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THERMAL ECOLOGY OF POECILIOPSIS GRACILIS IN THE PÁNUCO BASIN

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Abstract: Specimens of P. *gracilis* were collected in the Santiago River under the Jihuico bridge, which were acclimatized and maintained at 25 °C to carry out the necessary evaluations in the laboratory. The critical temperatures (Tcmin: 9.2 °C, Tcmax: 40.8°C) and the preferred temperature range (Tp: 27.9 – 29.2 °C) were determined. Likewise, performance tests were carried out under different thermal treatments to generate a locomotor performance curve. With the best model for the performance curve, it was determined that the temperature range in which organisms can swim from their maximum speed to 80% of this value is B_{80}: 21.4 to 35 °C. Additionally, data from the National Collection of Mexican Fresh water Fishes (ENCB-IPN-P) were consulted to obtain records of the species in the Pánuco basin and the availability, use and preference of habitat was calculated, the thermal limits in field for the species 17.2 – 32.5 °C, with a thermal preference range of 27.5- 30 °C. The thermal preference ranges and the range of optimal temperatures estimated with field data coincide with those obtained in the laboratory.
Keywords: Thermal ecology, *Poeciliopsis gracilis*, critical temperature, preferred temperature, optimal temperature.

INTRODUCTION

Poeciliopsis gracilis (Heckel, 1848) is a small-sized viviparous fresh water fish that belongs to the Poeciliidae family, native to the Papaloapan basin (Miller et al., 2005). According to the Eschmeyer catalogue, it is distributed from Mexico to Honduras on the Atlantic slope and was introduced in California, the USA and Venezuela (Fricke et al., 2020). In recent years it has extended its distribution area towards the interior of the continent, translocating to the rivers of the Pánuco and Balsas basins (González et al., 2017). This species has been widely studied in Mexico, however, there are no studies that address the thermal aspects of the species, these are of great importance since they are among the priority factors to understand how a species adapts and uses its environment (Payne et al., 2016).

In aquatic environments, temperature is the factor that has the greatest effect on the development, growth and survival of fish while other factors may or may not be present to exert their effects (Hernández-Rubio and Figueroa-Lucero, 2013). For all practical purposes, fish are considered obligate thermoconformists, that is, their specialized metabolic or behavioral attributes have little significant influence on the maintenance of body temperature, therefore, it fluctuates depending on the temperature of the aquatic environment. (Coutant, 1976).

Poeciliopsis gracilis (Heckel, 1848), is a small species; in its description the maximum size of males in the adult stage is 30 to 40 mm and 50 to 60 mm in females (Miller et al., 2005), although Álvarez del Villar (1970), mentions a maximum standard length of 73 mm, its body is elongated and slightly robust, light yellow in color, with eight to ten large dark spots of irregular shape whose diameter is almost as large as the orbit of the eye located on the middle side of the body, the belly is light in color, the pectoral and pelvic fins are slightly yellow, the anal fin has a dark edge, it is a viviparous species, whose reproduction occurs throughout the year, as long as the habitat allows it. be favorable (Álvarez del Villar, 1970., Huidobro, 2000).

The set of studies that relate the effect of temperature to the development of organisms or to the interaction between them is called thermal ecology (Angilletta et al., 2002; Gómez-Hoyos et al., 2016.). Two main aspects of thermal ecology play a central role in shaping distribution patterns:

1) temperature tolerance and

2) temperature-dependent effects on performance (Angilletta et al., 2002).

The effects of temperature on performance within the tolerance range can be visualized using the performance curve as a function of temperature. Thermal performance curves are graphs of the reaction norm to which one or more characters of a product are linked. organism (jumping capacity, reaction speed, swimming speed, resistance, oxygen consumption, etc.) with its response to different temperatures, thus allowing us to describe the phenotypic plasticity of response of organisms to the conditions of the environment that they encounter. surrounds (Huey and Stevenson, 1979).

Habitat is defined as: the place where there are resources, physical and biotic conditions that allow the survival, reproduction and establishment of an organism (Lubin et al., 1993). Habitat use is the way in which an organism takes advantage of all its physical and biological components (Montenegro and Acosta, 2008). Habitat preference is evaluated at the population level and is understood as a consequence of selection. Preference is a hierarchical process that involves genetic and learned decisions, which result in the asymmetric use of resources in a non-random manner, by each individual (Krausman, 1999; Underwood et al. 2004), an ideal habitat is one that favors the development, growth and reproduction of a species (Persson, 2003). Habitat preference is evaluated through the organism's use of some resources, therefore, it is an indirect evaluation of its evolutionary history (Litvaitis et al., 1994).

In this project, some aspects of the thermal ecology of Poeciliopsis gracilis are analyzed, with the intention that the results obtained can be used as inputs in mixed ecological niche analysis that can be used to locate sites of high suitability for the establishment of the species. in basins of the country.

METHODS

Using electrofishing, specimens of P. gracilis were collected in the Santiago River, located in the Metztitlán Valley, Hidalgo state, under the bridge in the town of Jihuico (20°32'30"N and 98 °43'35"W), the temperature recorded in the water at the time of capture was 25°C.

The organisms were acclimatized to laboratory conditions trying to maintain the levels of temperature, hardness and O_2 concentration recorded at the time of collection. These conditions were monitored and kept constant during the time in which the tests were carried out.

DETERMINATION OF CRITICAL TEMPERATURES

To determine the minimum and maximum critical temperatures (Tcmin and Tcmax), 20 adult organisms of each sex, with similar sizes, were randomly selected (males between 3 and 4 cm, females between 3 and 3.6 cm). Adult males were identified by the presence of a well-defined gonopodium, and non-gravid females were selected (they did not present an abdominal protuberance or gravid point) (Pérez-Osorio et al., 2014).

Once the organisms were selected, the method proposed by Elliot (1981) was followed, which consists of increasing or decreasing the temperature of the organism at a rate of 3 °C per minute, this way acclimatization of the fish to intermediate temperatures is not allowed. Critical temperatures were calculated as the arithmetic mean of the collective thermal points at which each organism presents loss of balance or disorganized locomotor movements (Lowe and Vance, 1955).

DETERMINATION OF PREFERRED TEMPERATURES UNDER LABORATORY CONDITIONS

To determine the preferred temperatures under laboratory conditions, ten adult males of similar sizes (3.5 - 4.0 cm) and ten non-pregnant adult females of similar sizes (3.5 - 4.0 cm) were used.

- 4.0 cm). To develop the test, a device similar to the one proposed by Buckle et al. was implemented. (2003). The test was carried out by placing the organisms in the center of the system, with a temperature of 25° C and allowing them to explore the system for half an hour. After this time, their position and temperature were recorded every five minutes for half an hour (Pulgar et al., 2005). Once all the tests were carried out, the mean and standard deviation were obtained. To determine if there were significant differences between preferred temperatures of males and females, an ANOVA test was carried out using the Past 3.21 software (Hammer et al., 2001). To verify the normal distribution and homoscedasticity of the data, Shapiro-Wilk and Levene tests were performed using the same program. Finally, the interval of preferences and avoidance was determined according to the interquartile method proposed by Hernández- Rodríguez and Bückle-Ramírez (2010), which considers the distance between the third quartile (Q3) and the first quartile (Q1), that is: Q3 - Q1.

DETERMINATION OF OPTIMAL TEMPERATURAS

The optimal temperature was obtained through locomotor performance tests as a function of temperature, applying a mechanical stimulus to the tail fin of each organism. The tests were carried out with ten organisms of each sex, of similar sizes and same reproductive conditions (non-pregnant females), considering that the same specimen cannot take part in continuous tests, in order to avoid its acclimatization (Botero, 2004).

Five temperatures were selected between Tcmin and Tcmax (15 °C, 20 °C, 25 °C, 30 °C and 35 °C). The fish with which each test was developed were placed in a fish tank at the maintenance temperature with oxygen supply and temperature monitoring. The fish tank was brought to the test temperature. This process was carried out for one hour to allow the fish to have a long acclimatization process and was kept constant for two hours prior to the test (LeRoy et al., 2017).

After the acclimatization time had elapsed, the fish were subjected to a modification of the mechanical stimulus test proposed by Gerick et al. (2014). They were placed in the container (one at a time) for one minute to allow the organism to explore the new space and at the same time to avoid the stress of the change of environment; later, the mechanical stimulation was performed. The test was repeated three times to find the best answer. Each test was filmed from the top of the lane, the videos were analyzed frame by frame and the best performance was recorded, evaluated as the greatest distance (cm) traveled in the first second immediately after the stimulus.

The estimation of the best model for the locomotor performance curve was performed by fitting generalized linear models (GLM), generalized additive models (GAM) and generalized mixed additive models (gamm). The analyzes were carried out using the "glm", "gam" and "gamm" functions of the MGCV library (Wood, 2011) and the gam library (Hastie, 2019), and the use of the "ISLR" libraries was also required. (Gareth et al., 2017), "voxel" (Garcia de la Garza et al, 2018), tidyverse (Wickham et al., 2019), and ggplot2 (Wickham, 2016) using the R-project v3.4.3 program (R Core Team, 2019): The response variable of the analyzes was the performance data, considered as the maximum distance traveled in the first second immediately after the mechanical stimulus and the predictive variable was the temperature of the treatments evaluated. The analyzes considered, in addition to the treatment performance and temperature data, the sex and pattern length of the fish (SVL). Different tests were carried out to explore the suitability or not of linear or non-linear functions for each term, in addition to exploring random effects. The resulting models were chosen by means of the lowest value of the Akaike information index or AIC (Akaike Information Criterion by its acronym in English) and an anova test, tests carried out through the aforementioned bookstores.

From the best model, the R2 value was verified, which allowed us to determine the amount of information that the model is capable of explaining, and therefore the predictive power of the generated model, in addition to the explained deviance, which is a measure of the variability of the model. Subsequently, from the best model generated, the optimal temperature range B80 was obtained, which corresponds to those temperatures at which the organisms reach their maximum performance up to 80% of this value. This interval was calculated using the predict and range functions included in the libraries mentioned above.

DETERMINATION OF THERMAL LIMITS AND PREFERRED TEMPERATURES IN THE FIELD

To obtain the thermal limits and thermal preference in the field, the method proposed by Jowett and Richardson (2008) was used; the available thermal habitats, as well as the number of P. gracilis organisms present in said habitats, were consulted in the database. from the National Collection of Mexican Fresh water Fishes of the ENCB (ENCB-IPN-P). All those records corresponding to the Pánuco basin that present the number of captured specimens and temperature at the time of collection were selected, as well as all the collections in the basin that had a temperature record, but that did not have the presence of the species.

These data were entered into the HABSEL software, with which a matrix of habitat use in terms of temperature was created. With this matrix, a habitat use curve was generated.

$$w_i = \frac{u_i / \sum_{i=1}^{n} u_i}{a_i / \sum_{i=1}^{n} a_i}$$

In the above equation wi is the foraging proportion for n habitat categories, ui is the number of organisms in each habitat category, Σui is the total number of organisms in all habitat categories, ai is the number of samples from the category i and Σai is the total number of samples (Manly et al., 1993).

Foraging proportion quantifies the strength of selection for a particular category of habitat. A value of w = 1 indicates neutral selection; Habitats with w < 1 are used less frequently than expected by chance and habitats with w > 1 are used more frequently than expected by chance (Jowett and Richardson, 2008).

RESULTS

DETERMINATION OF CRITICAL TEMPERATURES FOR *P. GRACILIS*

The range of minimum critical temperatures ranges from 7.2 °C observed in a 3 cm long male organism to 10.4 °C in a 3.4 cm male, however, most specimens are located in the range of 8.5 to 10 °C While the range of maximum critical temperatures is distributed from 38.9 °C in a 3.2 cm male to 41.6 °C in a 3 cm male, observing that the majority of specimens are located in the range of 40 to 41.5. (Figure 1).

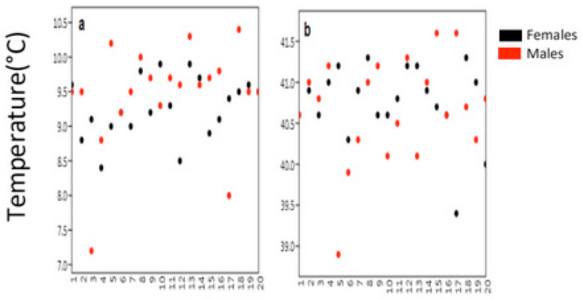

Figure 1. Distribution of critical temperatures in females and males at) Tcmin, b) Tcmax.

The minimum critical temperatures present normality and homoscedasticity (Shapiro-Wilk females= 0.96 males= 0.87; Levene f= 0.70), they do not present significant differences between males and females (Student's T p= 0.524), so it can be considered a single minimum critical temperature that corresponds to 9.27 °C. In the same way, the maximum critical temperatures (Shapiro-Wilk females = 0.87 males = 0.93; Levene f = 0.64) so a single maximum critical temperature (Student's t p = 0.552) of 40.8° C was considered for the entire population.

During the development of this test, two repetitive patterns were identified in the behavior of the fish that are not reported in other studies of critical temperatures for the Poeciliidae family: 1) the accumulation of blood in the cephalic area, which translates into a strong reddish tone both in the head and in the opercula, this coloration was seen 5 °C before the organisms reached the Tcmin and Tcmax and 2) at temperatures close to the critical ones (between 1- 1.5°C of the Tcmin and Tcmax) organisms, both male and female, make a jump to the surface.

DETERMINATION OF THE THERMAL LIMITS AND PREFERRED TEMPERATURES OF P. GRACILIS IN THE FIELD

The review of data in the National Collection of Mexican Fresh water Fish (ENCB-IPN-P) showed 241 sites in the Pánuco basin that have temperature data, of these sites 141 show the presence of the species, a total of 3553 organisms were recorded With this information, the preference table was created using the HABSEL program. The software automatically generates the frequency distribution so that the intervals adjust the behavior of the counts to a normal distribution. In this case, the available temperatures were divided into 10 intervals to obtain preference through the standardized selection index.

The range of available temperatures recorded in the Pánuco basin was 12.2°C to 33.3°C. In the organisms, three temperature intervals were recorded with a higher frequency of appearance than the other seven (20- 22.5 °C, 22.5- 25 °C and 25- 27.5 °C), the interval of greatest availability offered by the environment is that of 22.5- 25 °C (73 sites), this is the most used (1112 organisms collected), the interval of 27.5- 30 °C, only has 14 available sites; However, 313 organisms were collected in it, the same as in the interval of 30-32.5 °C (4 sites available), so it has a disproportionate use (89 organisms collected), so this thermal habitat is occupied in a greater proportion. than that found in the environment, the standardized index of selection or preference obtained through the HABSEL program indicates an active search by the organisms towards this temperature, which is why it was determined as the preferred temperature range.

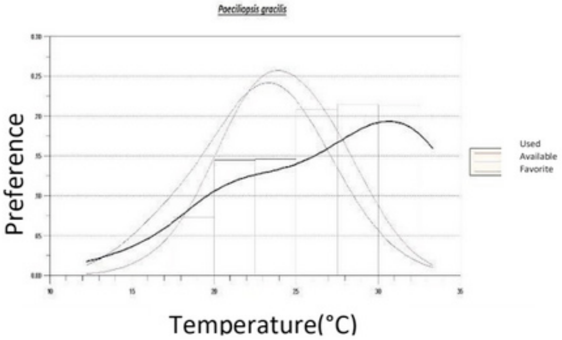

Figure 2. Thermal habitat preference curve of Poeciliopsis gracilis in the Pánuco basin obtained with information from collections carried out between 1998 and 2018, deposited in the ENCB-IPN-P.

The blue curve shows the range of temperatures available in the Pánuco basin. At the top of the red curve the most used temperature interval is observed (22.5-25 °C), the black curve corresponds to the preferred temperature modeled from the selection index, the graph shows that the preference of the organisms is due to warm temperatures (27.5-32.5 °C), with greater preference in the range of 27.5 to 30 °C without occupying sites where its performance may be diminished (32.5- 35 °C).

The organisms recorded in their preferred temperature ranges determined for the field were collected in the central and central-eastern zone of the basin, 218 organisms in Platón Sánchez, Veracruz (21° 17' 05"N, 98° 27' 12" O) at 344 m s. n. m. at 28.6 °C, 115 organisms in Santa María River, Querétaro (20°30'11.3"N, 98°42'17.5"W) at 894 m above sea level. n. m. at 27.5 °C, 51 organisms in the Jalpan River, Querétaro (21° 23' 16"N, 99° 35' 10"W) at 542 ms. n. m. at 27.5 °C, 47 organisms in El Trapiche, Querétaro (21° 19' 54"N, 99° 31' 33"W) at 1800 m above. n. m. at 27.9 °C and 45 organisms in the San Pedro stream, Hidalgo (20°30'11.3"N, 98°42'17.5"W) at 542 m s. n. m. at 28.1°C.

DISCUSSION

Poeciliopsis gracilis presents a thermal tolerance interval in the laboratory of 31.5 °C (Tcmin 9.27 °C, Tcmax 40.8 °C), which coincides with that recorded by Hernández-Rodríguez and Bückle-Ramírez (2010) for Poecilia sphenops at different acclimatization temperatures. : at 26 °C a range of 9.9 °C - 40.5 °C and at 29 °C a range of 10.5 °C - 40. 9 °C. However, these results may vary depending on the acclimatization temperature since, as demonstrated by Currie et al. (1998) tests with different acclimatization temperatures of three species tolerant to thermal variations result in narrower thermal intervals when the acclimatization temperature approaches the critical temperatures and wider as it moves away from them. In the case of the critical temperatures obtained for P. gracilis, they were determined for a batch of fish whose maintenance temperature was 25 °C. This temperature was chosen because it was determined in the field at the time of capture and as was later observed. This is practically halfway through the available thermal range (12.2- 33.5 °C) in the Pánuco basin, so if additional tests are carried out with different acclimatization temperatures, then significant differences will appear in the critical temperatures and The thermal limits, however, the latter will be narrower than those initially determined, in such a way that the thermal tolerance interval reported in this test could represent the respective range of the species in the Pánuco basin.

The TCmin reached by Poeciliopsis gracilis in this study was 9.2 °C with acclimatization to 25 °C, this temperature is lower than that reported by Martínez et al. (2016) for Poecilia caucana acclimated to 25 °C (Tcmin: 13.41 °C) and 20 °C (Tcmin: 12.5 °C). There are reports of lower Tcmin within the Poeciilidae family, such as the case of Poecilia sphenops, which has a Tcmin of 7.5 °C (Bierbach et al.,

2010) and Poecilia latipinna, whose Tcmin is 7.6 °C (Hernández Rodríguez et al., 2002), however, the lowest reported within the family corresponds to Poeciliopsis occidentalis with Tcmin of 4.8 ºC (Bulger and Schultz 1982), so it would be expected that if P. gracilis were acclimated to temperatures lower than 25 °C its Tcmin could be less.

The Tcmax achieved by P. gracilis in this study was 40.8 °C while the highest Tcmax reported among the Poeciliidae family is 42.3 ± 0.4 °C and corresponds to Gambusia affinis acclimated to 35 ºC (Otto, 1973). Poecilia caucana is capable of tolerating temperatures higher than 40 °C (Martínez et al. 2016), so it can be considered that under conditions of acclimatization to higher temperatures P. gracilis would be able to reach a higher Tcmax, since the family Poeciliidae is characterized by having great tolerance to high temperatures (Martínez et al., 2016).

The preference range determined in the laboratory for P. gracilis is 27.9 to 29.2 °C for males and females, unlike what was reported for Poecilia sphenops, which has preferred temperatures of 29.5 °C for females and 25.5 °C for males (Hernández -Rodríguez et al., 2002; Hernández-Rodríguez and Bückle-Ramírez, 2010), this is biologically relevant, since it implies the optimization of the thermal resource for P. gracilis at the time of reproduction, favoring sexual fitness at the same time or very close moments (Cosson, 2000).

The optimal temperature of P. gracilis determined in the laboratory was 29.94 °C, which coincides with the range reported for the entire Poeciliidae family (26 to 30 °C) (Page and Burr, 2011). However, the optimal temperature range (B80) is much larger than that of the family. The width of this interval can largely explain the disproportionate increase in the number of organisms recorded in the basin in the last 40 years (Soto-Galera et al., 2011), since according to the data recorded for the basin the interval of Optimal temperatures cover 82% of temperatures available in the environment. The wide availability of these temperatures is important since in this range organisms are capable of reaching up to 80% of their maximum performance. This factor can have a significant effect on growth and reproduction, as well as on their ability to survive (Valbuena-Villarreal and Cruz-Casallas, 2006).

Thermal determinations of P. gracilis in the Pánuco basin show a field tolerance interval of 13 °C (17- 30 °C), which coincides with the generality of poecilids in their preference for warm waters (Meffe and Snelson, 1989).

The thermal niche study in the field covered most of the available pools and the entire temperature range in the basin. The results do not only apply to the population studied in the laboratory, but also coincide with the rest of the organisms of the species. in the Pánuco basin, so what was obtained in the laboratory can be extrapolated to the other populations of the basin. Neubauer and Andersen (2019) mention that the temperatures that maximize performance in nature (optimal temperatures) depend on several ecological factors such as food availability, habitat availability, community structure, among others. In the current study, the effect of temperature was isolated, controlling and standardizing possible physical variations (hardness, ammonium, dissolved oxygen). However, in the basin organisms are exposed to a greater number of factors such as: presence of contaminants, presence of predators, availability of food or shortage of shelters. The fact that the range of preferred temperatures determined for the field and laboratory coincide suggests that the species in the field has few ecological pressures that could limit its performance in the basin, a reason that could be key along with the availability of optimal thermal habitat for the species. great colonization capacity of the species in recent years.

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