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LENGTH-WEIGHT RELATIONSHIP, CONDITION FACTOR AND SEX OF *Pseudoxiphophorus bimaculatus* IN A BORDO AT MORELOS STATE

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Abstract: The *Poeciliidae* family belongs to the order Cyprinodontiformes, which are small fish that inhabit freshwater bodies and are recognized as ornamental fish for aquariums and to eliminate mosquitoes and their larvae. The objective was to determine the relationship between length and weight, condition factor's and sex ratio of Twospot livebearer and its relationship with environmental factors. Monthly samplings were carried out from March 2017 to February 2018 and captured with 0.005 m mesh net. For each organism the total weight (Lt), parent weight (Lp), total weight (Pt) was measured and sex determinate. Additionally, the physical and chemical parameters of the water were analyzed. It was captured 374 fish *Pseudoxiphohporus bimaculatus*, with size ranges for females from 2.3 to 8.6 cm (total weight of 0.22 to 7.62 g) and males from 2.6 to 5.8 cm (weight of 0.18 to 2.84 g). The largest capture was obtained in May. 54.8% were female (205 organisms), 31.3% were male (114 individuals) and the remaining were undefined (55 fish). The sex ratio favors the female (1.8:1). The length-weight relationship for the female sex indicates positive allometric growth (b = 3.09) and the same for the male, but the latter with a tendency to isometry (b=·3.003) with greater growth in weight than in size. The relative condition factor indicates good conditions in July for both sexes. The microreservoir present adequate conditions for the development and reproduction of *P. bimaculatus*.

Keywords: Sexual dimorphism, condition, allometry

INTRODUCTION

Fish are the most abundant vertebrates on the planet, constituting more than half of all vertebrates in the world (Nelson *et al.,* 2016). Currently, about 32,000 named species are recognized and about 200 species are found annually (Fricke *et al.,* 2024). It is estimated that about 41.2% of fish species are strictly freshwater, this diversity is very large since only about 0.0142% of the total water on our planet is freshwater (Burr and Warren, 2014; Fricke *et al.,* 2024).

Mexico has a rich and diversified freshwater fish fauna which includes more than 503 described species (Miller *et al.,* 2009; Nelson *et al.,* 2016). The Poeciliidae family is found within the order Cyprinodontifor0mes (Lucinda and Reis, 2005). Representatives of this family are distinguished by being small, laterally compressed fish that inhabit freshwater and brackish water bodies. They are distributed from the eastern United States of America to northeastern Argentina. They comprise about 42 genera and 353 species, most of them small. They have sizes between 30-70 mm in total length, and can reach maximum sizes of up to 200 mm in some species (Miller *et al.,* 2009; Nelson *et al.,* 2016).

The Poecilid fishes have been introduced into freshwater systems in Mexico, mainly by aquarists (Mejía-Mojica, 1992; cited in Ramírez-García *et al.,* 2018), for the control of mosquitoes and their larvae (Miller *et al.,* 2009), for experimental studies and as forage species (Gómez-Márquez *et al.,* 1999;), and this process has been facilitated by their viviparous reproductive strategy (Miller *et al.,* 2009; Greven, 2011; Nelson, 2016).

The central region of Mexico, specifically the central plateau and the Balsas basin, is one of the main centers of cultivation, importation and sale of many of these species in Mexico, and *P. bimaculatus* was accidentally introduced as a product of aquaculture activities (Contreras-

MacBeath *et al.,* 1998; cited in Contreras-MacBeath *et al.,* 2014).

Pseudoxiphophorus bimaculatus (Heckel, 1848), synonym of *Heterandria bimaculata* (Agorreta *et al.,* 2013), is native to the Atlantic slope, in the middle and lower parts of streams from the Misantla River, Ver. in Mexico to the Prinzapolka River in Nicaragua, known from Campeche, Chiapas, Oaxaca, Quintana Roo, Tabasco, Veracruz and Yucatan (Miller *et al.,* 2009). It has been introduced into several basins in Mexico, including the upper Balsas River basin (Espinoza *et al.,* 1993; cited in Ramírez-García *et al.,* 2018), where it has reached a high degree of invasion in this region (Contreras-MacBeath et al, 2014) and in the Teuchitlán River, Jal. (Ramírez-García *et al.,* 2018).

The *P. bimaculatus*, commonly called "spotted guatopote", is a viviparous species that begins its reproductive stage when it reaches a size of approximately 22 mm in standard length in males and 27 mm in females (Gómez-Márquez *et al.,* 1999). It prefers environments such as springs, streams, lagoons, rivers and swampy ponds, clear lentic waters, but sometimes turbid or muddy, as well as well-shaded, slow-moving, more or less deep water, around fallen leaves and piles of organic matter or riparian plants on the water (Miller *et al.,* 2009).

It is an herbivorous species and also feeds on insects (carnivorous-insectivorous) and can occasionally consume eggs and larvae of other fishes (Trujillo-Jiménez and Toledo, 2007). Its reproduction takes place in the months of July-September (summer), during the rainy season in the state of Morelos. An adult female can release several broods in the same reproductive season, but the broods do not overlap, that is, they do not present superfetation (Gómez-Márquez *et al.,* 1999).

There are few studies on *P. bimaculatus* such as those cited by Gaspar-Dillanes (1987), Gómez-Márquez *et al.* (1999), Trujillo-Jiménez and Toledo (2007), Agorreta *et al.* (2013) and Ramírez-García *et al.* (2018). In the Amate amarillo reservoir, studies have been carried out on tilapia and water quality, leaving aside the characterization of fish such as *P. bimaculatus*, which have great value as a forage species and, being part of these ecosystems, have a very important ecological function in the aquatic environment, because they are part of the trophic web and energy flows that occur in it. The objective of this study was to investigate some aspects of the biology of this invasive species *P. bimaculatus* and its relationship with environmental factors.

MATERIAL AND METHODS

The Amate amarillo bordo is located in the municipality of Ayala, in the central-eastern part of the state of Morelos at the coordinates 18°44′ north and 98°58′ west, at 1267 m.a.s.l. (INEGI, 2021). It has an area of 7.84 ha and a perimeter of 1379 m (Figure 1). The climate of this area is warm subhumid with summer rains Aw0′′(w)(i')g, with an average annual temperature of 24°C and a precipitation of 894 mm (INEGI, 2021). The predominant type of vegetation in this state is Low Deciduous Forest and grassland (INEGI, 2010).

Figure 1. Location of the Amate amarillo bordo in the Municipality of Ayala, Mor.

Sampling was carried out monthly from March 2017 to February 2018. A single sampling station was established that was georeferenced using a GPS, located in the southwestern part of the system, being the deepest part. At the site, the parameters of ambient temperature (thermometer); water temperature and dissolved oxygen (HANNA brand oximeter, model H19146); pH and electrical conductivity (HANNA multiparameter, model HI 991300); transparency and depth (Secchi disk) were evaluated. A Van Dorn bottle was used to take water samples and they were collected at five depths (0.30, 1, 2, 3 and 4 m). Subsequently, the collected water samples were poured into one-liter polyethylene bottles that were stored in a cooler at 4 °C until their analysis in the laboratory. After sampling, alkalinity (indicator method) and total hardness (complexometric method) were determined using conventional techniques (Gómez *et al.,* 2014).

Fish were collected using a 10 m seine net with a mesh size of 0.005 m, at the littoral zone of the system. The fish were then placed in a bucket and 3 to 4 drops of clove essence were added to anesthetize the organisms (García-Gómez *et al.,* 2002), then they were placed in 10% formalin for preservation. For fish biometry, a caliper (0.1 mm precision) was used to measure the standard length (Lp), total length (Lt) and height (A) of each of the organisms; with the help of an analytical balance (0.1 mg precision) the total weight (Pt) was obtained.

To determine the sex and gonadal maturity of the organisms, a ventral cut was made from the anal opening to the shoulder girdle to expose the gonads. Using a stereoscope, the stages of ovarian development were determined in females and, in males, by observing the gonopodium, considering the proposal made by Contreras-MacBeat and Ramírez-Espinoza (1996). This species is characterized by presenting sexual dimorphism.

The Analysis of Covariance (ANCOVA) was applied to determine if there were differences between the size and weight between the sexes; if there were, then the length-weight relationship was made by sex, in addition to calculating the relationship for the total population.

The length-weight relationship was analyzed using the potential type equation:

$$P=aL^b$$

where P is the weight of the individual in grams; L is the length of the organism in cm; a and b are constants which are estimated by linear regression analysis using the least squares method. This equation can be transformed into linear form by using logarithms (base 10) where b is a constant that determines the proportionality of the increases in length with respect to weight and log a is the intercept (Salgado *et al.,* 2005).

$$\text{Log } P = \log a + b \log b.$$

Since length is a linear magnitude and weight is equal to the cube of the size, if an individual maintains its shape as it develops, then the type of growth is isometric (b=3). When b<3, individuals present negative allometric growth, that is, they grow more in size. On the other hand, when b>3, individuals preferentially increase their weight and it indicates that it is a positive allometric growth (Froese, 2006; Nehemia *et al.* 2012). The type of growth (allometric or isometric) was obtained with the value of the slope of the length-weight graph, and to know if this value has a statistical difference, a test was made using the t-Student statistic (Salgado *et al.,* 2005; Pauly 1984).

$$t - Student = \frac{dslogLt}{dslog\ Weight} * \left[\frac{|b-3|}{\sqrt{1-r^2}}\right] * \sqrt{(n-2)}$$

Where dslogLt is the standard deviation of the logLt values, dslogPeso is the standard deviation of the logWeight values, n is the number of fish used in the calculation and R^2 is the coefficient of determination. The value of b is different from 3 if the t value is greater than the t values in the table for n-2 degrees of freedom (Pauly, 1984).

To determine the sex proportion monthly and total, the statistical test of chi-square distribution ($p<0.05$) was performed, to which the Yates correction was applied (Guerra, 2014).

The condition factor was used as an indicator of the welfare status of the organisms. This relative condition factor (Kr) or Le Creen (1951), expresses in fish, the relationship between the observed weight and the calculated weight according to the following mathematical expression:

$$Kr = \frac{P\ obs}{aLt^b}$$

where Pobs is the weight of the fish in grams and aLt^b, the weight calculated based on the length-weight relationship.

RESULTS

The Amate amarillo bordo is a system with a maximum total depth of 4.1 m in September and a minimum depth of 1.5 m in April, with a maximum water temperature of 24.01 °C (April) and a minimum of 18.2 °C (February), with thermal stratification during the sampling hours throughout the study. Regarding dissolved oxygen, the aquatic system has good oxygenation with an average of 7.7 mg L-1, with no anoxia detected at the bottom of the bordo and a clinograde curve behavior (higher concentration of dissolved oxygen on the surface and lower at the bottom). Transparency (visibility to the Secchi disk) recorded maximum values of 0.45 m during the rainy months (June to September).

During the study, a maximum pH value of 9.14 was obtained in October and a minimum of 7.6 in August; in the other months, there were values that ranged from neutral to slightly alkaline pH. Regarding total alkalinity, the maximum value was in June with 288 mg $CaCO_3$ L^{-1} and the minimum in February with 121 mg $CaCO_3$ L^{-1}; therefore, the aquatic system has a high productivity. The total hardness of the edge showed a variable behavior during the study, with a maximum value of 285 mg $CaCO_3$ L^{-1} and the minimum of 121 mg $CaCO_3$ L^{-1} (Table 1), which means that the water in this system is hard and the organisms have an efficient metabolism based on the water quality. When applying the Kruskal-Wallis statistical test to the data of the physical and chemical variables, it showed that, between the sampling months, there is a significant difference ($P<0.05$), which indicates that there was always variability in the behavior of each of the variables.

Parameters	Maximum	Minimum	Average
Water Temperature (°C)	24.01	18.2	22.1
Depth(m)	4.1	1.5	2.5
Transparence (m)	0.45	0.25	0.37
Dissolved oxygen (mg L^{-1})	12.3	3.71	7.7
Total alkalinity (mg $CaCO_3$ L^{-1})	288	164	218
Total hardness (mg $CaCO_3$ L^{-1})	285	121	195
pH	9.14	7.6	8.4

Table 1. Physical and chemical parameters recorded for the yellow Amate edge

A total of 370 individuals were captured, 203 fish were females and 113 were males, and 54 individuals whose sex could not be determined. The size range for females was 2.3 to 8.1 cm (total weight 0.18 to 7.62 g) and for males 2.6 to 5.8 cm (weight 0.22 to 2.34 g) (Figure 2). The largest catch was obtained in May and the smallest amount in September and October.

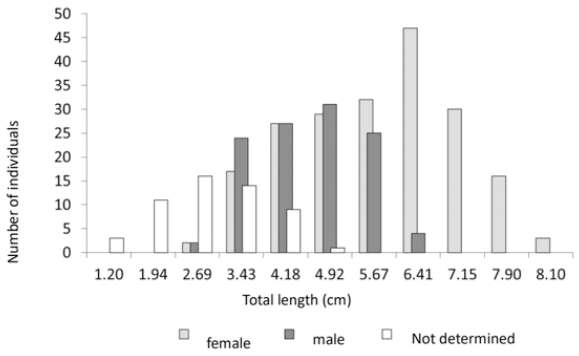

Figure 2. Size frequency distribution for the population of *P. bimaculatus*

The sex ratio was 4.2:1 (F:M) (χ^2=187.36; p<0.05) and there was female dominance (Figure 3) during the rainy season (August and September) and the cold season (December and January) of the study, except for October, where males dominated. No statistically significant differences were recorded in the other months.

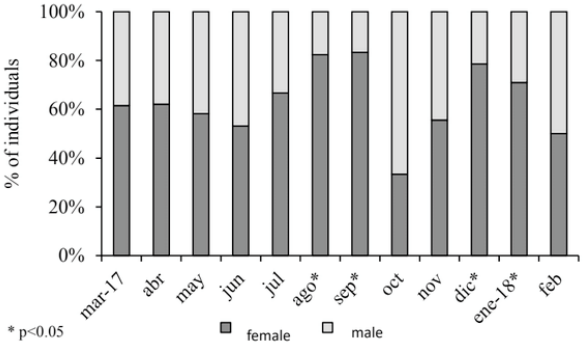

Figure 3. Temporal variation in the female-male ratio for *P. bimaculatus*

An analysis of covariance (ANCOVA) was performed to determine if there are differences between weight and length, taking into account sex, and it was found that there is a significant difference between males and females with respect to length and weight (F= 32.01, p<0.05). Because there are statistical differences between sexes, the length-weight relationship (total length) was performed independently.

The relationship between total length and standard length was estimated and a linear behavior was obtained between the variables and the model that represented this relationship was for the entire population Lp = 0.8648 Lt – 0.0557; R^2= 0.9927, which indicates that there is a high association between these variables, therefore, through one the value of the other variable can be obtained. For males and females, they are mentioned below:

Females: Lp = 0.8555 Lt - 0.1263; R^2 = 0.9907. (t-Student=2.6868; p<0.05)

Males: Lp = 0.8434 Lt - 0.0879; R^2 = 0.9712. (t-Student=3.8219; p<0.05)

Regarding the length-weight relationship for combined sexes of *P. bimaculatus*, it is represented by a potential model, where the value of the slope for the population (b=3.15) indicated that the type of growth is positive allometric, this indicates that the growth of the organisms tends to increase more in weight than in size (Figure 4), and perhaps this growth is influenced by the size and also by the state of maturation of the gonads of the females.

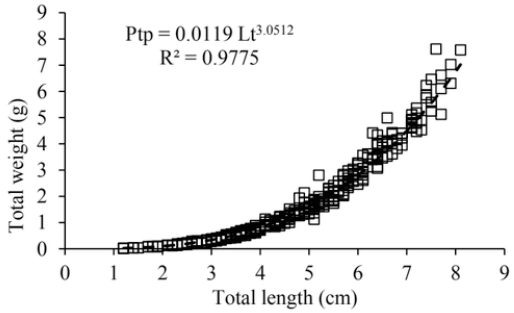

Figure 4. Length-weight relationship for combined sexes of *P. bimaculatus*

The total length-total weight length relationship for males and females (Figure 5) showed a potential type trend with negative allometric growth for males (t-Student=3.8219; p<0.05), while females showed positive allometric growth (t-Student=2.6868; p<0.05).

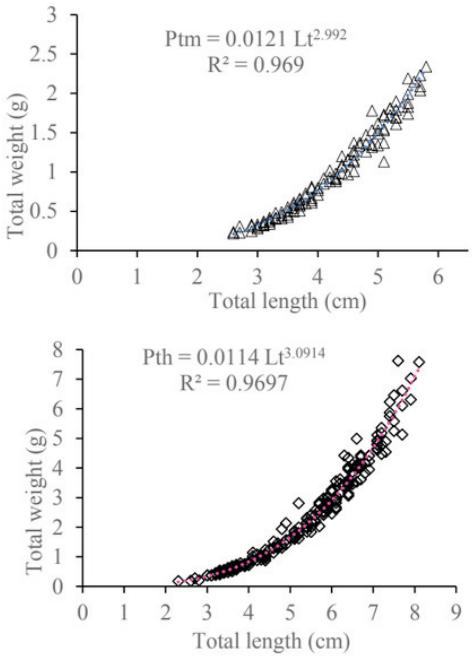

Figure 5. Length-weight relationship for males (Ptm) and females (Pth) of *P. bimaculatus*

The Le Cren condition factor (Kr) for males and females of *P. bimaculatus* increased during the months of June to August, coinciding with the increase in temperature, while, as the temperature decreased in the months of October to January, the condition factor decreased proportionally (Figure 6). The condition trend was to decrease towards the end of the study with maximums during the rainy season (greater than one) and minimums during the dry season. However, when associating the condition factor with environmental factors using Spearman's correlation, no significant relationship was found. Only a graphic relationship was observed between females and water temperature.

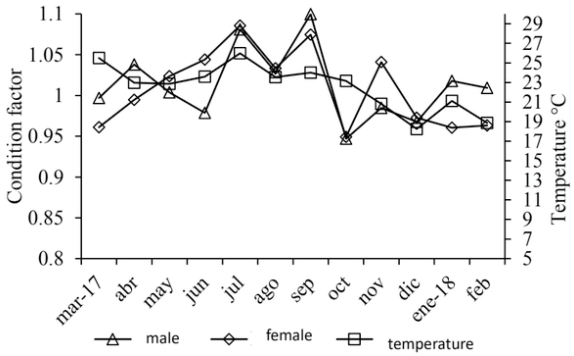

Figure 6. Relationship between water temperature and condition factor for males and females of *P. bimaculatus*

DISCUSSION

The state of Morelos has around 163 bodies of water (Torres-Albarrán *et al.*, 2023), of which 124 systems are natural reservoirs (lakes) and artificial ones (dams, microreservoirs, ponds, bordos, etc.) that can be permanent or temporary and depend on the time of year, as well as the objective for which they were built (Granados-Ramírez *et al.,* 2014). The Amate amarillo bordo is located in the warm zone, where temperatures are relatively constant and is characterized by having two well-established seasons, dry season and rainy season. This microreservoir is a small body of water of less than 10 hectares, shallow with a depth of 1.5 m during the dry season and up to 4.1 m during the rainy season, with an average transparency of 0.37 m, warm, well-oxygenated and highly productive waters, conditions that allow the development of *P. bimacualtus*. The hydrological behavior of this aquatic system is characterized by a dilution stage, due to the increase in volume during the rainy season (May-September) and a concentration stage during the dry season (March-May).

Gómez-Márquez *et al.,* (2013) mention conditions similar to those reported in this study. Likewise, Gómez-Márquez *et al.* (1999), report that *P. bimaculatus* can develop in temperatures of 23 to 29°C according to

the study carried out in El Rodeo, Mexico. Olinger *et al.* (2016) cite that *P. bimaculatus* can be present in temperatures below 16.5 to 25 °C in Honduras. However, Snelson (1989) mentions that the water temperature suitable for poecilids is 21 to 30°C, so the temperature of the Amate amarillo bordo is suitable for the development and reproduction of *P. bimaculatus*.

Regarding the maximum total length recorded in this study, that for the females was 8.1 cm and that for the males was 5.8 cm; this size of fish was larger than those reported in other water systems. Miller *et al.* (2009) report that the maximum size for *P. bimaculatus* is 6 to 7 cm. Gómez-Márquez et al (1999) cite that *P. bimaculatus* can reach standard length sizes of 6.9 cm for females and 6.0 cm for males. Ramírez-García *et al.* (2017) and Carbajal-Becerra *et al*. (2020), report a maximum size of 7.6 cm for females and 4.93 cm for males.

Lengths greater than those reported in this study were mentioned by Olinger *et al.* (2016), with values of 9.1 cm for females and 6.3 cm for males. The difference in size of individuals may be due to factors such as feeding, competition, capture area, mesh size, as well as environmental factors of the aquatic system such as water temperature, dissolved oxygen, water chemistry and the type of aquatic system being studied, lotic or lentic systems (Johnson and Bagley, 2011).

During the study period, it was observed that *P. bimaculatus* is a dimorphic species, with well-marked secondary sexual differences, a characteristic that it shares with all members of the Poeciliidae family (Pires *et al.,* 2011). It was recorded that females are larger than males, which tend to be smaller. Farr (1989) and Johnson and Bagley (2011) mention that the differences in size of the Poeciliidae family can be explained in terms of the fact that females take longer to mature and continue to grow throughout their life, whereas males mature quickly until they form the gonopodial structure and, once the gonopodium has been developed, males show lower growth rates, since they allocate their energy to reproduction.

Of the total number of organisms captured, the largest number corresponded to females. The monthly variation of this proportion showed that females dominated during the sampling period except in the months of October and February and the largest number of females was recorded during the month of August. Johnson and Bagley (2011) cite that there is evidence that in some species, densities change over time, typically in response to environmental changes such as variations in rainfall, food, etc.

Sex ratios in Poeciliidae species within the Cuautla River basin are generally skewed in favor of females (Contreras-Mac-Beath and Ramírez-Espinoza, 1996; Lorán-Nuñez *et al.,* 2013). The female:male sex ratio of *P. bimaculatus* at the Amate amarillo bordo was 1.8:1 (χ^2=24.5994; p<0.05). Gómez-Márquez *et al.* (1999) report a sex ratio of 1.7:1 at El Rodeo, Morelos. Ramírez-García *et al.* (2018) report a sex ratio of 1.9:1 at the Tecuchitlán River, Jalisco.

However, Olinger *et al.* (2016) cite a 1.1:1 sex ratio at Cusuco National Park, Honduras. Snelson (1989) and Johnson and Bagley (2011) mention that the populations of many poecilidae are biased in favor of females, because there is a difference in differential mortality between the sexes, since females have high survival rates due to their large size, weight and less attractive color than males.

The relationship between total length and total weight was potential, with positive allometric growth for females (b= 3.09) and negative allometric growth for males (b=2.99). This implies that fish grow more in length than in biomass in the early stages of growth, to avoid being preyed upon, and then grow

predominantly in weight than in length for the purpose of reproduction. Some species of the Poecilidae family have been recorded to show positive allometric growth (Ayala and Vera, 2007; Sánchez and Sastré, 2004). In contrast, Gómez-Márquez *et al.* (1999), Olinger *et al.* (2016), Gómez-Márquez *et al.* (2016) and Ramírez-García *et al.* (2018), report a slope value of less than 3 for both sexes, considering them as organisms with negative allometric growth.

Tesch (1968) mentions that the positive allometric growth type indicates greater growth in weight than in length. This type of growth is shown by females, since they reach larger sizes than males. In viviparous fish species, females are larger, in which fertility is directly related to the size of the organism. Gómez-Márquez *et al.,* (2016) cite that these differences could be due to the fact that the length-weight relationship can be influenced by sex, gonadal maturity, geographic location and environmental conditions.

The results of this study indicate that there was no high variation in the relative condition factor (Kr) for *P. bimaculatus* between sexes. Low Kr values for males and females were observed during the cold dry season (November-February), while high values were observed during the rainy season (May-September). In addition, Kr values for both sexes were close to 1.0, which suggested that *P. bimaculatus* individuals were in good condition during the study. This variability in the species could be due to differences in the size or nutritional condition of the organisms as mentioned by King (2007) and Ricker (1975).

The *P. bimaculatus* broods and juveniles showed a preference for clear, high-temperature, and highly oxygenated water in the shallow areas where they were observed. It was recorded that mature and adult fish were present in turbid waters, with high levels of suspended solids and high alkalinity. Therefore, this aquatic system has the appropriate conditions that allowed have good development and growth of the fish.

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