



REPRODUCTIVE BIOLOGY OF *Goodea atripinnis* (JORDAN, 1880)
(CYPRINODONTIFORMES: GOODEIDAE) UNDER CONTROLLED CONDITIONS

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ABSTRACT

The study was conducted to investigate the reproductive biology of *Goodea atripinnis* under controlled conditions in the Aguascalientes state. Fifty wild brooders were collected, acclimatized and reared to obtain two F1 that were cultured until reaching sexual maturation. Then, two males and one female were placed in 40-L aquariums maintained at an average water temperature of $24.4 \pm 0.37^\circ\text{C}$ under a 14 h light: 10 h dark photoperiod. The courtships description was obtained by observations and digital photographs. Furthermore, the gestation period, fertility, total weight (TW, g) and total length (TL, mm) of the both F1 offspring were recorded. Additionally, the TL and TW distributions, TL-TW relationship, sex ratio and size at first maturity were evaluated in each F1. During courtship, both brooders performed a quiver display before copulating. The females gave birth at eight and nine months of age. Females compared with males, reached a higher TW and TL. The TW-TL relationship differed significantly between the F1 sexes. The male: female sex ratio was 2.7:1.0 in the first F1 and 0.96:1.0 in the second one. The mature females of both F1 displayed an average of 24 offspring, with a mean TL of 15.4 ± 1.3 mm and TW of 0.040 ± 0.013 g. During their first year of life, the females gave birth twice with a 45-day interval. The sexual maturity size (L_{50}) of brooders averaged 42.0 mm for males and 47.9 mm for females. The results demonstrated a high reproductive potential in *G. atripinnis*.

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1 Introduction

The family Goodeidae includes 16 genera and 41 viviparous species distributed over the central Mexico plateau (Domínguez-Domínguez et al., 2010). The pioneer studies on these species were focused on their general biology (Mendoza, 1962; Fitzsimons, 1972; Kingston, 1979). In Mexico, most research has focused on various viviparous fish such as *Girardinichthys* spp. (Díaz-Pardo & Ortiz-Jiménez, 1986; Macías-García & Saborío 2004; Navarrete-Salgado et al., 2007; Cruz-Gómez et al., 2010; Cruz-Gómez et al., 2011; Gómez-Márquez et al., 2013; Cruz-Gómez et al 2013), *Hubbsina turneri* (Moncayo-Estrada, 2012), and *Zoogoneticus quitzeoensis* (Ramírez-Herrejón et al., 2007).

Studies on *Xenotoca variata* (García-Ulloa et al., 2011) and *Skiffia multipunctata* (Kelley et al., 2005; Kelley et al., 2006) under culture conditions were performed to observe the reproductive behavior and recognize the effects of captivity conditions on this behavior. In Europe, viviparous fishes, such as *Ataeniobius toweri*, *Ameca splendens* and *Chapalichthys pardalis*, have been maintained under culture for study and preservation (Koldewey et al., 2013). The above-mentioned works, combined with histological descriptions, have improved the comprehension of reproductive events (Koya et al., 2003; Ortiz-Ordóñez et al., 2007; Uribe et al., 2005; Uribe et al., 2006; Uribe et al., 2010a; Uribe et al., 2010b; Uribe et al., 2011; Uribe et al., 2012; Uribe et al., 2014).

In Aguascalientes State, the previous studies have focused on the classification and distribution of native freshwater fishes (Fitzsimons, 1972). In 1981, 23 species belonging to 19 genera and eight families were reported (Rojas-Pinedo, 1981), but in 1996, only 18 species belonging to 16 genera and eight families were reported (Martínez-Martínez & Rojas-Pinedo, 2008). Recently, a study on the identification, distribution and genotypes of native ichthyofauna of Aguascalientes was conducted, and six native species were described, including *G. atripinnis* (Arroyo-Zúñiga, 2015).

Currently, there are few studies regarding *G. atripinnis*. This species is not included in the red list of threatened Mexican species but is one of the two species (together with *Poeciliopsis infans*) that has experienced drastic changes and environment modifications in recent years. Other viviparous fishes, such as *Allotoca dugesii* and *Xenotoca variata*, have probably been eliminated from the state (Martínez-Martínez & Rojas-Pinedo, 2008). The anthropogenic impacts on continental aquatic systems can be considered permanent in many cases and will soon be problematic from the standpoint of restoration. Thus, the performance of studies focused on the maintenance, preservation and rearing of native fish species for propagation and repopulation purposes is very important (García-Ulloa et al., 2011). The goal of the present research was to describe the reproductive biology of the viviparous fish *G. atripinnis* in captivity under controlled conditions of photoperiod and water temperature.

2 Materials and methods

2.1 Collection and quarantine of wild brooders

Wild brooders of *G. atripinnis* were collected on May 5, 2013, in Arroyo Viejo Agua Zarca in San José de Gracia Municipality (22°07'13.6''N, 102°30'19.6''W) in Aguascalientes State. No specific permissions were required for collection at this location, because this species it is considered as a least concern (LC) in The International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Snoeks et al., 2009) and this species is not listed in The Norm Official Mexicana number 059 of 2010, from the Secretaria del Medio Ambiente and Recursos Naturales (SEMARNAT) (D.O.D.L.F., 2010), and their populations are abundant in Aguascalientes State. The fish were captured using a fishing basket and net with a mesh size of 0.5 and 1.0 cm, respectively and these methods are not invasive and not involved endangered on native fish species. Fifty brooders exhibiting sexual dimorphism were selected and introduced into 40-L plastic bags containing water from the collection site and 100 g of iodine-free marine salt and 1 mL of anti-stress solution (Neutra Stress, Grupo Acuario, Mexico) per 10 L of water. Oxygen was injected as described by García-Ulloa (2011). The captured brooders were transported to the Aquaculture Unit (AU) in the Agricultural Science Center (ASC) at the Autonomous University of Aguascalientes in Jesus María Municipality, Mexico.

The *G. atripinnis* brooders were placed under quarantine conditions in two 60-L plastic containers with chloride-free water and constant aeration at room temperature. During the quarantine period, the fish were treated with antibacterial and antifungal substances to avoid infectious diseases. The fish were fed commercial food (Wardley Tropical Fish Flake Food, Wardley-Hartz Company, USA) containing 44% crude protein, 10% crude lipids and 2% crude fiber) daily at 9:00 and 14:00 h. The quarantine time ended when no infectious diseases were registered, and the brooders accepted balanced food.

2.2 Experimental conditions

After the quarantine period, 25 wild brooders were placed in each of two 200-L semicircular plastic containers. Each container was constantly aerated using a ¼-hp Sweetwater blower (Aquatic Ecosystem, FL, USA). The fish were fed to satiety daily at 9:00 and 14:00 h with the Wardley Tropical Flake Food (Wardley-Hartz Company, USA). Under these conditions, two F1 broods were obtained in July of 2013 from two different females; the first one included 26 and the second 56 offspring. The offspring composing each F1 brood were placed in 60-L plastic receptacles with continuous aeration at room temperature and fed twice daily; 50% of the water of each receptacle was changed every week with chloride-free water. Each fish was weighted monthly for ten months using a digital balance Precisa XT 220A, (Precisa Gravimetrics, USA) with a precision of 0.0001 g to obtain the total weight (TW in

g). At each weighting, the total length (TL in mm) of each fish was measured using a 6" Petrucci vernier caliper with a precision of 1 mm. Furthermore, the water temperature and dissolved oxygen (DO) level were monitored daily with a digital dissolved oxygen meter (model YSI 550A, Yellow Springs Instruments, USA). During the experimental period, the DO averaged 6.86 ± 0.98 mg/L, and the water temperature fluctuated from 18 to 21°C. Both F1 broods exhibited sexual morphometric characteristics at six months of age, when virgin males and females were selected, separated and placed in seventeen 40-L glass aquarium in groups of two males and one female per aquaria. Every aquarium was equipped with a ground filter and a 100-watt heater to maintain high DO levels and a water temperature of 24.0°C. A photoperiod of 14 h of light and 10 h of darkness was maintained during the experimental period throughout twelve months. The water temperature and DO in each aquarium were monitored daily. Every two weeks, the pH was registered with a digital pH meter (Waterproof pHTestr 20, Oakton Instrument, USA), and total alkalinity and hardness were checked with Aquacheck test strips (Hach Company, USA). The following conditions were maintained throughout the experiment: the water temperature at 24.4 ± 0.37 °C; DO at 6.5 ± 0.8 mg/L, pH at 8.6 ± 0.18 , total alkalinity at 192 ± 24 mg CaCO₃/L and total hardness at 88.5 ± 35 mg CaCO₃/L.

Photographs taken with a digital camera (Nikon Coolpix P600, Japan) were used for all the aquarium observations regarding the sexual courtship behavior of males and females as well as to describe the fin pigmentation changes in both sexes and the abdominal modifications that occurred in females after copulation. Moreover, the following data were recorded: the total number of offspring per female; the survival rate, TW and TL of the offspring; the TW and TL of each female before and after birth; the number of brooders produced by each female; and the number of days between birth events.

2.3 Biological parameters

Diagrams of the TL distribution were elaborated (Salgado-Ugarte et al., 2005). The TW-TL relationship was calculated

by paired-curve analysis to test for significant differences between males and females in each F1 brood. For discerning the growth of fish during the experiment, the following formula was employed (Ricker, 1975; Salgado-Ugarte et al., 2005):

$$TW = a TL^b.$$

Where TW and TL are the total weight and total length of the fish, respectively, and a and b are constants estimated by linear regression analysis.

The sex ratio was calculating using the total number of fish of each sex and dividing each of these two values by the total number of females or males, whichever was smaller. The value obtained was analyzed under the null hypothesis of a 1:1 ratio using a χ^2 test and 95% confidence level (Daniel, 2002). The batch fecundity was calculated as BF= number of young * 100/TL (Grier et al., 2005). The sexual maturity size (L_{50}) for males and females was estimated as the size at which 50% of the fish were sexually mature in every class frequency (Pratt & Otake, 1990). The graphs were elaborated with the software GraphPad Prism version 5.0 (GraphPad software Inc., USA).

3 Results

3.1 Courtship

Courtship behavior began one month after virgin brooders of *G. atripinnis* were introduced into the aquarium. In both sexes, a black coloration developed in the anal, dorsal and caudal fins. The male placed his head in front of a female and quivered his body awaiting a response. When the female quivered too, both brooders performed side-by-side synchronized swimming along the aquarium. Copulation most likely occurred at this time but was not clearly observed due to its short duration. After copulation, the black color was missing from the fins (Figure. 1).

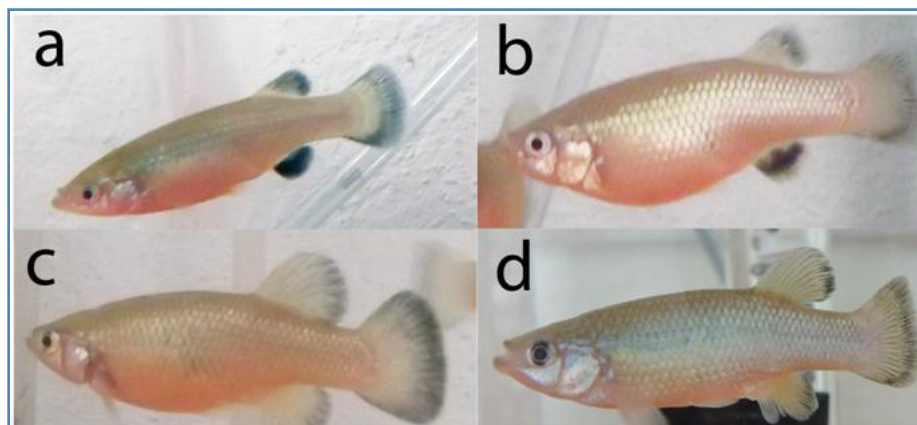


Figure 1 Fin pigmentation in brooders of *G. atripinnis* during courtship and aggression behaviors between congeners. (a) Female with pigmented fins before courtship, (b) female with unpigmented fins after courtship, (c) male with pigmented fins before courtship and (d) male with unpigmented fin after courtship.

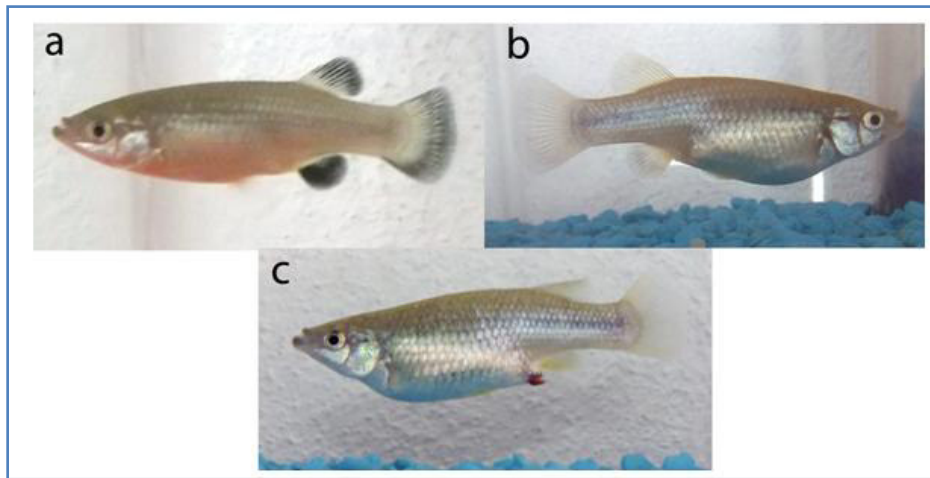


Figure 2 Characterization of abdominal morphology during gestational periods in a (a) female at the beginning of gestation, (b) female during the middle of gestation and (c) female at the end of gestation.

The males demonstrated territorial and aggressive behavior when two of them were introduced in the same aquaria. Commonly, the larger male attacked the smaller fish, causing lesions and provoking death ($n = 5$). In February and early March of 2014, the first gravid females were detected. Three states could be differentiated in the females: a) the beginning of gestation, when the females, some which conserved the black fins, lacked an obviously enlarged belly but evidenced abdominal morphological changes; b) the middle of gestation, when females did not exhibit black fins but displayed a large belly as a result of an increased egg size and the presence of embryos; and c) the end of gestation, when abdominal enlargement was advanced, and embryos could sometimes be observed moving inside the abdominal cavity (Figure. 2).

Gravid females initiated the liberation of offspring in the middle of March of 2014 at an age of eight months for the first F1 brood and nine months for the second F1 brood. The liberation was influenced by factors such as the maturation of embryos and the stress caused by the presence of males or a transfer from one aquarium to other.

3.2 TL and TW distributions

Three-month-old males and females of the first F1 brood reached an average TL of 37.2 ± 8.2 and 37.2 ± 4.9 mm, respectively. Two-month-old males and females of the second F1 brood reached a TL of 30.0 ± 2.3 mm and 32.2 ± 1.8 mm, respectively. The TW was 0.82 ± 0.26 g for males and 0.84 ± 0.30 g for females. For the second F1 brood, the TW reached 0.35 ± 0.08 for males and 0.41 ± 0.08 g for females.

At eight months, males and females of the first F1 brood showed a TL of 49.9 ± 2.1 mm and 56.6 ± 6.4 mm, respectively, and respective TWs of 2.11 ± 0.66 g and 3.01 ± 0.87 g. The males of the second F1 brood presented a TL of 48.5 ± 3.5 mm and a TW of 1.79 ± 0.33 g. The females of the second F1 brood exhibited a TL of 57.5 ± 3.8 mm and a TW of 3.27 ± 0.56 g. The Mann-Whitney U test indicated TL and TW significantly differences between the sexes in both the first ($P < 0.03$) and second ($P < 0.001$) F1 broods.

3.3 TW-TL relationship

For the first F1 brood, a significant difference was evident in the TW-TL relationship of males and females ($F=4.7$; $P < 0.05$); the growth equation was $TW = 0.00173TL^{2.9454}$ for males and $TW = 0.0175TL^{2.9443}$ for females. For the second F1 brood, the TW-TL relationship was $TW = 0.0095TL^{3.2958}$ for males and $TW = 0.0071TL^{3.4697}$ for females, with a significant difference between the sexes ($F= 32.11$, $P < 0.01$) (Figure. 3).

3.4 Sex ratio

Males of *G. atripinnis* exhibited a species-distinctive andropodium, which could be identified during the first two and half months of life. Of the 26 fish in the first F1 brood, 19 (73%) were males and seven (26%) were females, with a sex ratio of 2.7 males: 1 female; a χ^2 test indicated significant deviation from a 1:1 sex ratio ($\chi^2 = 2.7$, $P < 0.05$). The fish of the second F1 brood comprised 53 organisms: 26 (49%) were males, 27 (51%) were females and the resulting sex ratio was 0.96:1, respectively, which was not significantly different from a 1:1 ratio ($\chi^2 = 0.009$, $P > 0.05$).

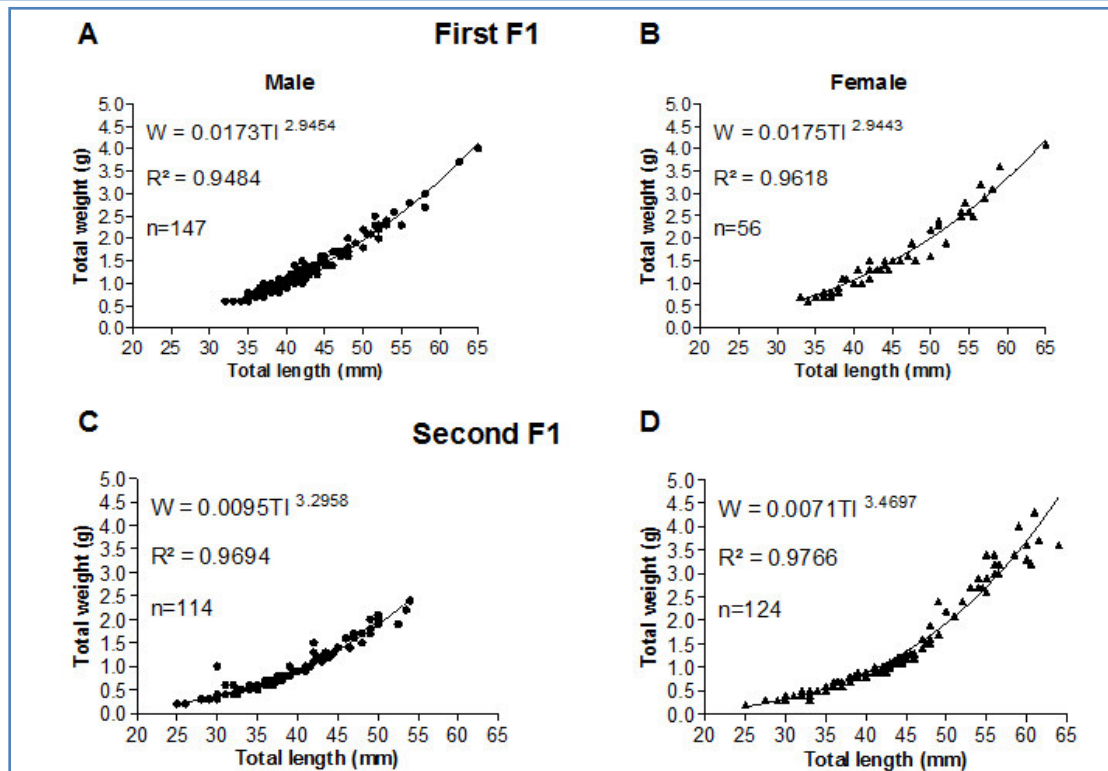


Figure 3 Total weight-total length relationship for male and female *G. atripinnis* of both F1 broods. Negative allometric development shown by (A) males and (B) females of the first F1 brood and positive allometric development shown by (C) males and (D) females of the second F1 brood.

Table 1 Fertility and size characteristics of *G. atripinnis* females.

Female number	Aquarium number	Number of offspring	Offspring survival (%)	TW of offspring (g)	TL of offspring (mm)	TL of females (mm)	TW *(g)	TW **(g)
1	2	25	100	0.044	15.9	56	3.4	2.4
2	2	18	100	0.039	15.6	61	4.3	2.8
3	3	35	100	0.046	16.4	62	5.3	4.0
4	3	15	87	0.033	17.7	70	6	4.9
5	4	38	100	0.037	14.9	61	4.45	3.3
6	5	28	96	0.030	14.7	63	4.9	3.4
7	6	26	100	0.058	14.9	61	4.3	3.2
8	6	33	100	0.027	14.1	62	5.2	4.2
9	6	45	100	0.033	14.4	72	7.7	5.7
10	9	11	100	0.064	16.9	60	3.7	3.1
11	10	14	93	0.062	18.1	58	3.5	2.7
12	11	33	97	0.025	12.9	56	3.4	2.5
13	11	29	97	0.036	15.2	64	4.4	3.8
14	12	18	100	0.061	17.5	60	4.9	3.0
15	15	15	100	0.053	16.5	56	3.4	2.6
16	15	18	28	0.020	13.1	60	3.6	3.0
17	16	25	100	0.032	13.6	58	3.7	2.8
18	16	11	55	0.033	14.4	60	5.2	3.3
19	17	25	100	0.040	15.9	61	4.6	3.4
Total		462						
Average		24.31	92.26	0.040	15.40	61.10	4.52	3.37
SD		9.64	18.74	0.013	1.51	4.17	1.08	0.84

SD = Standard deviation, * before giving birth, ** after giving birth.

3.5 Fecundity

The first birth in the second F1 brood was recorded at a parental age of eight months. The average number of offspring per female was of 24.32 ± 9.64 . At birth, the offspring measured 15.4 ± 1.3 mm for TL and 0.040 ± 0.013 g for TW. During the birth, the offspring survival rate was $92\% \pm 19\%$. The TL of the pregnant females ranged from 46 to 72 mm. The females in gestation had an average TL of 61.11 ± 4.18 mm. An average weight loss of 1.15 ± 0.25 g occurred each time a female gave birth (Table 1).

3.6 Gestational period

The females of *G. atripinnis* exhibited an average of two births during their first year, with a range of one to four births. The period between the first and second births averaged 45 days (± 11.2) (Table 2).

3.7 First maturation size

The L_{50} for the TL of males and females of the first F1 brood was 42.8 and 48.9 mm, respectively, whereas in the second F1 brood, this value was 41.3 mm in males and 47.4 mm in females. The average TLs obtained for each sex indicated a

first maturation size of 42 mm for males and 47.9 mm for females (Figure. 4).

4 Discussions

4.1 Courtship

The presence of two males in each aquarium provoked an increase in aggression and competence by the female. The males situated their heads on the caudal fin of their competitor, shifted in circles and nibbled. This behavior has been described in some fishes of the family Goodeidae, with large males displacing small ones through a brief persecution, after which the fighting stops (Kingston, 1979).

The introduction of two males for each female is recommended in *G. multiradiatus* because the presence of one male is insufficient to guarantee female fecundation (Macías-García, 1994). In *G. atripinnis*, suggestions have been made that pairing only one male and female of equal size is adequate to assure courtship and fecundation. The selection of males by females has been observed in *G. multiradiatus*; the presence of males 75% of the female size inhibits courtship. The use of two males and one female is also recommended to stimulate courtship because in some viviparous species, females select the male for copulation (Kingston, 1979; Kelley et al, 2005).

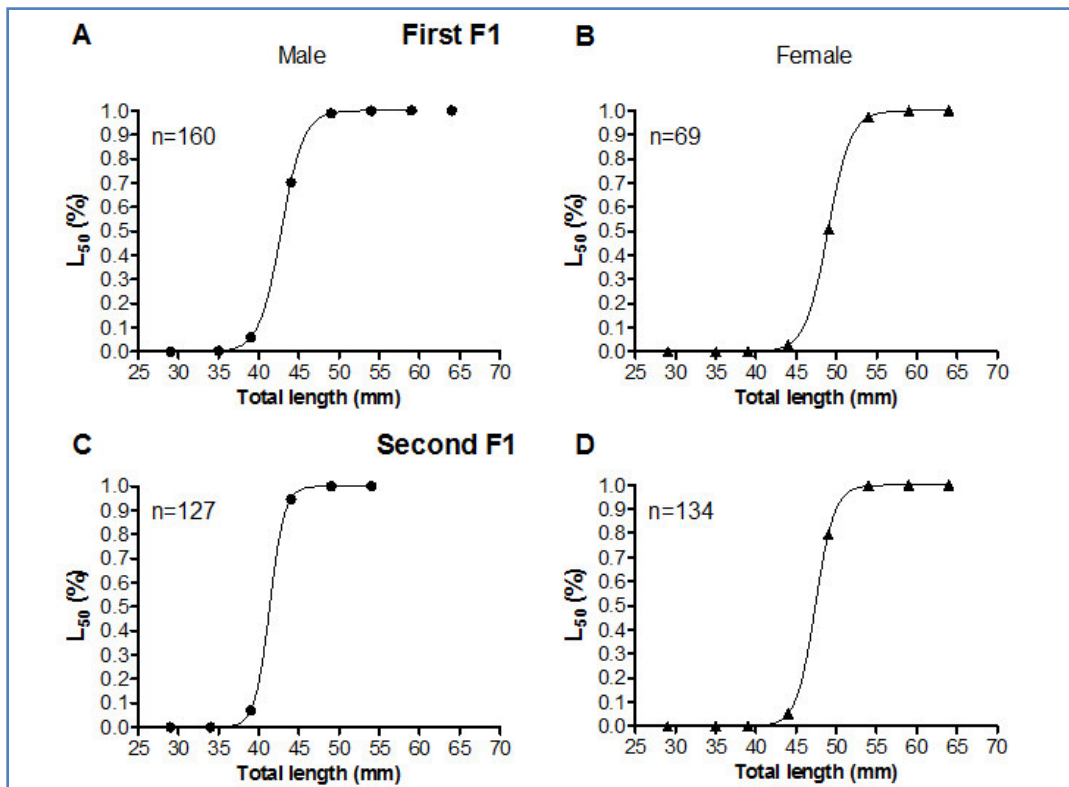


Figure 4 The size of the first state of maturation in males and females of *G. atripinnis* for the first and second F1 broods. A - Males of the first F1 brood matured at a TL of 42.8 mm, whereas. B - females of the first F1 brood matured at a TL of 48.9 mm. In the second F1 brood, (C) males matured at a TL of 41.3 mm, whereas (D) females attained maturity at a TL of 47 mm.

Table 2 Birth numbers, parturition dates and inter-birth periods for *G. atripinnis* females.

Aquarium	Number of births	1st birth	2nd birth	Days between births
2	2	04/28/2014	06/05/2014	38
3	3	03/30/2014	05/17/2014	48
4	2	03/13/2014	05/14/2014	52
5	2	05/04/2014	07/15/2014	72
6	4	03/11/2014	04/19/2014	39
9	2	04/14/2014	05/15/2014	31
11	2	03/21/2014	05/06/2014	46
12	2	03/30/2014	05/22/2014	53
15	2	03/17/2014	04/21/2014	35
16	3	03/14/2014	05/04/2014	51
17	4	03/20/2014	05/02/2014	43
Mean				46.1
SD				11.2

SD = Standard deviation.

The table only includes the females with two or more parturitions.

In *G. atripinnis*, three reproductive phases viz a) orientation, b) display and c) copulation have been reported. The first phase is subdivided into watching and following. The sexual behavior perceived in the present study was similar to that observed previously (Nelson, 1975); the male positioned himself in front of the female displaying his rigid head toward the aquaria wall and tilting his tail toward the female. Once the female retained a static position, the male entered a display phase and initiated body quivering in an S form, keeping the same position as that assumed in the observation phase. Sometimes, the female displayed quiver movements, indicating her receptivity. Such movements are similar to those reported in other viviparous fishes and are named conduct quiver behavior because the fishes vibrate their entire body rapidly in small amplitudes in an S or C shape (Kingston, 1979). Of the five displays mentioned, the most commonly observed in the present study was a sigmoid display with head tilting. However, the body of males was generally positioned with the head pointed toward the tail of the female or close to the aquaria wall, or vice versa. After displaying, the male approached the female keeping a head-to-head position to realize a synchronic swing, finally moving his body toward the caudal fin of the female. In the present study, it was not possible to observe copulation. However, this event is reportedly of short duration in certain species, lasting two to five seconds in *G. atripinnis* and approximately 0.6 to 1.7 seconds in *Xenotoca eiseni* (Greven & Brenner, 2010). The synchronization of the male and female is necessary for a successful copulation because the andropodium is not an intromittent organ and male must concealment female for copulation (Nelson, 1975).

Females of *G. atripinnis* can be classified as receptive and non receptive (Nelson, 1975; Greven & Brenner, 2010). When females were receptive during the present study, it was necessary to reintroduce a male after each birth. This condition has also been reported in *Allophorus robustus* and *Neophorus diazi* in that each cohort requires a separate insemination

(Mendoza, 1962). This condition may occur in *G. atripinnis* because the Goodeidae family does not present superfetation, which is exclusive to the Poeciliidae family (Turner, 1933; Burns, 1985; Macías-García, 1994; Contreras Mac Beath & Ramírez-Espinoza, 1996; Gómez-Márquez et al., 2008; Uribe et al., 2010a) and has only been observed as an occasional event in *Girardinichthys viviparus* (Díaz-Pardo & Ortiz-Jiménez, 1986).

Females of *G. atripinnis* maintained under laboratory conditions are not carnivorous (Kingston, 1979). Some viviparous fish, such as *Ameca splendens* and *Atenobious toweri*, can live in colonies and are not predators. In contrast, *Chapalichthys pardalis* show cannibalistic behavior, and for this reason, the separation of gravid females before birth is recommended (Koldewey et al., 2013).

Females of *G. atripinnis* exhibited two types of breeding stimulation in the present experiment. First, the females gently bit offspring, provoking them to swim to the surface of the water column. Large females suctioned offspring with their mouth and then expelled them with force. In the second behavior, females used their caudal fin to move the water column and stimulate the offspring. In both behaviors, the females incited fingerlings to swim reducing the mortality rate with this action.

4.2 Distribution of TW-TL

The TW-TL relationship results obtained in the present experiment suggest that *G. atripinnis* may attain a TL of 56 mm by nine months of age, with a tendency to reach 70 mm within one year. *G. luitpodii* (*G. atripinnis*) reached a TL of 78 to 85 mm by one year of age (Mendoza, 1962), whereas *Allophorus robustus* presented a TL ranging between 60 and 90 mm (Casebolt et al., 1998; De Lapeyre et al., 2010). Under natural conditions in the epicontinental waters of Aguascalientes State, the TL of wild *G. atripinnis* males and

females measured from 100 to 110 mm, and similar results were reported for this species in Lake Patzcuaro in Michoacan State, where mature females reached a TL generally ranging from 90 to 110 mm but exceeding this size in exceptional cases (Mendoza, 1962).

Fishes of the family *Goodeidae* show sexual dimorphism (Díaz-Pardo & Ortiz-Jiménez, 1986). *G. atripinnis* exhibit two features that allow the recognition of sexual dimorphism: a) one is the modification of the anal fin in males to generating an andropodium that can be recognized as an anal fin divided in two portions (Uribe et al., 2010b), and b) the other feature is the difference in TL between the sexes, with adult females 20% longer compared with males (Kobelkowsky, 2005).

The fingerlings obtained in the present study showed similar morphological characteristics between the sexes during the first months of life. During the second and third months, sexual dimorphism characterized by andropodium formation and the manifestation of fin coloration changes began. These characters facilitated sex separation. The TL differences between the sexes began in the first F1 brood at six months of age and in the second one after five months of age. A rapid increase in female size provided greater longevity and better resistance to reproductive stress, assuring good development and health for future cohorts (Gómez-Márquez et al., 2013).

The Goodeidae family exhibits great size diversity. In small fish such as *Skiffia multipunctata*, the TL averaged 25 mm in males and 28 mm in females; in Mexico, the genus *Allotoca* includes seven species usually showing a TL from 34 to 54 mm. *Allotoca catarinae* reach a TL of 47 mm in males and 63 mm in females (Domínguez-Domínguez et al., 2005). Among the medium-sized fishes, *Girardinichthys viviparus* reach an average TL of 49 mm in males and 61 mm in females (Gómez-Márquez et al., 2013). In *G. multiradiatus*, males manifest a TL of 42 mm, and females reach 48 mm (Domínguez-Domínguez et al., 2005), with a maximum of 49 mm recorded in wild populations (Cruz-Gómez et al., 2011). Among the largest fishes, *Chapalichthys encaustus* can attain an average TL of 63 mm in males and 61 mm in females, whereas in *Allophorus robustus*, the males measure 102 mm, compared with 93 mm in females (Domínguez-Domínguez et al., 2005). *G. atripinnis* is considered a large species in the Lerma-Chapala-Santiago basin, with a TL ranging between 66 and 83 mm in males and females, which makes this species relevant for consumption. This species is consumed by the rural population in some Mexican states, such as Michoacan (Kelley et al., 2005; Colon et al., 2009).

A search of the mainstream scientific literature resulted in no data regarding the TW of this species; for this reason, a comparison with other species of the family Goodeidae was not possible. However, it is important and necessary to register these data to increase the understanding of this group of viviparous fishes.

4.3 TW-TL relationship

During the experiment, *G. atripinnis* showed a coefficient of “b” significantly different from three other species displaying allometric growth (Ricker, 1975; Salgado-Ugarte et al., 2005). The organisms of the first F1 brood presented a “b” value below three, revealing negative allometric growth, which indicates a higher than proportionate increase in TL with increasing TW. The second F1 brood showed a value exceeding three and indicating positive allometric growth and a proportionately greater increase in TW with increases in TL (Salgado-Ugarte et al., 2005). Although both F1 broods were cultured under the same conditions, other factors might have influenced the growth rate. Some factors affecting growth rate in fishes have been divided into the following categories: a) intrinsic factors such as genetics, physiology, maturation stage, health state and behavior and b) extrinsic factors such as water temperature, accessibility of food, dissolved oxygen concentration and the presence of toxic metabolites (Hepher & Pruginin, 1985).

High variability has been observed for “b” estimation among different populations of the same species, probably due to TL variations, the procedure used to measure this variable, and the nutritional condition of the population (Ricker, 1975; Frota et al., 2004). In *Hubbsina turneri*, the TW-TL relationships demonstrated a wide dispersion of data for large fish, which was most likely associated with the reproductive season (Moncayo-Estrada, 2012). A similar case was reported in *Amecca splendens*, for which differences in the TW-TL relationship in diverse locations were most likely due to the environmental conditions (Ortiz-Ordóñez et al., 2007).

4.4 Sex ratio

The sex ratio variability detected for *G. atripinnis* was similarly reported for *G. multiradiatus* in San Miguel Arco Reservoir in Mexico State, where a male: female ratio of 3:1 was reported (Navarrete-Salgado et al., 2007), and in Villa Victoria Reservoir, also in Mexico State, where a male: female ratio of 1:2.77 was found (Cruz-Gómez et al., 2011). In San Martín Village Reservoir in Querétaro State, Mexico, a male: female ratio of 1:1.7 was registered (Cruz-Gómez et al., 2005). A proportionately higher number of females are commonly found in natural habitat, such as the male: female ratio of 1:2.4 reported for *G. viviparus*, a species living in urban lakes in Mexico City, Mexico (Gómez-Márquez et al., 2013). In Lake Cuitzeo in Michoacan State, Mexico, females of *Hubbsina turneri* were recorded in high abundance, with a male: female ratio of 1:41M (Moncayo-Estrada, 2012). Out of captivity, females of several species of viviparous fish are the more abundant sex (Macías-García et al., 1998).

The 1:1M *G. atripinnis* sex ratio recorded in the second F1 brood in the present experiment has also been documented for *H. turneri* in Lake Zacapu, Michoacán, Mexico, where a ratio close to 1:1M was registered (Moncayo-Estrada, 2012).

Similar ratios have been reported for *Zoogoneticus quitzeensis* in the reservoir of Mintzita in Morelia, Michoacan, Mexico (Ramírez-Herrejón et al., 2007).

In natural populations, selection processes influence the sex ratio toward 1:1 (50% males and 50% females), preserving a steady evolutive strategy (Maynard, 1978). In Aguascalientes State in the natural distribution area of *G. atripinnis*, the predators do not have a specific affinity for one particular gender, nor is the competence for niche and food important because *G. atripinnis* share the habitat with other species, such as *Yuriria alta*, *Algansea tincella* and *Scartomyzon austrinus*. However, upholding low fecundity but a high survival rate of fingerlings can be a strategy for tolerating adverse conditions. A great variety of mechanisms for sexual determination have been mentioned in relation to the differential distribution of sex, including genetic causes and environmental factors, such as water temperature, pH and social behavior (Devlin & Nagahama, 2002; Van Aerle et al., 2004; Guerrero-Estévez & Moreno-Mendoza, 2012). In the present experiment, water temperature most likely played a role in male production; however, the sex ratio approaches 1:1 because multiple factors are in equilibrium (Valenzuela et al., 2003).

The sex ratio is influenced by genetic, environmental and social factors, although the effects of these factors could differ between species (Rosenfeld & Roberts, 2004; Guerrero-Estévez & Moreno-Mendoza, 2012). In the case of genetic factors, there is no simple model that is useful for all fishes. In *Gambusia affinis*, reports of heterogametic females exist. In *Xiphophorus* spp., both heterogametic females and males exist, supporting the idea of a recent sex chromosome divergence (Barollier et al., 1999). The available sexual studies are specific for each species and sometimes for a particular population of fishes. The influence of water temperature on the sex ratio has been confirmed in 59 different species belonging to 13 families of fishes (Ospina-Álvarez & Piferrer, 2008). However, insufficient studies exist for viviparous fishes, and the mainstream has been focused on members of the Poeciliidae family, in which males are not abundant when the fish are maintained in high-temperature water (Sullivan & Schultz, 1986; Römer & Beisenherz, 1996).

4.5 Fertility

This is the first report of *G. atripinnis* fertility in Aguascalientes State. The average offspring number was of 24.32 ± 9.64 per birth. This result was similar to that reported for *G. luitpoldii* (*G. atripinnis*) studied in Lake Patzcuaro, namely, 19.1 offspring in the 44 ovaries examined (Mendoza, 1962), and differed from the maximum of 60 embryos reported for *G. atripinnis* (Uribe et al., 2005). The small differences mentioned could be the result of the rearing conditions in captivity compared with those under natural conditions (Navarrete-Salgado et al., 2007). Differences in the fertility of viviparous fish have been attributed to a relationship between TL and age, which was demonstrated in *G. viviparous*, as was a relationship between the number of embryos and TL (Cruz-

Gómez et al., 2011). In *G. atripinnis*, an average of 30 offspring per brood has been reported, but a high number were frequently found when the maternal TL was high (White & Turner, 1984). In a wild female with a TL of 111 mm, 110 fingerlings were reported in one brood. In the current study, this relationship was not observed, perhaps because all the females presented a similar TL.

Schoenherr (1977) determined that fertility in viviparous fish is the total number of embryos present within the female at the time of his capture and preservation, therefore in this study the fertility was determined as the number of embryos obtained for each birth. The partial fecundity in viviparous fishes is defined as the number of young counted in the ovary during dissection or in a brood at birth, suggesting that this term may be expressed as the number of fingerlings in relation to the female standard length (SL), TL (in mm or cm) or TW (in g) $\times 100$ to evaluate and estimate intraspecific fecundity (Grier et al., 2005). In *G. atripinnis*, reports of an average fecundity of 55 to 60 embryos exist (Uribe et al., 2005), whereas the average in the present study was 40 embryos despite a similar TL for females.

The offspring born of *G. atripinnis* in the present study registered an average TL of 15.4 ± 1.3 mm, with a TW of 0.040 ± 0.013 g, respectively. These data were obtained from 19 births and differed from those reported in *G. luitpoldii* (*G. atripinnis*) in Lake Patzcuaro, where the high TL measured was of 23.7 mm, and some of the fish reached a maximum of 31.2 mm before giving birth (Mendoza, 1962). In other studies, juveniles of the same species showed a TL of 20 mm, a longitudinal arrangement in the ovary, and a distribution aligned in parallel along the main ovarian axis in two ovarian chambers (Uribe et al., 2005). This change in TL was observed in offspring born with different sizes and pigmentation in aquariums and occurred because of matrotrophic development in this species, allowing accessibility to nutrients during the embryonic developmental phase while the embryos remain in the reproductive tract (Lombardi & Wourms, 1988; Hollenberg & Wourms, 1995). In *G. atripinnis*, the increase in embryonic mass results from the maternal transfer of nutrients during the gestational period.

4.6 Pregnancy period

Females in this experiment presented sequential births with a gestation period of 45 days (± 11 days). This result is consistent with the gestation period reported in Goodeidae fish under laboratory conditions, where a gestation period of 30 to 90 days occurred and was influenced by the number of daylight hours, the water temperature and the nutritional status of females. Furthermore, not all females in one population are pregnant simultaneously because eggs are not synchronically produced (Kingston, 1979). For *G. atripinnis*, the gestation period has been determined to last 60 to 75 days (Mendoza, 1962) or two months (Kingston, 1979). In comparison, the gestation period was shorter in the present study because the water temperature influenced embryonic development. In

Xenotoca variata, a small variation in temperature between two and three °C generated important changes in breeding performance (García-Ulloa et al., 2011).

G. luitpoldii (*G. atripinnis*) can produce one birth annually at the end of the dry season between April and June (Mendoza, 1962; Orbe-Mendoza, 2002). This timing coincides with that found in the present study; however, an average of two births per female and as many as four births were observed in the present study. A similar pattern was reported in *H. turneri* and was attributed to individual condition, habitat characteristics and the length of the gestational period (Moncayo-Estrada, 2012).

The reproductive period of *G. atripinnis* has been reported to last three months from April to June (Mendoza, 1962), but lasted seven months from March to October in the present study, in similarity with a third study reporting an April to September reproductive period (Bárragan & Magallón, 1994). This species could most likely reproduce all year as long as the water temperature remained optimal. Such a result has been reported in *A. splendens* in the Ameca River, México, where the water temperature varied from 25.0 to 28.2 °C, allowing multiple reproductive cycles (Ortiz-Ordóñez et al., 2007). However, only one reproductive event was recorded annually in cold waters (Díaz-Pardo & Ortiz-Jiménez, 1986). Other species, such as *G. multiradiatus* inhabiting San Miguel Arco Reservoir in México State and San Martín Reservoir, Querétaro, México, also presented one reproductive season (Navarrete-Salgado et al., 2007; Cruz-Gómez et al., 2010; Cruz-Gómez et al., 2011). The variation in the reproductive cycle of *G. atripinnis* and other Goodeids may be considered evidence for the plasticity of populations acclimated to different geographic conditions and the particular characteristics of new habitats (Del Mar Torralva et al. 1997).

4.7 Size at first reproduction

In Goodeidae fish, scientific reports on sexual maturity are scarce. In particular, the *G. atripinnis* maturation period was described as two years (Mendoza, 1962); however, under the rearing conditions of the present study, maturation occurred at only seven to eight months of age. For males of this species, early sexual maturation can be attributed to photoperiodic and water temperature conditions that induce gametogenic development. The reproductive season can vary with environmental conditions, such as water temperature and feeding strategies, but not all the organisms reproduce simultaneously, even those of the same size or age (Salgado Ugarte et al., 2005; Uribe et al., 2010b; Cruz-Gómez et al., 2011).

In the present study, the males of *G. atripinnis* developed sexually at a lesser TL and age compared with females; males and females in both the F1 brood attained sexual maturity at an average TL of 42 and 48.3 mm, respectively. The size at the first reproduction can vary among populations of the same species; for example, the first reproduction of *G. multiradiatus*

females occurred at a TL of 32 mm (Cruz-Gómez et al., 2011), and 30 mm at Villa Victoria Dam and Lake Texcoco, respectively, both in the State of México (Díaz-Pardo & Ortiz-Jiménez, 1986). Another example involves *H. turneri* females, which reached sexual maturity at an SL of 30 mm in Lake Zacapu, Michoacan State, Mexico; however, the minimum SL for reproduction was 25.9 ± 0.21 mm in Lake Cuitzeo, Michoacan, Mexico (Moncayo-Estrada, 2012).

Conclusions

Under the experimental conditions of the present study, *G. atripinnis* showed a sex ratio near 1:1M. The average TL of nine-month-old fish was 49.2 mm in males and 57.05 mm in females; the average TW was 1.95 ± 0.22 and 3.14 ± 0.18g, respectively.

Under a 14 h light: 10 h dark photoperiod and an average temperature of 24 °C, sexual maturity occurred at a TL of 42 mm in males and 48.3 mm in females. The females first gave birth between eight and nine months of age, with the possibility of year-long reproduction under stable photoperiodic and thermal conditions.

G. atripinnis females can copulate as many as four times during the reproductive season, with an average of two times. The gestational period lasted ninety days, with an average production of 24.32 ± 9.64 fingerlings per female in the first year of life. The fingerlings reached an average TL of 15.4 ± 1.3 mm and TW of 0.040 ± 0.013 g.

The information generated in the present study establishes a foundation upon which to initiate the rearing of *G. atripinnis* under controlled conditions. This study has shown the relevance of promoting reproductive studies on native fishes, especially those with scientific and ecological value that has been underestimated.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise

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