

Research Articles

Reproductive cycle of *Aplodinotus grunniens* females (Rafinesque, 1819) in the Usumacinta River, Mexico

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ABSTRACT. The freshwater drum *Aplodinotus grunniens* is a species widely distributed in North America. In the Mexican southeast, this species occurs in the Usumacinta River, where it supports an artisanal fishery. In this regard, the present study was conducted to supply detailed information on the female reproductive cycle of this species. Calculations of gonadosomatic (GSI) and hepatosomatic (HSI) indexes, histological and visual staging of ovaries as well as the staging of oocyte development were applied together to determine the reproductive changes during an annual cycle. The histological analysis revealed the presence of spawning capable females throughout the year, and the distribution frequencies of oocyte diameters displayed the continuous occurrence of mature oocytes, with great data dispersion and different modal groups. These observations were further corroborated by the presence of alternating peaks and drops in the GSI and HSI, which were correlated with the occurrence of maturing and spawned females. These data, taken together, reveal that *A. grunniens* from the Usumacinta River is a partial spawner with asynchronous ovarian development and undetermined fecundity that reproduces year-round, but with spawning occurring preferably during the rainfall-flooding season (June to November). The Usumacinta River seems to be a permanently favorable environment for sheltering, feeding and growth of *A. grunniens* broods, especially during the flooding season. The information generated in this study will be useful for the development of management strategies in the wild as well as for the exploration of *A. grunniens* as a potential species for aquaculture production in southern Mexico.

Keywords: *Aplodinotus grunniens*; reproductive biology; histology; oogenesis; partial spawner; fecundity

INTRODUCTION

The freshwater drum *Aplodinotus grunniens* is the only species of the *Aplodinotus* genus that has permanently colonized freshwater environments (Nelson, 1994). The species is widely distributed in North America, from southern Canada, through USA and Mexico up to Guatemala, in Central America (Chao, 2002; Sluss & Harrel, 2006). In spite of its wide distribution, only *A. grunniens* from northernmost populations inhabiting temperate zones have been subject of study, resulting in several studies about a range of topics such as diet, re-

production, sexual dimorphism, growth, age and morphological variations among river ecosystems (Daiber, 1952; Rypel, *et al.*, 2006; Rypel, 2007; Davis-Foust *et al.*, 2009; Jacquemin & Pyron, 2013; Jacquemin *et al.*, 2014).

Regarding reproduction, it was reported that *A. grunniens* occurring in various lakes located within USA and Canada, spawns between May and July, while in the Tennessee River the species spawns in September (Wallus & Simon, 2006). Oocyte development was also described in *A. grunniens* specimens from the Red Lakes in Minnesota, USA, an area conside-

red as the northernmost limit in the range of distribution of the species (Palmer *et al.*, 1995). By contrast, in populations inhabiting the tropical zone of its range, the reproductive biology of *A. grunniens* has been poorly investigated. In the case of *A. grunniens* population(s) occurring in the Usumacinta River, in the Mexican southeast, only two studies have been conducted until date; one focused on determining the potential of the species for aquaculture (Chávez *et al.*, 1989) and another one that characterized the embryonic development using captive organisms (Hernández-Gómez *et al.*, 2013). In the Mexican southeast, *A. grunniens* supports an artisanal fishery due to the quality and taste of its meat (NOM-037-PESC-2004). However, some studies have suggested that the abundance of several fish species in the Usumacinta River has been reduced in the last years, due to a generalized degradation of the Usumacinta basin caused by demographic expansion, environmental pollution and landscape modifications (Cabrera & Cuc, 2002; Alvarez-Merino *et al.*, 2014). This study was carried out to determine the developmental phases of female gonads throughout an annual cycle, using organisms inhabiting in the middle part of the Usumacinta River in Tabasco, southern Mexico, in view of the pivotal role that the understanding of reproduction plays for the development of fisheries management and fish conservation strategies (*e.g.*, to establish catch and size limits as restrictions in the catch season).

MATERIALS AND METHODS

Study area and fish sampling

The study was carried out from January to December 2013. The sampling area was delimited from the Usumacinta River Canyon “Boca Del Cerro” (17°25'33"N, 91°29'29"W) up to the locality of El Copo (17°23'33"N, 91.28'23"W). This area was selected for the study because the abundance of the resource supports the only artisanal fishery for the species in the region. During low river levels (December-June), specimens were captured using an 80 m long dragging net with 3 m height and 2 cm of net opening. During the flood months (July-November), on the other hand, captures were conducted using hooks, baited with freshwater shrimp (*Procambarus llamasii*). Eighty-eight adult females were sampled, having an average weight of 452.4 g (± 198.75) and a total length (TL) of 33.52 cm (± 3.61). We aimed to sample 10 females per month; however, during June-October, when river depth increased, and sampling activities turned difficult, sample size varied from one to five specimens. Collected fish were sacrificed with an overdose of

clove oil (0.10 mL L⁻¹), and suffering was reduced as much as possible.

Specimen processing

For each female, the total length was measured using a conventional ichthyometer (± 0.1 cm). The total weight (TW) and gonadal weight (GW) were registered with a ScoutTMPro™ digital scale (± 0.01 g). The length and width of the ovaries were measured using a metric vernier caliper (the nearest 0.1 mm). The gonadal volume (GV) was estimated using the formula of an ellipsis $GV = (4/3 \pi) a^2 \times b$; where “a” is the diameter in the central portion of the gonad and “b” is the length (Méndez-Marin *et al.*, 2012). Sex was identified through macroscopic examination. For the histological processing, right ovaries were sampled by making three 3 cm-thick cuts (anterior, middle and posterior) and quickly fixed in Bouin solution. The small ovaries were immersed 24 h and largest ones 48 h. Afterwards, samples were washed in tap water to remove excess fixative. Samples were dehydrated in growing concentrations of ethanol (30, 50, 70, 96 and 99%), cleared in xylene by 1 h (small gonads) or 1.5-2 h (large gonads), and finally embedded in paraffin (Paraplast). A series of 7 μ m-thick cuts were obtained using a Reichert-Jung™ Model I sliding microtome. The selected sections were stained with hematoxylin-eosin, according to Humason (1979) and Aguilar *et al.* (2013).

Microscopic analysis

The histological analysis of *Aplodinotus grunniens* ovaries was carried out using a light microscope (Zeiss™ Axiostar Plus model) coupled to a digital camera (Zeiss™ Axiocam MRc 5 model). Three histological slides from the middle portion of the ovary of each female were selected to determine the phases of gonad development. Gonads were classified as regenerating, developing, spawning capable, and regressing, according to the histological arrangement of somatic and germinal elements in the ovary. Also, the steps of oogenesis characteristic of each gonadal phase were described, and the diameters of the most frequent oocyte steps registered ($n = 30$ in all cases). Only oocytes showing a germinal vesicle were measured. Measurements were done using the same Zeiss™ optic microscope linked to the AC AxioVision r4.5 program, designed to carry out morphometric measurements with an accuracy of $\pm 0.1 \mu$ m.

Classification of the ovarian cycle and oogenesis

The ovarian cycle of *A. grunniens* was classified in reproductive phases, according to Brown-Peterson *et al.* (2011) as immature (never spawned), regenerating, developing, spawning capable and regressing. Oocyte

staging was performed following Grier *et al.* (2009), who classified oogenesis in stages and steps, as follows: 1) Oogonial proliferation (OP); 2) Chromatin nucleolus (CN); 3) Primary growth (PG), further subdivided into the steps one nucleolus (PGon), multiple nucleoli (PGmn), perinucleolar (PGpn), oil droplets (PGod), and cortical alveoli (PGca); 4) Secondary growth (SG), further subdivided into early secondary growth (SGe), late secondary growth (SGl), and full grown oocytes (SGfg); 5) Oocyte maturation (OM), further subdivided into eccentric germinal vesicle (OMegv), germinal vesicle migration (OMgvm) and germinal vesicle breakdown (OMgvb); and 6) Ovulation (OV). Although follicular atresia is not considered as part of oogenesis, this feature was also present in all the reproductive phases analyzed in this study and occurred in primary and secondary growth oocytes. In this study, the term *phase* was restricted to designate the reproductive condition of the ovaries during the annual cycle, and the term *stage* was restricted to describe the events of oogenesis that are common to all fish, that is, OP, PG, SG and OM. Finally, the term *step* was used to designate the sub-stages occurring during the main stages of oogenesis.

Environmental conditions

Data for river level, rainfall and water temperature were obtained from CONAGUA (Mexican National Water Commission) weather station number 30019, located at the village of Boca Del Cerro, close to the study zone.

Data analysis

In order to determine the best indicators of the annual reproductive cycle of females, the gonadosomatic index (GSI) was calculated, with the equation: $GSI = Wg/Wt \times 100$; where Wg: weight of the gonads (g) and Wt: total weight of the specimen (g) (Rossendillum *et al.*, 1987). The hepatosomatic index (HSI) was calculated with the equation: $HSI = Wh/Wt \times 100$; where Wh: weight of the liver (g), and Wt: total weight of the specimen (g) (Rodríguez-Gutiérrez, 1992). All the statistical analyses were carried out with a confidence level of 95%, using the Statgraphics Centurion™ v.16 software.

RESULTS

The species presents paired elongated ovaries suspended from the dorsal body wall by the mesovarium (Fig. 1a). The ovaries communicate with the exterior through a common gonoduct, opening at the genital pore. Ovaries of *Aplodinotus grunniens* are cyst ovarian type; they have a central cavity toward eggs released after ovulation. From the inside out, the

ovarian wall is made up of germinal epithelium, ovarian stroma, smooth muscle, connective tissue and a peritoneal epithelium. (Figs. 1b-e). Sampled females presented four of the five reproductive phases described by Brown-Peterson *et al.* (2011): regenerating, developing, spawning capable and regressing. The absence of young females in the immature phase may have resulted from a bias in the sampling method or the use of different ecosystems at this stage of development. Macroscopically, the ovaries ranged in texture and appearance from dough-like consistency in the regenerating phase, to granular and densely packed in the spawning capable phase. The reproductive phases observed were present throughout the year, indicating continuous reproduction. Below, we described the reproductive phases of *A. grunniens* females based on the gross morphology and histological characteristics of the gonads. The oocyte stages representative for each gonadal phase were also described.

Regenerating

This phase occurred more frequently during April and September (Fig. 2). It was observed in females with sizes between 29.03 and 36.56 cm (average = 31.82 ± 2.44 cm TL), whose total weights ranged between 222.61 and 583.80 g (338.49 ± 109.56 g). The average GSI of females was 1.02 ± 1.28 . In this phase, ovaries had an elongated shape, and they occupied approximately 1/3-1/2 of the coelomic cavity. Ovaries were hardly vascularized with discreet blood capillaries. Their color ranged from cream to reddish, mainly towards the cephalic region (Fig. 3a). Oocytes in these ovaries were indistinguishable with the naked eye. The ovarian length varied from 34.96 to 70.20 mm (56.17 ± 6.69 mm) and total weight between 0.30 and 4.9 g (2.31 ± 1.14 g). Ovaries had an average volume of $1,333.62 \pm 1,837.06$ mm³. During this reproductive phase, ovaries contained primary growth oocytes including one nucleolus (PGon), multiple nucleoli (PGmn), perinucleolar (PGpn) and oil droplet (PGod) steps (Figs. 3b-i). The oocytes presented a densely basophile cytoplasm during the earlier steps, but they turned more acidophilic as development progressed. From all the oogenic steps identified, the multiple nucleoli (PGmn) was the most frequent (42.7%), with a diameter ranging from 50 to 150 μ m (Fig. 3f).

Developing

This phase presented its highest frequency in August, October and November (Fig. 2), with females ranging from 29.13 to 40.45 cm TL (35.51 ± 3.07 cm). Total weights varied between 232.71 and 997.82 g (572.20 ± 187.39 g). The average GSI of females was 2.73 ± 1.60 . Ovaries were larger than in the previous phase; they

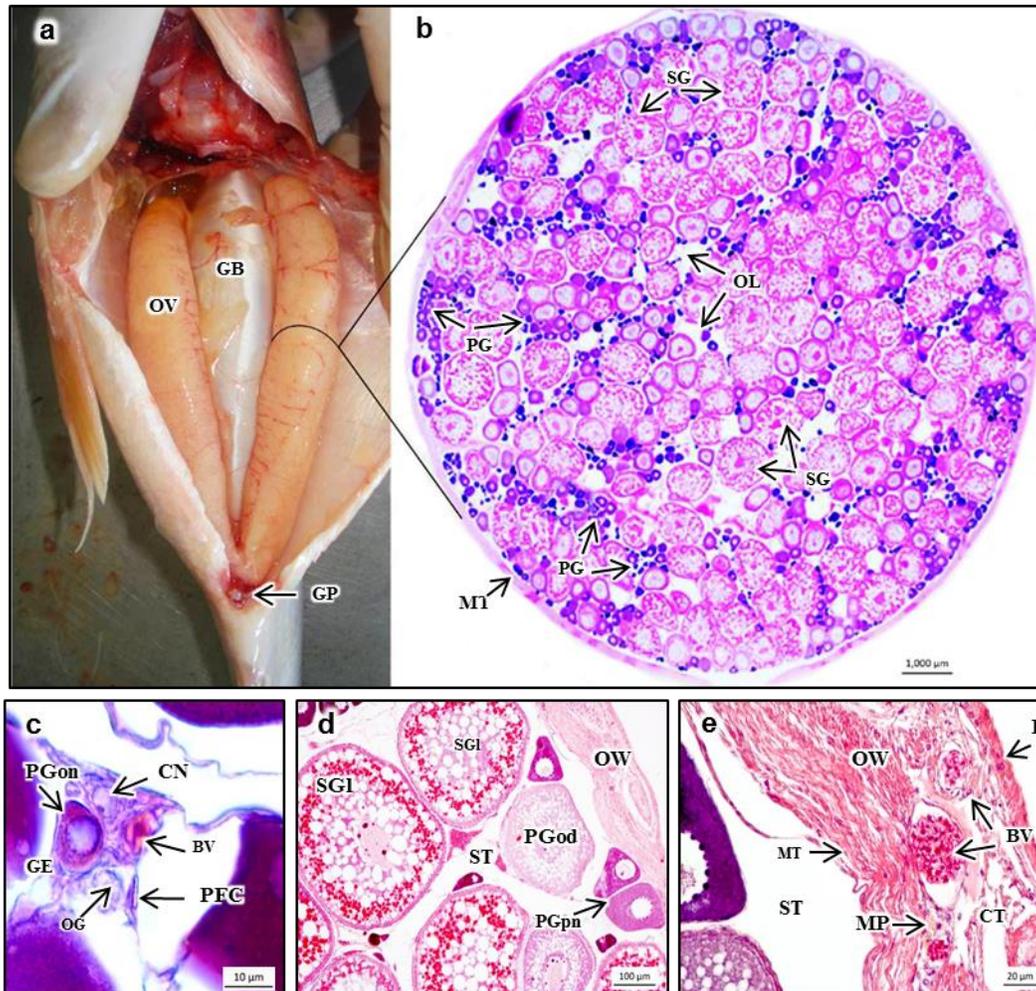


Figure 1. Gross morphology and anatomical location of *Aplodinotus grunniens* ovaries. a) The ovaries are paired organs located in the abdominal cavity. They communicate with the exterior through the genital pore (GP); b) histological cross section of a developing ovary showing a mass of oocytes in primary (PG) and secondary (SG) growth stages. The large number of oocytes has drastically reduced the ovarian lumen (OL). From the inside out, the ovaries consist of the following histological layers: the germinal epithelium, the ovarian stroma and the ovarian wall, described in c, d and e images, respectively; c) the germinal epithelium (GE) is made of germ cells such as oogonia (OG), chromatin nucleolus (CN) and primary growth one nucleolus oocytes (PGon) as well as pre-follicular cells (PFC); d) the ovarian stroma (ST) consists of extracellular fluid and connective tissue, where follicles at different stages of development are immersed; e) the ovary is enclosed by the ovarian wall (OW), which consists of both muscular (MT) and connective tissue (CT), surrounded by an external epithelium, the peritoneum (P). Muscular tissue of the ovarian wall (MT), blood vessels (BV), primary growth perinucleolar oocyte (PGpn), primary growth oil droplet oocyte (PGod), late secondary growth oocyte (SGI), ovarian stroma (ST), melanomacrophage (MP). Hematoxylin and eosin stained.

were homogenous in shape but with a slight dilatation in the cephalic region, occupying around 2/3 of the coelomic cavity. Vascular circulation was located ventrally, going across each ovary lengthwise (Fig. 4a). Oocytes were visible with the naked eye; they looked as granules ranging from milky to yellowish (Fig. 4a). Ovarian length during this phase varied from 47.55 to 100.66 mm (76.68 ± 14.21 mm), weighing between 5.60 and 32.90 g (16.19 ± 7.45 g), and with an average

volume of $4,516.42 \pm 2,751.84$ mm³. In this phase, oocytes in almost all the developmental stages/steps were observed, including primary growth oocytes such as multiple nucleoli (PGmn), Perinucleolar (PGpn), oil droplet (PGod) and cortical alveoli (PGca); and secondary growth oocytes in early secondary growth (SGe), late secondary growth (SGI) and full grown (SGfg) steps (Figs. 4b-e). The secondary growth stage of oogenesis was characterized by the gradual accumu-

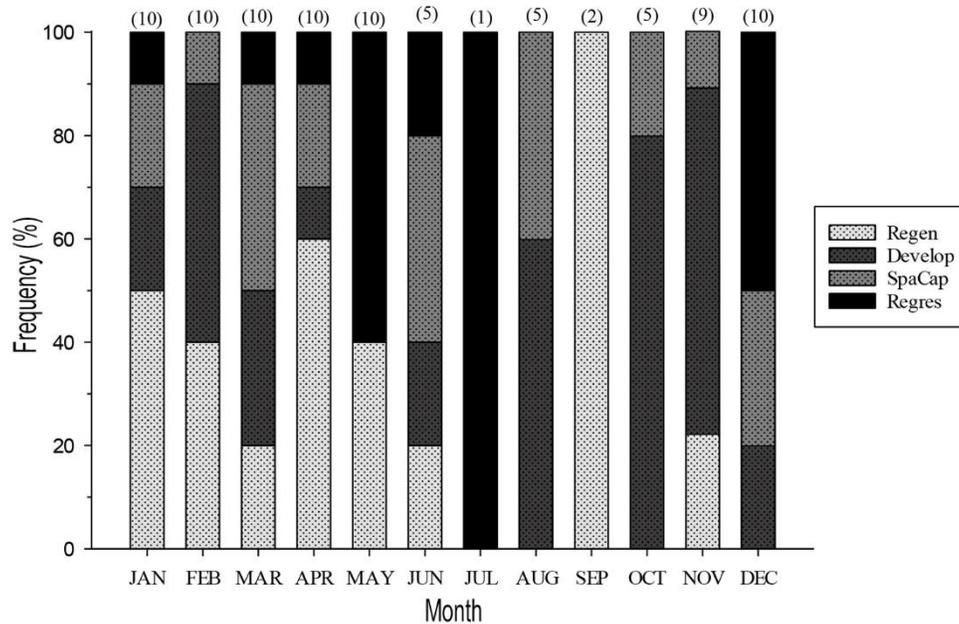


Figure 2. Monthly frequency distribution of the different reproductive phases from *Aplodinotus grunniens* females captured in the middle Usumacinta River. Regen: regenerating, Develop: developing; SpaCap: spawning capable, Regres: regressing. The number of females sampled per month is indicated in parenthesis.

ation of oil droplets and yolk globules through the ooplasm, causing a significant increase in oocyte size (Figs. 4e-g). From all the oocyte steps observed, the early secondary growth (SGe) was the most frequent step (27.7%), with oocytes ranging from 200-500 μm (Figs. 4a-b).

Spawning capable

This phase occurred more frequently in March, June and August, (Fig. 2), corresponding to females between 28.15 and 39.34 cm TL (33.09 ± 3.56 cm), with a total weight ranging from 231.10 to 698.56 g (437.44 ± 163.44 g). Females at this stage had an average GSI of 3.77 ± 1.48 . Ovaries were homogenous in their shape; they occupied most of the coelomic cavity and showed lesser vascularization when compared with the previous phase (Fig. 5a). At a gross level, oocytes had spherical morphology and coloration ranging from yellow to orange. Ovary length in this phase varied between 45.61 and 111.29 mm (80.73 ± 15.30 mm), weighing between 8.42 and 41.30 g (18.19 ± 10.02 g) and with an average gonad volume of $5,788.17 \pm 4,510.08$ mm³. In this phase, most oocytes corresponded to the oocyte maturation stage (Figs. 5b-g); however, a few primary and secondary growth oocytes were also observed, of which some become atretic (Figs. 5b-c). The oocyte maturation stage appeared represented by the following steps: eccentric germinal vesicle (OMegv), germinal vesicle migration (OMgvm)

and germinal vesicle breakdown (OMgvb) (Figs. 5e-g). The OMeqv step was the most frequent (45%). These oocytes had a maximum diameter of 620 μm , and their cytoplasm appeared filled with merging oil droplets and yolk globules (Fig. 5e). In the germinal vesicle breakdown step (OMgvb) oil droplets merged into a single oil globule and the cytoplasm became filled with a continuous yolk mass, in such a way that the germinal vesicle was no longer visible (Fig. 5g).

Regressing

Ovaries, in this phase, presented their highest frequency in May and July (Fig. 2). Females had between 27.03 and 41.23 cm TL (33.40 ± 4.55 cm) and a total weight ranging from 173.41 to 717.52 g (399.64 ± 177.67 g). Females had an average GSI of 0.70 ± 0.63 . Generally, these ovaries were flaccid, with hemorrhagic appearance towards the caudal region, and occupying 1/3 of the coelomic cavity (Fig. 6a). Ovaries ranged from 40.12 to 77.31 mm (63.19 ± 11.03 mm), with a weight between 1.01 and 10.42 g (3.52 ± 2.60 g), and an average volume of 814.53 ± 490.91 mm³. At the histological level, the Regressing phase was characterized by presenting a thickened ovarian wall, numerous blood vessels, muscle bundles (Figs. 6b,e,f), scattered oogonia and primary growth oocytes in one nucleolus (PGon), multiple nucleoli (PGmn), perinucleolar (PGpn), cortical alveoli (PGca) and oil droplet (PGod) steps, which appeared separated by abundant

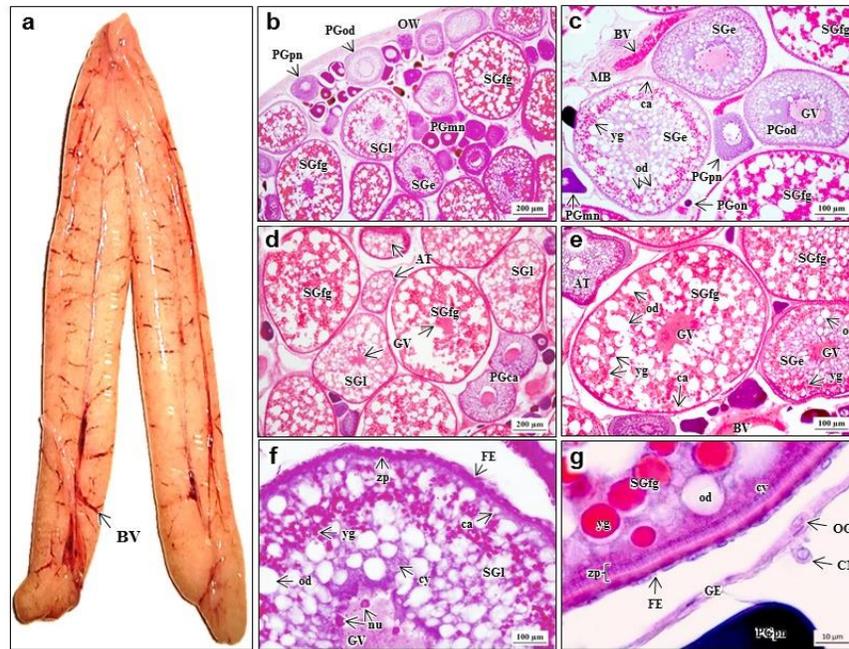


Figure 4. *Aplodinotus grunniens* ovaries during the developing phase. a) At this phase, gonads show gold-sand color and notorious blood vessels (BV); b) cross-section of an ovary showing oocytes in different stages of development. There is a predominance of secondary growth oocytes (SG) in early (SGe), late (SGL) and full grown (SGfg) steps. The co-occurrence of developing primary growth oocytes such as multiple nucleoli (PGmn), perinucleolar (PGpn) and oil droplet (PGod,) is indicative of asynchronous ovarian development; c) magnified view of PG (PGon: one nucleolus, PGpn: perinucleolar and PGod: oil droplet step) and SG (SGe, Early and SGfg, full grown) oocytes; d-e) SG oocytes (SGe, SGL and SGfg) gradually increase their sizes due to the massive accumulation of yolk globules (yg) and oil droplets (od), which start merging in larger inclusions. Regardless of this, the germinal vesicle (GV) remains central. Some atretic follicles (AT) are observed; f) during the Secondary growth late step (SGL), oocyte cytoplasm (cy) is filled with yolk globules (yg) and oil droplets (od); g) detail of the peripheral ooplasm of a Secondary growth full grown oocyte (SGfg) showing oil droplets (od) and yolk globules (yg). There is a strip of cytoplasm (cy) immediately over the zona pellucida (zp). The oocyte is surrounded by a flat follicular epithelium (FE). An oogonium (OG) and a chromatin nucleolus oocyte (CN) are observed in the germinal epithelium (GE). Ovarian wall (OW), blood vessel (BV), germinal vesicle (GV), nucleoli (nu), zona pellucida (zp), follicular epithelium (FE), cortical alveoli (ca), primary growth perinucleolar oocyte (PGpn). Hematoxylin and eosin stained.

follicles in regressing ovaries demonstrated that spawning occurred in May, July and September. All these data, taken together, suggest that *A. grunniens* inhabiting the Usumacinta River reproduces year-round, with spawning events occurring preferably during the second semester of the year, from June to November.

Environmental parameters

Water temperature ranged from 24°C in December to 29°C in May. Rainfall ranged from 1.5 mm in March to 19 mm in November. The level of the Usumacinta River reached its lowest value in April, with 12 m.a.s.l, getting the largest depth (18.17 m) in October (Table 1). Rainfall was high along the second semester of the year initiating in June with 10.7 mm and ending in December with 14 mm.

Table 1. Average monthly values of river level, rainfall and water temperature registered in the study site. Data for river level and rainfall were obtained from the CONAGUA meteorological station number 30019; m.a.s.l: meters above sea level.

Month	River level (masl)	Rainfall (mm)	Water temperature (°C)
January	15.12	5.3	25
February	13.75	3.6	26
March	12.43	1.5	24
April	12.00	4	27
May	12.77	5.9	29
June	17.31	10.7	27
July	16.90	5.7	27
August	16.69	8.5	27
September	18.01	11.2	26
October	18.17	15	25
November	14.73	19	26
December	13.53	14	24



Figure 5. *Aplodinotus grunniens* ovaries during the spawning capable phase. a) Ovaries show a sweet-corn color and large blood vessels (BV). Oocytes are visible with the naked eye; b) cross section of an ovigerous lamellae showing oocytes in different stages of development: primary growth oocytes in one nucleoli (PGon) and multiple nucleoli (PGmn) steps as well as secondary growth oocytes in late (SGl) steps; c) in spawning capable ovaries there is a predominance of mature oocytes (Oocyte maturation stage), with the eccentric germinal vesicle (OMegv) and germinal vesicle breakdown (OMgvb) being the most common steps; d) an oocyte in the OMEgv with its characteristic eccentric germinal vesicle (GV). Oil droplets (od) have fused in several oil globules of middle size; e) a germinal vesicle migration (OMgvm) oocyte showing a flattened germinal vesicle (GV) being pushed towards the animal pole by a coalescent oil globule (og). The oocyte is surrounded by primary (PGod, oil droplet and PGpn perinucleolar steps) and secondary growth (SGfg, full grown) oocytes; f) in OMgvm oil droplets (od) continue merging in a huge oil globule. In this oocyte the germinal vesicle (GV) has reached the animal pole, where the rupture of the germinal vesicle will subsequently take place; g) an oocyte in the final step of maturation, the germinal vesicle breakdown (OMgvb), where the GV is no longer recognizable. The fusion of yolk globules and oil droplets is completed. PGmn, primary growth multiple nucleoli oocyte; SGfg, secondary growth full grown oocyte; MB, muscle bundle; AT, follicular atresia; OW, ovarian wall; OL, ovarian lumen, GE, germinal epithelium; zp, zona pellucida. Hematoxylin and eosin stained.

DISCUSSION

The results indicate that *Aplodinotus grunniens* is an iteroparous species that reproduce year-round, with peaks of gametogenesis occurring in January, June and October, and with spawning occurring in May, July, September and November. The histological analysis revealed the co-occurrence of oocytes in various developmental stages throughout the year, ranging from primary growth to oocyte maturation stages, consistent with the definition of asynchronous ovarian development (Wallace & Selman, 1981). It means that females can shed oocyte batches at different moments

of the year. Asynchronous development of oocytes is associated with an indefinite fertility strategy, where the potential annual fertility is not fixed before spawning, but instead, primary growth oocytes are continuously recruited for maturation in order to be released along the reproductive season (Murua & Saborido-Rey, 2003). In addition to those features mentioned above, the absence of spacing (hiatus) among oocyte diameter frequencies separating mature from primary growth oocyte stocks, clearly shows that the production of new mature oocytes is continuous. The occurrence of multiple oocyte stages has also been reported in the same species inhabiting in USA and

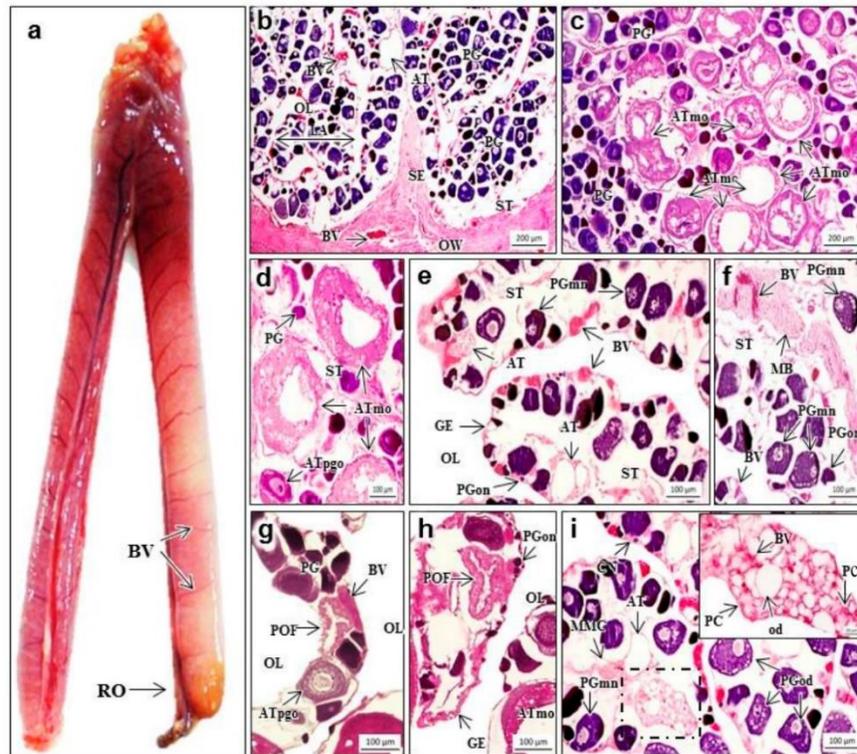


Figure 6. *Aplodinotus grunniens* ovaries during the regressing phase. a) Macroscopic appearance of an ovary in the regressing phase. The color ranged between pink and plumb. In some cases, residual mature oocytes (RO) were seen; b) histologically, the ovaries show a thickened ovarian wall (OW) with large septa (SE) protruding into the ovarian stroma (ST); c) many atretic follicles in several stages of degeneration occur during this phase, mainly non-ovulated mature oocytes (ATmo) that are resorbed by atresia. Primary growth oocytes start to develop at the same time, chiefly PG one nucleolus (PGon) and multiple nucleoli (PGmu) oocytes; d) amplification of some atretic mature oocytes (ATmo). The ooplasm is liquefied and oocyte morphology turns irregular. Atretic primary growth oocytes (ATpgo) were observed as well; e) regressing ovaries were characterized by abundance of small blood vessels (BV), mainly in the germinal epithelium (GE), which contained primary growth one nucleolus (PGon) and multiple nucleoli (PGmn) oocytes. Atretic follicles (AT) are scattered in the ovarian stroma (ST), surrounded by developing oocytes in primary growth (PG); f) these ovaries presented spaced oocytes (PGon and PGmn) and muscle bundles (MB) in the ovarian stroma (ST); g-h) ovigerous laemella displaying postovulatory follicles (POF) next to the ovarian lumen (OL); i) follicular atresia (AT) was the most common event in regressing ovaries. The inset is an amplification of the dotted area and depicts an advanced atretic mature oocyte being resorbed by phagocytic cells (PC) and blood vessels (BV). Melanomacrophage centers (MMC) seems to be involved in this process as well. Ovarian lumen (OL), ovigerous lamellae (LA), primary growth oocytes (PG), blood vessel (BV), follicular atresia (FA), ovarian stroma (ST), chromatin nucleolus oocyte (CN), primary growth oil droplet oocyte (PGod), oil droplet (od). Hematoxylin and eosin stained.

Canada (Daiber, 1953; Palmer *et al.*, 1995). As a matter of fact, the majority of species belonging to the Sciaenidae family that occur in the tropical and subtropical regions such as the white seabass *Atractoscion nobilis*, the corvina drum *Cilus gilberti*, the seatrout *Cynoscion nebulosus* and the brown meagre *Sciaena umbra*, all are considered asynchronous and iteroparous (Brown-Peterson *et al.*, 1988; Cárdenas, 2012). However, there are several saltwater species from the same family that use synchronous spawning as reproductive strategy, such as the red drum *Sciaenops ocellatus*, the croaker *Argyrosomus regius* and the Shi

drum *Umbrina cirrosa* (Wilson & Nieland, 1994; Mylonas *et al.*, 2004; Shabana *et al.*, 2012; Gil *et al.*, 2013). The GSI and HSI showed alternating peaks and drops caused by the presence of gametogenic and spawned females, respectively, pointing out that *A. grunniens* reproduce continuously, especially during the second semester of the year (June-November). These results, taken together with the occurrence of asynchronous ovarian development, indefinite fertility, and partial spawning, indicate that *A. grunniens* from the Usumacinta River reproduce year-round. Probably due to the existence of favorable environmental condi-

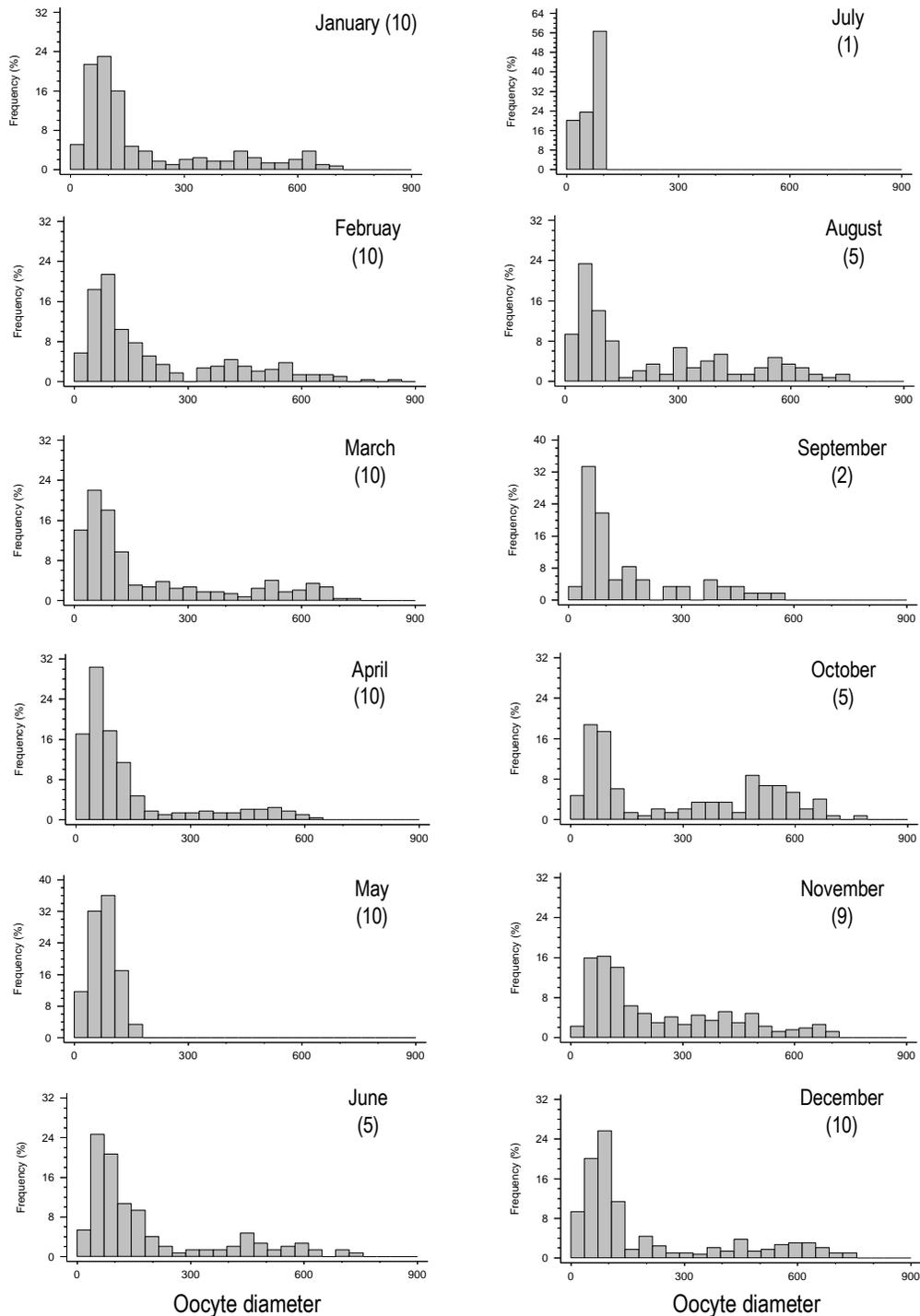


Figure 7. Monthly size frequency distributions of the oocyte diameters as measured at the microscope (n = 30 oocytes from each female). The number of females sampled per month is indicated in parenthesis.

tions for the development and survival of offspring, especially in the rainfall-flooding season, when heavy rainfalls cause the rising of the river and the flooding of riverside areas.

During the reproductive cycle of *A. grunniens*, four reproductive phases of the female gonad were identified; regenerating, developing, spawning capable and regressing, with almost all the stages of oogenesis co-occurring in the same gonad throughout the year. In

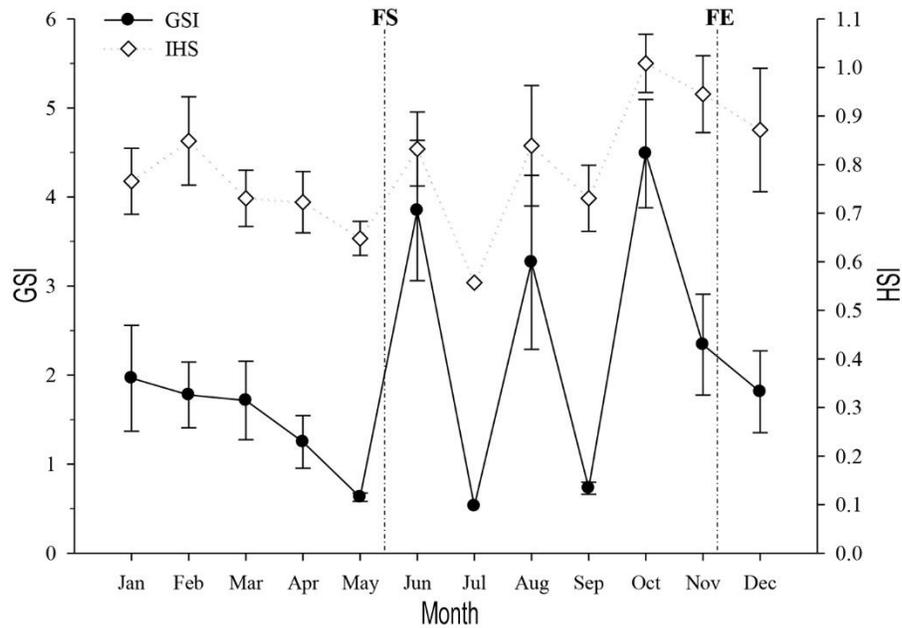


Figure 8. Reproductive indices of *Aplodinotus grunniens* females and duration of the flooding season. GSI: gonadosomatic index, HIS: hepatosomatic index. Dotted lines indicate the beginning (FS) and the end (FE) of the flooding season.

in general, in terms of gonadal stages, for the same species but inhabiting the Lake Erie, between USA and Canada, the stages of oogenesis described in the present study were similar to those described for other species of the same family. Such as *Sciaenops ocellatus*, *Sciaena umbra*, the whitemouth croaker *Micropogonias furnieri*, roncador *Umbrina robinsoni*, yellowfin croaker *Umbrina roncadorensis*, silver croaker *Bairdiella chrysoura*, croaker *Argyrosomus regius*, yellow croaker *Larimichthys polyactis*, southern kingcroaker *Menticirrhus americanus* and South American silver croaker *Plagioscion squamosissimus* (Valdebenito *et al.*, 1995; Félix *et al.*, 2009; Grau *et al.*, 2009; Miller *et al.*, 2009; Hutching & Griffiths, 2010; Lim *et al.*, 2010; Grier, 2012; Gil *et al.*, 2013).

The occurrence of post-ovulatory follicles (POFs) in fish is the most reliable evidence for determining spawning (Ganias, 2012). Daiber (1953) and Palmer *et al.* (1995) confirmed ovulation in *A. grunniens* based on the presence of POFs as indicators of spawning. In this study, POFs were present year-round and coincided with drops in the GSI, which is explained by the releasing of egg masses from the ovaries. Post-ovulatory follicles were mainly identified in regressing ovaries (usually termed as post-spawning ovaries in the fisheries literature) after cessation of spawning. Besides using POFs, we used other histological criteria as indicators of spawning, such as the presence of atretic follicles, a thickened ovarian wall and broad dispersion of primary and secondary growth oocytes (Brown-

Peterson *et al.*, 2011). Although POFs were present in all the regressing ovaries. Their number was much smaller in comparison with the number of mature oocytes observed in spawning capable ovaries. This small quantity of POFs in regressing ovaries might be due to the fast degeneration of these structures, since according to the literature POFs in the ovary of sciaenids last around 24 h, after which they degenerate rapidly (Saborido-Rey, 2008; Hutchings & Griffiths, 2010). Further studies must be focused on determining the timing and sequence of morphological events involved in the degeneration of POFs in *A. grunniens*.

The nutritional condition of the females determines the spawning season of a species, so they have to accumulate enough energy reserves in order to support gametogenesis and carry out a successful spawning (Saborido-Rey, 2008). In this study, data from gonadal histology, GSI and HSIs strongly suggest that *A. grunniens* from the Usumacinta River reproduce year-round, pointing out favorable conditions for growth and reproduction throughout the year. However, our results also show an increased channeling of energy for reproduction towards summer-autumn that might be related to an increase in food availability for offspring during the rainfall-flooding season (June-November), when the increase in river flow causes the flooding of riverside areas. From these observations, it is clear that the reproductive strategy of *A. grunniens* from the Usumacinta River differs with *A. grunniens* specimens from northern populations, such as those from the Red

Lakes, USA (Palmer *et al.*, 1995) and several other sciaenids species that reproduce either in spring or summer, such as the brown meager *Sciaena umbra*, *Bairdiella chrysoura*, *Larimichthys polyactis*, *Menticirrhus americanus*, *Sciaena umbra* and *Umbrina roncadorensis* (Ferreira *et al.*, 2011; Grammer *et al.*, 2009; Grau *et al.*, 2009; Miller *et al.*, 2009; Lim *et al.*, 2010).

We observed an association between environmental parameters, increases in the GSI, frequency of spawning capable ovaries (reproductively active females) and the frequency of mature oocytes larger than 600 μm . The moment and duration of spawning events in sciaenid fishes have been linked to water temperature, beginning with the rise of water temperature and ending when it drops (Hutchings & Griffiths, 2010). *A. grunniens* adapts to highly diverse environmental conditions as shown by its wide geographical distribution. The reported temperature in spawning grounds ranges from temperate (18.9-24.5°C) in the Lewis and Clark Lake in USA (Wismer & Christie, 1987) to mid (24-27°C) in the Missouri River in the US (Braaten & Guy, 2004) to tropical (26-29°C) in this study. According to Valdebenito *et al.* (1995), the reproductive cycle of fishes is mainly regulated by environmental factors, predominantly temperature and photoperiod, which are important for maturity and spawning. Additionally, in river ecosystems different annual nutrient cycles coincide with the rise of river level and flooding, creating conditions that are favorable for the survival and growth of larvae and juveniles, as described for *Sciaena umbra* and *Umbrina robinsoni* (Grau *et al.*, 2009). The rainy season is an environmental factor that influences the reproductive cycle of a species, because increased flows and flooded areas may constitute sheltering and nursing places for developing larvae and juveniles (Peña-Mendoza *et al.*, 2011).

CONCLUSIONS

The contribution of this research on the reproductive cycle of *Aplodinotus grunniens* females in the Usumacinta River must be considered for the development of a management plan in the river and its influences zones, with an emphasis on the highest reproductive activity period, which coincides with the highest fishing activity in the zone. The freshwater drum *A. grunniens* has a high biological, ecological and nutritional value in the region and that is why management measures should be implemented to guarantee the permanency of this valuable resource. Moreover, the understanding of the reproductive dynamics of this species will allow developing strategies for its captive management for aquaculture purposes.

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