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Research Article

Preference, tolerance and resistance responses of *Poecilia sphenops* Valenciennes, 1846 (Pisces: Poeciliidae) to thermal fluctuations

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ABSTRACT. *Poecilia sphenops* was acclimated to two thermal fluctuations, each following a symmetrical and asymmetrical cycle. The critical maximum temperatures of the fish were significantly different ($P \leq 0.001$) between the thermal fluctuations: 40°C for the 20-29ºC thermal cycle and exceeding 42°C for the 26-35ºC cycle. Fish acclimated to the 20-29ºC thermal cycle were more resistant to cold, with a critical thermal minimum of 10ºC, unlike specimens of the 26-35ºC regime, with a critical minimum of 11.7°C. In both cycles, the upper incipient lethal temperature interval was 38.8 to 39.5ºC and the lower incipient lethal temperature interval was 10.8 to 11.8°C. Females preferred temperatures between 30 and 31°C, in all but the 26-35ºC symmetric cycle. Males preferred a temperature between 23.8 and 24.2°C (symmetrical cycle). The high and low avoidance temperatures of male and female fish acclimated to the 20-29ºC cycle were, respectively, 14 and 16ºC, compared to the 26-35°C treatment, in which case these values were 8.4 and 11.4ºC. The results indicate that *P. sphenops* is perfectly adapted to the marked seasons of rain and drought of their habitat.

Keywords: thermal fluctuations, behaviour, fishes, *Poecilia sphenops*.

Respuesta de preferencia, tolerancia y resistencia de *Poecilia sphenops* Valenciennes, 1846 (Pisces: Poeciliidae) a fluctuaciones térmicas

RESUMEN. *Poecilia sphenops* fue aclimatada a dos fluctuaciones térmicas cada una con un ciclo simétrico y asimétrico. La temperatura crítica máxima de los peces fue significativamente diferente ($P \leq 0.001$) entre las fluctuaciones y se observó a 40°C en el ciclo 20-29°C; para el ciclo 25-35°C fue mayor a 42°C. Los peces aclimatados a la fluctuación 20-29°C fueron más resistentes al frío con una temperatura crítica mínima de 10°C, en contraste a los 11.7°C del régimen 26-35°C. En ambos ciclos la temperatura letal incipiente superior tuvo un intervalo de 38.8 a 39.5°C y la temperatura letal incipiente inferior fue de 10.8 a 11.8°C. La temperatura preferida de las hembras fue de 30 a 31°C, con excepción del ciclo simétrico 26-35°C. La temperatura preferida de los machos aclimatados al ciclo simétrico fue de 23.8 a 24.2°C y de 27.4 a 29.4°C en el ciclo asimétrico. Las temperaturas de evitación de los machos y las hembras aclimatados a la fluctuación 20-29°C tienen un intervalo de 14 a 16°C comparado con el intervalo de 8.4 a 11.4°C del tratamiento 26-35°C. Los resultados indican que *P. sphenops* está perfectamente adaptado a las marcadas estaciones de lluvia y sequía de su hábitat.

Palabras clave: fluctuaciones térmicas, comportamiento, peces, *Poecilia sphenops*.

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INTRODUCTION

Aquatic organisms react in the presence of thermal variations avoiding lethal temperatures and preferring others, which allow them to survive and reproduce (Brett, 1956). In a graphic model enclosing a tolerance and resistance area, Fry (1947) and Brett (1956) demonstrated the existing relationship among the organism's responses (preferred, avoided, lethal and critical temperature) and the constant acclimation temperature.

The tolerance area comprises the low and high avoided temperatures that are less frequented by the organisms as well as the preferred temperature or final preferendum described by Fry (1947). This area is limited by the lower and upper incipient lethal temperatures (LILT and UILT) defined as the temperature beyond which 50% of the population survives an indefinitely long exposure (Fry, 1947).

Thermal tolerance of an organism is based on the lethal incipient temperature; however, fish avoid temperatures exceeding the thermal preference limited by the low and high avoidance temperatures delimiting the thermal preference area (Giattina & Garton, 1982). This zone reflects the optimum temperature for the biological processes, which occur inside the thermal interval of the species habitat where they become more efficient near the final preferendum (Crawshaw, 1977).

The resistance area is limited by the critical thermal minimum and maximum (CTMin and CTMax), characterized by the interaction of temperature and time. These responses are used as stress and adaptation indicators for invertebrate and vertebrate poikilotherms (Paladino *et al*., 1980).

Research has been carried out in different species to relate the effect of fluctuating temperatures with different process such as, the thermoregulatory behavior, thermal tolerance, metabolism, growth rate and mortality, development time, maturity among others (Feldmeth *et al*., 1974; Otto, 1974; Hokanson, 1977; Diana, 1984; Hernández-Rodríguez *et al*., 2002; Hernández-Rodríguez & Bückle-Ramírez, 2002; Rahel, 2003; Pörtner *et al*., 2004; Carveth *et al*., 2006; Widmer *et al*., 2006). Their results are very important because several species of the same environment were contrasted. In the present work we only used *Poecilia sphenops* as a target species particularly by his extended geographical distribution.

With base in the limits of the temperature polygon proposed by Fry (1947) and Brett (1956), the behavior response of an organism, is a tool that allows predicting the individual's behavior in a thermolabil environment (Anguilletta, 2009), its limits, as well as the optimum temperature interval in which the species can be cultivated, and a guidance for evaluating and recommending temperature regimens (Armour, 1991). This work evaluated and compared the experimental responses of *Poecilia sphenops* thermal fluctuations, versus previous investigations carried out in the same species (Hernández-Rodríguez & Bückle-Ramírez, 1998; Hernández-Rodríguez *et al*., 2002) acclimated to constant temperatures regimes to predict their environmental performance capacity.

MATERIALS AND METHODS

P. sphenops 2 to 6 cm total length was collect in Piedra Azul dam in the Municipality of Teotitlan del Valle (17°02'N, 96°30'W), Oaxaca, Mexico. In the laboratory, two thousand fish were distributed in three fiberglass circular tanks 380 L each where they stayed in quarantine at the site's temperature (26°C) and exposed to natural photoperiod. After quarantine, organisms were exposed to fluctuating acclimation temperatures to encompass the prevailing temperature of the dam and particularly the extreme temperature of the shallow area, each one constituted by two independent cycles. We choose the symmetric cycle (SC) proposed by Feldmeth and Stone (1974) used in *Cyprinodon nevadensis amargosae* and we add an asymmetric cycle (AC) to identify the change in species tolerance and resistance. The criteria to establish the thermo cycles were based in the environmental thermal fluctuations. The populations of *P.ephenops* that inhabit the dam in Oaxaca tolerate prolonged periods of drought and rain. In June-October (Summer-Autumn) prevail high temperatures and lower in November-December (late Autumn-Winter) averaging 19 to 28ºC throughout the year. In the shallow areas of the dam where the reproducers are located (Barón, 2001) higher temperatures have been register (35ºC).

Average weight and standard length of females and males acclimated to the 20-29 and 26-35°C both, exposed to the SC and AC are shown in Table 1. Four circular tanks of 380 L were used, two of them were modified to generate cycles of 24 h (Fig. 1). The 20-29ºC thermal fluctuation (TF) applied to fish (N = 400) (aprox. 2 fish per litre) of the symmetrical cycle (SC) of 5-7-5-7 h, began with the heating period 20-29°C in 5 h, then temperature was maintained at 29°C for 7 h, followed by a cooling period 29-20°C in 5 h keeping temperature at 20°C for 7 h before beginning the new cycle. Fish (N = 400) of the asymmetric cycle (AC) 4-10-5-5 h, experienced the heating period 20-29°C in 4 h, followed by 10 h at 29°C, followed by a cooling period 29-20°C in 5 h and maintaining temperature at 20°C for 5 h before beginning the new cycle.

Two tanks, each one with a 1000 watts heater connected to an automatic clock controller (ChronTrol®) began the fish heating period at the established time. Heating began at 6:00 h A.M. and when the water temperature reached 29°C, the controller kept it constant until the starting of the cooling period, which was made replacing 17°C water from the storage tank (Fig. 1b). Water replacement for the SC was 550 mL min^{-1} and 660 mL min^{-1} for the

Table 1. Mass and standard length measures of organisms acclimated to the fluctuating temperatures. N = 400 per tank. Average ± standard deviation.

Tabla 1. Medidas de masa y longitud estandar de los organismos aclimatados a las fluctuaciones de temperatura. N = 400 por estanque. Promedio ± desviación estandar.

Fluctuating temperature (°C)	Sex	Mass (g)	Standard Length (cm)
Symmetric 20-29	Females	2.26 ± 0.56	4.3 ± 0.38
	Males	0.97 ± 0.31	3.3 ± 0.34
Asymmetric 20-29	Females	1.78 ± 0.88	3.95 ± 0.78
	Males	0.98 ± 0.31	3.34 ± 0.32
Symmetric 26-35	Females	2.40 ± 0.48	4.43 ± 0.26
	Males	0.91 ± 0.30	3.19 ± 0.37
Asymmetric 26-35	Females	1.02 ± 0.28	3.30 ± 0.25
	Males	0.71 ± 0.19	2.94 ± 0.26

AC. In the tanks, a central drainage functioned as level controller (Fig. 1); the surplus water was received in one of the lower tanks and returned again to the 17°C storage tank.

We applied the same cycle's design and the same amount of fish per tank for the 26-35ºC TF. For these cycles, water temperature in the storage tank was cooled to 10ºC (Neslab RTE-220). Timing for heating and cooling periods was the same as for the 20-29°C TF.

The organisms of the two TFs were fed *ad libitum* twice a day with catfish food containing 35% protein. Daily, faeces, death and sick organisms were subtracted and measured the dissolved oxygen concentration (Oxymeter YSI 50B), pH (Orion SA 23), ammonium concentration (Nessler method), salinity (Lapcomp SCT-100) and water hardness (Hach 5B) as $CaCO_3$. In each tank a thermograph

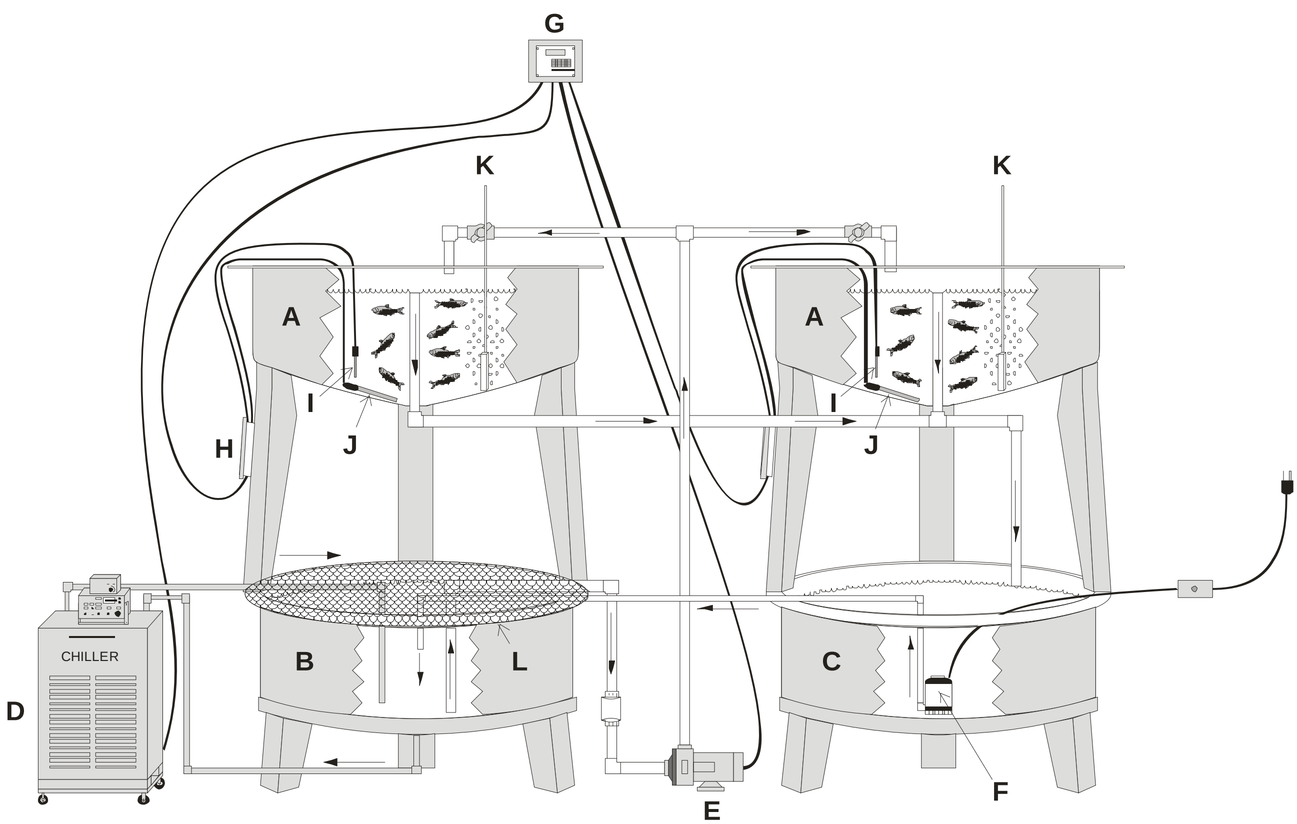

Figure 1. System used in the acclimation of *Poecilia sphenops* to fluctuating temperatures. A: acclimation tanks, B; tank for water supply (17ºC), C: reception tank for the water of the acclimation tanks, D: cooling unit (chiller), E: motor-pump, F: submerged motor-pump, G: control clock (timer, ChronTrol ®); H: electronic regulator of the thermocouple and the heater, I: thermocouple, J: 1000 watts titanium heater, K: air, L: isolating cover.

Figura 1. Sistema usado para la aclimatación de *Poecilia sphenops* a temperaturas fluctuantes. A: estanque de aclimatación, B: estanque para el suministro de agua (17ºC), C: estanque de recepción para el agua del estanque de aclimatación, D: unidad de enfriamiento (chiller), E: motobomba, F: motobomba sumergible, G: reloj control (timer, ChronTrol ®), H: regulador electrónico de la termocupla y el calentador, I: termocupla, J: calentador de titanio de 1000 vatios, K: aire, L: tapa aislante.

registered the temperature every 30 min for 30 days in order to know the variation of the fluctuating acclimation temperatures. The measured physico-chemical water parameters for the 20-29 and 26-35ºC TFs are presented in Table 2.

The experiments initiated after 30 days acclimation period of adjustment to the fluctuating temperatures and 12 h light: 12 h darkness photoperiod. Organisms were not fed 24 h prior to the experiment to avoid post-digestive processes to interfere with behavior responses.

Critical thermal maximum and minimum (CTMax and CTMin), upper and lower incipient lethal temperatures (UILT and LILT), and preferred (PT) and avoided temperatures (AT) experiments were carried out with four repetitions. All experiments began one hour after fish had reached the cycle's respective maximum temperature.

The resistance of *P. sphenops* was evaluated with CTMax trials, by increasing the water temperature 1°C min^{-1} according with Paladino *et al*. (1980) and Lutterschmidt & Hutchison (1997) until the responses, muscular spasms (MS) and loss of equilibrium (LE) were observed. Experiments began with the temperature of the aquarium at the highest cycle temperature (29 or 35°C). The 10 fish used for each experiment of the 20-29°C TFs (N = 40) had a weight of 0.76 ± 0.12 g and a standard length of 2.99 ± 0.12 cm. Fish of the 26-35°C TFs (N = 40) had a weight of 1.08 ± 0.23 g and a standard length of 2.44 ± 0.20 cm.

Ten fish were used for each CTMin experiments, the weight was 1.31 ± 0.37 g and their standard length 3.53 ± 0.29 cm. Behavior responses of sporadic quick activity with muscular spasms (SA+MS) and cold coma (CC) were examined decreasing the aquarium temperature in average 1.15 ± 0.14ºC min^{-1}. For the fish thermal tolerance studies, we applied the model of Kilgour *et al*. (1985) to asses the upper incipient lethal temperature (UILT). The experimental temperatures where 50% of the mortality occurred were established, based on the temperatures where the CTMax behavior responses (MS and LE) were observed and, 1°C was added or subtracted to the CTMax (Table 3). Ten organisms of each TF cycle were placed in each aquarium were temperature remain constant for 48 h experimentation. The hour that each fish entered the aquariums and their moment of death was registered (Table 3).

To study the low incipient lethal temperature (LILT), a horizontal acrylic tank was used (describe in Bückle *et al.,* 2003) conditioned with 5-20°C thermal gradient established based on the temperatures in which the CTMin responses (SA+MS and CC) were observed. Five to six experimental temperatures were determined for each TFs cycle (Table 3). Each experimental temperature was represented by eight fish confined inside the gradient in perforated plastic baskets and placed in the chamber matching the experimental temperature. The gradient temperature was registered with a thermograph (Stanford Research Systems, Mod. SR 630) every two minutes during the 45 min. The LILT was established when coma response took place, and immediately after, fish were returned to perforated baskets in the corresponding temperature fluctuation tank to evaluate their recovery during the following 48 h.

The preferred temperature (PT) or final preferendum and the low and high avoided temperatures (LAT and HAT) were studied with the acute method in the same horizontal acrylic thermal gradient. In previous experiments we observed thermal preference among sexes (Hernández-Rodríguez *et al*., 2002). Therefore 10 males or females separately were chosen at the moment of maximum temperature of the 29 or 35°C TFs exposure and placed in the chamber of the thermal gradient matching the same temperature. The trials began 40 min later to reduce handling stress. Temperature and organisms location in the gradient cameras was registered every 10 min during two hours. The LAT and HAT were estimated with the temperature less frequented by fish throughout the experiment.

Data analyses were performed using SIGMASTAT Version 3.1 software (SPSS, Chicago, IL, USA). The analysis of the results was made with the variance (ANOVA) test at a significance level $\alpha = 0.05$. The data didn't have a normal distribution, non parametric tests of variance analysis were applied by ranges (Kruskal-Wallis) and multiple comparisons (method of Dunn and Student-Newman-Keuls) (Zar, 1984).

RESULTS

The responses LE and MS were significantly different ($P \leq 0.001$) between the thermal fluctuations 20-29 and 26-35ºC. The fish acclimated to the 26-35ºC thermal fluctuation increase the responses MS and LE in 2ºC. There exist no significant differences when LE and MS responses are contrasted between cycles of the same TF (Fig. 2a).

Fish responses exposed to CTMin were significantly different ($P < 0.05$, Dunn´s Method test). The SA+MS and CC responses of fish acclimated to the cycles of 20-29°C and 26-35°C TFs were statistically different. Fish acclimated to the SC and

Table 2. Physical and chemical parameters in the acclimation tanks of *Poecilia sphenops*. Average ± standard deviation.

Tabla 2. Parámetros físicos y químicos en los estanques de aclimatación de *Poecilia sphenops*. Promedio ± desviación estandar.

Acclimation to fluctuating temperatures (°C)	Dissolved oxygen (mg L^{-1})	pH	Hardness ($CaCo_3$) (mg L^{-1})	Ammonia (NH_4^+) (mg L^{-1})	Salinity (‰)	Temperature Min. and Max. (°C)
Symmetric 20-29	6.56 ± 0.54	7.9 ± 0.12	350 ± 12.1	------	0.66 ± 0.1	21.20 ± 0.56 28.99 ± 0.22
Asymmetric 20-29	6.24 ± 0.24	7.8 ± 0.13	376 ± 10.1	------	0.54 ± 0.4	21.12 ± 0.77 29.25 ± 0.26
Symmetric 26-35	5.53 ± 0.23	7.7 ± 0.15	278 ± 21.4	1.32 ± 0.33	0.61 ± 0.1	25.37 ± 0.49 34.82 ± 0.40
Asymmetric 26-35	5.25 ± 0.23	7.6 ± 0.15	270 ± 18.4	0.97 ± 0.07	0.60 ± 0.1	25.72 ± 0.25 34.03 ± 0.21
Potable H_2O	------	7.3 ± 0.26	231 ± 10.1	0.07 ± 0.02	0.50 ± 0.1	------

Table 3. *Poecilia sphenops* acclimated to fluctuating temperatures and exposed to experimental temperatures (°C) to identify the low and upper incipient average lethal temperature. Average ± standard deviation.

Tabla 3. *Poecilia sphenops* aclimatada a fluctuaciones de temperatura y expuesta a temperatures experimentales (°C) para identificar la temperatura letal incipiente superior e inferior promedio. Promedio ± desviación estandar.

	Symmetric cycle 20-29°C	Asymmetric cycle 20-29°C	Symmetric cycle 26-35°C	Asymmetric cycle 26-35°C
High experimental Temparatures (°C)	39 ± 0.1	39 ± 0.1	39 ± 0.1	------
	40 ± 0.1	40 ± 0.1	40 ± 0.1	40 ± 0.1
	41 ± 0.1	41 ± 0.1	41 ± 0.1	41 ± 0.1
	42 ± 0.1	42 ± 0.1	42 ± 0.1	42 ± 0.1
	------	------	43 ± 0.1	43 ± 0.1
	------	------	44 ± 0.1	44 ± 0.1
Low experimental Temperatures (°C)	16.4 ± 0.67	16.2 ± 0.53	16.4 ± 0.44	17.4 ± 0.08
	14.4 ± 0.52	14.2 ± 0.50	14.7 ± 0.29	15.2 ± 0.27
	10.8 ± 0.43	10.9 ± 0.47	13.6 ± 0.38	13.6 ± 0.34
	7.6 ± 0.53	7.6 ± 0.23	11.3 ± 0.12	11.8 ± 0.73
	5.5 ± 0.25	6.2 ± 0.16	9.8 ± 0.41	9.9 ± 0.70
	------	------	------	8.1 ± 0.23

AC of the 26-35°C TF were less resistant to cold in 1.3-1.6°C compared to the organisms exposed to the 20-29°C TF (Fig. 2b). Similar temperatures were observed for the SA+MS and CC responses, and the differences were less than 0.6°C (Fig. 2b). The upper incipient lethal temperature (UILT) values of the fish acclimated to both cycles and TFs had a thermal interval of 38.8 to 39.5ºC (Table 4a).

The low incipient lethal temperature (LILT) of fish exposed to the 20-29ºC and 26-35°C TFs differ in only 1°C (Table 4a), the thermal fluctuation do not affect the low temperature responses. However, organisms in the SC and AC of the 20-29°C exposed to the bath temperature of 5.5 and 6.2°C died. Most fish in the SC and AC of the 26-35°C that were abruptly exposed to cold bath temperatures, a sanguine spill in the mouth and others, a corporal bend took place and fish mortality was 62.5% and 66% when exposed to 9.8°C bath temperature.

Females preferred temperature around 30°C in both TFs was not significantly different ($P \leq 0.001$). Males preferred temperature (mean 28ºC) increase by the effect of the AC of the TFs compare to the SC (mean 24ºC) (Table 4a).

The thermal fluctuations modified the fish avoided temperature interval delimited by LAT and HAT. In

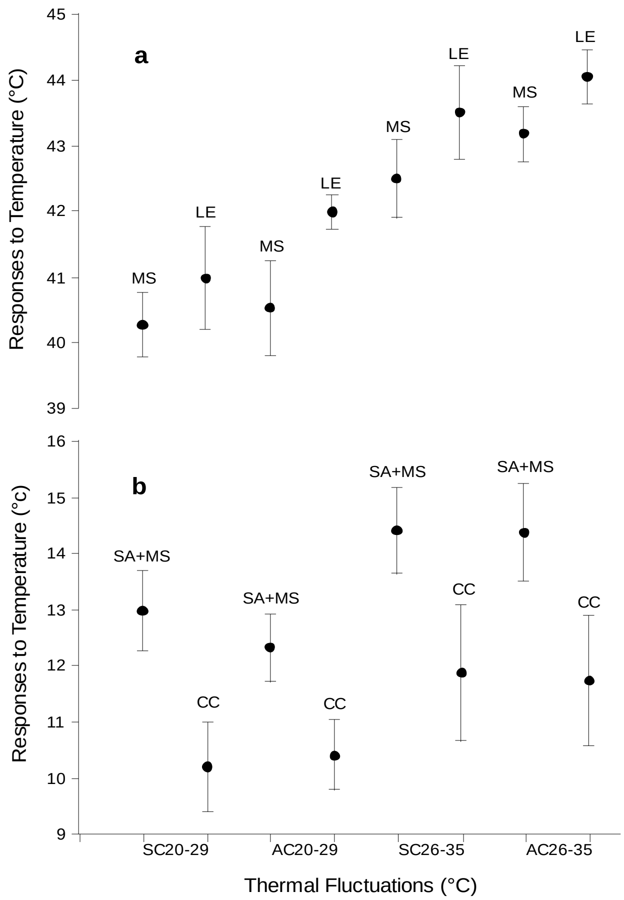

Figure 2. Relationship among the high (a) and low (b) temperatures that caused the stress responses in *Poecilia sphenops* acclimated to 20-29 and 26-35ºC thermal fluctuations of the symmetrical cycle and asymmetric cycle. Muscular spasms (MS), loss of equilibrium (LE), sporadic activity with muscular spasms (SA+MS) and, cold coma (CC). Each value corresponds to the average of four repetitions (± standard deviation).

Figura 2. Relación entre las temperatures altas (a) y bajas (b) que causaron las respuestas de estrés en *Poecilia sphenops* aclimatada a las fluctuaciones térmicas 20-29ºC y 26-35ºC del ciclo simétrico y asimétrico. Espasmos musculares (MS), pérdida de equilibrio (LE), actividad esporádica con espasmos musculares (SA+MS) y estado de coma al frío (CC). Cada valor corresponde al promedio de cuatro repeticiones (± la desviación estándar).

females and males acclimated to 20-29ºC was more than 14ºC, and in the 26-35ºC cycle the interval decrease in 4ºC and 6.7ºC respectively (Table 4a).

DISCUSSION

The thermal fluctuations, each one, with an asymmetric and symmetrical cycle provided important knowledge over the resistance and tolerance of *P. sphenops*. The critical thermal maximum used as a stress and adaptation indicator, in this species is characterized by the muscular spasm according to the analysis carried out by Lutterschmidt and Hutchison (1997). The vicinity among the responses of muscular spasm and loss of equilibrium represents the thermal transition area that allows the organisms to escape from the stress caused by high temperatures and it constitutes the threshold of thermal resistance.

P. sphenops acclimated to the 20-29ºC and 26-35°C TFs increased their resistance in 2°C compared with the results of the constant temperatures experiments 20 to 35°C, where we observed that for each 3°C increment, CTMax increase 1°C, and it was constant in 32 and 35°C (Hernández-Rodriguez & Bückle-Ramirez, 1998). In ecological terms, it represents the thermal response capacity of the species to temperature variations and only when acclimated to constant temperatures we can establish his resistance limit and the relative change in °C. *P. sphenops* that experience great thermal fluctuations in the environment of the dam is capable to increase their resistance in 3.0°C (AC of the TFs) (Table 4).

The responses of increased activity+muscular spasm and cold coma of fish acclimated to the thermal fluctuation had very similar values and the transition among the temperatures of these responses is 2.0°C, which allows them to escape from the stress caused by the decrease of the environmental temperature, before the cold coma takes place. Fish acclimated to the thermal fluctuations did not resist temperatures under 10°C. In this study fish exposed to cold did not die after they lost the equilibrium and remained in lethargy, and the time in stupor was fundamental for their recovery, when returned to the fluctuating acclimation temperatures. *P. sphenops* acclimated to the thermal fluctuations experienced a primary coma according to the description of Pitkow (1960) as a result of the continuing temperature decrease, where the time of exposure was decisive for survival and the immediate recovery.

There are few works related with the organism's critical thermal minimum characterized by the loss of equilibrium (Becker & Genoway, 1979) or when the animals enter in coma (Pitkow, 1960). The values of the critical thermal minimum that Barrionuevo & Fernandes (1995) obtained in *Prochilodus scrofa* acclimated at 15-35°C were 5-14.5°C. In *P. sphenops* acclimated to the 20-29ºC and 26-35°C TFs; the cold coma occurred at a mean temperature of 10.3 and 11.8°C respectively. However, when *P. sphenops* was acclimated to constant ambient of 20 to 26°C the TCMin was 7.7 to 9.9°C (Hernández-Rodriguez & Bückle-Ramirez, 1998).

Table 4. Thermal responses of the tolerance and resistance zones of *Poecilia sphenops* females (F) and males (M) acclimated at different temperature regimen. CTMax and CTMin, critical thermal maximum and minimum; UILT and LILT, upper and lower incipient lethal temperatures; HAT and LAT, high and low avoided temperatures; PT, preferred temperature; SC, symmetrical cycle; AC, asymmetric cycle. a) cyclic acclimation, b) constant acclimation (Hernández-Rodríguez, 1998; Hernández-Rodríguez & Bückle-Ramirez, 2002; Hernández-Rodríguez *et al.*, 2002). The numbers in black correspond to the final preferendum.

Tabla 4. Respuestas térmicas de la zona de tolerancia y resistencia de hembras (F) y machos (M) de *Poecilia sphenops* aclimatadas a diferentes regimenes de temperatura. CTMax y CTMin, temperatura critica máxima y mínima; UILT Y LILT, temperatura letal incipiente superior e inferior; HAT y LAT, temperaturas de evitación superior e inferior; PT, temperatura preferida; SC, ciclo simétrico; AC ciclo asimétrico. a) ciclos de aclimatación; b) aclimatación constante (Hernández-Rodríguez, 1998; Hernández-Rodríguez & Bückle-Ramirez, 2002; Hernández-Rodríguez *et al.*, 2002). El número en negritas corresponde al valor del preferendum final.

Thermal regimen		CTMax	UILT	HAT		PT		LAT		LILT	CTMin
				F	M	F	M	F	M		
a) Cyclic acclimation temperature (°C)											
20-29	SC	40.2	39.4	35.5	34.4	30.0	24.2	19.5	18.5	10.8	10.2
	AC	40.5	38.8	35.1	34.3	30.0	29.4	21.0	18.9	10.9	10.4
26-35	SC	42.5	38.9	31.2	28.5	24.0	23.8	19.8	20.1	11.3	11.8
	AC	43.1	39.5	34.9	31.4	31.0	27.4	24.7	22.0	11.8	11.7
b) Constant acclimation temperature (°C)											
20		37.5	37.9	28.4	31.5	26.5	29.5	25.0	28.2	7.5	7.7
23		39.0	38.2	36.4	31.5	32.3	30.0	28.2	25.3	9.0	8.0
26		40.0	38.9	35.7	33.9	30.0	24.9	21.6	17.3	9.8	9.9
29		40.9	39.3	32.7	36.0	29.5	28.3	19.4	22.8	11.6	10.5
32		41.9	39.9	32.9	27.0	25.5	25.0	21.5	16.9	12.9	12.2
35		42.0	39.9	32.7	33.8	27.2	25.0	14.7	14.0	12.5	14.1
						29.2	**25.6**				

In this species, prolonged exposures to low constant acclimation temperature (20°C), cold resistance increase 2.5ºC (Tables 4a and 4b).

The increasing resistance in *P. sphenops* after exposed to the critical thermal maximum and minimum experiments subsequent to the thermal fluctuations reveals an adjustment of the fish homeostatic mechanisms reflected in their capacity of thermal response. The thermoresistence of fish exposed to increasing and decreasing temperatures can be consider as an approach to select more resistant species to the environmental thermal variations.

The daily and seasonal temperature fluctuations influence the critical thermal maximum temperature evidencing the differences and adjustments in the tolerance to high temperatures and in the distribution of several species such as *Etheostoma flabellare*, *E. blennoides*, *E. caeruleum* and *Morone saxatilis* (Hlohowskyj & Wissing, 1985; Cox, 1978). The acclimation capacity of the organisms confronting different temperature regimens differs among species, which indicates that the thermal ability can be correlated with the environmental thermal stability (Barrionuevo & Fernandes, 1995).

Currie *et al*. (2004) found that CTMax and CTMin of *Ictalurus punctatus, Micropterus salmoides* and *Oncorhynchus mykiss* exposed for 24 h to a sine-wave trajectory “10ºC thermo period” for 32 consecutive days, did not reveal “increase or decrease differences between the same species acclimated to a constant temperature equal to the mean of the thermoperiod”. Their results differ from other research therefore they consider that “the explanation may be the characteristics of thermo periods including shape, peak, minimum temperature and amplitude”. We agree that thermo cycles characteristics are very important for analyzing, comparing and explaining results but also associate with the fact that this type of response is species specific.

The upper incipient lethal temperature of *P. sphenops* exposed to the 20-29 and 26-35°C TFs had an interval of 38.8-39.5°C, these values are similar when this species is acclimated at constant temperatures, 26 to 35°C (Table 4b) (Hernández-Rodriguez, 1998). Similar results have been obtained in *Salvelinus fontinalis*, *Cyprinodon nevadensis*, *Salmo gairdneri* and *Lepomis gibbosus*, where the resistance to extreme temperatures was associated with the thermal history or with the fish acclimation conditions (Brown & Feldmeth, 1971; Fahmy, 1972; Hokanson, 1977; Becker & Genoway, 1979).

The lower incipient lethal temperature of *P. sphenops* acclimated to both thermal fluctuations was 10.8-11.8°C and they do not resist less than 10°C, however, they increased its resistance in 0.67 and 0.95°C respectively. The thermal differences of this response were of 3.3 and 1.0°C compare with fish acclimated from 20 to 26°C constant temperatures (7.5-9.8°C) respectively. In this species, the acclimation to constant and fluctuating temperatures is a determining factor to identify the thermal points that characterize this type of response. This response has been studied in few species, and the values obtained are dependent of the thermal history and the acclimation temperature. The LILT values varied depending on the methodology used by the authors that can imply, an abrupt thermal change or gradual decrease of 1°C day^{-1} until the organisms loses the equilibrium or they fall in coma.

The results of experiments with the low and high lethal temperatures vary due to the grade of eurythermality and stenothermality among species, because the organisms resistance depends on the thermal history (acclimation or acclimatization), age and time-space distribution. Fry (1967) mentioned that lethal temperatures are important for the physiologic analysis of poikilotherm organisms because it provides a detailed pattern of responses that can be used as an immediate ecological index, since the animals can find lethal temperatures in the habitat as well as thermal fluctuations overcoming their tolerance limits.

Fish behaviour studies, included in the tolerance area of the response polygon; consider the preferred temperature as one of the most important responses because the organisms choose the temperatures that could be the appropriate for their physiologic processes efficiency. In *P. sphenops* acclimated a constant and fluctuations temperature, the thermal preference was influenced by the sex (Tables 4a and 4b) (Hernández-Rodríguez *et al*., 2002). Females had a final preferendum of 31.6ºC during the day and 28.9ºC in the night. Males preference was 26.9ºC during the day and 25.5ºC in the night (Hernández-Rodríguez, 1998).

In general, females of *P. sphenops* prefered higher temperatures than males which could be associated with the grade of sexual maturity studied in other species. For instance, in *Gambusia sp*. and *Dionda nubila* has been pointed out that the resistance to heat may be concatenated with sex and it is suggested that, fish sexual hormones could be related with the thermoregulation behaviour (Hagen, 1964; Baker *et al*., 1970). In this conduct Johansen and Cross (1980), studied the effect of steroid hormones on the sexual maturation of *Poecilia reticulata* that influenced the males preferred temperature by choosing lower temperatures (24.5°C) than females (28.2°C) and juveniles (28.1°C). The authors also comment that the male's androgenic hormone induces the preferred temperature decrease, which was verified treating juveniles and females with several testosterone concentrations. A similar situation in *P. sphenops* is probable; however, it is necessary more studies in this theme.

For many species, the preferred temperature is mainly a function of the thermal acclimation history of the individuals. In this context, the preferred temperature varies markedly among the species in direct or inversely proportional correspondence to the acclimation temperature. This correlation is an adaptative response, where the exposure to a new temperature causes a compensatory physiologic adjustment by increasing the metabolic efficiency to the new condition generating an appropriate adjustment in the preferred temperature (Kelsch & Neill, 1990).

The thermoregulatory behaviour of the organisms is extremely variable, because of the numerous factors like age, sex, time of the year and food availability, among others that influence the organism's response in such a way that, each species is characterized by a preferential interval or a final preferendum where the biological processes are at their maximum efficiency. When the eurythermic species behaviour is associated with the geographical distribution the panorama of influences is expanded with ecological, social and genetic factors. On the other hand in the stenothermic species, the temperature effect at physiologic level can be the dominant factor that governs its distribution (Cherry *et al*., 1975, 1977).

The preferential orientation that the organisms use to select a thermal interval in the environment, serves them to find and adapt to the temporary (provisional) space of their niches. In this theme, the response of thermal avoidance of *P. sphenops* that were acclimated to the 20-29°C TF had an avoidance range over 14°C; and in the symmetric and asymmetric cycles of 26-35°C TF the avoided temperatures had an interval of 9.3 and 11.4°C respectively. The fish acclimated to the TFs of the SC and AC enlarged the avoidance interval. Most of the investigations related with the avoided temperatures have been made in organisms acclimated to constant temperatures. Eurythermic species like the ciprinidae, centrarquidae and ictaluridae acclimated from 6-30°C are characterized for having relatively wide intervals of low and high avoided temperatures (Cherry *et al*., 1975, 1976, 1977). Jander (1975) based on the lifestyle of the species, considers that the organisms that minimize the effective distance of a primary resource (food, refuge, reproduction and other) they maximize their effective distance of a stress source (temperature, predators and other) they can respond better to the environmental variation compare with other animals that do not use this mechanism.

The capacity of adaptation to thermal changes is a highly complex problem, because the physiologic adjustments of the organisms happen at all levels of its biological organization with compensatory and other non-compensatory responses, the latter usually associated to seasonal rhythms as, food availability, reproduction, migration and molt, among other (Cossins & Bowler, 1987).

The knowledge of the relationship between the constant or fluctuating acclimation temperature and the thermoregulatory behaviour of the species allows understanding part of the biology, geographical distribution and adaptation capacity of *P. sphenops* to the thermal changes that can occur in the habitat. This ability could explain the wide geographical distribution of *P. sphenops* and the high reproduction rate. The behaviour pattern observed in this investigation including the results of the constant temperatures experiments, characterizes it as a species with a great plasticity response towards the thermal fluctuations that occurred in the dam with a daily monthly average maximum temperature from 26 to 36°C and minimum from -0.2 to 5°C. *P. sphenops* is perfectly adapted to the marked rain and drought seasons of their habitat, via increasing or decreasing their preference intervals, tolerance and resistance conferring them a better adjustment capacity towards the temperature variation.

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REFERENCES

Anguilletta Jr., M.J. 2009. Thermal adaptation. A theoretical and empirical synthesis. Oxford University Press, London, 289 pp.

Armour, C.L. 1991. Guidance for evaluating and recommending temperature regimes to protect fish. U.S. Fish Wildl. Serv., Biol. Rep., 90(22): 13 pp.

Baker, C.D., W.H. Neill Jr. & K. Strawn. 1970. Sexual difference in heat resistance of Ozark minnow, *Dionda nubila* (Forbes). Trans. Am. Fish. Soc., 99: 588-591.

Barón-Sevilla, B. 2001. Inducción hormonal del sexo en *Poecilia sphenops*. Tesis de Doctorado en Ciencias, Universidad Nacional Autonóma de México, 85 pp.

Barrionuevo, W.R. & M.N. Fernandes. 1995. Critical thermal maxima and minima for curimbatá, *Prochilodus scrofa* Steindachner, of two different sizes. Aquat. Res., 26: 447-450.

Becker, C.D., R.C. Genoway & M.J. Schneider. 1977. Comparative cold resistance of three Columbia River organisms. Trans. Am. Fish. Soc., 106: 178-184.

Becker, C.D. & R.G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environ. Biol. Fish., 4: 245-256.

Brett, J.R. 1956. Some principles in the thermal requirements of fishes. Quart Rev. Biol., 31: 75-87.

Brown, H.J. & C.R. Feldmeth. 1971. Evolution in constant and fluctuating environments: thermal tolerances of desert pupfish (*Cyprinodon*). Evolution, 25: 390-398.

Bückle-Ramírez, L.F., B. Barón-Sevilla, M. Hernández-Rodríguez, A. Ledo-Martell, J.R. Solis-Aguilar B. Pérez-Estrada & A. Hernández-Santiago. 2003. Sistema de temperatura, oxígeno y salinidad para la experimentación en ecofisiología. Hidrobiológica, 13: 277-287.

Carveth, C.J., A.M. Widmer, S.A. Bonar & J.R. Simms. 2006. An examination of the effects of chronic static and fluctuating temperatures on the growth and survival of spikedace, *Meda fulgida*, with implications for management. J. Therm. Biol., 32: 102-108.

Crawshaw, L.I. 1977. Physiological and behavioral reactions of fishes to temperature change. J. Fish. Res. Bd. Can., 34: 730-734.

Cherry, D.S., K.L. Dickson & J. Cairns Jr. 1975. Temperature selected and avoided by fish at various acclimation temperatures. J. Fish. Res. Bd. Can., 32: 485-491.

Cherry, D.S., R.K. Guthrie Jr., J.H. Rogers, J. Cairns Jr. & K.L. Dickson. 1976. Response of mosquito fish (*Gambusia affinis*) to ash effluents and thermal stress. Trans. Am. Fish. Soc., 105: 686-694.

Cherry, D.S., K.L. Dickson, J. Cairns Jr. & J.R. Stauffer. 1977. Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. J. Fish. Res. Bd. Can., 34: 239-246.

Cossins, A.R. & K. Bowler. 1987. Temperature biology of animals. Chapman & Hall, London, 339 pp.

Cox, D.K. 1978. Acclimation states of juvenile striped bass held in constant and fluctuating temperature regimes. In: J.H. Thorp & J.W. Gibbons (eds.). Energy and environmental stress in aquatic systems. Published Technical Information Center U.S. Department of Energy, pp. 703-713.

Currie, R.J., W.A. Bennett, T.L. Beitinger & D.S. Cherry. 2004. Upper and lower temperature tolerance of juvenile freshwater game-fish species exposed to 32 days of cycling temperatures. Hydrobiologia, 532: 127-136.

Diana, S.J. 1984. The growth of largemouth bass, *Micropterus salmoides* (Lacépède), under constant and fluctuating temperatures. J. Fish Biol., 24: 165-172.

Dhillon, R.S. & M.G. Fox. 2007. Growth-independent effects of a fluctuating thermal regime on the life-history traits of the Japanese medaka (*Oryzias latipes*). Ecol. Fresh. Fish, 16: 425-432.

Fahmy, F.K. 1972. Heterogeneous acclimation of fish to temperature. Can. J. Zool., 50: 1035-1037.

Feldmeth, C.R., E.A. Stone & J.H. Brown. 1974. An increased scope for thermal tolerance upon acclimating pupfish (*Cypronodon*) to cycling temperatures. J. Com. Physiol., 89: 39-44.

Fry, F.E.J. 1947. Effects of the environment on animal activity. University of Toronto Studies, Biol. Ser. 55, Ontario Fisheries Research Laboratory Publication, 68: 1-62.

Fry, F.E.J. 1967. Responses of vertebrate poikilotherms to temperature. In: A.H. Rose (ed.). Thermobiology. Academic Press, New York, pp. 375-409.

Giattina, J.D. & R.R. Garton. 1982. Graphical model of thermoregulatory behavior by fishes with a new measure of eurithermality. Can. J. Fish. Aquat. Sci., 39: 524-528.

Hagen, D.W. 1964. Evidence of adaptation to environmental temperatures in three species of *Gambusia* (Poeciliidae). Southwest Naturalist, 9: 6-19.

Hernández-Rodríguez, M. 1998. Comportamiento termorregulador de *Poecilia sphenops* (Pisces: Poeciliidae) aclimatada a temperaturas constantes y fluctuantes. Thesis, Centro de Investigación Científica y de Educación Superior de Ensenada, B.C. México, 136 pp.

Hernández-Rodríguez, M. & L.F. Bückle-Ramirez. 1998. Critical thermal maximum and minimum of *Poecilia sphenops* acclimated to different temperatures. Riv. Ital. Acquacolt., 33: 61-71.

Hernández-Rodríguez M. & L.F. Bückle-Ramirez. 2002. Temperature tolerance polygon of *Poecilia sphenops* (Pisces, Poeciliidae). J. Therm. Biol., 27(1): 1-5.

Hernández-Rodríguez, M., L.F. Bückle-Ramirez & S. Espina. 2002. Temperature preference in *Poecilia sphenops* (Pisces, Poeciliidae). Aquac. Res., 33: 933-940.

Hlohowskyj, I. & T.E. Wissing. 1985. Seasonal changes in the critical thermal maxima of fantail (*Etheostoma flabellare*), greenside (*Etheostoma blennioides*), and rainbow (*Etheostoma caeruleum*) darters. Can. J. Zool., 63: 1629-1633.

Hokanson, E.F.K. 1977. Temperature requirements of some percids and adaptations to the seasonal temperature cycle. J. Fish Res. Bd. Can., 34: 1524-1550.

Jander, R. 1975. Ecological aspects of spatial orientation. Ann. Rev. Ecol. Syst., 6: 171-88.

Johnstone, C.H. & F.J. Rahel. 2003. Assessing temperature tolerance of Bonneville cuttthrat trout based on constant and cycling regimes. Trans. Am. Fish. Soc., 132: 92-99.

Johansen, H.P. & A.J. Cross. 1980. Effects of sexual maturation and sex steroid hormone treatment on the temperature preference of the guppy, *Poecilia reticulata* (Peters). Can. J. Zool., 58: 586-588.

Kelsch, W. & H.W. Neill. 1990. Temperature preference versus acclimation in fishes: Selection for changing metabolic optima. Trans. Am. Fish. Soc., 119: 601-610.

Kilgour, M.D., R.W. McCauley & W. Kwain. 1985. Modeling the lethal effects of high temperature on fish. Can. J. Fish. Aquat. Sci., 42: 947-951.

Lutterschmidt, I.W. & H.V. Hutchison. 1997. The critical thermal maximum: data to support the onset of spasms as the definitive en point. Can. J. Zool., 75: 1553-1560.

Otto, G.R. 1974. The effects of acclimation to cycle thermal regimes on heat tolerance of the western mosquito fish. Trans. Am. Fish. Soc., 2: 331-335.

Paladino, F.V., J.R. Spotila, J.P. Schubauer & K.T. Kowalski. 1980. The critical thermal maximum: a technique used to elucidate physiological stress and adaptation in fishes. Can. Biol. Rev., 39: 115-122.

Pitkov, R.B. 1960. Cold death in the guppy, *Lebistes reticulatis*. Biol. Bull., Mar. Biol., Lab. Woods Hole, 119: 231-245.

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Pörtner, H.O., F.C. Mark & C. Bock. 2004. Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. Resp. Physiol. Neurobiol., 141: 243-260.

Widmer, A.M., C.J. Carveth & S.A. Bonar. 2006. Upper temperature tolerance of Loach Minnow under acute, chronic, and fluctuating thermal regimes. Trans. Am. Fish. Soc., 135: 755-762.