REPRODUCTIVE BIOLOGY OF THREE NATIVE LIVEBEARER FISH SPECIES (ACTINOPTERYGII: CYPRINODONTIFORMES: GOODEIDAE) IN THE TEUCHITLÁN RIVER, MEXICO

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Background. The Ameca River basin in central Mexico, especially the Teuchitlán River, hosts a rich native and endemic ichthyofauna. The biological traits of these species, however, have not been fully studied, and their habitat has been altered by anthropogenic activities. The aim of this study was to evaluate the reproductive cycle of three native goodeids, and to describe the variation in the reproduction of each species. The results of this study have important conservation implications and can be used to support specific conservation actions, trying to protect specific areas where native species are reproducing, aimed at maintaining biological diversity in the Teuchitlán River.
Materials and methods. This two-year study investigated the fertility, size at first maturity (L_{50}), sex ratio, gonad maturity stage, and gonadosomatic index of three native livebearer fish species, *Goodea atripinnis* Jordan, 1880; *Ameca splendens* Miller et Fitzsimons, 1971; and *Zoogoneticus purhepechus* Domínguez-Domínguez, Pérez-Rodríguez et Doadrio, 2008. Environmental variables were evaluated, with respect to the reproductive variables, using non-metric multidimensional scaling (NMDS) analysis.
Results. Three hundred and eighty-three specimens of *G. atripinnis*, 319 of *A. splendens*, and 170 of *Z. purhepechus* were examined. *Goodea atripinnis* was widely distributed along the river, presenting a complete size structure. The endemic species (*A. splendens* and *Z. purhepechus*) showed lower abundance downstream. The native species presented two reproductive periods: January through March and July through September. The sex ratio is 1 ÷ 1 (female ÷ male) and the fertility was lower compared to other species in other river basins. The NMDS analysis showed that the native species are associated with clean, deeper waters that present higher dissolved oxygen and a neutral pH.
Conclusion. The lower population abundance of native species downstream in the river is due to the fact that the goodeid species are less tolerant to pollution and cannot reproduce successfully in polluted water. These species have to adapt to the anthropogenic activities that have modified the river, affecting their habitat. In spite of this perturbation, there are no specific conservation actions underway to maintain biological diversity in the Teuchitlán River.

Keywords: goodeids, livebearers, reproductive habitat, Teuchitlán River

INTRODUCTION

The central region of Mexico features a depauperate freshwater fish fauna, except several endemic groups. The Goodeidae (Actinopterygii: Cyprinodontiformes) constitute the most diverse component of this region. Goodeids comprise approximately 40 to 45 species, four of which (from the Great Basin of the USA) are oviparous and belong to the subfamily Empetrichthyinae, while

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the remaining species are livebearers belonging to the subfamily Goodeinae that inhabit drainage basins of the Mexican high plateau and its periphery. Goodeids fulfil many different ecological roles and thus possess unique and varied morphological and life-history specializations (Webb and Miller 1987). Goodeid reproduction is unique among the co-occurring groups; for example, during gestation, the majority of goodeids develop structures known as trophotaeniae, which are an embryonic trophic adaptation consisting of a simple surface epithelium surrounding a highly vascularized core of loose connective tissue. The trophotaeniae are the chief sites of nutrient absorption in goodeid embryos. Through this nutrient uptake, massive weight gain can take place during embryonic development (Hollenberg and Wourms 1994).

The majority of goodeid species are critically endangered and some are now considered extinct (Domínguez-Domínguez et al. 2005, 2008). The reasons for the decline of this group of livebearing fishes in Mexico include the arid/semiarid conditions in the northern and central parts of the country, overexploitation of water, the introduction of invasive species, habitat destruction, and water pollution caused by a diverse array of human activities (Contreras-Balderas 2005).

The Ameca River basin in central Mexico (Pacific Slope) is of great biological importance since it has been identified as one of the richest areas for native and endemic ichthyofauna in Mexico (Miller and Smith 1986). The upper part of the Ameca drainage basin, mainly the Teuchitlán River, is inhabited by several endemic species that are not found in any other aquatic systems of central Mexico (Domínguez-Domínguez et al. 2008). In contrast, the Ameca River has a drainage basin that is among the most heavily disturbed by human activities in Mexico. Water pollution, reduced groundwater and surface water levels, basin deforestation, habitat modification and fragmentation, the introduction of exotic species, and overfishing have all contributed to the severe degradation of the aquatic systems (Domínguez-Domínguez et al. 2008). The Teuchitlán River, in the upper part of the Ameca basin, has been particularly impacted by anthropogenic activities that have modified the aquatic ecosystem. These activities include reservoir construction, water extraction, and municipal and industrial pollution. There has been a significant consequent decrease in native fish species in the Teuchitlán River. Previously, the area hosted twenty native and six non-native fish species, the majority of which were described taxonomically during the last decade. However, the Teuchitlán River system currently harbours only four native fish species, including the endemic *Ameca splendens* Miller et Fitzsimons, 1971 and the natives *Zoogoneticus purhepechus* Domínguez-Domínguez, Pérez-Rodríguez et Doadrio, 2008 and *Goodea atripinnis* Jordan, 1880, as well as seven non-natives (López-López and Paulo-Maya 2001).

Previous studies have provided some life-history information for each of the three goodeid species included in this study; however, much information is still required. *Ameca splendens* has a restricted distribution and is endemic to the state of Jalisco, Mexico. The species is mainly herbivorous, grazing on filamentous algae and diatoms, but can also consume mosquito larvae, copepods, oligochaetes, small insects, and spiders that fall on to the water surface (Miller et al. 2005). Reproduction occurs from midwinter to early spring, but the reproductive period can be greatly extended. In aquarium stocks, the fecundity of this species ranges from 1 to 17. Maximum known length is 90 mm SL (Miller et al. 2005). *Goodea atripinnis* is the most widespread goodeid species, occurring throughout central Mexico. The species inhabits a variety of different habitat types, including lakes, ponds, springs, outflows, and streams (De la Vega-Salazar 2006, Miller et al. 2005). Its diet consists of filamentous green algae, micro-crustaceans, and molluscs (Miller et al. 2005). Its reproduction period is long, occurring from January to July, and it produces a large number of embryos (167) (Miller et al. 2005). Maximum length is 200 mm SL. *Zoogoneticus purhepechus* is a recently described species (Domínguez-Domínguez et al. 2008), formerly recognized as *Zoogoneticus quitzeoensis* (Bean, 1898). To date, no autecological studies have been conducted on this species, and it is restricted to particular river drainage basins in the lower Lerma, upper Ameca, Armeria, and Santiago rivers basins and Chapala Lake, Mexico (Domínguez-Domínguez et al. 2008).

In spite of their high species richness and endemicity, goodeids have largely been ignored in terms of conservation efforts. However, the documented extinctions and extirpations have led to some level of legal protection, and these fishes have caught the attention of aquarists worldwide (De la Vega-Salazar et al. 2003, Domínguez-Domínguez et al. 2005). Understanding the basic aspects of their life history represents one of the first steps in the development of conservation plans for any species. In general, little life history information is available for the majority of goodeid species.

The aim of this study was, therefore, to conduct a comprehensive study to evaluate the reproductive cycle of three native goodeids (*G. atripinnis*, *A. splendens*, and *Z. purhepechus*) and to describe annual variations in the reproduction of each species and their associations with the habitat characteristics of the Teuchitlán River, Mexico. We hypothesized that the native species (especially the endemics), present in this river, will be affected by anthropogenic activities along the river, and will present low fertility rates, incomplete size structure, and low abundances at the majority of modified sites. The results of this study have important conservation implications and can be used to support specific conservation actions to maintain biological diversity in the Teuchitlán River.

MATERIALS AND METHODS

The Teuchitlán River is 1274 m long with a maximum width of 29.6 m, and is located in Jalisco State, Mexico, where it flows from its headwater springs to the La Vega dam (Fig. 1). Five study sites with different habitat characteristics were selected: two springs located at the headwaters, a third on the first stretch of the river, and two

sites downstream that are polluted by sewage. This study was conducted over a bi-annual cycle with bi-monthly sampling conducted from January 2015 to November 2016.

Fish collection. Fish were captured using a seine net (4.5 m long, 2.3 m high and with the mesh size of 1.35 mm) and electrofishing (DC-backpack electrofisher model ABP-3, ETS electrofishing systems, LLC, average power ~200 W, peak voltage ~250 V, peak current ~10 A). Captured fish were preserved in 70% ethanol and transported in plastic containers to the laboratory, where they were identified, quantified, measured (0.01 mm), and weighed (0.001 g), following the criteria of Cruz-Gómez et al. (2013). Voucher specimens were deposited in the Colección de Peces de la Universidad Michoacana (CPUM), under the following catalogue numbers: *A. splendens* (12536, 12537); *Z. purhepechus* (12540, 12551), and *G. atripinnis* (12538, 12550).

Data analysis. The following reproductive variables were assessed: fertility, size at first maturity, sex ratio, gonad maturity stage, gonadosomatic index (GSI), condition factor (*K*), size structure, and reproductive habitat. The ovaries of each female were removed and embryonated eggs and embryos quantified. A fertility (*F*) model was obtained with the data from the embryonated eggs and embryos and was adjusted to the potential model of Schoenherr (1977)

$$F = aL^b$$

where *a* is a coefficient and *b* is coefficient in the potential model. Size at first maturity (L_{50}) was related to the standard length using the logistic regression model to fit sigmoid curves, according to the following equation:

$$M(L) = 1 \cdot (1 + e^{(-aL + b)})$$

Confidence limits were derived by Bayesian inference based on stochastic simulation. Sex ratio was described per site and season following the criteria of Sparre and Venema (1997). The statistical significance of the sampling site ratio results was established by fitting to a Chi-squared test (χ^2), using a *P*-value of < 0.05. Gonad maturity was estimated with the criteria proposed by Ramírez-Herrejón et al. (2007) (Table 1). The gonadosomatic index (GSI), an estimator of reproductive condition, was calculated by dividing the gonad mass by total body mass × 100 (values in grams) (Zeyl et al. 2014). The condition factor was assessed with Fulton's condition factor (*K*). Population length structure was analysed by sampling site, grouping the data into standard length ranges following the criteria of Sturges (1926), while an analysis of variance allowed the identification of significant differences among sampling sites per size, species and sex. A Tukey–Kramer test (*P* < 0.05) was used to determine these significant differences. Model growth was evaluated by linear regression, calculating the *a* and *b* values of the equation

$$W = aL^b$$

where *W* is the body weight, *L* is the standard length, *b* is the growth exponent or length–weight factor, and *a* is a constant. The *a* and *b* values were estimated using a linearized form (Froese 2006).

The physical and chemical characteristics of the water, such as water temperature (°C), depth (mm), transparency (mm), pH, dissolved oxygen (mg · L^{-1}), total alkalinity (mg · L^{-1}), chlorophyll *a* content (µg · L^{-1}), total hardness (mg · L^{-1}), turbidity (NTU) and the sedimentary, dissolved and total solids (mg · L^{-1}), were evaluated following the criteria of the American Public Health Association, American Water Works Association, and the Water Environment Federation (Rice et al. 1995).
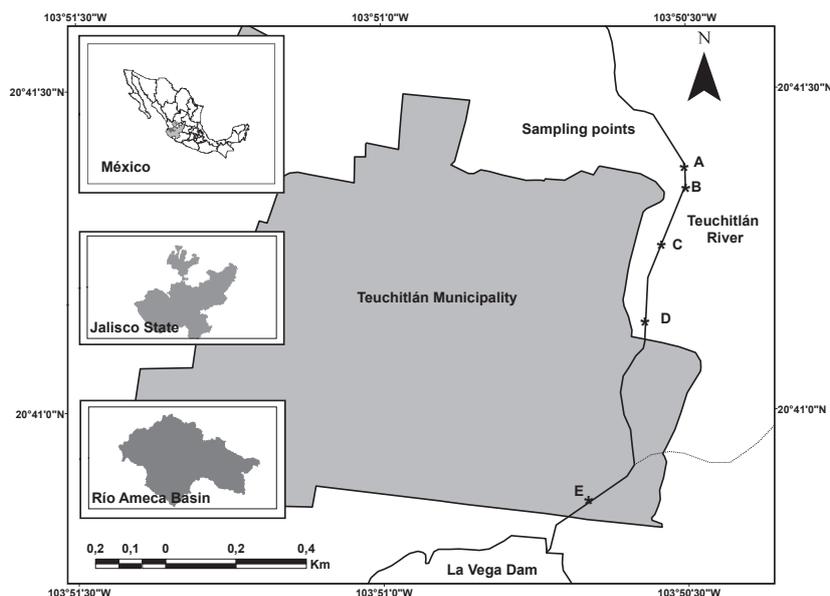

Fig. 1. Location of the Teuchitlán River (Mexico) and study sites (A, B, C, D, and E)

Gonadal maturity stages of livebearer fish (Ramírez-Herrejón et al. 2007)

	Female			Male	
Phase	Name	Description	Phase	Name	Description
I	Immature	Small ovaries, <6 mm long, reaching 30%–50% of visceral cavity; with packed eggs	I	Immature	Testis thin and yellowish occupying ~25% of visceral cavity
II	Developing eggs	Ovaries longer than in previous stage (10 mm); eggs enclosed in ovarian tissue	II	Developing juvenile	Turgid and yellow testis occupying <25% of visceral cavity
III	Free eggs	Ovary with free eggs and embryos (~2 mm long); enclosed within common membrane	III	Juvenile	Turgid and yellow testis occupying <50% of visceral cavity
IV	With embryos	Ovary with embryos standard length > 3.5 mm	IV	Immature	Whitish translucent testis occupying ~50% of visceral cavity; fish reaches sexual maturation
V	After spawning	Ovaries having flaccid walls and few visible eggs, with rupture at end of gonad	V	Mature	Turgid, whitish opaque testis occupying > 50% of visceral cavity
VI	In recess	Recovery after spawning, without embryos; turgid ovaries >6 mm long	VI	In recess	Flaccid and transparent testis occupying > 50% of visceral cavity; corresponds to semen ejaculation phase

Ordering of the environmental variables of the sampling sites, with respect to the reproductive variables of the species, was carried out using a non-metric multidimensional scaling (NMDS) analysis with Bray–Curtis distance. This analysis was conducted using the metaMDS function of R (R Development Core Team 2016) Vegan package (Oksanen 2016).

RESULTS

Environmental variables. The physicochemical variables showed the lowest water depth values at site A in the dry season and at site E in the rainy season. The highest values of depth were at site B in the dry and rainy seasons. Over the two years of study, the temperature ranged from 24.4 ± 1.9 to 27.9 ± 0.8°C. The pH (6.3~6.9) indicated slightly acid water, with moderate electrical conductivity in the sampling site near to the La Vega dam. The headwaters (springs) sites (A and B) presented the greatest transparency, which then decreased downstream. Dissolved oxygen concentrations ranged between 3.5 mg · L⁻¹ at site E (rainy season) and 6.1 mg · L⁻¹ at site B (dry season). Chlorophyll *a* presented its minimum value of 0.6 µg · L⁻¹ at site B and maximum value of 10.7 µg · L⁻¹ at site E, both in the rainy season. The total hardness values indicated that the waters were soft (Table 2). The habitat characteristics along the Teuchitlán River differed among sites. Site B showed high impacts of anthropogenic activities that have transformed the spring into a pool, which lacks the emergent or floating vegetation that can serve as a habitat or shelter for the epifauna. Site A contains organic material at different degrees of degradation, submerged trunks and emergent and floating vegetation, and the site has been dammed with masonry. Site C marked the beginning of the river, and showed abundant vegetation covering the left bank, while the right bank had been impacted by the construction of a path and the beginning of the settlement of the population of Teuchitlán. The site comprised different kinds of ponds, both shallow (<0.5 m) and deep (>0.5 m). Sites D and E presented a low and homogeneous substrate available for the epifauna, composed mainly of silt and clay covering the river bottom. The construction of bridges was observed; however, the ponds are very extensive and of more than 40 m in width, which acts to produce little oxygenation in the water. Moreover, these sites are polluted by wastewater discharge.

In total, 383 specimens of *Goodea atripinnis* were examined in this study, as well as 319 of *Ameca splendens* and 170 of *Zoogoneticus purhepechus* (Table 3).

Goodea atripinnis. The size structure of females of *G. atripinnis* ranged from 19.36 mm SL to 123.33 mm SL, with the majority of individuals in the size range of 39 to 60 mm SL (Table 4). There were significant differences in size among sampling sites for both females ($F = 15.42$, $P < 0.0001$) and males ($F = 6.05$, $P < 0.0001$). Site E (mean ± standard deviation = 57.46 ± 1.55) presented the greatest size, and site C (39.64 ± 3.69) the smallest size for females. For males, sites B (52.85 ± 1.43) and E (52.60 ± 2.42) presented similar mean size values but differed from those of site A (42.97 ± 1.84). In all sampling sites, the fertility of *G. atripinnis* was 9 ± 2.47; however, site A presented the highest (13 ± 5.67) and site B the lowest (7 ± 2.68) fertility values. Females began their reproduction at size 43.02 ± 8.9 mm SL. The mean length of mature males was similar to that of the females. However, across sampling sites, males of *G. atripinnis* reach L_{50} at 36.01 mm SL and females at 30.09 mm SL, both at site C (Table 5). The sex ratio (female ÷ male) was 1.3 ÷ 1 at site A ($\chi^2 = 14.72$, $P > 0.0116$), 1 ÷ 1 at site B ($\chi^2 = 11.38$, $P > 0.0443$), 2 ÷ 1 at site C ($\chi^2 = 6.6$, $P > 0.2521$), 2.5 ÷ 1 at site D ($\chi^2 = 10.08$, $P > 0.0729$), and 1.6 ÷ 1 at site E ($\chi^2 = 14.64$, $P > 0.0119$). All gonadal stages were present for *G. atripinnis* at all sites, although few individuals were found in stage VI. Mature individuals (stages III, IV, and V) were more frequent in the headwaters (site B). We found a higher frequency of immature individuals (stages I and II) in the middle portion of the river (sites C and D) (Fig. 2). Bi-monthly variation in GSI for females varied among sites, with a reproductive peak in the headwaters occurring in March, and downstream in September and November.

Physical and chemical water characteristics for the dry and rainy seasons at each study site in the Teuchitlán River, Jalisco, Mexico, during 2015 and 2016

Parameter	Site and season									
	A		B		C		D		E	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
DO	5.0 ± 0.3	5.9 ± 0.3	6.1 ± 0.1	5.9 ± 0.2	5.0 ± 1.3	4.2 ± 0.5	4.9 ± 0.9	3.7 ± 1.1	4.5 ± 1.3	3.5 ± 1.0
AL	99.1 ± 1.4	110.9 ± 12.4	100.1 ± 6.4	109.8 ± 11.4	99.1.0 ±1.4	112.0 ± 5.1	127.5 ± 32.5	122.1 ± 1.3	118.6 ± 50.6	125.2 ± 13.5
CL	1.7 ± 1.5	3.1 ± 3.6	0.7 ± 0.3	0.6 ± 0.5	2.7 ± 1.9	5.1 ± 5.3	1.3 ± 1.3	4.1 ± 3.2	2.2 ± 0.6	10.7 ± 7.9
HA	48.5 ± 5.7	48.8 ± 3.3	48.3 ± 2.9	49.2 ± 4.9	46.5 ± 3.0	49.4 ± 1.7	52.9 ± 4.0	54.1 ± 1.4	52.5 ± 2.5	60.8 ± 11.3
pH	6.8 ± 0.2	6.7 ± 0.2	6.9 ± 0.1	6.8 ± 0.2	6.8 ± 0.3	6.5 ± 0.3	6.8 ± 0.1	6.5 ± 0.3	6.8 ± 0.4	6.3 ± 0.1
TU	64.1 ± 18.7	9.0 ± 6.3	0.8 ± 0.4	1.5 ± 0.8	14.6 ± 6.8	19.1 ± 9.8	8.1 ± 2.9	11.6 ± 8.6	11.7 ± 5.0	37.8 ± 21.9
SE	0.6 ± 0.7	1.3 ± 1.8	0.1 ± 0	1.1 ± 1.9	0.7 ± 0.9	1.8 ± 1.5	0.5 ± 0.7	0.8 ± 1.3	0.9 ± 1.3	0.1 ± 0.1
DEE	39.6 ± 4.7	41.2 ± 3.9	97.9 ± 3.4	98.2 ± 2.0	42.1 ± 4.7	48.2 ± 7.6	68.1 ± 11.1	60.5 ± 5.1	60.1 ± 34.8	39.1 ± 18.2
TRA	28.0 ± 4.1	41.2 ± 3.9	97.9 ± 3.4	98.3 ± 2.0	40.5 ± 7.5	42.4 ± 9.4	68.1 ± 11.1	57.8 ± 8.2	60.1 ± 34.8	37.7 ± 19.6
TEM	27.5 ± 0.9	27.1 ± 0.6	27.1 ± 0.4	27.0 ± 0.4	26.3 ± 1.3	26.6 ± 1.3	24.6 ± 2.5	26.4 ± 1.7	24.4 ± 1.9	27.9 ± 0.8
DIS-SOL	101.1 ± 2.0	102.3 ± 2.0	100.1 ± 0.6	99.5 ± 8.2	99.3 ± 6.2	103.3 ± 5.6	104.7 ± 2.0	122.7 ± 0.9	96.1 ± 13.1	129.8 ± 10.1
SUS-SOL	55.1 ± 30.3	11.1 ± 8.3	2.4 ± 2.3	2.5 ± 1.6	17.3 ± 13.8	21.8 ± 8.3	279.3 ± 478.0	10.1 ± 10.6	298.3 ± 500.5	19.4 ± 11.9

A, B, C, D, E are sampling sites; elevation (m above sea level) was 1266 for A, B, A, and 1265 for D and E; DO = dissolved oxygen [mg · L⁻¹], Al = total alkalinity [mg · L⁻¹], Cl = Chlorophyll a [μg · L⁻¹], HA = total hardness [mg · L⁻¹], TU = turbidity [NTU], SE = sedimentation, DEE = depth [cm], TRA = transparency [cm], TEM = water temperature [℃], SUS-SOL = suspended solids [mg · L⁻¹], DIS-SOL = total dissolved solids [mg · L⁻¹].

Table 3
Number of specimens of *Ameca splendens*, *Zoogoneticus purhepechus*, and *Goodea atripinnis* captured per site from the Teuchitlán River, Mexico, in 2015 and 2016

Site	*A. splendens*		*Z. purhepechus*		*G. atripinnis*	
	♀	♂	♀	♂	♀	♂
A	83	62	42	43	63	47
B	63	48	32	24	77	77
C	25	8	21	8	8	4
D	21	9	0	0	25	10
E	0	0	0	0	45	27
Total	192	127	95	75	218	165
Grand total	319		170		383	

The GSI for males was not consistent with that for females and a reproductive peak was found in July to September in the headwaters, but in March and September at the sites downstream (Fig. 3). Condition factors did not show a clear relation with GSI. *K*-condition data showed low values downstream. Both sexes presented positive allometric growth (Table 6). The non-metric multidimensional scaling (NMDS) analysis for mature individuals revealed a strong relation with the environmental variables, including higher pH, suspended solids, and turbidity, found at the headwater sites (A and B). Juveniles were associated with the lower part of the river (site E), which presented the highest values of sedimentary solids, chlorophyll *a*, and temperature (Fig. 4).

Ameca splendens. Size structure for females was represented by a range of 15.13 mm to 59.26 mm SL, with the majority of individuals found between 26 and 31 mm SL. The males were between 15.15 mm and 57.95 mm SL (Table 4), with the greatest frequency of individuals between the sizes of 31 and 37 mm SL. There were significant differences in size among sampling sites in both females ($F = 33.55$, $P < 0.0001$) and males ($F = 18.22$, $P < 0.0001$). For female size, all of the sampling sites differed statistically from one another: site A (30.61 ± 0.90), site B (38.04 ± 1.03), site C (23.42 ± 1.64), and site D (43.92 ± 1.79). Males presented similar values to the females at site D (42.24 ± 2.33), while the sexes at A (32.82 ± 0.89), sites B (39.58 ± 1.00), and C (24.15 ± 2.47) differed from one another. Across all sampling sites, the endemic species *A. splendens* possessed a fertility value of 7 ± 3.25. Site C showed the lowest fertility value (5 ± 9.95) and site B the highest (7 ± 4.8). Reproduction began at the mean size of 34.26 ± 9.57 mm SL for females and 31.59 ± 11.69 mm SL for males. Size at first maturity varied across sites, with individuals reaching maturity at 27.04 mm SL for females at site C, and 31.95 mm SL for males at site A (Table 5). The sex ratio was 1.14 ÷ 1 at site A ($\chi^2 = 6.19$, $P > 0.2875$), 1.3 ÷ 1 at site B ($\chi^2 = 13.27$, $P > 0.0209$), 3.12 ÷ 1 at site C ($\chi^2 = 2.20$, $P > 0.8119$), and 2.33 ÷ 1 at site D ($\chi^2 = 2.60$, $P > 0.7603$). It was not possible to capture a sufficient number of individuals in the lower section of the river (site E) for inclusion in this analysis. Site B showed the most complete structure of gonadal stages, with a high frequency of juvenile and mature stages. Immature individuals dominated in the four sites (Fig. 2). The values of GSI for females showed a reproductive peak in January and November for site A, March and July for site B, March for site C, and January for site D. Males showed a similar tendency in their GSI values (Fig. 3). Condition factors showed a similar tendency during the peaks of GSI values in both sexes (Fig. 3). Both sexes presented negative allometric growth (Table 6). The NMDS analysis showed that the mature individuals (stage IV and V) were established in the headwaters of the river (site B) and downstream (at site D) where the environmental variables showed the highest values of transparency, depth, pH, and

Table 4

Standard length for females and males of *Goodea atripinnis*, *Ameca splendens*, and *Zoogoneticus purhepechus* from the Teuchitlán River, Mexico

Species	Site	Minimum size [mm]	Maximum size [mm]	Mean size [mm]
G. atripinnis Female	A	19.36	94.41	43.24
	B	36.33	57.97	49.60
	C	22.62	66.24	39.64
	D	40.02	66.62	54.57
	E	28.52	78.17	57.46
G. atripinnis Male	A	24.83	67.43	42.97
	B	36.73	123.33	52.85
	C	23.14	53.84	36.11
	D	39.24	59.68	50.61
	E	38.45	65.46	52.60
A. splendens Female	A	15.13	48.30	30.61
	B	17.03	59.26	38.04
	C	15.54	49.63	23.42
	D	33.63	54.99	43.92
A. splendens Male	A	15.15	57.95	32.82
	B	31.18	48.91	39.58
	C	15.63	39.7	24.15
	D	33.62	48.55	42.24
Z. purhepechus Female	A	15.70	36.01	26.78
	B	18.17	39.30	31.88
	C	15.23	33.67	23.10
Z. purhepechus Male	A	16.46	33.59	25.43
	B	21.41	39.37	31.72
	C	18.22	41.43	26.72

Table 5

First maturity size L_{50} (SL, in mm) per study site for *Goodea atripinnis* (Ga), *Ameca splendens* (As) and *Zoogoneticus purhepechus* (Zp) from the Teuchitlán River, Mexico

Site	♀ Ga	♂ Ga	♀ As	♂ As	♀ Zp	♂ Zp
A	37.72	37.82	ID	31.95	28.03	25.03
B	46.05	49.27	38.39	40.12	38.42	32.19
C	30.09	36.01	27.04	ID	ID	ID
D	51.41	47.23	45.84	39.36	ID	ID
E	49.85	45.08	ID	ID	ID	ID

ID = insufficient data; logistic model: $M(L) = 1 \cdot (1 + e^{(-aL + b)})$

Table 6

Standard length and the length – weight relation parameters for *Goodea atripinnis*, *Ameca splendens*, and *Zoogoneticus purhepechus* from the Teuchitlán River, Mexico

Species	Sex	Standard length Mean ± SD	LWR a	b	R^2
G. atripinnis	♀	47.26 ± 8.2	0.13	4.11	0.78
	♂	49.59 ± 11.75	0.12	3.89	0.73
A. splendens	♀	33.58 ± 10.11	0.07	1.53	0.80
	♂	35.53 ± 8.31	0.07	1.54	0.81
Z. purhepechus	♀	27.68 ± 6.51	0.04	0.79	0.84
	♂	27.58 ± 5.74	0.05	0.98	0.90

SD = standard deviation, LWR = length–weight relation, a = intercept, b = the slope of the linear regression, R^2 = correlation coefficient, $b > 3$ values denote positive allometry, $b < 3$ denote negative allometry, $P < 0.01$.

hardness. At sites D and E, the juvenile (stages I and II) organisms were associated with the highest values of dissolved solids, sedimentary solids, and turbidity (Fig. 4). *Zoogoneticus purhepechus* was the most common species in the headwaters (sites A, B, and C), but an insufficient number of individuals were captured from the downstream sites for analysis. The size structure ranged from 15.70 to 41.43 mm SL for females and 16 to 41 mm SL for males (Table 4). The majority of individuals were between 23 and 30 mm SL. There were significant differences in size among sampling sites for females ($F = 16.21$, $P < 0.0001$) and males ($F = 18.22$, $P < 0.0001$). All of the sampling sites differed statistically from each other in terms of female size: site A (26.78 ± 0.87), site B (31.88 ± 1.00), and site C (23.10 ± 1.23). Males presented similar sizes at site A (25.43 ± 0.76) and C (26.72 ± 1.78), but these both differed from those of site B (31.72 ± 1.02). Fertility across all of the sampling sites was 8 ± 3.17; however, site C presented the highest fertility (11 ± 10.95) and site A the lowest (5 ± 2.64). Reproduction began at size 33.22 ± 7.34 mm SL for females and 28.61 ± 5.06 mm SL for males. Standard length at first maturity varied slightly according to site and was 28.03 mm SL for females and 25.03 mm SL for males at site A (Table 5). Sex ratios were 0.97 ÷ 1 at site A ($\chi^2 = 2.03$, $P > 0.8449$), 1.3 ÷ 1 at site B ($\chi^2 = 16.21$, $P > 0.0062$), and 2.6 ÷ 1 at site C ($\chi^2 = 13.07$, $P > 0.0227$). Mature individuals were present in higher frequencies in the headwater sites (site B), while immature individuals dominated at site A (Fig. 2). At site B, the GSI and K values for both sexes showed a similar tendency, with two reproductive peaks in March and November. Site A also presented two reproductive peaks, but in July and November for females. The highest GSI values for males were presented in March, while the values for K peaked in May. The females presented two reproductive peaks during March and September, at site C. The GSI values for males coincided with the reproductive peak of females in September and the K values were low at this site (Fig. 3). Both sexes presented negative allometric growth (Table 6). The NMDS analysis results showed that mature organisms were correlated with headwater site parameters (sites A and B) with high values of depth, transparency, dissolved oxygen, and pH. Juveniles were related to site A, with high values of dissolved solids and high turbidity in the water (Fig. 4).

DISCUSSION

The presently reported study documents the reproductive cycle of three native goodeids in the Teuchitlán River, Mexico. Seasonal and inter-annual variations in the spatial distribution, life history stages, and size structure of the species reflect and are influenced by anthropogenic activities along the river (Table 7). Both *Ameca splendens* and
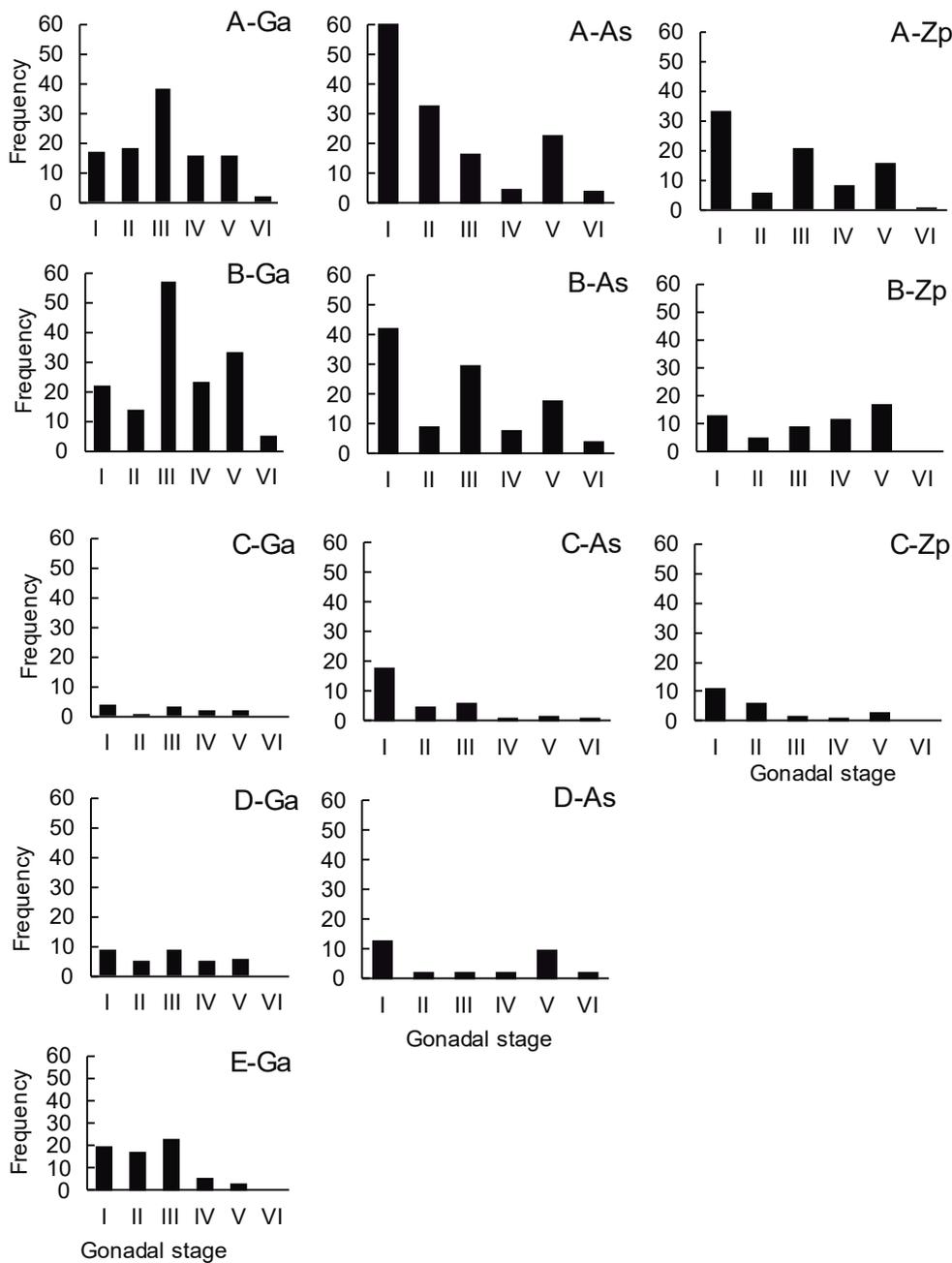
Fig. 2. Relative frequency of gonadic maturity stages of *Goodea atripinnis* (Ga), *Ameca splendens* (As), and *Zoogoneticus purhepechus* for each study sites (A, B, C, D, E) during 2015–2016 in the Teuchitlán River, Mexico

Zoogoneticus purhepechus occurred in lower abundance than *Goodea atripinnis* throughout the river basin and their abundances decreased progressively downstream. The population density in the lower portion of the river was very low. Abundance differences could be explained by the greater environmental tolerance of *G. atripinnis*, which seems to be a generalist in terms of its use of habitat. Both *A. splendens* and *Z. purhepechus* seem to be less tolerant to environmental stress, and are less abundant in more disturbed environments, as reported by López-López and Paulo-Maya (2001) and Varela-Romero et al. (2002).

Fertility. The native fish species in Teuchitlán River show relatively low mean fertility compared to the same species in other aquatic systems and also in comparison to other goodeid species. Other goodeids, such as *Alloophorus robustus* (Bean, 1892), *Allotoca diazi* (Meek, 1902), *G. atripinnis*, and *Ilyodon whitei* (Meek, 1904), presented mean values of 20 to 50 embryos per female in other river basins (Mendoza 1962, Uribe-Aranzábal et al. 2006). Furthermore, species of *Allodontichthys* had 11 to 30 embryos (Lyons et al. 2000) and *Zoogoneticus quitzeoensis* (Bean, 1898), *Hubbsina turneri* de Buen, 1940, and *Girardinichthys multiradiatus* (Meek, 1904) had a mean number of embryos per female that was fewer than 20 (Ramírez-Herrejón et al. 2007, Moncayo-Estrada 2012, Cruz-Gómez et al. 2013). Other exotic species in the
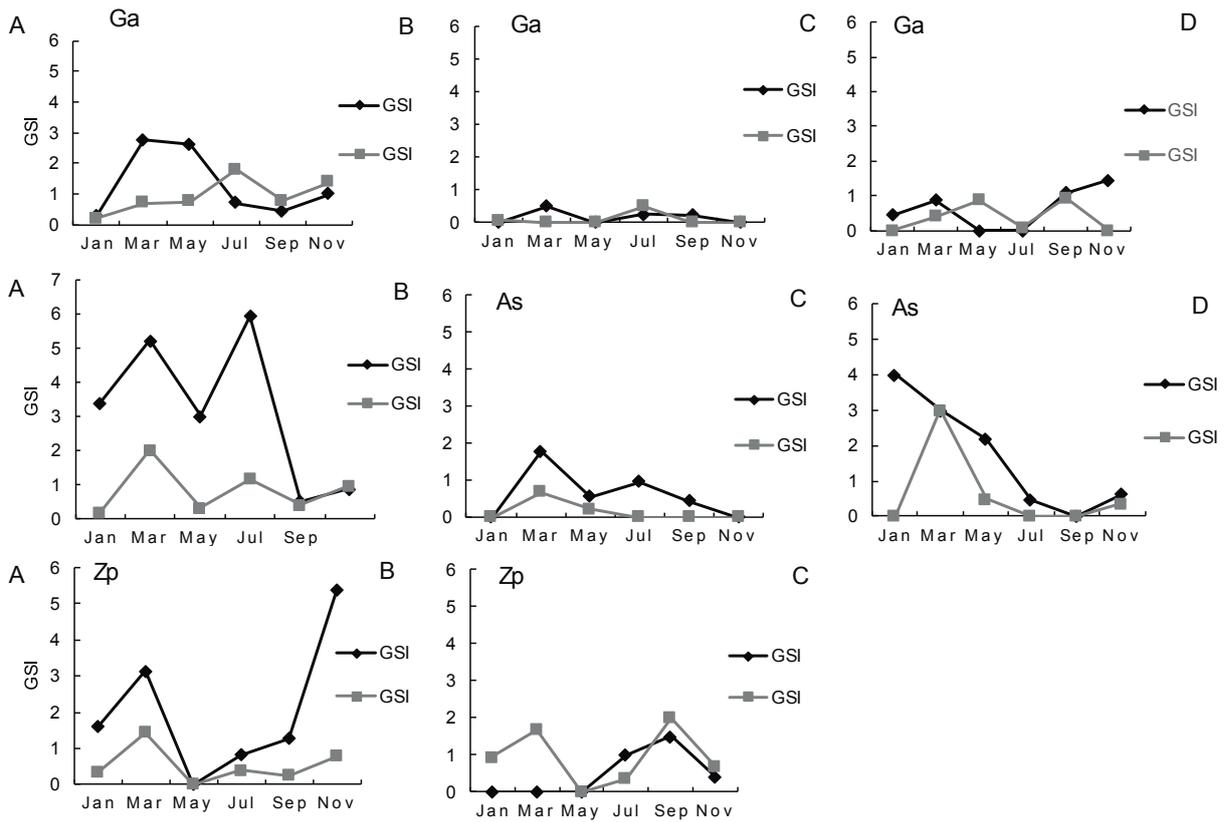
Fig. 3. Bimonthly variation in the gonadosomatic index (GSI) values for each sampling site (A, B, C, D, E) for females (black line) and males (gray line) of *Goodea atripinnis* (Ga), *Ameca splendens* (As), and *Zoogoneticus purhepechus* (Zp) in the Teuchitllán River, Mexico
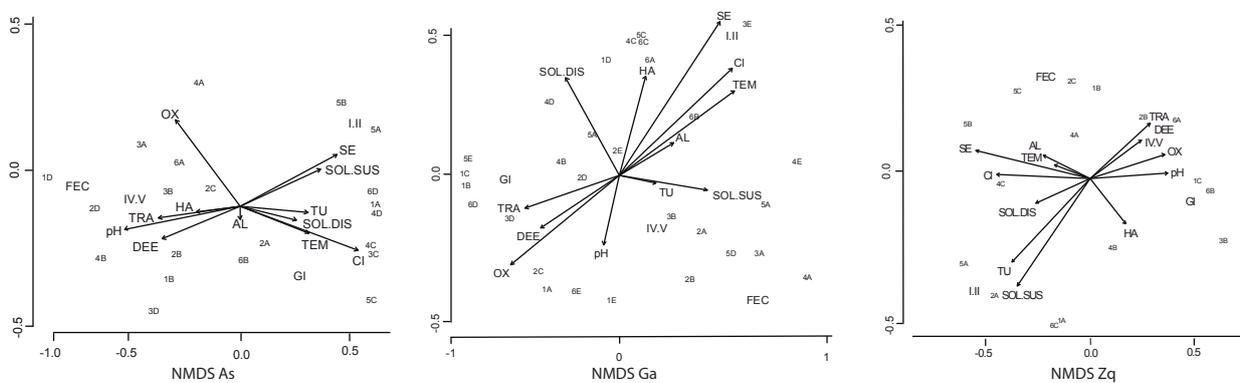

Fig. 4. Non-metric analysis of multidimensional scaling (NMDS) for *Ameca splendens* (AS), *Zoogoneticus purhepechus* (Zp), and *Goodea atripinnis* (Ga) from the Teuchitlán River, Mexico; abbreviations: sites (A, B, C, D, E), months (1 = January, 2 = March, 3 = May, 4 = July, 5 = September, 6 = November); reproductive variables (I.II = immature and juvenile in growth fish, IV.V = fish in mature and mature fish, GSI = gonadosomatic index, FEC = fecundity); physical and chemical water characteristic for each study sites (DO = dissolved oxygen, Al = total alkalinity, Cl = chlorophyll *a*, HA = total hardness, pH = pH, TU = turbidity, SE =, DEE = deep, TRA = transparency, TEM = water temperature, SOL.SUS = suspended solids, SOL.DIS = total dissolved solids

Teuchitlán River, such as *Poecilia sphenops* Valenciennes, 1846 and *Pseudoxiphophorus bimaculatus* (Heckel, 1848), produce a greater number of embryos (*P. sphenops* with mean of 31 embryos per female) than the native goodeids (Ramírez-García et al. 2018), which is likely to contribute to an increase in exotic fish stocks.

Size at first maturity (L_{50}). Environmental variables (high values of dissolved oxygen, low values of alkalinity, high water temperature, and low dissolved solids) are directly related to gonad development (Salgado Ugarte et al. 2005). We found that females mature earlier than males in the headwaters, which may be advantageous since maturation at a smaller size means a greater production of offspring over the entire lifespan. At the same time, this explains why populations of native species decrease downstream. Ramírez-García et al. (2018) stated that, in exotic species

Summary of reproductive cycle information pertaining to *Ameca splendens*, *Goodea atripinnis*, and *Zoogoneticus purhepechus* from the Teuchitlán River, Mexico

Characteristics	Ameca splendens	Goodea atripinnis	Zoogoneticus purhepechus
Size at sexual maturity	♀♀ ≈34.26 ± 9.58 mm ♂♂ ≈31.59 ± 11.69 mm	43.02 ± 8.9 ♀ + ♂	♀♀ ≈32.22 ± 7.34 mm ♂♂≈28.61 ± 5.06 mm
Fertility	6.00 ± 2.66 embryos	7.00 ± 1.49 embryos	6.00 ± 1.59 embryos
Spawning period	March and July	March (at springs) September and November (downstream)	March and November
Sex ratio (♀ ÷ ♂)	1 ÷ 1	1 ÷ 1	1 ÷ 1
Maximum size (TL)	♀ 59.26 mm ♂ 57.95 mm	♀ 94.41 mm ♂ 123.33 mm	♀ 39.30 mm ♂ 41.43 mm
Type of growth	Negative allometric	Positive allometric	Negative allometric

TL = total length.

(*Pseudoxiphophorus bimaculatus* and *Poecilia sphenops*) from the Teuchitlán River, the males reached maturity first at a smaller size than the females in all of the sites sampled, since these exotic species are well established along the river. Presentation of different sizes at first maturity is known in other goodeid species (*G. atripinnis*, *G. multiradiatus*, *Z. quitzeoensis*, *H. turneri*, *A. robustus*, and *A. diazi*) in different aquatic systems (Mendoza 1962, Moncayo Estrada et al. 2001, Ramírez-Herrejón et al. 2007, Salazar-Tinoco et al. 2010, Cruz-Gómez et al. 2011). **Sex ratio.** The observed proportion of females was greater downstream (sites C, D, and E), whereas the headwater populations showed nearly equal proportions for females and males. Ramírez-García et al. (2018) stated that, in exotic species from the Teuchitlán River, female *Pseudoxiphophorus bimaculatus* generally dominated throughout the year (~2 ÷ 1), while, for *Poecilia sphenops*, the sex ratio was generally ~1 ÷ 1. Sex ratios tend to be 1 ÷ 1 in cases where multiple factors in the aquatic system are in equilibrium (Valenzuela et al. 2003). Several authors have determined the equality of sex ratios for other species of goodeids (Moncayo Estrada et al. 2001, Ramírez-Herrejón et al. 2007). The pressures of natural selection direct populations towards equal sex ratios, providing an evolutionarily stable strategy (Maynard 1978). However, several species in the family Goodeidae present a female-biased population structure (Navarrete-Salgado et al. 2007, Cruz Gómez et al. 2010, Cruz-Gómez et al. 2011, 2013, Moncayo-Estrada 2012). In goodeids and poeciliids (viviparous and ovoviviparous species), the sex ratio generally favours females in wild populations, thus ensuring reproduction.
Reproductive period. The native fish species in the Teuchitlán River presented at least two reproductive peaks, depending on the portion of the river, as a reproductive strategy to adapt to variations of the habitat. However, the native species presented lower abundances than the exotic species such as *Poecilia sphenops* and *Pseudoxiphophorus bimaculatus,* which presented high reproductive output and iteroparous spawning, permitting population increase and demonstrating their effective exploitation of environmental resources (Ramírez-García et al. 2018). In other goodeid species such as *Alloophorus*

robustus, *G. atripinnis*, *Allotoca diazi*, and *Girardinichthys multiradiatus*, the reproductive season was related to the increase in water levels and temperature (Mendoza 1962, Gómez-Márquez et al. 1999, Cruz-Gómez et al. 2013), as was the case in the headwater of the Teuchitlán River.
Type of growth. The positive allometric growth in *G. atripinnis* and the negative allometric growth in *Z. purhepechus* and *A. splendens* can be related to variations in food availability, intra- and inter-specific competition, water temperature and dissolved oxygen at the different sites, according to Hepher and Pruginin (1985). The exotic species present in the Teuchitlán River, *Poecilia sphenops* and *Pseudoxiphophorus bimaculatus*, also presented allometric growth; however, these species presented negative growth at some sites of the river, (i.e., the headwater for *P. sphenops* and all of the sites apart from A for *P. bimaculatus*). This indicates that their weight increased to a greater proportion in relation to standard length. Positive growth indicated that the organisms presented the highest increase in weight in relation to standard length (Froese 2006, Ramírez-García et al. 2018).
Environmental and reproductive variables. Gonadal maturity stages showed differences in the habitat that are associated with ontogenic movements to deeper waters over the course of individual development, while pH and dissolved oxygen had stronger influences than the temperature on the mature organisms. Juveniles showed a persistent preference for downstream habitats throughout the season, which can be related to protection from predators and availability of food.
Goodea atripinnis is a well-established species along the river, showing a complete structure of sizes, and a frequency of gonadal stages at all five sites sampled in this study. This species matures earliest in the headwaters of the river, and later in the downstream reaches, showing that the environmental conditions act to affect its reproduction. Mature individuals seem to prefer the headwaters (springs), while the juveniles are more frequent downstream where there is a higher proportion of sedimentary solids, higher temperatures and a greater concentration of chlorophyll *a* (Fig. 4).
Ameca splendens presented lower abundances in the lower portion of the river. The females of this species

mature earlier in the upstream reaches (site C) and the females at site D mature at larger sizes. The males mature earliest at site A. A complete structure of sizes of *A. splendens* occurs all along the river, except at site E. However, mature individuals are more frequent at sites A, B, and D. Two reproductive peaks occur at the springs but only one notable peak occurs downstream (January to March). Immature organisms seem to prefer the environmental conditions of sites A and B (clear, deep waters, more dissolved oxygen in the water and neutral pH), whereas the mature organisms are associated with sites D and A (higher transparency, greater depth, harder water, and neutral pH).

Zoogoneticus purhepechus was the least abundant of the native species of the Teuchitlán River. Size at maturity was evaluated in the headwater springs only and showed that site A presented the best conditions for early maturity in both sexes. There were more mature individuals at site B, and more juveniles at site A and two reproductive peaks were found at site C (March to September). In the springs, one reproductive peak occurred from September to November. Mature organisms are associated with clear and deeper waters, higher dissolved oxygen and neutral pH. Juveniles are associated with sites with higher turbidity and dissolved solids in the water. This is similar to the results of Ramírez-Herrejón et al. (2007), who described the reproductive habitat of *Z. quitzeoensis* in La Mintzita. This species prefers shallow, neutral pH, warm and clear waters with abundant vegetation. First maturity occurs at 30 mm SL, the sex ratio is 1 ÷ 1, and fecundity ranges from 6 to 10 embryos per female with a reproductive peak presented in winter.

The Teuchitlán River has been altered by the extraction of water for human uses and introduction of exotic species, which has caused habitat loss, reduction in the density of native fish populations and low reproduction in many species compared to populations found elsewhere. The endemic species *A. splendens* and *Z. purhepechus* could face an elevated risk of extinction. However, our results provide baseline data with which to design a management and conservation plan for the native species of the Teuchitlán River and to promote the aquacultural research of species endemic to Mexico for conservation purposes.

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