maturity based on external morphology of the ovary, colour and size of oocytes, % coverage of ovary in the coelomic cavity, and histologic features are described in Table 2 and Fig. 7. Validation of oocyte diameters used to assign stages of ovarian maturity shows markedly higher frequency of the spawning/hydrated oocyte size range (3-4 mm) in the stage 4 ovaries. Spawning capable (stage 3) ovaries have a conspicuously higher number of maturing oocyte size range (1-2.5 mm) and a smaller number of immature oocytes (≤ 1 mm). Maturing (stage 2) ovaries exhibit marked abundance of the early vitellogenic (< 1 mm) and maturing

oocytes (Fig. 8). This validation confirms the macro-scale classification and demonstrates that ovaries of the classified size ranges have distinctly distributed percentages of oocyte sizes.

The minimum length to reach sexual maturity, by which an ovary attains highly vitellogenic and hydrated appearance, was mainly found in females of 26.7 cm SL and onwards. However, incidences of precocious maturation were seen in smaller females at 19 cm SL during the peak spawning season. The macroscopic and histological analyses of the ovary show that *P. disjunctivus* females from Marikina River have a short reproductive season, initially taking place from June to September coinciding with the rainy season (Fig. 9). The number of spawning stage ovaries peaked in July and subsequently declined in October along with increasing occurrence of spent/regressing ovaries. Females had a slow turnover of primary growth oocytes from the previous spawning season from October, 2010, peaking on January 2011 where fully spent ovaries were seen. Nonetheless, a few late stage recrudescence and mature females were observed from March to April serving as evidence of imminent recovery towards the start of another reproductive season. However, this initial sign of recovery declined in May following the warmest month recorded during the study period. Late stage recrudescence then progressed to spawning-capable stage by June in time for the rainy season.

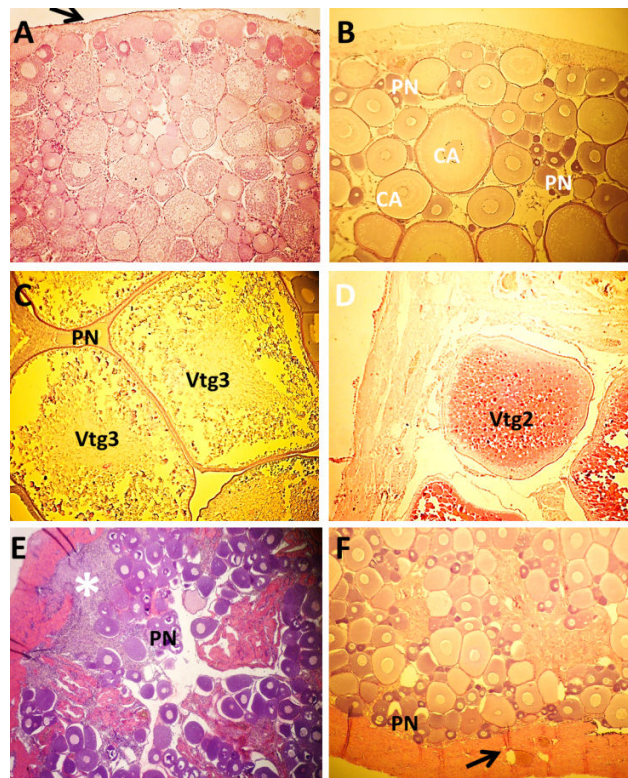


Fig. 7. Stages of ovarian maturity in *P. disjunctivus*. A: Immature stage dominated by PG oocytes. B: Maturing stage. C: Spawning capable stage characterised by presence of mostly vtg3 oocytes. D: Section of a recently spawned ovary. E: Spent ovary characterised by thick ovarian lining. F: Late regeneration stage characterised by thick ovarian wall (black arrow) and abundant PG oocytes. Scale bar: A,B;E,F-10 μ m; C,D-200 μ m. PN: perinucleolar stage, CA: cortical alveoli, vtg2: migratory nucleus stage, vtg3: ripe/mature stage.

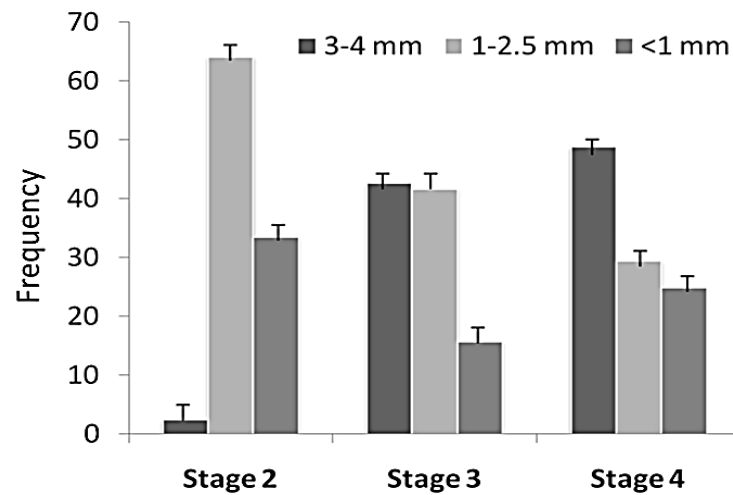


Fig. 8. Macroscale validation of oocyte diameters for ovarian stages according to its frequency in *P. disjunctivus*. Stage 2: developing/maturing stage, Stage 3: mature stage, Stage 4: actively spawning stage

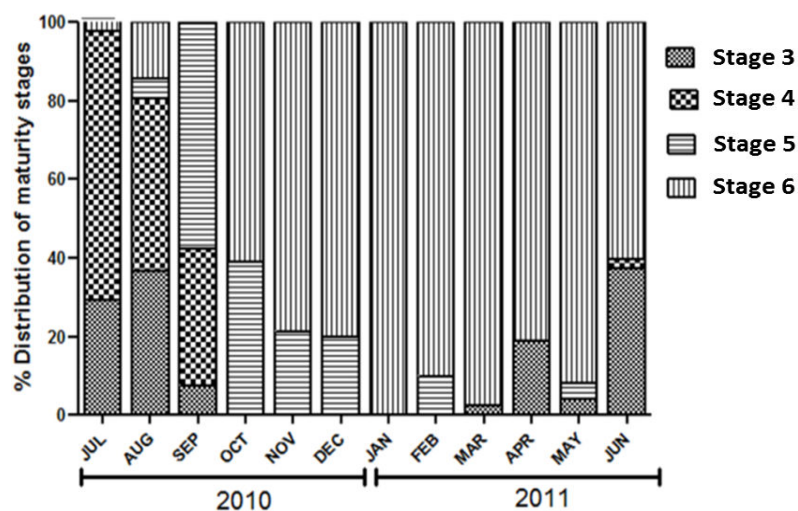


Fig. 9. Percentage (%) distribution of maturity stages in *P. disjunctivus* ovaries from July 2010- June 2011. Stage 3: spawning capable, Stage 4: actively spawning, Stage 5: Spent, Stage 6: Regenerating stage. % distribution of Stage 1 (immature) and Stage 2 (maturing) females were not included in this figure to provide focus on the interplay of the reproductive phenology of adult-sized *P. disjunctivus* females.

Discussion

During the peak of the reproductive season, gravid females can be distinguished from males through their swollen abdomen; however, there were no other defined differences apart from the sizes of males and females. Other reproductive features to distinguish the two sexes were lacking because actual male-female sexual interactions in the field are difficult to observe and catfishes in general are difficult to breed under culture conditions (Legendre et al. 1996).

Gibbs et al. (2008) has reported that *P. disjunctivus* from Volusia Blue Springs, Florida were spawning capable starting at 300 mm SL. However, from this present study, the minimum length of females to reach sexual maturity starts at 26 cm SL, although several females as small as 19 cm SL were observed to have spawning capable ovaries during the peak spawning season. This new size range wherein *P. disjunctivus* is capable of reproducing has important implications on its invasive nature, considering that females can grow up to 52 cm TL in Marikina River which is rich in organic matter and detritus, and without predators to control the population. Further, the spawning activity observed in this study (July to September) is also consistent with the study of Gibbs et al. (2008) where the GSIs of female *P. disjunctivus* at Volusia Blue Springs, Florida were prominently elevated starting the month of May; eventually peaking in July and extending towards September.

Most catfish families anticipate the monsoon season by the preceding dry summer when photoperiod is long and temperatures are warm (Legendre et al. 1996). In most siluriforms, the final stimulus to spawn is associated with the rise in water level and flooding (Bruton 1979; Legendre et al. 1996) although occasional observations of sexually mature individuals may be found in lagoons and enclosures provided with unrestricted feeding (Richter et al. 1987).

The present data indicate that the female *P. disjunctivus* prefer the rainy season for spawning and elevated temperatures for recrudescence. Nonetheless, the drop in water level and the consequent elevated water temperature during the hottest month of 2011 may have a limiting effect on the reproduction of females.

Tropical freshwater systems have relatively stable temperatures, and months exhibiting high temperatures and decline in water levels could be avenues for possible population control since spawning does not occur or is largely reduced during the dry seasons. Aggressive catching/harvest of these fishes during these months may be exerted to curb their population especially in Marikina River because natural predators feeding on mature *Pterygoplichthys* are practically absent except for few residents living near the river that catch large-sized *Pterygoplichthys* for food. In Palizada River, Mexico, however, the common snook *Centropomus undecimalis* (Bloch 1792) preys on *P. pardalis* (Toro-Ramirez et al. 2014).

Gibbs et al. (2008) observed a strong asymmetry in the size of the mature ovary, with the left lobe being larger than the right. In the present study, the connective tissue/sphincter joining the left and right ovaries at the post-ovarian sinus was naturally loose, having no tight barrier that separated oocytes from the left and right ovaries. This loose end allows for the transfer or displacement of mature oocytes at the posterior end of the ovary on either side of the gonad without causing injury to the displaced oocyte. This could also be the reason why there were no differences in the oocyte diameters in the left and right ovaries. Gibbs et al. (2008) has mentioned that ovaries may have been constrained by the bands of connective tissue while Rounsefell (1957) demonstrated a similar form of asymmetry in three female salmonids species as caused by crowding of the intestine.

Pterygoplichthys disjunctivus is a gonochoristic iteroparous species which go through multiple reproductive cycles in a lifetime. The cystovarian type ovary, in which the ovarian lumen is continuous with the gonoduct is a common feature for most teleosts (Nagahama 1983). The three modal oocyte diameters and the group synchronous mode of oocyte development in *P. disjunctivus* indicate that the fish is a determinate batch spawner. However, observation of frequency of spawning within a single reproductive season has been difficult in the field. Gibbs et al. (2008) reported that *P. disjunctivus* ovaries contain multiple oocyte classes but was not able to observe completely spent females in their study. Completely spent ovaries were predominantly seen in September and October in this study.

Oocyte diameters of *P. disjunctivus* from Volusia Springs Texas were larger (max 3.8mm) (Gibbs et al. 2008) compared to those observed in this study (max: 3.6 mm; mean 3.2 mm). Comparison with other relative loriciid catfishes from the Parana, Brazil has shown that the mean thickness of the zona radiata (4.56 μm) in the ripe oocytes of the janitor fish were comparable with *Hypostomus ternitzi* (Boulenger 1895) (3.97 μm) and *Megalancistrus aculeatus* (Perugia 1891) (4.8 μm) where males also guard their broods in excavations. Similarly, *Lophiosilurus alexandri* Steindachner 1876, whose eggs are also deposited in nests and are incubated by males also exhibit a thin zona radiata (1.35 μm) (Barros et al. 2007). Interestingly, the zona radiata and granulosa layer of *P. disjunctivus* was largely comparable to that of *Rhinelepis aspera* (Spix and Agassiz 1829), a broadcast spawner with no parental care at 5.1 μm and 20.96 μm respectively (Suzuki et al. 2000).

The thin zona radiata in *P. disjunctivus* seen in this study may be compensated by the nest guarding behaviour of males, protecting the eggs from injury or abrasion as eggs are protected inside burrows until hatching. Accordingly, species with a thick zona radiata are capable of releasing eggs in a variety of substrates due to the mechanical protection of this layer (Coward et al. 2002) apart from its role in the control of passage of substances to the interior of part of the oocytes during vitellogenesis and adherence of eggs to the substrate during spawning. The thick granulosa in a mature oocyte is an advantage to the successful survival of the embryos as columnar follicular cells surrounding the mature oocyte have greater synthesising capacity for mucosubstances from the zona radiata, which is of great advantage for the egg's adhesiveness (Santos et al. 2006; Barros et al. 2007; Melo et al. 2011). The long recrudescence period of the ovaries of *P. disjunctivus* may also be attributed to the large amount of yolk present in the follicle, consistent with other species with large yolky oocytes (Rastogi 1967).

It has been proposed that spawning pattern of a population is dependent on the reproductive performance and is affected by environmental parameters (Ricklefs and Wikelski 2002; Young et al. 2006), which in turn, influence fitness and population density. The rich nutritional supply by the yolk during late vitellogenesis, along with parental care to compensate for a thin zona radiata, provides *P. disjunctivus* with a high degree of adaptive and reproductive success. This survival efficiency is further enhanced because *P. disjunctivus* is not a promising fishery resource because of

its hard body cover and tolerance to poor water conditions, making it unappealing for consumption by the general public.

Conclusion

This study for the first time described the ovarian histomorphology of the non-native suckermouth sailfin catfish *P. disjunctivus* in a tropical setting. Results show that females are spawning capable at smaller sizes compared to their congeners. The three oocyte diameters in vitellogenic ovaries and the short spawning season suggest that the females were iteroparous batch spawners. Female *P. disjunctivus* have a relatively short spawning period coinciding with the rainy season followed by a short regression stage which overlapped with the long recrudescence stage progressing towards in the dry months of the year. This reproductive phenology data provide a possible avenue for mechanical control to curb the population of this invasive species in Marikina River.

Acknowledgements

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