Critical thermal limits of *Poecilia caucana* (Steindachner, 1880) (Cyprinodontiformes: Poeciliidae)

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Although temperature has far-reaching effects on fish biology, the thermal tolerance ranges of most freshwater fish species are unknown. This lack of information precludes forecasting responses to climatic change and does not allow for comparative analyses that may inform evolutionary and biogeographic studies. We used the critical thermal methodology to quantify acclimation capacity and thermal tolerance in the Neotropical freshwater species *Poecilia caucana*. For fish acclimated to 20˚C, 25ºC, and 28ºC, critical thermal minima (CT_{min}) were 12.52 ± 0.62ºC, 13.41 ± 0.56ºC and 14.24 ± 0.43ºC respectively, and critical thermal maxima (CT_{max}) were 38.43 ± 0.64ºC, 40.28 ± 0.92ºC and 41.57 ± 0.27ºC, respectively. Both CT_{min} and CT_{max} changed with acclimation temperatures, indicating that *P. caucana* was effectively acclimatable. Relative to values reported for other freshwater fish species, the acclimation capacity of *P. caucana* for CT_{min} was low, but it was average for CT_{max}. The data, together with similar work in other species, can be used in analyses focusing on broad ecological and evolutionary questions.

Aunque la temperatura tiene grandes repercusiones en la biología de los peces, se desconocen los rangos de tolerancia térmica de la mayoría de los peces dulceacuícolas. Esta falta de información impide pronosticar respuestas al cambio climático y limita los análisis comparativos que podrían enriquecer estudios evolutivos y biogeográficos. Utilizamos la metodología del crítico térmico para cuantificar la capacidad de aclimatación y la tolerancia térmica en la especie neotropical dulceacuícola *Poecilia caucana*. Para peces aclimatados a 20˚C, 25ºC y 28ºC, los críticos térmicos mínimos (CT_{min}) fueron $12,52 \pm 0,62$ºC, $13,41 \pm 0,56$ºC y $14,24 \pm 0,43$ºC, respectivamente, y los críticos térmicos máximos (CT_{max}) fueron $38,43 \pm 0,64$ºC, $40,28 \pm 0,92$ºC y $41,57 \pm 0,27$ºC, respectivamente. Tanto el CT_{min} como el CT_{max} cambiaron significativamente con las temperaturas de aclimatación, indicando que *P. caucana* es efectivamente aclimatable. Comparada con otras especies de peces dulceacuícolas, la capacidad de aclimatación de *P. cuacana* fue baja para CT_{min} y promedio para CT_{max}. Estos resultados, en conjunto con los datos de otras especies, pueden ser utilizados para responder preguntas ecológicas y evolutivas más generales.

Keywords: Acclimation capacity, Colombia, Physiological ecology, Thermal tolerance range.

Introduction

Temperature influences crucial organismal traits such as energy use, growth, reproduction, and development (Johnston & Bennett, 1996). Temperature is especially important for ectotherms, including fishes, because of its influence on metabolic rate and physiological efficiency (Hoffman *et al.*, 2012; Ohlberger *et al.*, 2012). Some fish traits influenced by temperature are foraging pattern and foraging ability (Persson, 1986), locomotor capacity (Bennett, 1990), sexual selection (Johansen, 1985), mating preferences (Johansen, 1985), and life-history traits such as age at sexual maturity (Kuparinen *et al.*, 2011). Accordingly, temperature has been suggested to be the abiotic factor with strongest effect on fish biology (Brett, 1971).

When organisms are exposed to changes in temperature, they may display various strategies to stay within tolerance limits so that vital biological processes and physiological rates are maintained. These strategies vary from behavioral (moving to more comfortable sites), to physiological (*e.g.*, phenotypic plasticity in enzyme conformation or plasmatic membrane fluidity; Di Prisco & Giardina, 1996; Gracey *et al.*, 1996), to evolutionary (*e.g.*, evolution of dormancy stages; Marshall & Sinclair, 2011). One of the first steps to study the effect of temperature on the biology of any species is to determine its range of thermal tolerance. However, this sort of information is still lacking for most freshwater fish species.

Collecting data on thermal tolerances of organisms is especially relevant given the urgency to determine the ability

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of species to persist in the face of ongoing anthropogenic climate change (Sala *et al.*, 2000; Stillman, 2003; Pörtner & Farrell, 2008). The need for this kind of data is particularly pressing for tropical species because these may show overall higher susceptibility to climatic changes (Deutsch *et al.*, 2008; Sheldon *et al.*, 2011). However, data on temperature tolerance ranges are especially scarce for tropical freshwater species. In addition, the limited existing data are difficult to examine in a comparative framework because studies used dissimilar methodologies that measure different aspects of physiological limits and experimental parameters such as time, rate of temperature increase, and endpoint of the experiment are not consistent across studies. The lack of basic information and consistent methodology has thus prevented the analysis of thermal tolerance of fishes in broad ecological and evolutionary contexts (*e.g.* Ghalambor *et al.*, 2006; Araújo *et al.*, 2013).

We estimated the range of temperature tolerance for the Cauca Molly, *Poecilia caucana* (Steindachner, 1880), a Neotropical Poeciliid species from the Magdalena River basin of Colombia. We assessed thermal tolerance using the critical thermal methodology, a non-lethal procedure that requires relatively few individuals (Lutterschmidt & Hutchinson, 1997; Beitinger *et al.*, 2000). We measured critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) on fish obtained in the field and acclimated to three different temperatures in the laboratory. Using these data, we estimated the capacity of this species to vary its CT_{min} and CT_{max} values when acclimated to different temperatures. Despite Colombia's rich freshwater fish fauna (Maldonado *et al.*, 2008), this is the first study to report thermal tolerance ranges for any of its fish species. In addition, we estimated the thermal tolerance polygon for *P. caucana* and compared it to existing data on other tropical fish species. Although the available data are still too limited to reach any definitive conclusions about patterns of variation in thermal tolerance among tropical fishes, our work is one the first efforts to synthetize the existing information (Prodocimo & Freire, 2001).

Material and Methods

Fish collection and acclimation period. Adult males and females of *P. caucana* were collected in September 2013 with a seine in a man-made pond in the municipality of Guapotá, Santander, Colombia (6˚20'30N 73˚18'25W, 1350 m elevation). We assume that thermal tolerances of these fish should be similar to those from other natural populations of this species because the pond was connected to a natural stream, which connects the pond population to other natural populations, and because this species prefers low-flow habitats similar to the source pond (Maldonado-Ocampo *et al.*, 2006). Surface temperature was measured *in situ* three times to calculate a mean value (25.13 ± 0.21ºC). Fish were transported to the Universidad de los Andes in Bogotá less than 24 hours after capture

to be kept at constant conditions (25ºC) for one week in three 42-l fish-tanks in the laboratory. Fish were then transferred to different 42-l fish-tanks (65 fish per tank) with constant aeration and experienced three different acclimation temperatures (T_a): 25ºC (the average field temperature), 20ºC (the most common temperature in studies of critical thermal limits), and 28ºC (the highest stable temperature with the equipment available to us). The different acclimation temperatures were chosen to assess the acclimation ability of the species and to determine whether critical thermal limits depend on the conditions to which fish were acclimated. Two replicate tanks were set at each temperature for a total of six tanks in which we allowed fish to acclimate for at least 30 days (Lutterschmidt & Hutchinson, 1997). During this time, temperature was kept constant with Shark H-229 thermo regulators. Fish were fed *ad libitum*, taking care to remove excess food; 25% of the water was changed in each tank every two days with treated tap water and commercial API Stress Coat.

Estimation of critical thermal limits. CT_{max} and CT_{min} are physiological upper and lower limits that are determined by acclimating animals to different temperatures and then exposing them to a constant linear increase or decrease in temperature until a predefined sub lethal endpoint is reached. In fishes, the characteristic sub lethal endpoint is typically recognized by loss of equilibrium and a halt in opercular movements (Lutterschmidt & Hutchinson, 1997). CT_{max} was defined as the temperature at which acclimated fish subjected to increasing temperature showed loss of equilibrium (LOE; Beitinger *et al.*, 2000; Currie *et al.*, 1998). LOE is not lethal, as most fish survive the experiment (94% in this study). The water temperature was changed at a constant rate of 0.3˚C/min, either heating it using four Shark H-229 thermo regulators, or cooling it with a MC-1/4HP AquaEuroUSA Cooling Chiller. The volume of water in the experimental tanks was 30 l and 10 l for the CT_{min} and CT_{max} tests, respectively. These volumes were established given our ability to modify temperatures based on our equipment. Styrofoam was used as insulation, covering the sides and bottom of the experimental tank. Experiments started at the same temperature at which fish were acclimated; after LOE was reached, fish were immediately transferred to water with the same temperature as that in their respective acclimation tanks. A total of 399 fish were tested, 183 in CT_{max} trials and 216 in CT_{min} trials. For each trial the number of fish tested ranged between 8 and 24.

Once we determined CT_{max} and CT_{min}, we estimated a thermal tolerance polygon, the thermal area in which the species may survive (Lutterschmidt & Hutchinson, 1997; Beitinger *et al.*, 2000). We measured acclimation capacity as the change in critical thermal limit divided by the change in acclimation temperature ($\Delta CT/\Delta T_a$); this quantity indicates the degree to which organisms are able to adjust their thermal sensitivity given changes in environmental

temperature (Stillman, 2003). Because no statistical difference was found between replicate tanks (t-test, *p*>0.05), all the data for each acclimation temperature were pooled when testing for acclimation capacity. We tested the effect of acclimation and fish tanks on CT_{min} and CT_{max} using one-way within-subjects ANOVA and Tukey's multiple comparisons tests implemented in R 3.0.1. (R Development Core Team, 2015).

Finally, based on a literature review, we compared the estimates of thermal tolerance for *P. caucana* with data for 13 species in six orders of fishes. These species had reports of mean (and standard deviation, SD) CT_{min} and CT_{max} for at least two acclimation temperatures. We conducted randomizations to test whether the acclimation capacities for both CT_{min} and CT_{max} differed between *P. caucana* and other freshwater species. We performed four separate randomizations of the slopes of the lines describing acclimation capacity, *i.e.* the line connecting CT_{min} or CT_{max} (Y-values) at different T_a (X-values), two for CT_{min} and two for CT_{max}. With the first randomization for each CT we tested whether the slopes estimated for *P. caucana* were higher than those estimated for other species, and with the second we tested whether the slopes of *P. caucana* were lower than those of other species. In each of these randomizations, p-values were calculated as the number of times that simulated slopes were either lower or higher in *P. caucana* than in each of the other species, divided by the total number of runs (10,000 times). We adjusted significance values due to multiple comparisons by performing a false discovery rate test (Verhoeven *et al.*, 2005), which determines an alpha value that decreases from 0.05 in proportion to the number of statistical tests performed, in this case 13 (one for each comparison between *P. caucana* and the 13 species having CT mean and SD data).

Results

For fish acclimated to 20°C, 25°C, and 28°C, CT_{min} were 12.52 ± 0.62°C, 13.41 ± 0.56°C and 14.24 ± 0.43°C respectively, and CT_{max} were 38.43 ± 0.64°C, 40.28 ± 0.92°C and 41.57 ± 0.27°C, respectively (Fig. 1, 2). We found that *Poecilia caucana* is acclimatable with respect to thermal tolerance: both CT_{min} and CT_{max} increased significantly with acclimation temperature (one-way ANOVAs, p<0.001; Fig. 1). The area comprised by the thermal tolerance polygon was 214.65°C^2. The acclimation capacity of the CT_{min} of *P. caucana* was 0.17, on the lower end of that observed in all 13 other species it was compared to, and significantly lower than that of *Micropterus salmoides* (p<0.001), *Ictalurus punctatus* (p<0.001), *Pangasius pangasius* (p<0.001), *Carasius auratus* (p = 0.025), and *Danio rerio* (p = 0.020; Fig. 3). The acclimation capacity of the CT_{max} of *P. caucana* was 0.31. This value was similar to that observed in all 13 other species it was compared to and not significantly lower than that reported for any other freshwater species (Fig. 3),

but was statistically higher than that of four other species (*Oncorhynchus mykiss*, p = 0.018; *Labeo rohita*, p = 0.015; *Cirrhinus mrigala*, p<0.001; and *P. pangasius*, p = 0.007).
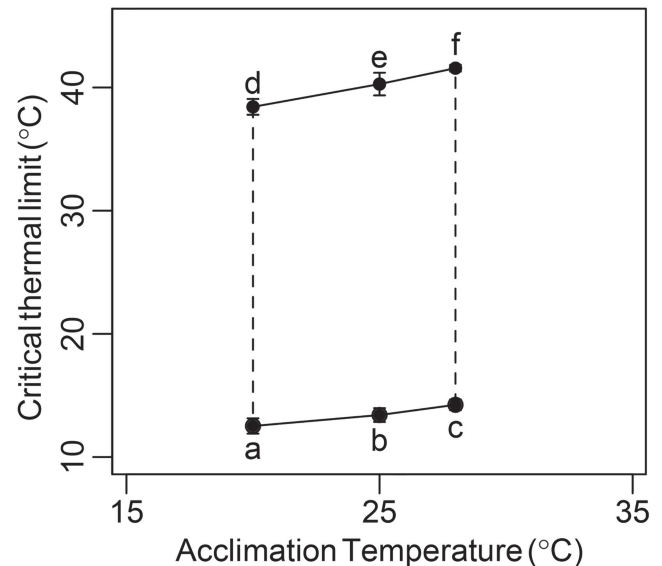
Fig. 1. Thermal tolerance limits of *Poecilia caucana*. Values represent mean ± SD in critical thermal limits estimated for each acclimation temperature. Letters represent significant differences in the Tukey post-hoc test using α=0.05.

Discussion

Poecilia caucana showed a high upper thermal tolerance (CT_{max}) relative to other freshwater fishes, *i.e.* it has a CT_{max} higher than 40°C (Fig. 2). High upper thermal tolerance is characteristic of other members of the family Poeciliidae and in general of members of the order Cyprinodontiformes (Fig. 2; Beitinguer *et al.*, 2000: table 8). The highest documented CT_{max} of any Poeciliid is 42.3±0.4°C, observed in *Gambusia affinis* acclimated at 35°C (Otto, 1973). Our data suggest that *P. caucana* may tolerate higher temperatures if acclimated at higher temperatures than those used in this study, because after acclimation at 25° it showed a higher CT_{max} than *G. affinis* (40.28±0.92°C vs. 38.0±0.4°C; Otto, 1973). Moreover, the CT_{max} value for *P. caucana* is only slightly lower than that reported for some species of Cyprinodontiformes with the highest heat-tolerance (*e.g.*, *Cyprinodon* sp., CT_{max} = 41.7±0.19°C at T_a=25°C; Otto & Gerking, 1973). Therefore, *P. caucana* may be among the species with the highest CT_{max} in the order, but this needs further tests with individuals acclimated to higher temperatures than those available to us, and more studies should be conducted on other species in the order (*e.g.*, species inhabiting thermal springs likely have greater CT_{max} values than those reported so far for this group).

In contrast to its high tolerance for high temperatures, *P. caucana* was relatively intolerant to cool temperatures, *i.e.*, CT_{min} in all treatments was higher than 10°C (Figs. 1-2). Other poeciliids tolerate lower temperatures. For

example, the CT_{min} of *Poeciliopsis occidentalis, Poecilia sphenops*, and *Poecilia latipinna* were 4.8ºC, 7.5ºC, and 7.6ºC, respectively (Bulger & Schultz, 1982; Hernández *et al*., 2002; Bierbach *et al*., 2010). Because all these species occur at higher (*i.e.* cooler) latitudes than *P. caucana,* the data appear consistent with the hypothesis that thermal tolerance ranges are narrower in organisms from lower latitudes (Janzen, 1967; Sheldon & Tewksbury, 2014). In a forthcoming study, we test this hypothesis in a phylogenetic framework.

Relative to other freshwater fish species, the acclimation capacity of *P. caucana* was low for CT_{min} (0.17) and close to average for CT_{max} (0.31; Fig. 3). Compared to other poeciliids, *P. caucana* has a lower heat acclimation capacity than *G. affinis*, although estimates for the latter species vary considerably between two studies (*i.e.*, 0.34 vs. 0.43; Otto, 1973, 1974). This variation may be explained by environmental differences between populations or by differences in the range of acclimation temperatures (the first study used a wide range from 5 ºC to 35 ºC and the second a range from 25 ºC to 35 ºC). Thus, comparative studies should bear in mind that data collected from thermal tolerance experiments depend strongly on experimental conditions (Terblanche *et al*., 2007).

To our knowledge there are no functional studies of *P. caucana* that may allow one to explain the limited tolerance to cold temperatures of the species. A possible mechanism involved is oxygen limitation, proposed as the strongest physiological barrier establishing thermal limits (Pörtner & Knust, 2007). There are, however, multiple other mechanisms that may be involved, including reductions in heart function, mitochondrial respiration, membrane static order (fluidity), action potential generation, protein synthesis, heat-shock protein expression, and protein thermal stability (Somero, 2002).

A limitation to any ecophysiological study arises from the geographical origin of the studied species. As with most studies reporting critical thermal limits (Das *et al*., 2004; Dalvi *et al*., 2009; Barrionuevo & Fernandes, 1995; Currie *et al*., 1998; Prodocimo & Freire, 2001), we focused on a single population of *P. caucana*. Because populations might be locally adapted or show long-term effects of diet and other environmental factors (Feminella & Mattheus, 1984; Atwood *et al*., 2003; Narum *et al*., 2013), the conclusions that follow regarding vulnerability to climate change are preliminary and subject to revision once more populations of this species are assessed. The information is nonetheless the best available so far for *P. caucana*.
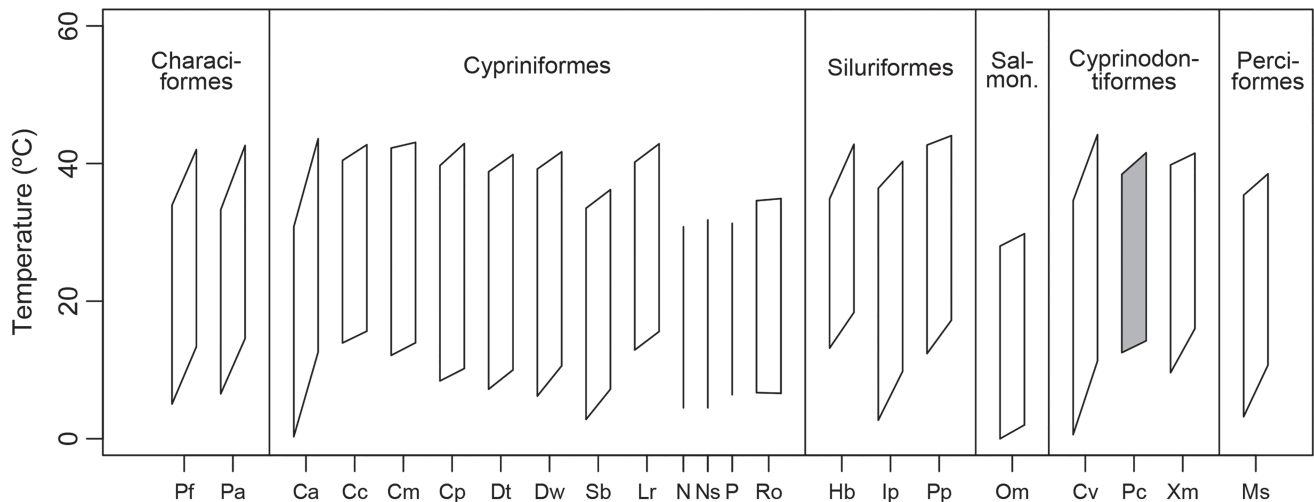
Fig. 2. Comparison of Critical Thermal (CT) limits for *Poecilia caucana* (Pc, grey polygon) and a sample of species with CT_{min} and CT_{max} data available in the literature. Polygons are formed by two vertical lines representing the CT tolerance ranges observed at the lowest and highest acclimation temperatures used in each study. The polygons have an arbitrary width that does not reflect the range of acclimation temperatures used among different studies. The top and bottom lines of each polygon join, respectively, the CT_{max} and CT_{min} values for each species. Three species (N, Ns, and P) having data for only one acclimation temperature are represented by vertical lines. Pf = *Prochilodus scrofa* (currently *Prochilodus lineatus*), fry, and Ps = *P. scrofa*, adults (Barrionuevo & Fernandes, 1995); Ca = *Carassius auratus* (Ford & Beitinger, 2005); Cc = *Catla catla* and Cm = *Cirrhinus mrigala* (Das *et al*., 2004); Cp = *Cyprinus carpio* (Chatterjee *et al*., 2004); *Danio rerio*, transgenic breed, and *D. rerio*, wild (Cortemeglia & Beitinger, 2005); Sb = *Siphateles bicolor* (McClanhan *et al*., 1986); Lr = *Labeo rohita* (Chatterjee *et al*., 2004; Das *et al*., 2004); N = *Notropis chrysocephalus*, Ns = *N. spilopterus*, P = *Pimephales notatus* (Hockett & Mundahl, 1989); Ro = *Rhinichthys osculus* (Kaya *et al*., 1992); Hb = *Horabagrus_brachysoma* (Dalvi *et al*., 2009); Ip = *Ictalurus punctatus* (Currie *et al*., 1998); Pp = *Pangasius pangasius* (Debnath *et al*., 2006); Om = *Oncorhynchus mykiss* (Currie *et al*., 1998); Cv = *Cyprinodon variegatus* (Bennett & Beitinger, 1997); Pc = *Poecilia caucana* (this study); Xm = *Xiphophorus maculatus* (Prodocimo & Freire, 2001); Ms = *Micropterus salmoides* (Currie *et al*., 1998).
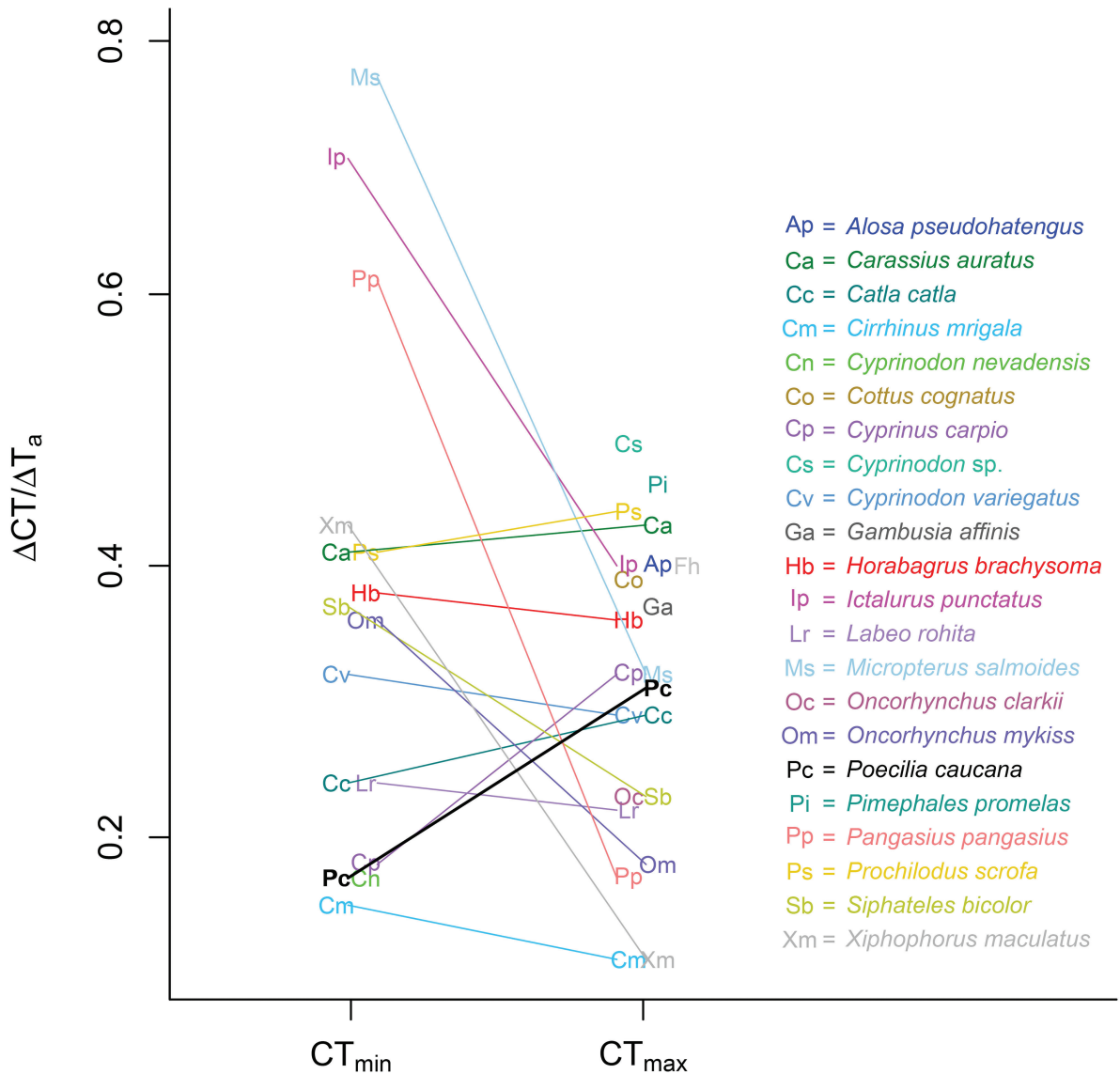
Fig. 3. Comparison of acclimation capacity for *Poecilia caucana* (Pc, bold letter and thicker line) and a sample of species with CT and acclimation temperature values available in the literature. Lines join acclimation capacities for CT$_{min}$ and CT$_{max}$ of the same species, when both values are available. Symbols are jittered in the horizontal axis to avoid crowding. Abbreviations as in Fig. 2 except for Ap = *Alosa pseudoharengus* (Otto *et al.*, 1976); Cn = *Cyprinodon nevadensis* (Feldmeth *et al.*, 1974); Cs = *Cyprinodon* sp. (Otto & Gerking, 1973); Fh = *Fundulus heteroclitus* (Bulger & Tremaine, 1985); Ga = *Gambusia affinis* (Otto, 1973, 1974); Oc = *Oncorhynchus clarkii* (Heath, 1963).

Our literature survey revealed that most available data on thermal tolerance in fishes are biased to the temperate zone (and focus on the family Cyprinidae), an effect of greater research efforts in North America and Europe. Thermal tolerance data remain lacking for most freshwater fish species despite their extreme diversity (Lundberg *et al.*, 2000) and despite how essential such data are for understanding the natural history and range limits of species, and hence their potential responses to environmental change. In the case of *P. caucana*, for example, the relatively low tolerance to cool temperatures we observed may explain why this species is restricted to warm elevations below 1500 m (Maldonado *et al.*, 2006). Thermal tolerance data may also be useful to estimate the ability of species to tolerate changes in water temperature associated with global warming or with industrial discharges. It appears unlikely that the temperature changes projected even under the most pessimistic global warming scenarios (Walters *et al.*, 2012) pose a risk for the viability of populations of *P. caucana*. On the other hand, streams can reach high temperatures due to wastewater from industrial plants (*c.* 50ºC in some sites; Cairns, 1969), which may threaten its local persistence. More studies on the thermal biology of *P. caucana* and other tropical fish species are needed to be able to forecast effects and manage populations in the face of global change.

Acknowledgements

Adolfo Amézquita at the Departamento de Ciencias Biológicas of the Universidad de los Andes provided equipment for experimentation. Juan Pablo Bueno, Laura Natalia Céspedes, Juliana Cuccaro, Juan Fernando De la Hoz, Ana Maria Galeano, Santiago Herrera, David Ocampo, Ángela Perilla and Simón Quintero helped throughout experimentation and fish maintenance. Jorge Mejía facilitated access to the field site in his property. Ángela Celis, Egna Mantilla, Julián Lozano, members of the Laboratorio de Biología Evolutiva de Vertebrados at Universidad de los Andes, and two anonymous reviewers made valuable comments on the manuscript.

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