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Permanent germinal epithelium and reproductive cycle of *Atractosteus tropicus* (Lepisosteiformes: Lepisosteidae) males, Tabasco, Mexico

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Abstract: The tropical gar *A. tropicus* plays an important ecological role as it regulates other fish stocks in different water bodies in Southeastern México. Nevertheless, wild populations are declining, and one conservation alternative is the aquaculture production and basic knowledge of reproductive biology; for males, this requires the study of germ and somatic structures of testes, to characterize the reproductive cycle, and to provide basic knowledge for exploitation and conservation models and strategies. With this aim, a total of 24 males with an average sL = 47.2 cm were collected from wild populations from the Laguna Pomposú, municipality of Jalpa de Mendez (18°19' - 93°01'12" W), Tabasco, Mexico. Fish were collected with a trawl net and were transported live to the Tropical Aquaculture Laboratory, División Académica de Ciencias Biológicas (DACBiol), Universidad Juárez Autónoma de Tabasco (UJAT). Males were killed by prolonged immersion in MS222. Testes samples were collected from each specimen and were processed using the standard histological procedures, that consisted of dehydration in an ascending ethanol series, xylol, embedding in paraffin, sectioning at 7 µm, and staining with hematoxylin-eosin (HE). The diameter of 20 seminiferous tubules (Dst), height of germinal epithelium (Hge), gonadosomatic index (GSI) and gonad volume (gV) were determined monthly. Based on morphometric and morpho-physiological characteristics, the testes consisted of a network of anastomosed tubules with non-restricted cystic spermatogenesis, and a permanent germinal epithelium. This is the first report of a permanent germinal epithelium in *A. tropicus*. Five reproductive classes were histologically identified: Class I Regressed; Class II Early Maturation; Class III Mid Maturation; Class IV Late Maturation; Class V Regression. Monthly GSI, gV and Dst values were lower in January and February, the testis showed spermatozoa remains and a regenerating discontinuous germinal epithelium. In March spermiogenesis increased and proliferation of spermatogonia decreased. Male tropical gar followed a seasonal reproductive cycle, indicated by the monthly variation of the reproductive classes and the reproductive season processes observed, and for which temperature and rainfall seem to stimulate reproductive activity and spermiation. Rev. Biol. Trop. 64 (4): 1597-1609. Epub 2016 December 01.

Key words: *Atractosteus tropicus,* germinal epithelium, spermatogenesis, spermiation, testes.

The state of Tabasco is located in Southeastern Mexico and has a wide variety of water bodies (Río González, Chibirital-loncho and Pantanos de Centla, among others) where *A. tropicus* plays an important predatory ecological role as a regulator of other fish stocks. This resource constitutes a traditional fishery, but unfortunately it is unregulated. Overfishing and various anthropogenic changes on habitat quality and availability have contributed to the decline of the tropical gar populations in Tabasco (Alemán & Contreras, 1987).

Aquaculture production represents an option for conservation of aquatic organisms, but the information of the tropical gar is limited and requires research to improve captive handling and care. Another option for this species conservation is the development of management plans and regulations for its wild populations. According to Pérez and Páramo (1998) the gonadosomatic index is a macroscopic indicator of maturation in females, but for males is not the case. Likewise, Márquez, Contreras, Hernández and Hernández (2003) performed histological analyses of ovaries and testes, and found that females are previtellogenic in April, and by June they showed the highest number of vitellogenic oocytes, whereas males mature before females.

The reproductive cycle of *A. tropicus* has been described by various authors such as Reséndez and Salvadores (1983), Mora, Cabrera, and Galeano (1997), and Martínez (2007) based on females data. Nevertheless, a complete description of the reproductive cycle requires of complete anatomy and physiology studies of the reproductive organs in both sexes of wild populations. Besides, Le Gac and Loir (1999), Ravaglia and Maggese (2002) stated that it is essential to describe gametes development in males, which are important from an ecological, conservation and management point of view. According to Méndez-Marín, Hernández-Franyuti, Álvarez-González, Contreras-Sánchez and Uribe-Aranzábal (2012) understanding gamete development in both sexes is required to define both the reproductive behavior and seasonality.

The purpose of this project was to characterize testicular germ and somatic structures, and to identify the reproductive cycle of *A. tropicus* males. This is important for the understanding of the reproductive processes of the tropical gar, and to contribute with basic information for future research in aquaculture and environmental sciences, to support management plans and conservation models.

MATERIAL AND METHODS

Collection and biometrics: Three sexually mature *A. tropicus* males were collected monthly with a minimum size of 36 cm standard length (Lp), which was reported as an initial maturation size by Reséndez and Salvadores (1983), from a wild population in Laguna de Pomposú, municipality of Jalpa de Méndez (18°19' N - 93°01'12" W), Tabasco, México. The study was conducted from October 2009 to September 2010. Fish were collected with a 50 m by 2.5 m trawl net with and 3 cm mesh size, performing three repetitions in three random points of the lagoon. Fish were transported alive in plastic containers to the Tropical Aquaculture Laboratory, DACBiol, UJAT.

By prolonged immersion in tricaine methane sulfonate (MS222) the specimens were killed in accordance with the Manual Handling of Animals for Experiments and Education of Falconi et al. (2010). Fish were weighed (W_T), measured for standard length (sL) and gonads were removed and weighed (W_G), after a ventral incision. Sex was identified by gross inspection of the gonads (Ferrara & Irwin, 2001).

Sample processing: Three cm thick sections of the anterior, middle and posterior regions of both testes in all fish were fixed in 10 % neutral buffered formalin and Bouin´s solution. Samples were dehydrated in ethyl alcohol and xylol for one hour, and were embedded in paraffin. A microtome Reichert-Jung (model Hn40) was used to make 7 µm thick sections that were stained with hematoxylin-eosin (Humason, 1979; Hinton, 1990; Aguilar-Morales, Coutiño-Bello, and Salinas-Rosales, 1996). The morphological measurements were obtained with the aid of a microscope Zeiss (model Axiostar plus) coupled with a camera Zeiss (model Axiocam MRc 5), and the computer program for morphometric measurements AC AxioVision Release 4.5.

Examination of the testes: In order to characterize morphological changes, testicular germ and somatic structures for each fish, the diameter of 20 seminiferous tubules (Dst) and the height of the germinal epithelium (Hge) were measured, and the gonadosomatic index GSI = WG/WT(100) and the gonad volume gV = 4/3 πa2.b (Vazzoler, 1996; Hernández, 2003) were calculated. The identification of germ cells features was based on Wallace and Selman (1989) and Uribe, Grier and Mejía-Roa (2014).

Monthly values of Dst, Lp, GSI, gV were compared using one-way analysis of variance and Tukey's post hoc tests, when applicable, at p=0.005, using the Statistica Release 8 and SigmaPlot 11 statistical packages for Windows. Correlation analyses between sL and GSI; and gV and GSI were also performed.

RESULTS

Testes structures: A total of 24 males with an average sL = 48.17 cm (Table 1) were collected. The testis are paired and oval-shaped, the right one is lightly more cephalic than the left one, they are located in the abdominal cavity, surrounded by the tunica albuginea (Fig. 1 A). They consist of ducts or tubules, arranged in a radial system that originates the main duct (proximal area) and the secondary duct (middle area), towards the periphery of the testis (distal area), comprising a network of tubules that are ramified and are intercommunicated or anastomosed (tertiary duct) (Fig. 1 B, Fig. 1C, Fig. 1D). The testes have germinal epithelium composed of two compartments: interstitial, formed by the connective tissue, blood vessels and Leydig cells, a thin peritubular cover of myoid cells and collagenous fibers surrounding the tubules; and germinal, formed by the tubules comprising germ cells (spermatogonia, primary and secondary spermatocytes and spermatozoa) and somatic cells (Sertoli) lying on a basement membrane (Fig. 2 A, Fig. 2B). In the germinal compartment the spermatogenesis is generated.

Testes development: *A. tropicus* testes are cystic type, since during the development of spermatogenesis the Sertoli cells that surround the germ cells forming cysts. The cysts are distributed throughout all the tubule walls. This distribution is characterized as a non-restricted spermatogenesis of the anastomosed tubular type (Fig. 2 A, Fig. 2B). It shows a permanent germinal epithelium continuous or discontinuous depending on the reproductive condition, composed at all times of diploid spermatogonia, and which may divide mitotically that differentiate into spermatozoa (Table 2).

Reproductive classes: The reproductive stages of the spermatogenesis (spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa) are a commonly used indicator for depicting reproductive cycles

TABLE 1
Mean values and standard deviation of sL, WT, GSI, gV, Dst, of A. tropicus males October 2009 - September 2010

Months	n	sL X±SD	WT X±SD	GSI X±SD	gV X±SD	Dst X±SD
Oct	20	46.3±5.78	527.6±215	1.52±071	9 177.54±36	409.6±9.83
Dec	20	49.0	714.0	1.2	14 567.7	263.2
Jan	20	46.73±2.74	519.66±92	0.24±0.13	1 601.08±57	132.7±25.7
Feb	20	45.333±1.2	532±78.56	0.24±0.11	1 590.85±84	160.1±40.8
Mar	20	55.0	676.0	1.0	11 780.0	216.7
May	20	47.55±5.63	539.6±204	1.2±0.85	6 033.69±64	187.2±24.3
Jun	20	48±8.49	622±395.9	1.8±2.27	13 619.13±19	208.3±40.8
Jul	20	47.5	422.0	2.3	11 807.3	209.3

n=number of specimens, **sL**= standard length, **WT**= total Weight, **GSI**= gonadosomatic index, **gV**= volume of gonads, **Dst**= tubule diameter.

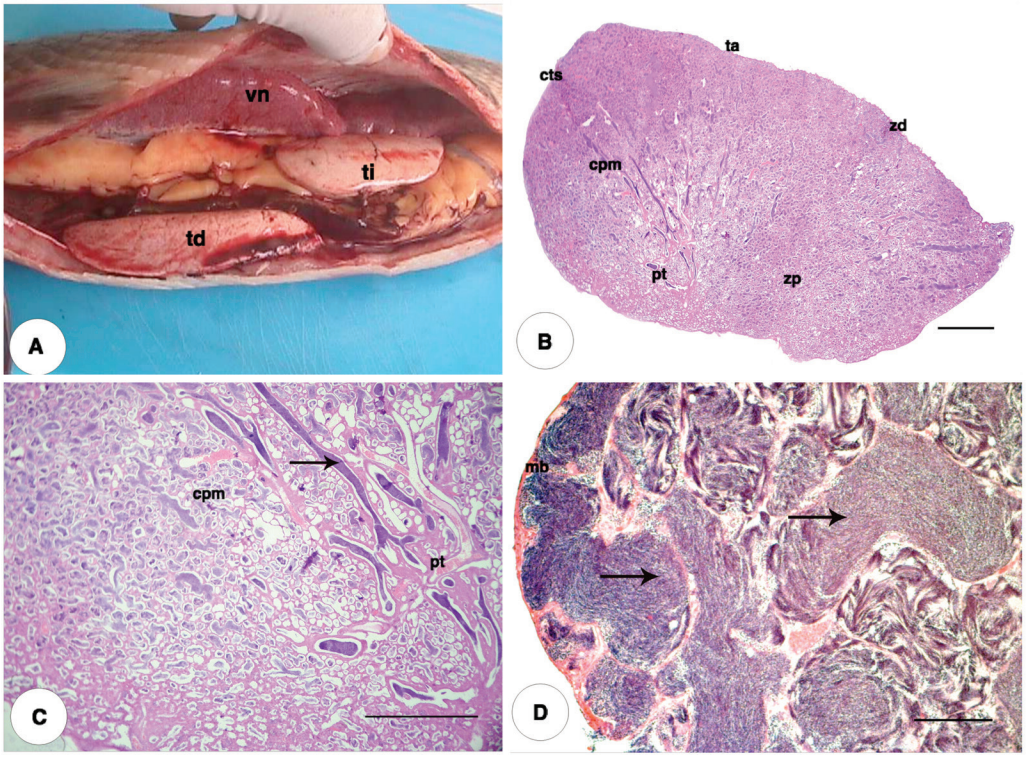

Fig. 1. A. Testes of A. tropicus in the celomic cavity; **B.** Longitudinal section of a testis (bar= 50µm); **C.** Detail of tubul of the proximal region (bar= 50µm); **D.** Anastomosed tubes (bar= 20µm): right testis (td), left testis (ti), air bladder (vn), principal tubules (pt), proximal region (zp), distal region (zd), primary tubules (cpm), secundary tubules (cts), tunica albuginea (ta), spermatozoa (→), basement membrane (mb).

TABLE 2
Average diameter and standard deviation of Dst and Hge for the reproductive classes of A. tropicus, October 2009-September 2010

Class	n	Dst (µm) X ± SD	Hge (µm) X ± SD
I	20	130.101±9.32	15.344±0.74
II	20	146.427±5.56	34.81± 1.28
III proximal	20	241.036±24.55	18.961±0.98
III distal continuous	20	287.92±37.95	24.724±1.34
III distal discontinuos	20	272.725±24.27	23.067±25.72
IV	20	327.867±20.13	6.678±0.32
V proximal	20	112.694±6.12	18.917±1.20
V distal	20	120.619±9.51	8.587±0.41

n=number of specimens, **Dst**= tubule diameter, **Hge**= height of germinal epithelium, **µm**= micron.

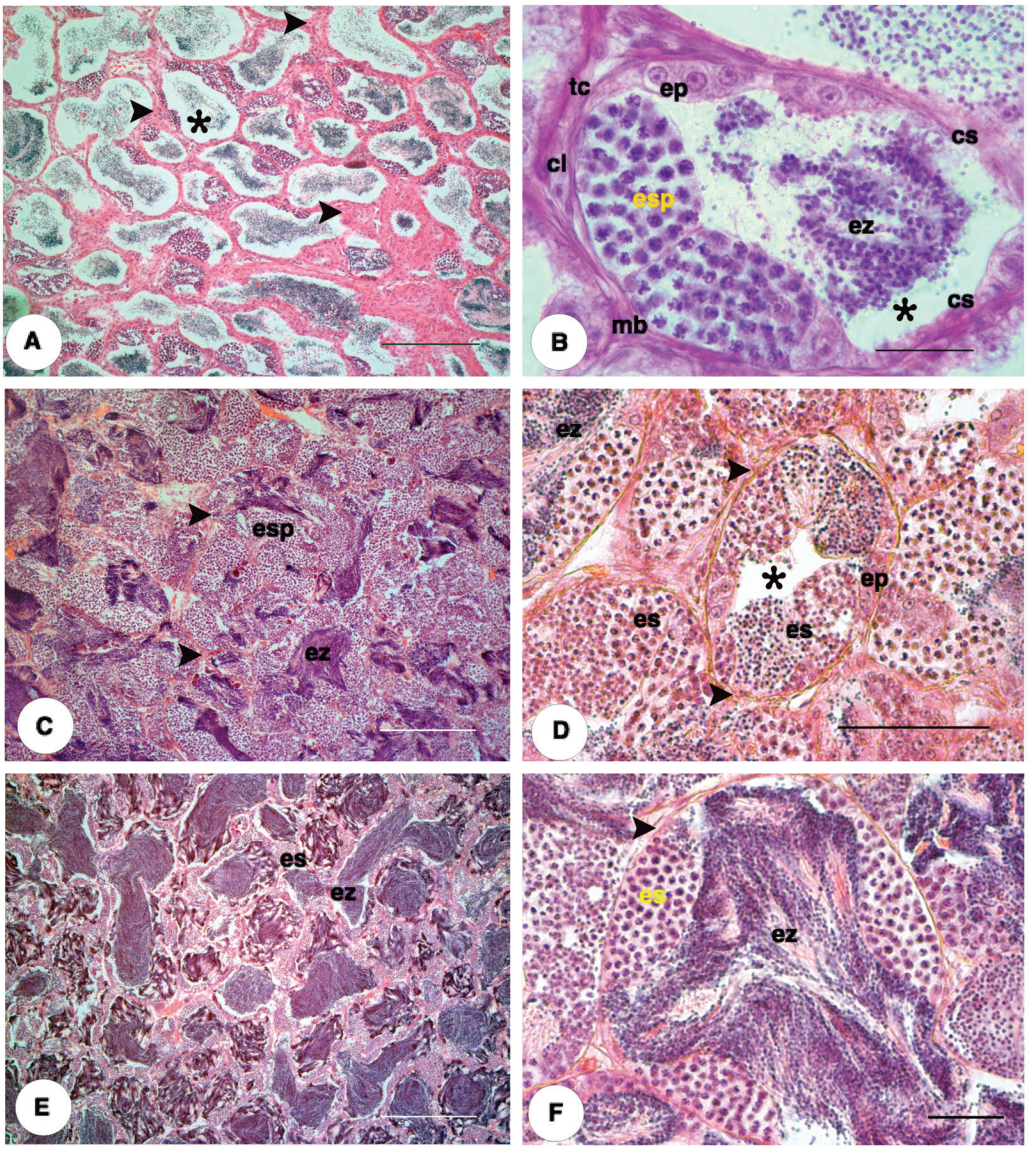

Fig. 2. A. CI. Recrudescence (bar= 100 µm) **B)** CI. Tubules in recrudescence (bar= 5 µm), **C)** C II Early maturation (bar= 50 µm), **D)** CII. Tubules with continuous germinal epithelium (bar= 5 µm), **E)** C III. Mid maturation (barra= 50 µm), **F)** C III. Tubules with continuous germinal epithelium, and discontinuous (bar= 5 µm): lumen (*), connective tisuue (tc), interstitial tissue (►), spermatogonia (ep), spermatozoa (ez), tunica albuginea (ta), principal tubules (pt), primary spermatocytes (esp), secundary espermatocysts (es), spermatids (et), Leydig cells (cl), Sertoli cells (cs).

(Grier & Taylor, 1998; Wallace & Selman 1989; Uribe et al. 2014). Nevertheless, histological and morphometric analysis of the germinal epithelium during annual seasonal have been used to document the reproductive condition. During the reproductive cycle of *A. tropicus*, the changes taking place allowed the definition of five reproductive classes:

Class I Regressed (Dst = 130.101±9.324 µm): The germinal epithelium activity occurs when spermatogonia are divided by mitosis. The testis tubules show regeneration

of the germinal epithelium (15.344±0.747 µm), due to the activity of the spermatogonia that are grouped in cysts delimited by Sertoli cells, of acidophilic coloration; they have round large nucleus and nucleoli, (103.309 ±9.836 µm). The interstitial tissue surrounding each tubule has a fibrous and thick appearance with Leydig cells (Fig. 2 A, Fig. 2B; Table 2).

Class II Early Maturation (146.427±5.694 µm): Tubules in the distal, middle and proximal areas show a continuous germinal epithelium, which is 34.81 µm in height, ±1.281 consisting of germ cysts in all stages of development (spermatogonia, primary and secondary spermatocytes and spermatids). They are distributed without interruption throughout all testis ducts. The main duct shows spermatozoa, indicating the beginning of early spermiation, few Leydig cells can be observed (Fig. 2C, Fig. 2D; Table 2).

Class III Mid Maturation: Tubules and the main duct can be observed with spermatozoa in the lumen to the increase of spermiation. Towards the distal area, the tertiary ducts show a continuous or discontinuous germinal epithelium; the continuous germinal epithelium (24.724±1.340 µm) shows a maximum spermatogenic activity with primary, secondary spermatocytes and spermatids; the discontinuous (23.067±25.724 µm) shows lower spermatogenic activity, but greater spermiation, finding abundant spermatozoa filling the tubules, with scarce spermatogonia. In the proximal area, the epithelium is discontinuous and thin (18.96±0.987 µm), consisting of scarce primary spermatocytes and spermatogonia. The interstitial compartment does not show significant changes (Fig. 2E, Fig. 2F; Table 2).

Class IV Late Maturation (327.867±20.132 µm): The spermatogenic activity decreases significantly, showing tubules with reduced germinal epithelium (6.678±0.326 µm) with the maximum amount of spermatozoa in the lumen, and significant increasing of the tubules diameter. The epithelium consists of Sertoli cells and scarce spermatogonia, condition that

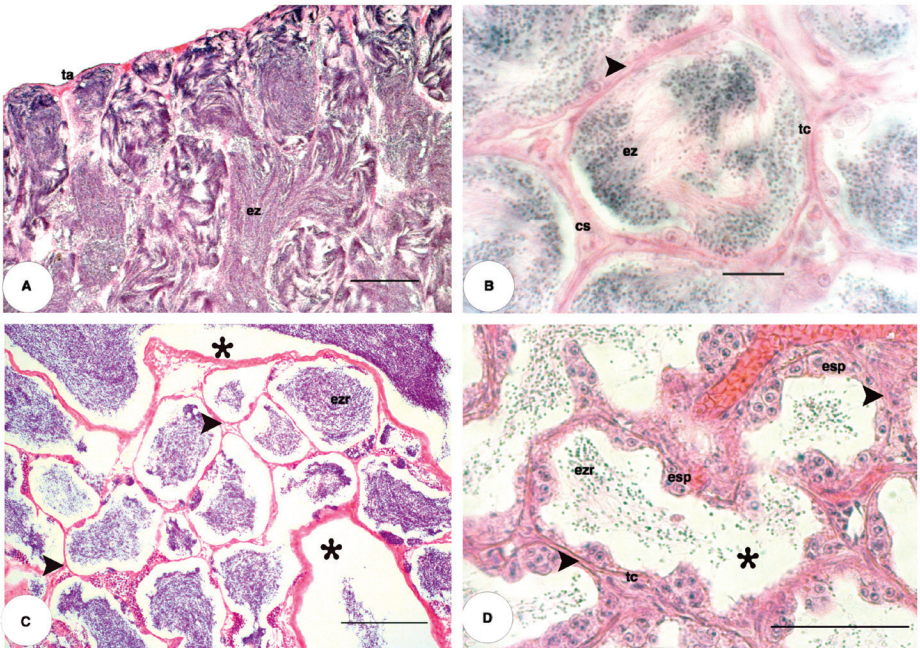

Fig. 3. A. Class IV. Late maturation (barra= 20 µm); **B.** C IV. Tubules with discontinuous germinal epithelium (bar= 5 µm); **C.** C V. Regression (barra= 100 µm); **D.** C V. Discontinuous germinal epithelium (bar= 10 µm): *lumen* (*), connective tisuue (tc), interstitial tissue (►), spermatogonia (ep), residual spermatozoa (ezr), tunica albuginea (ta), primary spermatocytes (esp), Sertoli cells (cs).

is observed throughout the whole tubul. The interstitial compartment is observed scarce and thin (Fig. 3 A, Fig. 2B; Table 2).

Class V Regression: After completing spermiation, the testis shows a reduction in the tubule diameter; towards the distal area shows remaining spermatozoa and a discontinuous and thin germinal epithelium (8.587±0.419 µm). The germinal epithelium consists of Sertoli cells and scarce spermatogonia. The proximal area increases the germinal epithelium height (18.917±1.207 µm) due to its regeneration, observing spermatogonia, cysts and agglomerations of residual spermatozoa. The interstitial compartment shows a fibrous and thick appearance, consisting of Leydig cells, blood cells and connective fibrous tissue (Fig. 3 C, Fig. 3D; Table 2).

Reproductive cycles (indicators and morphology): *A. tropicus* males showed an annual seasonal reproductive cycle, this condition was based on the monthly variation pattern observed on the gonad volume (gV), gonadosomatic index (GSI), and diameter seminiferous tubules (Dst). Besides, the indicators of the reproductive classes (Fig. 4, Fig. 5) were revealed by the changes in the morphology and morphometry of somatic and germ cells of the germinal epithelium. From January to February, the species showed the lowest values of gV (1 601.08±57), GSI (0.24±0.13), and Dst (132.7±25.7), observing empty lumen with discontinuous germinal epithelium in regeneration, the reabsorption of the residual spermatozoa, and the restart of the spermatogenesis, that occurred fast and in a short time. Therefore, March recorded a significant increase of the gV (1 1780), GSI (1) and Dst (216.7) indicators, explained by the increase of spermiogenesis and the reduction of spermatogenesis. The lack of data from August to September can be explained by the overfishing during the maximum reproductive activity.

The association between gV and GSI shown by the linear regression indicates that there is a significant association between these variables (r=0.88), providing the regression model: gV=334.057+6490.613*GSI ($p<0.005$); where an increase of gV leads to an increase in GSI. On the other hand, There is no significant association between sL and GSI (r= 0.21; Fig. 6).

DISCUSSION

A. tropicus is an organism with characteristics that place it as a link between more evolved

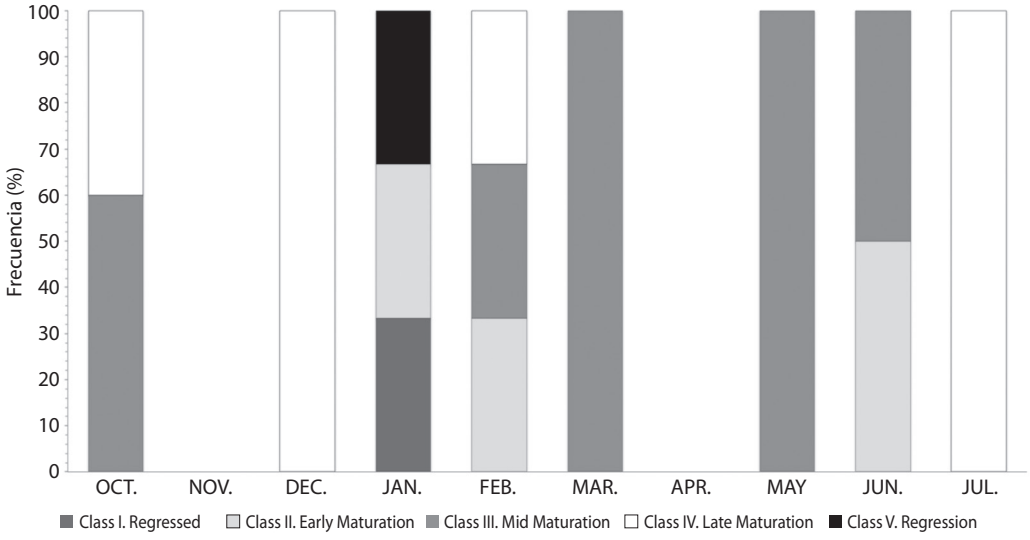

Fig. 4. Classes frequency of the reproductive cycle of A. tropicus males, October 2009-September 2010.

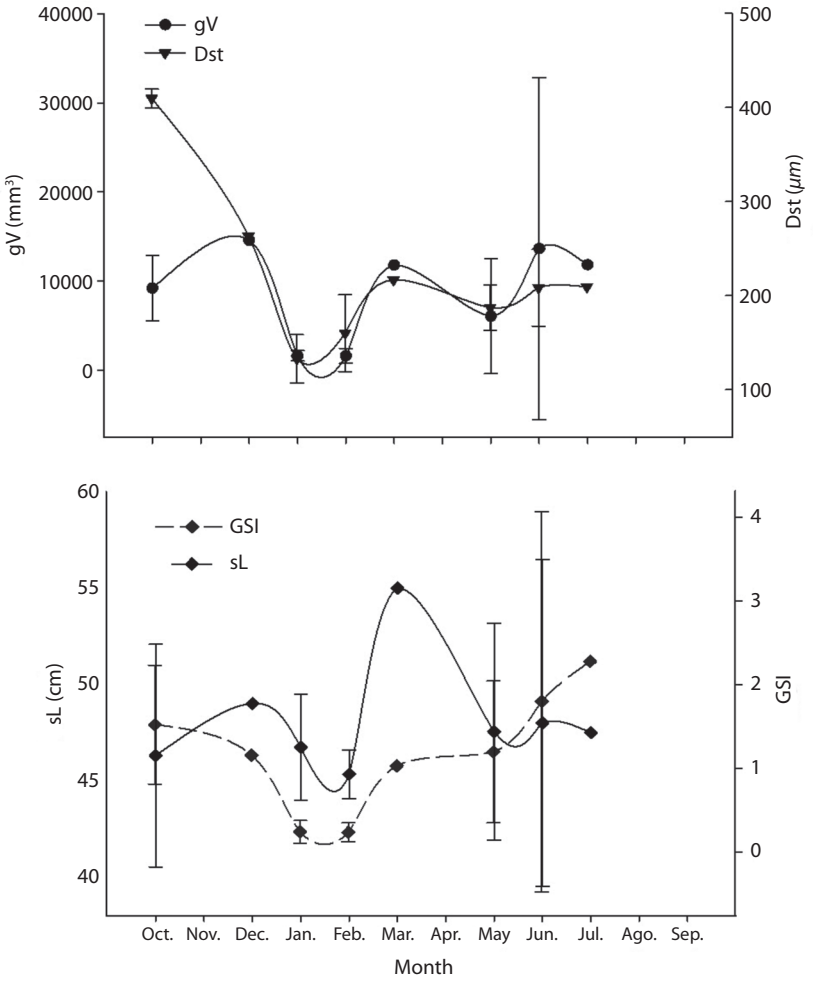

Fig. 5. Monthly variation in the gonadal volume "gV" and diameter of the tubules "Dst", gonadosomatic index "GSI" and standard length "sL" of *A. tropicus*, october 2009-september 2010.

and less evolved fish. Its external morphology, heterocercal fins, type of scale and its highly vascularized swim bladder, place it as a primitive fish (Páramo, 1982). Méndez-Marin and others (2012) stated that *A. tropicus* females show diverse reproductive characteristics, such as cyst ovaries, a condition observed in the Actinopterygii (Parenti & Grier, 2004). The analysis of morphological and physiological characteristics of the tropical gar testes structures suggests the presence of a permanent germinal epithelium. Therefore, in the chordate, a permanent germinal epithelium first appears in Holostei (Lepisosteiformes) reported by Grier and Taylor (1998) in *Centropomus undecimalis*. This is the first study that identifies this structure; suggested because the germ cells are always present, distributed discontinuously in the tubules throughout the months.

The arrangement of a germinal compartment is explained by its integration with germ and Sertoli cells, and the separation of the germinal and interstitial compartment, both separated by the basement membrane. These arrangements explain the evolution of the tubular and lobular testis (Grier, 1992, 1993; Parenti

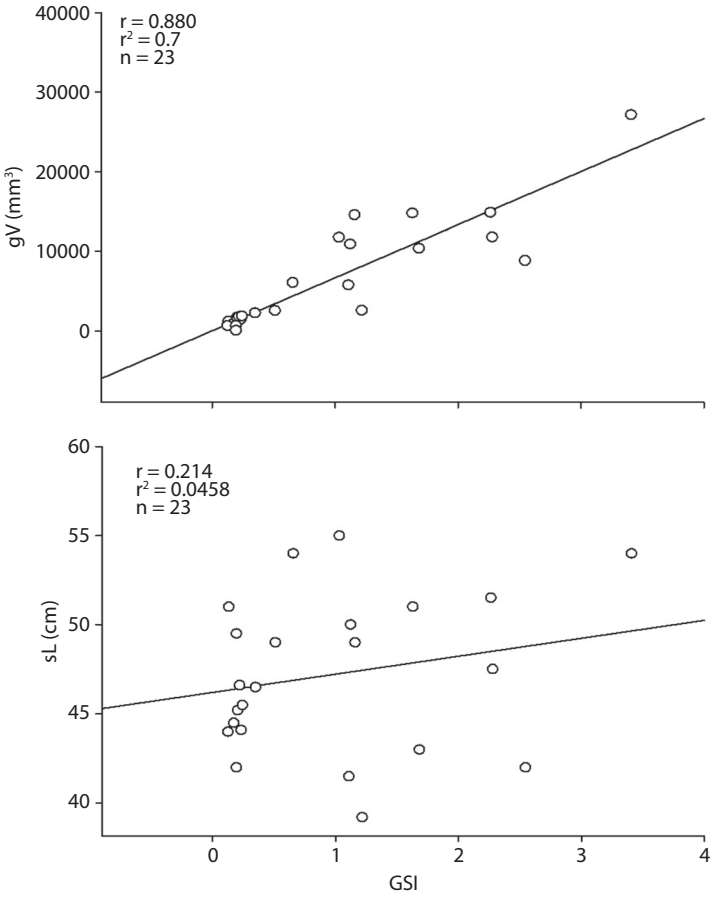

Fig. 6. A. Relationship between gonadal volume (gV) and gonadosomatic index (GSI); B. relationship between standard lengths (sL) and gonadosomatic index (GSI) of A. tropicus, October 2009-September 2010.

& Grier, 2004; Uribe et al., 2010). This condition is present in *A. tropicus* where testis comprises a network of tubules anastomosed with a germinal and interstitial compartment throughout its walls. However, spermatogenesis is not restricted to the periphery of the testis as occurs in atheriniform fishes, but spermatogonia are long the length of tubules (Uribe et al., 2014). It should be noted that restricted spermatogenesis is the evolution of the Sertoli cell/spermatid association, resulting in the formation of spermatozeugmata or spermatophores present in *C. undecimalis*, *Melanorivulus aff. punctatus*, *Poecilia latipinna*, *Goodea atripinnis*, *Ilyodon whitei*, and *Cichlasoma dimeru* (Grier, Linton, Leatherland, & Vlaming, 1980; Pudney, 1995; Grier & Taylor, 1998; Uribe et al. 2010; Rey, Da Cuña, Meijide, & Guerrero, 2012; Cassel, Mahmoud, Lúcia, & Adelina, 2013).

Spermatogenesis in *A. tropicus* is a dynamic process in which spermatogonia go through several development stages to form spermatozoa. The testicular morphological changes have originated the five classes of the reproductive cycle. When contrasting it to other teleosts, they show some similarity but with particular arrangements in spite of the taxonomic differences, *Morone saxatilis*, *Oxyeleotris marmoratus*, *Brycon henni*, *C. undecimalis*, *C. dimerus*, *Paralabrax maculatofasciatus* (Sullivan, Berlinsk, & Hodson, 1997; Grier & Taylor, 1998; Estrada & Uribe,

2002; Suwanjarat, Amornsakun, Thongboon, & Boonyoung, 2005; Montoya-López, Tabares, Echeverri, & Olivera-Ángel, 2006; Uribe et al. 2014). Prats (2003) states in *Atractosteus tristoechus* that the spermatogonial cells distribution is towards the testis periphery, and as they mature the cysts are distributed to the inside (restricted testis), description that does not correspond to the *A. tropicus*.

Grier and Taylor (1998) created a classification of five classes, such as the ones that were described in this study: regressed (Class I) distribution of abundant spermatogonia cysts in the tubules; early maturation (Class II) beginning of spermatogenesis, cysts in a greater number and sizes; mid maturation (Class III) testis with non-synchronous spermatogenesis, distal tubules with maximum spermatogenic activity and main tubule with greater spermiation; late maturation (Class IV) abundant spermatozoa in the lumen, maximum spermiation activity and reduction of spermatogenesis; regression (Class V) empty tubular lumen and quick regeneration of germinal epithelium. According to Carrillo and Rodríguez (2001) tubules with permanent germinal epithelium, is only present in mammals, birds, reptiles, and amphibians. Grier (1993) pointed out that the evolution of the permanent germinal epithelium coincides with the evolution of a permanent testicular compartment of the anastomosed tubules, since during regression and recrudescence a quick regeneration of the spermatogonia can be observed forming growing cysts (Uribe et al.2014), features present in *A. tropicus*, which confirms the hypothesis that has a permanent germinal epithelium.

The histological description and measurements of the gV, GSI and Dst, suggest a reproductive cyclical seasonality, due to the monthly variation matter explained in the maturation and reproductive season processes. This pattern shows a similarity with the one pointed out for *C. undecimalis* and *Gasterosteus aculeatus* (Grier & Taylor, 1998; Sokolowska & Kulczykowska, 2006) where throughout a reproductive cycle there are maturation indicators for the reproductive organs, which point out the spawning season. It should be noted that the identification of each stage of the germ cells defines the maturation of each gamete; then, classes describe events that occur during the reproductive development in the whole testis (Grier & Taylor, 1998). From a seasonal cycle point of view, the spermatogenesis of *A. tropicus*, restarts after completing the reproductive season, when carrying out spermiogenesis the tubules show a reduction of its diameter, the germinal epithelium is discontinuous and very thin, observing scarce spermatogonia in proliferation.

Environmental factors such as temperature, precipitation, and salinity, regulate the reproductive physiology of fish, such as the development of oogenesis and spermatogenesis, which determine the spawning seasons and the settlement of the reproductive cycle (Sokolowska & Kulczykowska, 2006; Lucano-Ramírez, Ruiz-Ramírez, González-Sansón, & Ceballos-Vázquez, 2011; Costa & Daniel, 2012; Correa-Herrera & Jímenez-Segura, 2013). This condition explains the results found in this research, where the lowest values of the GSI, gV, Dst reproductive indicators from January to February, coincide with the lowest averages for the temperature in the region. From March to July all values increase, which suggests that spermatogenesis development increases during the months with highest environmental temperature. It should be noted that from August to September there was no capture, due to seasonal overfishing. According to Martinez (2007) and Méndez-Marin et al. (2012) the maximum reproductive activity of this species takes place from August to November, which explains the presence of males with abundant spermatozoa and with reactivation of spermatogenesis in October. This monthly variation pattern showed some similarity with that of females, which showed synchrony in the development of spermatogenesis and oogenesis at a population level. It should be noted that in February males in advanced maturation were found, which can be deemed as a reproductive strategy for this species, since in case that there are females which have not spawned during

the previous months with the highest reproductive activity, there will be available males to carry out the reproductive activity. The present work studied reproductive aspects of male *A. tropicus*, with the main objective to contribute with the necessary information for sustainable management of this species fishery.

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RESUMEN

Epitelio germinal permanente y ciclo reproductivo en machos de *Atractosteus tropicus* (Lepisosteiformes: Lepisosteidae) Tabasco, México. *A. tropicus* tiene un papel ecológico importante, como regulador de otras poblaciones de peces, en los cuerpos de agua de México, pero sus poblaciones silvestres se reducen. Una alternativa de conservación es el cultivo, el cual requiere caracterizar el ciclo reproductivo por medio del estudio de estructuras germinales y somáticas de los testículos, conocimientos que son básicos para formar modelos de aprovechamiento y conservación. Se capturaron mensualmente tres machos sexualmente maduros (N = 24), con un promedio de sL = 47.2 cm en Laguna de Pomposú, Jalpa de Méndez (18°19´59" N - 93°01´12" W), Tabasco, México, de octubre 2009 a septiembre 2010. La técnica de captura fue red de arrastre, se transportaron vivos al laboratorio de acuicultura tropical, DACBiol, UJAT. Los machos recolectados se sacrificaron con baños de inmersión en sobredosis de MS222, los testículos se procesaron para análisis histológico. Se determinó mensualmente el diámetro de 20 túbulos seminíferos (Dst), altura de epitelio germinal (Hge), índice gonadosomático (GSI) y volumen de gónada (gV). Características morfo-fisiológicas del testículo muestran que está constituido de una red de túbulos anastomosados con espermatogénesis quística no restringida, y un epitelio germinal permanente, de nuestro conocimiento es la primera vez que se reporta este tipo de epitelio en Holostei (Lepisosteiformes: Lepisosteidae). Se identificaron cinco clases reproductivas: Clase I Recrudescencia, Clase II Madurez temprana, Clase III Madurez intermedia, Clase IV Maduración tardía, Clase V Regresión, que al contrastarlo con el valor mensual de los indicadores sexuales "GSI, gV, Dst" muestra un patrón de variación; durante enero-febrero se presentan valores bajos, se observa un epitelio germinal discontinuo en regeneración; durante marzo se incrementa la proliferación de espermatogonias disminuyendo la espermatogénesis. Los machos de *A. tropicus* muestran una actividad reproductora estacional anual, explicado por las variaciones mensuales de los indicadores reproductores, donde la temperatura y la precipitación parecen tener un papel importante como factores que estimulan la actividad reproductora y por tanto la espermiación.

Palabras clave: *Atractosteus tropicus*, epitelio germinal, espermatogénesis, espermiación, testículos.

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