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Research Article

Life-history traits of Balsas Splitfin Ilyodon whitei (Cyprinodontiformes: Goodeidae) Mexican endemic fish species

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Abstract

Ilyodon whitei, a small fish endemic to the Balsas River Basin, and even though it has been declared as Least Concern by the International Union for Conservation of Nature populations are severly fragmented and decreasing in many places. Despite the above, very little is known about the biological characteristics of this species. The aim of this study was to describe the population and reproductive structure of *Ilyodon whitei* in the Amacuzac River, Morelos, Mexico. To do this, we analyzed 1493 specimens from the Ichthyological Collection of the Centro de Investigaciones Biológicas, Universidad Estatal de Morelos, corresponding to five collection sites over one annual cycle. We evaluated size structure, sex ratio, length-weight relationship, gonadal maturity stage, size at first maturity, fertility, morphological indexes related to reproduction, and reproductive habitat. A spatial and seasonal pattern was observed in the size-class frequency distribution. The overall sex ratio was 1.2:1 (Females:Males). The results of the analysis of the length-weight relationship in both sexes by sites and seasons showed different types of growths. There was a spatial and seasonal pattern in the stages of gonadal development in both sexes. The length at maturity was 51.54 mm (females) and 48.12 mm (males). Fertility ranged between 9 and 42 embryos per female, averaging 18. Anthropogenic activities that have modified the Amacuzac River have caused the current population sizes of *Ilyodon whitei* to be very low. Since, as reported in the literature, goodeid species have a low tolerance to pollution and cannot reproduce successfully in disturbed waters, notwithstanding the foregoing, in Morelos there are no underlying specific conservation actions to maintain this species and the biodiversity of the Amacuzac River. There are no data available on aspects of reproduction of this species, so this study provides information for the first time, therefore, important information is provided that will allow carrying out strategies for the management and conservation of *Ilyodon whitei*.

Keywords Amacuzac River, Breeding habitat, Least concern, Fertility, Somatic indices

INTRODUCTION

The Central Mexican Plateau is of special conservation interest due to its high richness of freshwater fish. The family Goodeidae constitutes this region's most diverse fish component, with approximately 40 to 45 species (Ramírez-García et al., 2020). Four of those species are also found in the Great Basin of the United States (subfamily Empetrichthyinae). In contrast, the remaining species (subfamily Goodeinae) inhabit drainage basins in and around the Central Mexican Plateau.

On the Pacific slope, members of this family are distributed from the Aguanaval and San Pedro-Mezquital rivers southward to the Balsas River, while on the Atlantic slope, they are found in the upper reaches of the Salado and Pánuco river basins (Domínguez-Domínguez & Pérez Ponce de León, 2007). In all of these watersheds, human activities have severely altered freshwater ecosystems.

The introduction of exotic species and their parasites, pollution, water extraction, deforestation and overfishing have been the main causes of the decline in the populations of native fish species (Soto-Galera et al., 1999; Trujillo-Jiménez et al., 2010). All of these factors have affected the distribution of goodeid fishes in the region because the areas with a high number of species of the group are also those with the most human activities (Soto-Galera & Paulo-Maya, 1995; Domínguez–Domínguez et al., 2006). All this has made the subfamily Goodeinae one of the most endangered taxa in the world (Duncan & Lockwood, 2001).

All the watersheds in the State of Morelos, Mexico, are part of the Balsas River Basin, which is the largest river in southern Mexico and flows into the Pacific Ocean. In Morelos the basin is divided into three subbasins: The Nexapa or Atoyac, the Balsas-Mezcala and the Amacuzac (Contreras-MacBeath, 1995). Unfortunately, in some areas of the Amacuzac River, the Morelos state government built, without any preceding environmental study, sidewalls measuring 4 m high to prevent flooding by the heavy rains that affect the region. These infrastructure projects also included drainage and widening the riverbed in some areas, modifying the river environment. Following these projects, individuals of some fish species were no longer detected in areas where they had previously been captured, including the Balsas Splitfin, *Ilyodon whitei* (Meek 1904). This small fish is endemic to the Balsas River Basin, even though it has been declared Least Concern by the International Union for Conservation of Nature (Lyons et al., 2020) populations are severely fragmented and decreasing in many places (Koeck, 2019).

In a prospecting study carried out between 2015 and 2018 using capture and recapture techniques, we did not find the species in some of the locations reported in Trujillo-Jiménez et al. (2010) or Mejía-Mojica et al. (2012). Furthermore, Cordero-Martínez et al. (2022) mention that in ten study sites along the Amacuzac River during a one-year period (2019-2020), they captured only

14 specimens, evidencing low relative abundance and prevalence and suggesting possible reductions in their population sizes.

In general, little information is available on the life history of most goodeid species. This is the case of *I. whitei*, for which only aspects of its diet (Trujillo-Jiménez & Díaz-Pardo, 1996) are euryphagous and opportunistic since it tends either toward an herbivorous or carnivorous diet according to availability and histology of its ovaries are known (Uribe et al., 2006; 2014). Therefore, this study's main aim was to describe the population and reproductive structure of *I. whitei* in the Amacuzac River, Mexico. For some species and lineages, the situation in the wild is so dire that captive maintenance and breeding programs must be implemented as soon as possible to avoid their imminent extinction (Lyons et al., 2019), so with this study, results will be contributed important information that can be used to support conservation actions for the species.

MATERIAL AND METHODS

Río Balsas system with an area of 117,305 km^2, is the largest basin of Mexico's Pacific slope, encompassing parts of seven states. Flowing for 770 Rkm, it is also one of the longest rivers in México. It is delimited by two physiographic provinces: The Neovolcanic Axis to the north and the Sierra Madre del Sur to the south (Pease *et al.*, 2023). All the aquatic drainages in the state of Morelos are part of the Río Balsas system. This river is divided into three systems in the state: The Amacuzac, which has an area of 4303:39 km^2. The Nexapa or Atoyac with 673:17 km^2 and the Balsas Mezcala with 1:6 km^2 (Contreras-MacBeath et al., 1989).

The Amacuzac River generates a volume of approximately 4,216 million m^3, of which 3,432 represent runoff that can be used. Of these, 880 million m^3 are used in the agricultural, domestic, commercial and industrial sectors, so there is an availability of 2,552 million m^3. It has a total hydrological surface of 117,406 km^2, distributed in three subregions: Alto Balsas 50,409 km^2, Medio Balsas 31,951 km^2 and Bajo Balsas 35,046 km^2 (CONABIO y UAEM, 2004) and has a marked seasonality of rains and dry season. The prevailing climate is warm subhumid, mainly in the lower areas of the Amacuzac River (García, 1988). The dominant vegetation in the area is composed mainly of low deciduous forest (Aguilar, 1999).

We analyzed 1493 specimens preserved in 70% alcohol from the Ichthyological Collection of the Biological Research Center at the Autonomous University of the State of Morelos "Dr. Edmundo Díaz Pardo" (CICIB-UAEM, registration ke: MOR-CC-243-2011). Catalogue numbers: 3405 at 3451. The specimens were collected over the course of one yearly cycle (1999–2000), including sampling during the dry season (November–December 1999; January, March, April and May 2000) and the rainy season (August–October 1999; June–July 2000). These specimens were collected in five localities along a 60 km stretch of the Amacuzac River. The site E1, named "El

Estudiante", had the highest altitude (892 m) and site E5, "Las Huertas" was the lowest one, with 742 m (Figure 1). The substrate along the river the substrate was divided into sandy (E1 to E3) and muddy-sandy (E4 and E5). Based on the percentage of land uses in the area of influence of the study sites, along the Amacuzac River, Morelos, it was observed that site E1 presents 53.7% of natural vegetation, which increases downstream. (E5) to 86.95% (Bonilla, 2022), which allows there to be several microhabitats and therefore food availability.

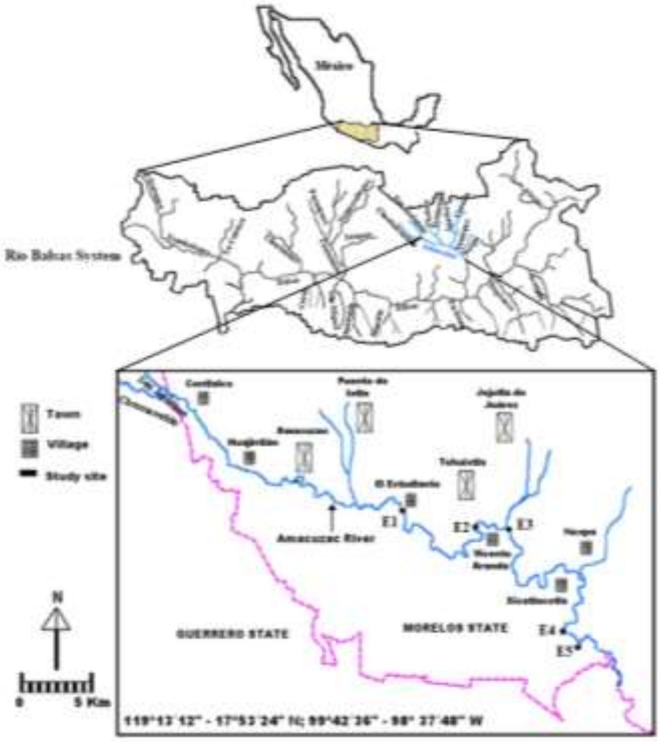

Figure 1. Location of the study localities: E1) El Estudiante, E2) Vicente Aranda, E3) Los Lagartos, E4) Las Granjas, E5) Las Huertas.

For each specimen, we measured the standard length (SL in mm) with a calliper (±0.1 mm); total weight (TW), eviscerated weight (EW), liver weight (LW), and gonadal weight (GW) were measured with a digital balance (0.01 g). Sex was determined by external sexual dimorphism and was subsequently corroborated by removing and examining the gonads.

The normal distribution of all variables was analyzed using the Kolmogorov-Smirnov test and the homogeneity of variance using the Levéne test. Since residuals had no normal distribution in any case, the Kruskal Wallis nonparametric analysis of variance and Dunn's tests (a posteriori) and the Chi-squared test (X^2) were applied to identify significant differences between sites and seasons. All statistics were performed with the XLSTAT-Pro 2016 software.

Population structure was analyzed using body size, based on the relative frequencies of SL classes at each study site. Nine length classes were established with intervals of 10 mm using the following code: Class 1 (11-20 mm), Class 2 (21-30 mm), and so on, up to Class 9 (91-100 mm) (Vitule et al., 2008). We evaluated whether there were significant differences in length-class frequencies among study sites and seasons using a non-parametric Kruskal Wallis analysis (α = 0.05) (Samat et al., 2008).

Sex ratio was determinated as the proportion of females to males, expressed as a percentage of the total sample. We analyzed the differences between study sites with Kruskal Wallis and Dunn tests (α = 0.05).

The length-weight relationship was obtained by linear regression, calculating the values of *a* and *b* in the equation: $W = aSL^b$; where W is the body weight, SL is the standard length, *a* is the origin of the function or condition factor and *b* is the allometry coefficient, this was performed for each sex. The *a* and *b* values were estimated using a linearized form (Froese, 2006).

Gonadal maturity stages were estimated by study sites and seasons, following the criteria proposed by Ramírez-Herrejón et al. (2007) (Table 1).

Table 1. Gonadal maturity stages of livebearing fish proposed by Ramírez-Herrejón et al. (2007).

Female		**Male**	
Phase	**Description**	**Phase**	**Description**
I Immature	Small ovaries, very thin < 6 mm long, occupying between 30% and 50% of the visceral cavity, with packed eggs.	I Immature	Small testes, very thin and yellowish occupying ~25% of visceral cavity.
II Developing eggs	Ovaries longer than in Stage I (10 mm); eggs enclosed in ovarian tissue.	II Developing juvenile	Turgid and yellow testes, reaching less than 20% of the visceral cavity
III Free eggs	Ovaries with free eggs in the ovarian lumen, display eggs that vary from stages close to fertilization to stages with embryos (~2 mm). The embryos are still enclosed in the same membrane.	III Juvenile	In conditions similar to stage II, occupying less than 50% of the visceral cavity. They retain their yellowish color.
IV With embryos	Ovary with embryos no completely formed, standard length >3.5 mm, eyes of the embryos are not completely developed, occupying 50% of the visceral cavity.	IV Maturing	Whitish translucent testis occupying 50% of visceral cavity; fish reaches sexual maturity.
V After spawning	Ovaries having flaccid walls and few visible eggs, with rupture at end of gonad.	V Mature	Turgid whitish opaque testis occupying occupying more than 50% of the visceral cavity.
IV In recess	Recovery after spawning, without embryos; turgid ovaries > 6 mm long	IV In recess	Flaccid and transparent testis; corresponding to semen ejaculation phase.

To estimate the size at first maturity where 50% of the individuals were sexually mature (L_{50}) of each sex, we used the probity method, performed in XLSTAT-Pro 2016 software. The analysis was performed on all fish analyzed and was calculated separately by sex and by study sites. Differences were determined using a Chi-squared test (X^2, $\alpha = 0.05$).

A fertility model (F) was obtained based on the data from embryonated eggs and embryos and fitted to the potential model of Schoenherr (1977). A correlation analysis (Pearson's coefficient) determined the relationship between F and biometric factors (SL and EW).

The gonadosomatic index (GSI) and hepatosomatic index (HSI) are indicators of the mobilization of energy reserves from the hepatopancreas to the gonads, which can be used to estimate reproductive activity. The GSI was calculated by dividing the gonadal mass by the total body mass × 100, while the HSI was calculated by dividing the liver mass by the total body mass × 100 (values in grams). Fulton’s condition (Froese, 2006) was calculated as $K = 100(W/SL^b)$, where W is wet body weight (g) and SL is length (cm), and the value of 100 is a scaling factor used to bring K closer to unity; b is the exponent of the arithmetic form of the relationship weight-length. We analyzed the differences between seasons and sites with a non-parametric Kruskal Wallis analysis ($\alpha = 0.05$).

The specimen samples have a database of physicochemical parameters: water temperature (ºC), dissolved oxygen (mg/L), hydrogen potential (pH), conductivity (µS/cm), total hardness ($CaCO_3$ mg/L), total alkalinity (mg/L), chlorine (mg/L). We applied Kruskal Wallis tests to describe differences among sites and seasons. We measured the relationship between physicochemical parameters and reproductive characteristics (gonadal stages, GSI, HSI and K) with correlation tests (Spearman correlation, $\alpha = 0.05$).

RESULTS

A spatial and seasonal pattern was observed in the size-class frequency distribution, which is summarized in Figure 1. At the study sites “El Estudiante” (E1), “Vicente Aranda” (E2) and “Los Lagartos” (E3), size classes 1 and 2 had their highest frequencies during the dry season. Class 1 had its highest frequencies in the E2 and E3 sites, and class 2 had two maximum values (E1 and “Las Huertas,” E5). Size classes 3, 4 and 5 were detected during both periods in all study sites. Class 3 presented two maxima (the E3 site during the rainy season and the Las Granjas E4 site during the dry season). The highest frequency of class 4 occurred at the E3 and E4 sites during the rainy season, while class 5 had high frequency values in all five study sites during the rainy season. Class 6 was recorded at all of the study sites during the rainy season and reached its highest values at study sites E1, E2 and E5. Classes size 7 and 8 were only recorded at some of the study sites, and the highest values were obtained during the rainy season (for class 7, at E1 and for class 8, at E1 and E5). Class 9 was found only at the E2 site during the rainy season and had a low percentage (Fig. 2). There were no significant differences between the seasons (K = 0 3.84, p = 0.92) however, when the analysis was done by separate seasons (dry K = 14.06; p = 0.04; rainfall K = 15.5, p =0.014) and sites (K = 15.5, p = 0.0002), there are significant differences.

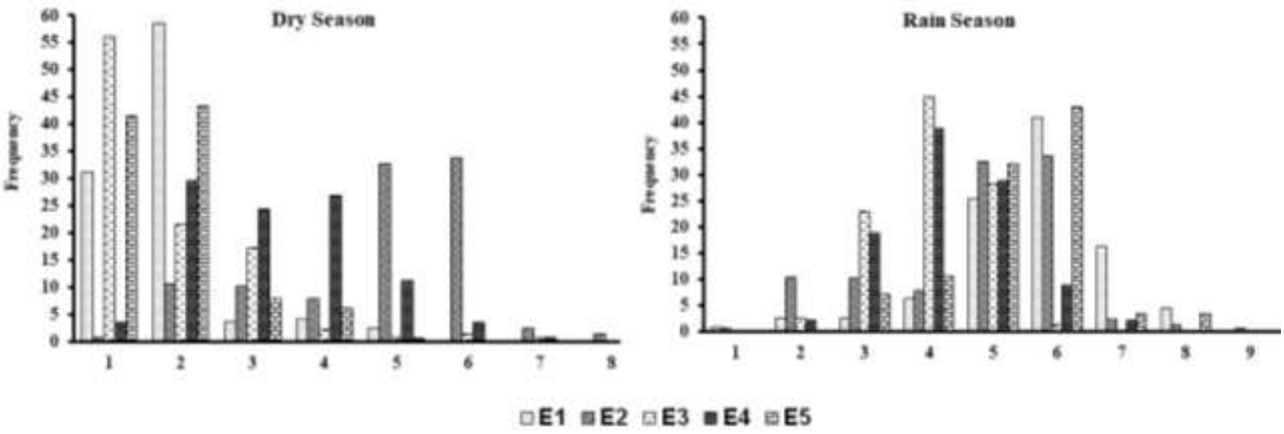

Figure 2. Size class frequency distribution by seasons and study sites. E1) El Estudiante; E2) Vicente Aranda; E3) Los Lagartos; E4) Las Granjas; E5) Las Huertas.

Of the 1,493 specimens collected, 359 (24.1%) were immature, 584 (39.1%) were mature females, and 550 (36.8%) were mature males. The sex ratio was 1.2:1 Female:Male. When considering the sex ratio at each study site separately, only the sex ratio at E1 differed from 1:1, having a significantly higher proportion of females than males, with a value of 2.8:1 (Female:Male) (Table 2). There were significant differences in sex ratio among study sites (α = 0.05, p = 0.020).

Table 2. Sex ratio of *Ilyodon whitei* at each study site. E1) El Estudiante; E2) Vicente Aranda; E3) Los Lagartos; E4) Las Granjas; E5) Las Huertas. *The sex ratio at E1 was significantly different from 1:1 (see results text).

Study sites	**Sex ratio (Female:Male)**
Overall	1.2:1
E1	2.8:1*
E2	1:1.1
E3	1:1.1
E4	1:1
E5	1.1:1

The results of length-weight relationship in females showed that the constant *b* had values below 3, indicating negative allometric growth in E1 (during both seasons), E2 in rains and E5 in dry season. In E2 (dry season), *b* had a value of 3, which corresponds to isometric growth. In E3 and E4, *b* was higher than 3 in both seasons, which corresponds to positive allometric growth. The analysis of the length-weight relationship in males showed negative allometric growth for E1 (dry season), E2 (both seasons) and E4 (rainy season). Meanwhile, specimens from E1 (rainy season), E4 (dry), E3 and E5 (both seasons) exhibited positive allometric growth (Table 3).

Table 3. Length-weight relationship in *Ilyodon whitei* at each study sites during each season. E1) El Estudiante; E2) Vicente Aranda; E3) Los Lagartos; E4) Las Granjas; E5) Las Huertas.

Study sites	Season	Female	Male
E1	Dry	$W = 0.0143L^{2.90}$ $R^2=0.8973$ (n=177)	$W = 0.0136L^{2.97}$ $R^2=0.9688$ (n=50)
	Rainy	$W = 0.0178L^{2.90}$ $R^2=0.9741$ (n=64)	$W = 0.0152L^{3.01}$ $R^2=0.9851$ (n=36)
E2	Dry	$W = 0.013L^{3.00}$ $R^2=0.989$ (n=127)	$W = 0.0145L^{2.91}$ $R^2=0.8722$ (n=132)
	Rainy	$W = 0.014L^{2.91}$ $R^2= 0.837$ (n= 140)	$W = 0.0146L^{2.97}$ $R^2=0.9887$ (n=158)
E3	Dry	$W = 0.0097L^{3.35}$ $R^2=0.989$ (n=74)	$W = 0.0111L^{3.18}$ $R^2=0.9855$ (n=64)
	Rainy	$W = 0.0096L^{3.18}$ $R^2=0.9383$ (n=27)	$W = 0.0098L^{3.18}$ $R^2=0.9554$ (n=49)
E4	Dry	$W = 0.0129L^{3.09}$ $R^2=0.9617$ (n=63	$W = 0.0107L^{3.24}$ $R^2=0.9935$ (n=49)
	Rainy	$W = 0.0106L^{3.14}$ $R^2=0.9749$ (n=35)	$W = 0.0232L^{2.70}$ $R^2=0.8999$ (n=49)
E5	Dry	$W = 0.0155L^{2.89}$ $R^2=0.9531$ (n=11)	$W = 0.0145L^{3.01}$ $R^2=0.9764$ (n=18)
	Rainy	$W = 0.0092L^{3.24}$ $R^2=0.8213$ (n=88)	$W = 0.0072L^{3.40}$ $R^2= 0.99$ (n=76)

A spatial and seasonal pattern was observed in the distribution of gonadal developmental stages of both sexes. Immature was recorded in all sites in the dry season; the highest frequency of immature individuals in both sexes was observed at E1 and the lowest in E4. Stage II was observed in both seasons and sexes, with maximum values in females in E4 (dry season) and E5 (rainy season); males had their highest percentage in E2, E4 and E5 (dry season). Stage III was demonstrated in both seasons and sexes. In females, there were two high values during the rainy season, in E2 and E3. The maximum values of the males were in E3 in the rainy season and in E4 they were recorded in both seasons. Stage IV in females was recorded in the rainy season at all study sites except E3; the highest value was observed in E1. In males it was observed in both seasons, with high values at all sites during rains and the highest value in E1. Stage V was recorded in females in E1 (rainy season), while in males it was observed in E1 (both seasons), with a relatively high value during the rains. Stage IV was not recorded at any study site in both sexes (Figure 3).

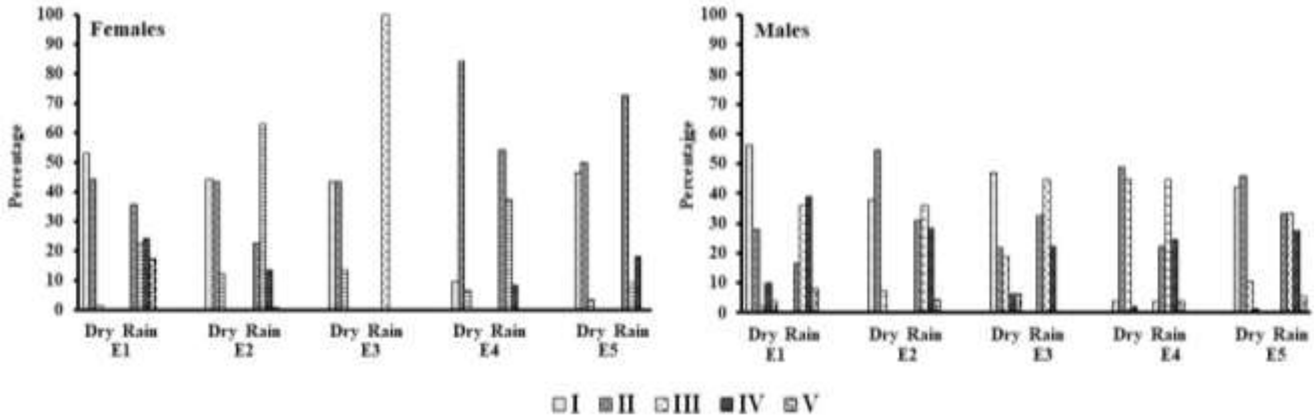

Figure 3. Gonadal maturation stages in percentages for sexes and study sites: E1) El Estudiante; E2) Vicente Aranda; E3) Los Lagartos; E4) Las Granjas; E5) Las Huertas.

The overall analysis of size at first maturity in 50% of individuals (L50) was 51.54 mm for females and 48.12 mm for males. When performing the analysis separately for each study site, females exhibited a higher value than males in E1, E2 and E4, while males present larger L50 in E3 and E5 (Table 4). There were no significant differences among study sites (X^2; p> 0.05).

Table 4. Estimated size at first sexual maturity by sexes and study site in *Ilyodon whitei*. E1) El Estudiante; E2) Vicente Aranda; E3) Los Lagartos; E4) Las Granjas; E5) Las Huertas. Values are given in percentages

Study sites	Female	Male
E1	61.05	54.36
E2	50.04	44.98
E3	38.33	50.56
E4	50.45	45.26
E5	48.54	53.91

Results of fertility showed that the number of embryonated eggs and embryos per female from ranged from 9 to 42, with an average of 18 per female. The highest value was detected in a female measuring 68.31 mm SL with a mass of 5.21 g collected at E1. Relation of F was not significantly related to SL (r=0.22, p>0.05) or mass (r=0.20, p>0.05).

Physiological condition in females were as follows, the gonadosomatic index (IGS) presented a reproductive peak at site E5 in the rainy season. The hepatosomatic index (HIS) in the dry season had high values, while in the rainy season it exhibited low values. Condition factor (Kn) had low values in both study periods. In males, the following values were recorded. IGS, unlike the females, had low values during both seasons. The IHS presented higher values in the dry season than in the rainy season. Kn exhibited values less than 2 in both study seasons (Figure 4). There was only a significant difference in the IHS index in females (Kn = 3.84, p = 0.0009) and in males (Kn = 3.84, p = 0.0009) by seasons.

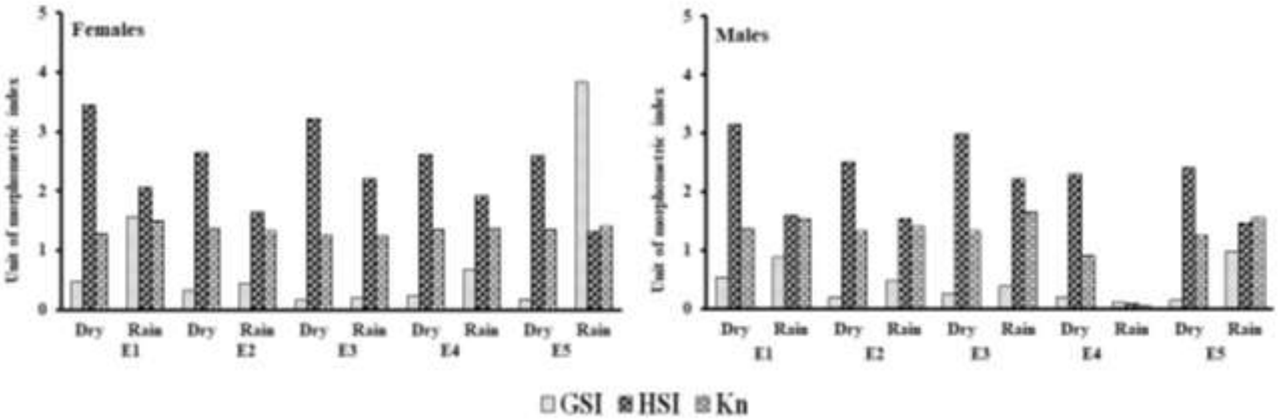

Figure 4. Morphological parameters of *Ilyodon whitei*. GSI, gonadosomatic index; HSI, hepatosomatic index; Kn, condition factor.

The data for the physicochemical parameters are shown in Table 5. Based on the Kruskal Wallis analysis, no significant differences existed between the year's seasons, but they were observed when study sites performed the analysis. The Spearman correlation showed no significant differences between physicochemical parameters and somatic indices related to reproduction. The Spearman correlation showed a significant relationship between GSI and Alkalinity (R^2 = -0.46 p = <0.0001), a significant relationship between the HSI and Alkalinity and chlorides (R^2 = 0.47, p = <0.0001, and R^2 = 0.44, p = <0.0001 respectively) and Gonadal maturation stages and chlorines (R^2 = -0.46, p =<0.0001). According to the ecological water quality criteria (CE-CCA-001/89; INE, 1989), all the parameters are within the permissible levels.

Table 5. Physical and chemical characteristics of water in Amacuzac River, Morelos, Mexico. Data by seasons and study sites. SD = standard deviation. Tem = water temperature (°C), DO = dissolved oxygen (mg L^{-1}), pH = hydrogen potential, Cond = conductivity (μS/cm), Hard= Total hardness ($CaCO_3$ mg/L), Alk = Total Alkalinity (mg L^{-1}) and Chlor = Chlorides (mg L^{-1}).

	Dry	**Rainy**	**E1**	**E2**	**E3**	**E4**	**E5**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Tem	24.5 ± 2.8	24.7 ± 1.7	23.2 ± 2.6	25.6 ± 2.3	25.2 ± 2.2	26.6 ± 1.8	23.5 ± 2.2
Min-max	19.8 - 30	21 - 27	19.8 - 28	23 - 30	22.8 - 29	22 - 28	20 - 27
DO	6.8 ± 1.7	8.6 ± 3.1	7.7 ± 2.4	8.6 ± 2.4	7.2 ± 3.2	7.6 ± 2.8	6.9 ± 2.6
Min-max	2.4 - 9.8	3.5 - 12.8	4.8 - 12.6	4.4 - 12.8	2.4 - 12.4	4.5 - 11.9	3.5 - 11.5
pH	7.3 ± 0.3	7.2 ± 0.6	7.3 ± 0.34	7.3 ± 0.21	7.1 ± 0.6	7.1 ± 0.7	7.2 ± 0.4
Min-max	6.6 - 8.1	5.5 - 7.9	7.0 - 8.0	6.9 - 7.5	6 - 8.2	5.5 - 8.1	6.6 - 7.9
Cond	386.7 ± 269.1	1702 ± 644.9	383.7 ± 276.5	613.2 ± 525.5	811.6 ± 678.1	935.3 ± 706.3	778.4 ± 668.8
Min-max	150 - 1559	230 - 1996	150 - 970	279 - 1715	270 - 1980	315 - 1996	268 - 1869
Hard	330.8 ± 300	203 ± 107.8	172.4 ± 154	308.3 ± 296.02	310.9 ± 265.4	285.3 ± 265.3	288.4 ± 232.5
Min-max	29.2 -816.8	40 - 400.4	36.1 - 544.5	30.03 - 816.8	44.04 - 400.4	46 - 780.8	29.2 - 400.4
Alk	204.7 ± 72.7	115.7 ± 52.2	126 ± 80.2	163.8 ± 71.5	197.6 ± 85.3	170.1 ± 71.1	164.5 ± 83
Min-max	20 - 322	44 - 231.5	20 - 266	55 - 242	81 - 322	71 - 279	54 - 291
Chlor	11.2 ± 2.9	7.7 ± 2.0	10.04 ± 5.1	8.9 ± 2.6	9.4 ± 2.4	9.5 ± 2.3	10.1 ± 2
Min-max	6.5 - 14.5	45235	5 - 19.5	8 - 13.5	6 - 12.5	6.5 - 13.5	7.5 -14.5

DISCUSSION

The Goodeinae subfamily has species with sizes no longer than 200 mm of standard length (Miller et al., 2005). *Ilyodon whitei* as well as other species *(Ameca splender, Xenotoca variata, Chapalichthys pardalis)* are considered medium-sized with a range of 90 to 101 mm, compared to most species with small sizes with around 42 mm *(Neotoca bileneata, Skiffia francesa, Skiffia lermae, Skiffia multipunctata*), and large sizes with values of 200 mm*: Goodea atripinnis* (Miller et al. 2005) and *Allophorus robustus* (Soto-Galera et al., 1990).Size-class frequency distribution of *I. whitei* evidenced the differential use of spatial resources (study sites) over time (study periods) in the longitudinal gradient of Amacuzac River. This may be the result of different factors such as period and location for reproduction, spatial-temporary variations in environmental conditions and food availability. The larger classes reached their maximum frequencies during the rainy season in upstream sites, which are deeper and have more stream and bank vegetation and a rocky substrate that can be used by fish as shelter or for feeding. Smaller size classes had higher frequencies at downstream sites, which are shallower, have slower currents, and where there are small ponds with submerged vegetation. Medium size classes were recorded along the entire river, but the highest percentages were in the middle during the rainy season. In this regard, Wootton & Smith (2014) mentions that the ichthyofauna of river systems tend to have changes in their spatial distribution patterns and habitat use due to temporal variations related to reproduction and/or foraging.

The values of slope *b* of the length-weight relationship show that at both sexes tend to have positive allometric growth year-round at downstream sites, while in upstream sites, they have negative allometric growth. These changes have been observed to depend on several factors, such as food availability, diet, dissolved oxygen concentration, temperature, presence of toxic metabolites, genetics, physiology, maturation stage, health status, behaviour, seasonality, sex, and intra- and interspecific competition at different sites (Froese, 2006; McPherson et al., 2011). Different types of growth have been reported, including allometric growth in *Girardinichthys multiradiatus* (Meek 1904) (Navarrete-Salgado et al., 2007); positive allometric growth in *Hubbsina turneri* (de Buen 1940), *Goodea atripinnis* Jordan 1880 (Moncayo-Estrada, 2012; Ramírez-García et al., 2020), and negative allometric growth in *Zoogoneticus purhepechus* Domínguez-Domínguez, Pérez-Rodríguez & Doadrio 2008, *Ameca splendens* Miller & Fitzsimons 1971 (Ramírez-García et al., 2020).

Fishes present diverse strategies to adapt to habitat variations and thus ensure reproduction. These include having at least two reproductive peaks: *A. splendens*, *Z. purhepechus*, *G. atripinnis; Allotoca zacapuensis* Meyer, Radda & Domínguez-Domínguez 2001*; Alloophorus robustus* (Bean 1892)*; Skiffia lermae* Meek 1902*; Xenotoca variata* (Bean 1887) (Ramírez-García et al., 2020; 2021), multiple reproductive cycles: *Girardinichthys viviparus* (Bustamante 1837) (Díaz-Pardo & Ortiz-Jimenez, 1986); *Allotoca dugesii* (Bean 1887) (Soto-Galera & Paulo-Maya, 1995) or a continuous reproductive period, as evidenced in the Balsas Splitfin. *Ilyodon whitei* had maximum spawning during the rainy season in the middle part of the river, where the river’s course covers terrestrial areas to form pools with vegetation, providing food, protection, and better environmental conditions that likey increase offspring survival. This coincides with what has been reported in other goodeids *Zoogoneticus quitzeoensis* (Bran 1898), *G. multiradiatus* (Macías-García & Saborio, 2004; Navarrete-Salgado et al., 2007); *H. turneri* de Buen, 1940 (Moncayo-Estrada, 2012).

The sex ratio recorded for the study sites was close to 1:1 (F:M), except at the E1 site, where females were dominant. Some species in this subfamily have a 1:1 ratio (*Z. quitzeoensis*; Ramírez-Herrejón et al., 2007; *H. turneri*, Moncayo-Estrada, 2012). However, in other studies, it is reported that the female ratio is higher (Ramírez-García et al., 2021). In this regard, Wootton & Smith (2014) report that the sex ratio in wild populations generally favours females to ensure offspring; likewise, foraging behaviour and high predation lead to higher mortality among males.

Males of *I. whitei* mature at small sizes than females at all study sites, which is consistent with other goodeid species: *Z. quitzeoensis* (Ramírez-Herrejón et al., 2007, 2021); *G. multiradiatus* (Navarrete-Salgado et al., 2007)*; A robustus*, *A: zacapuensi*, *X. variata*, *H. turneri*, *S lermae* and *Z. quitzeoensis* (Ramírez-Garcia et al., 2021). However, it does not coincide with the results reported by Ramírez-García et al. (2020), who found that in *G. atripinnis*, *A. splendens* and *Z. purhepechus*, females mature earlier than males which they consider that this characteristic may be advantageous since maturation at a smaller size may result in greater lifetime production of offspring. Wootton & Smith (2014) mention that fish modify their reproductive strategies between sexes, due to environmental pressures, water quality, flood cycles experienced by different rivers, and ecological and environmental pressures. predation.

The average fertility in *I. whitei* was 18 embryos, which does not coincide with that reported by Uribe et al. (2006) 30 embryos; however, those authors only reported data from three gravid females, so that small sample size may have resulted in bias. Similar fertility to our results has been reported in *H. turneri* (Moncayo-Estrada, 2012), *G. atripinnis* and *G. atripinnis* Jordan 1880, reported as *Characodon luitpoldii* (Mendoza, 1962), and *A. robustus* (Soto-Galera et al., 1990). The fertility of *I. whitei* is low compared to that reported in *Z. quitzeoensis* (Mendoza, 1962) and *G. viviparus* (Díaz-Pardo & Ortiz-Jiménez, 1986) but relatively high compared to *G. multiradiatus* (Ramírez-Herrejón et al., 2007), *A. splendens*, *G. atripinnis* and *Z. purhepechus* which have low average fertility (Ramírez-García et al., 2020).

Both sexes presented an increase in GSI during the rainy season at sites E1 and E5, which correlates with the presence of the gonadal maturation stage (stage V), with a decrease in the dry season, when there is a greater number of individuals of stage I, which suggests that spawning has already taken place. This coincides with *Z. quitzeoensis* where both sexes present high values in winter, a time when sexually mature specimens of both sexes are consistently present, and the highest frequency of females with embryonated ovaries and mature males is recorded (Ramírez-Herrejón et al., 2007). While *G. multiradiatus* shows high values of gonadal maturity in the rainy season (Cruz-Gómez et al., 2010), *H. turneri* has a reproductive period of eight months (March to October) with a peak in summer (June-August) that corresponds to the moment of the maximum value of GSI (Moncayo-Estrada, 2012). This study recorded a decrease in GSI and an increase in HSI were recorded during the dry season. This is interpreted as increased reserve materials stored in the liver for later use in gamete production. In females, low HSI values before and during reproduction may be due to the allocation of energetic materials stored in the liver to gonadal maturation and the reproductive event (Santos et al., 1996). The Kn values in both sexes exhibited high values in the rainy season, when the highest frequency of gonadal development was recorded in Stage V (i.e., active reproduction); this coincides with what was reported in *G. multiradiatus* (Cruz-Gómez et al., 2013) and *H. turneri* (Moncayo-Estrada, 2012).

In different viviparous fish species (*G. atripinnis*, *Allotoca diazi* and *G. multiradiatus*), temperature, pH and dissolved oxygen are closely related to reproductive periods (Mendoza, 1962; Moncayo-Estrada, 2012; Ramírez-García et al., 2020), however, in *Ilyodon whitei* there is no relationship between these parameters and reproductive peaks. Ramírez-Garcia et al. (2021) mentioned that the lack of correlation between indices related to reproduction and environmental variables may be related to the preproduction strategy of goodeids comprising viviparous species, which causes eggs and embryos develop inside the female's body; the various external environmental conditions less influence them.

Ilyodon whitei exhibits an opportunistic reproductive strategy that is associated with early maturity, small oocytes, small clutches and continuous spawning (Winemiller & Rose, 1992) and belongs to the internal carrier eco-ethological guild, which comprises viviparous species with iteroparous matrotrophic trophotenia. It is currently an LC species (IUCN 2020). In a study carried out by Pita (2019), it is mentioned that the Amacuzac River has been altered by the drainage and widening of the riverbed, modification of the river environment, extraction of water for human use, introduction of exotic species, and contamination of the river water and substrate with chlorpyrifos and heavy metals. These factors have caused habitat loss, reduction in the density of native fish populations and low reproduction in many species. Immediate corrective measures must be taken to prevent species loss, such as the maintaining breeding stock and rearing larvae under laboratory conditions. Some general aspects of fish conservation under field conditions, such as the elimination of exotic invasive (*Andinoacara rivulatus, Oreochromis mossambicus, Thorichthys helleri, Thorichthys maculipinnis, Tilapia zillii, Amatitlania nigrofasciata, Ictalurus punctatus, Pterygoplichthys disjunctivus, Pterygoplichthys pardalis, Pseudoxiphophorus bimaculatus, Poecilia reticulata, Poeciliopsis gracilis*); competitor species, could also be attempted to protect *I. whitei*. Zúñiga-Vega et al. (2022) mention that the demographic characteristics of most viviparous species remain completely unknown and quantitative information is urgently needed to guide future conservation strategies. Therefore, the information generated in this study and previous information on diet (Trujillo-Jiménez & Díaz-Pardo, 1996) and histology (Uribe et al., 2006, 2014) will contribute to the search for management and conservation options for this species. Therefore, important information is provided to allow the carrying out of strategies for managing and conserving of *Ilyodon whitei*. Currently, the members of the ichthyology laboratory of the Biological Research Center of the Autonomous University of the State of Morelos are carrying out conservation strategies for the species, such as maintaining broodstock in laboratory conditions. Likewise, several live specimens have been translocated to the Las Estacas Nature Reserve (Com. per. Dr. Topiltzin Contreras MacBeath), based on the Theory of Change (ToC) consisting of (1) restoration and reintroduction, (2) conservation management, (3) explorations, and (4) communication and outreach to gain support for conservation interventions. The framework has been developed as a tool for conservation advocates and policymakers to implement and monitor change that prevents extinctions, but also to seek and attract funding. It is also meant to guide different levels of government in setting priorities for conservation interventions (Contreras-MacBeath et al. 2022).

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Authors' Contributions

PTJ: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing-original draft, writing-review and editing.

KKBR: Data curation, formal analysis, investigation, methodology, writing-original draft, writing-review

RCB and MGBZ: Investigation, statistical analyses, writing-original draft, writing-review

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical approval

For this type of study, formal consent is not required, since collection specimens were used.

Data Availability

All data generated or analysed during this study are included in this published article

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