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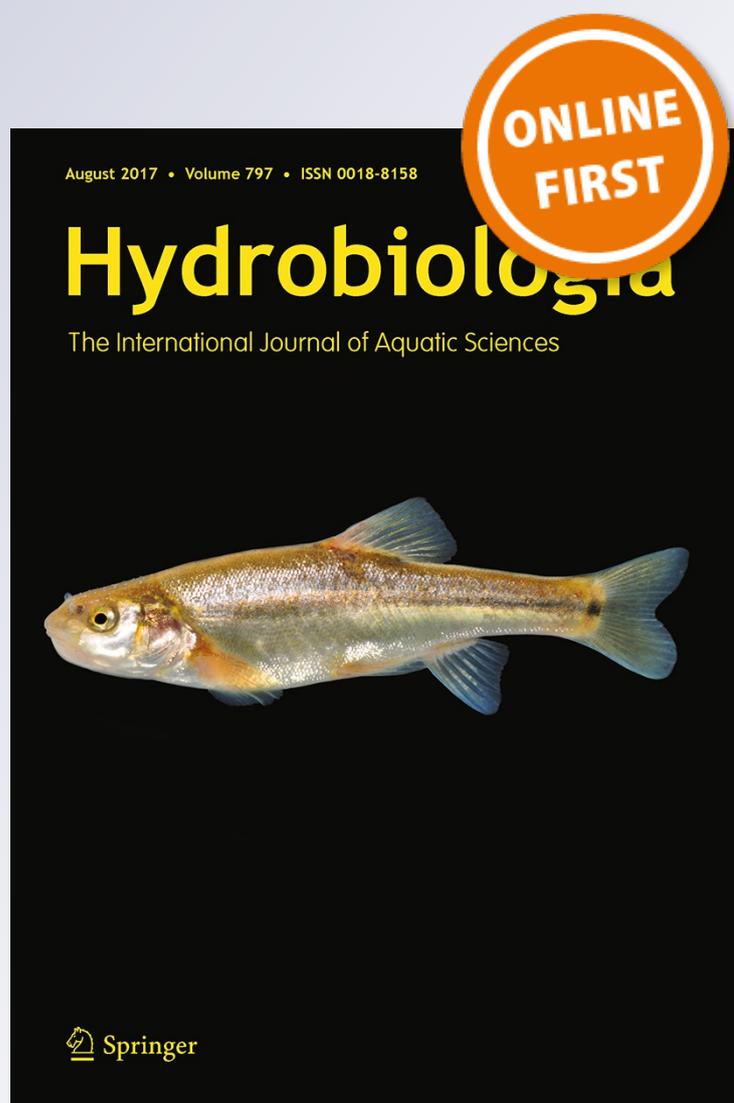
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Thermal ecology of *Galaxias platei* (Pisces, Galaxiidae) in South Patagonia: perspectives under a climate change scenario

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Abstract The native freshwater fish *Galaxias platei* shows a wide latitudinal distribution in Patagonia, being found on both sides of the Andes. Currently, climate change poses one of the main threats to native fish, and its effects are appearing faster in high southern latitudes. The aim of this work was to analyse the possible effects of climate change in *G. platei* through its thermal responses. We hypothesized that juveniles of this species would be affected by indirect rather than by direct consequences of climate change. We determined the thermal tolerance polygon using Critical Thermal Methodology and preferred temperatures using a thermal gradient. Additionally, we evaluated routine metabolic rate using stop-flow respirometry.

Results showed an intermediate to large polygon, with a non-negligible portion acquired through acclimation. Preferred temperatures and routine metabolic rates were positively related to acclimation temperature. Results suggest that *G. platei* thermal tolerance is dependent on its prior thermal history, have a eurythermal nature but maintain high levels of cold tolerance. Moreover, *G. platei* would be better suited at maintaining homeostasis at highest temperatures where more energy could be available for growth. This is the first time that thermal ecology data are registered for this species at its southernmost distribution.

Keywords Acclimation · Metabolic rate · Q_{10} · Thermal tolerance · Thermal preference

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Introduction

Tierra del Fuego is the southernmost region of the world with freshwater fish, albeit at a very low specific diversity: four native species of the family Galaxiidae (Cussac et al., 2004; Milano et al., 2006) and four species of salmonids introduced either accidentally (Fernández et al., 2010) or with economic purposes (Pascual & Ciancio, 2007). Among the galaxiids present in Tierra del Fuego is the big puyen, *Galaxias platei* Steindachner, 1898. This species is distributed in continental waters of Chile and Argentina, including Tierra del Fuego and Malvinas/Falklands (McDowall, 1971). Its origin, evidenced by similarities with galaxiids from Australia and New Zealand, is related to the Gondwanic radiation (Milano, 2003). The existence of the species in Patagonia, prior to the rise of the Andes, has been suggested by the presence of landlocked populations in most environments on both sides of the Andes (Milano, 2003). In the adult state, *G. platei* inhabits the benthic zone (Campos, 1970; McDowall, 1971); this feature allowed the species to survive in deep lakes in Patagonia during glaciations and made it the most successful dispersant species in Patagonia (Cussac et al., 2004; Zemlak et al., 2008). In freshwater systems threats to native biodiversity are acute, and invasive species are considered a primary threat (Clavero & García-Berthou, 2005). Accordingly, Correa and Hendry (2012) found that the abundance of *G. platei* was negatively associated with the abundance of salmonids.

Climate change also poses currently one of the main threats to native biodiversity (Heller & Zavaleta, 2009), and its various effects are appearing faster in high southern latitudes (Gille, 2002). In recent years, there has been great interest in the study of peripheral populations of different species. They are usually found lightly connected to central and continuous populations and occupy habitats at the edge of the species' geographic range, in more extreme environments (Sagarin & Gaines, 2002; Haak et al., 2010). They also tend to be smaller and potentially more prone to extinction due to demographic stochastic or catastrophic events (Lesica & Allendorf, 1995). Therefore, peripheral populations may become important for the species persistence in light of on-going anthropogenic actions, such as the introduction of non-native species, as well as in the context of climate change (Haak et al., 2010).

Fish are ectotherms, so their presence and success within a habitat depend strongly on environmental

temperature. Water temperature can affect the viability of populations at high latitudes through constraints to body size, due to the limited availability of food in winter (Shuter & Post, 1990), and directly by thermal lethality (Fry, 1971). Water temperature varies in space and time, which constitutes a crucial abiotic factor that shapes geographical and seasonal distribution of fish (Aigo et al., 2014). However, fish have evolved behavioural, physiological and biochemical responses to face the challenges that imply living in habitats with changing temperatures (Beitinger & McCauley, 1990; Beitinger & Lutterschmidt, 2011; Dabruzzi et al., 2012).

Behavioural thermoregulation is the first response to changes in environmental temperature and is characterized by preference and avoidance responses (Wallman & Bennett, 2006). This response defines fish distribution in heterothermic environments (Magnuson et al., 1979). Typically, fish living in thermally heterogeneous environments select a thermal optimum or preferred temperature in which their functions and metabolic processes occur at higher efficiency (Fry, 1971). Physiological responses are the second defence against extreme temperatures, and are exemplified by a readjustment in tolerance endpoints (Dabruzzi et al., 2012). Temperature tolerances and preferences are determined in part by the evolutionary history of species and population (Jobling, 1994; Beitinger & Bennett, 2000; Golovanov, 2006). Also, these may change during the course of growth and development (Jobling, 1994) and can be modulated by environmental conditions such as food availability, light, O₂, salinity, and distribution of competitors and predators (Jobling, 1996; Beitinger & Lutterschmidt, 2011). Typically, thermal tolerance polygons are used to quantify fish' thermal niche (Bennett & Beitinger, 1997) because they provide a convenient and useful comparative index of eurythermicity between species, and also define tolerances independent of previous thermal acclimation history as well as thermal tolerance gained (Eme & Bennett, 2009a). The final response occurs when temperatures reach sub-optimal values; species couple their metabolic rate with temperature (Ficke et al., 2007), since metabolic pathways are plastic and allow fish to adjust to changes in the environmental conditions (Sogard & Spencer, 2004), affecting their ability to grow, reproduce and maintain homeostasis. Ectotherm standard metabolic rates double with every 10°C increased in temperature,

but deviations from the norm may improve energy budgets of fishes living in heterogeneous thermal environments (Eme & Bennett 2009b; Di Santo & Bennett, 2011; Dabruzzi et al., 2012).

Previous results from Milano (2003) showed that *G. platei* from northern Patagonia did not show acclimation abilities, however, its thermal tolerance was broad, from 0 to more than 30°C, being much higher than the temperature experienced in the nature. This incomplete and inconclusive state of knowledge on the thermal biology of *G. platei* has led us to pursue our study.

While numerous current studies about changes in environmental temperature essentially intend to predict or document the change in the distribution of species, the aim of this work was to analyse possible effects of climate change in *G. platei*, a key species from Tierra del Fuego lakes, through its behavioural (temperature preferences), physiological (thermal tolerance ranges) and biochemical responses (metabolic rates). In the view of the wide latitudinal distribution of the species and that it inhabits freshwater bodies with thermal cycles of relatively high amplitude and duration, we hypothesized that *G. platei* have a eurythermal nature and a low thermal sensitivity, and that it would be affected by indirect (e.g. habitat degradation or changes in trophic structure) rather than by direct (lethality) consequences of climate change. The knowledge and understanding of the behavioural, physiological and metabolic thermal responses of juvenile *G. platei* will also provide concrete elements to analyse the vulnerability of the species to changing temperatures, to define its optimal thermal habitat and finally, to generate different scenarios of changes in the fish community (Pörtner & Peck, 2010).

Materials and methods

Fish collection and general experimental framework

Fieldwork was carried out in Lake Udaeta (54°33'S, 66°44'W) between October 2015 and January 2016. Water temperatures were recorded during 2015–2016, and particularly at the time of fish collection, using a multiparameter meter HANNA HI9828 (Hanna Instruments, Inc., Woonsocket, Rhode Island, USA). Water temperatures showed values between 3.9 and 14.9°C. These temperatures are consistent with those

from other lakes and ponds of Tierra del Fuego (Saad et al., 2013).

Galaxias platei juveniles were captured in the shallower littoral zone up to 1 m depth using a seine net (25 m long, 1.5 m high, and 3 mm stretched mesh). They were then carried alive to the laboratory in 100 l tanks containing aerated freshwater.

In the laboratory, they were transferred to aquaria containing freshwater at the same temperatures they were captured (8–12 ± 0.2°C). Dissolved oxygen was maintained near saturation conditions (11.00 ± 0.13 mg/l). They were left to acclimate for 2 weeks to captivity conditions before undergoing experimental acclimation and exposed to 'thermal tolerance', 'thermal preference' or 'metabolic rates' trials. The fish used in a trial were not used in other trials.

A total of 280 juveniles of *G. platei* were tested in this work. Groups of juveniles of homogeneous sizes (72.21 ± 9.84 mm; 2.44 ± 1.07 g) were selected and held for 20 days at a density of 1 individual per litre in 20-l aquaria with oxygen supply. Four aquaria were used for each acclimation temperature, using a total of 16. The fish were exposed to different acclimation temperatures (AT): 2 (n = 70), 4 (n = 70), 10 (n = 72) and 16°C (n = 68). These temperatures corresponded to extreme (2 and 16°C) and mean (4 and 10°C) water temperatures for winter and summer conditions, respectively, in Tierra del Fuego lakes (Saad et al., 2013; Lattuca personal communication). Different AT and experimental 12:12 h L:D diel photoperiod were reached at a rate of 1°C day⁻¹ and 1 h day⁻¹, respectively. During the acclimation period the feeding regimen consisted of chopped hake muscle offered ad libitum every 3 days; however, fish were not fed 24 h in advance of, or during experimental trials. Water quality was maintained by water changes (20–25% of total volume) every other day, and monitored in between water changes. Oxygen concentration was kept near saturation for each AT: 13.60 ± 0.15, 12.86 ± 0.16, 11.12 ± 0.14 and 9.85 ± 0.18 mg/l for 2, 4, 10 and 16°C, respectively. Furthermore, pH, ammonia, nitrite and nitrate were kept at 7.81 ± 0.25, <0.1 ppm, <0.3 and <12.5 mg/l, respectively. Oxygen concentration and pH were monitored using a multiparameter meter HANNA HI9828; ammonia was monitored with NH₃ Acuanalítica test kit; and nitrite and nitrate were monitored with NO₂⁻ and NO₃⁻ Tetra kits test, respectively. No visual signs of stress were observed during the acclimation period.

Thermal acclimation limits

The total thermal acclimation range of *G. platei* juveniles were estimated from chronic upper and chronic lower temperatures measurements as indicated in Dabruzzi et al. (2012). For this purpose, 12 juveniles of 70.03 ± 3.70 mm total length (TL) and 2.04 ± 0.47 g body mass (BM) were placed individually into 20 l tanks at $10 \pm 0.2^\circ\text{C}$, fed daily and the temperature was increased ($n = 6$) or decreased ($n = 6$) at a constant rate of 1°C day^{-1} until fish stopped feeding. The mean upper and lower feeding cessation temperatures were considered as the upper and lower chronic temperatures, respectively. Chronic upper and chronic lower temperatures defined the thermal acclimation limits of *G. platei* (29.50 ± 1.05 – $0.50 \pm 0.24^\circ\text{C}$), thus establishing a total acclimation range of 29.50°C .

Thermal tolerance

Thermal tolerance ranges were estimated on the basis of Critical Thermal Methodology (CTM) (Cox, 1974; Becker & Genoway, 1979) following acclimation. This methodology involves fish being initially acclimated at a predetermined temperature (2, 4, 10 and 16°C) and then subjected to a continuous dynamic temperature change until the point at which a predefined sub lethal endpoint is reached.

For Critical Thermal Maxima (CTMax) trials fish were placed individually ($n = 79$) into 250-ml plastic beakers filled with freshwater at appropriate AT and provided with aeration. Beakers were suspended in a 50-l water bath equipped with a water pump to recirculate water and prevent thermal stratification. The water bath was heated by means of a Techne TU-20D (Bibby Scientific Limited, Stone, Staffordshire, UK) in order to increase temperature inside beaks at a constant rate of $0.3^\circ\text{C min}^{-1}$ until loss of equilibrium (LOE), defined as the inability of fish to maintain dorso-ventral orientation for at least 1 min (Beitinger et al., 2000).

For Critical Thermal Minima (CTMin), fish were also placed individually ($n = 78$) into 250-ml plastic beakers filled with water at corresponding AT and provided with moderate aeration. Beakers were placed in a freezer unit and were left to decrease temperature at a constant rate of $0.3^\circ\text{C min}^{-1}$ until LOE was observed or temperature remains constant ($\sim 0^\circ\text{C}$).

When LOE was observed in both CTMax and CTMin, water temperature was registered inside beaks with a certified Lutron TM-917 digital thermometer provided with a Pt100 thermo resistance (Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan). Fish were also measured (total length TL, $\text{mm} \pm 0.1$ mm), weighed (body mass BM, $\text{g} \pm 0.2$ g) and then returned to acclimation temperatures to assess survival. Experiments were conducted between 9:00 and 14:00 local time.

The CTMax and CTMin of fish at each AT were calculated as the arithmetic mean temperature at which LOE was observed (Becker & Genoway, 1979; Beitinger et al., 2000).

The thermal tolerance niche for *G. platei* juveniles was determined constructing a thermal tolerance polygon using a modified version of the methods described by Bennett and Beitinger (1997). The polygon was constructed by connecting the CTMax and CTMin linear regressions (see below) generated over the total range of acclimation temperatures (Beitinger & Lutterschmidt, 2011). The figure produced expresses quantitatively the thermal tolerance zone in areal units, $^\circ\text{C}^2$ (Eme & Bennett, 2009a). The polygon was further divided into an intrinsic tolerance zone (i.e., thermal tolerance independent of previous thermal acclimation history) and acquired tolerance zones (i.e., thermal tolerance gained through acclimation) by drawing two boundary lines horizontally across the polygon at the lowest CTMax and highest CTMin constant temperature values (Fangue & Bennett, 2003; Eme & Bennett, 2009a).

Thermal preference

The preferred temperature (PT) of individual fish ($n = 80$) was measured in a custom-built horizontal thermal gradient tank, similar in design to that described by Bettoli et al. (1985) and Aigo et al. (2014). The system consisted of a horizontal 4-m length pipe of white polyvinyl chloride (PVC) with a 16-cm inside diameter and a 10-cm wide longitudinal upper opening which allowed fish observation. Inside the system containing 65 l of freshwater a temperature gradient was generated and maintained through two 0.7-cm diameter aluminium coils located from each end of the tube towards the centre, and through which circulated cold and hot water, respectively. The source of cold water was a LKB Bromma thermoregulator

(LKB Bromma, Sollentuna, Sweden) that maintained 0.2°C inside. The hot water source was a 20 l bath maintained at 28–40°C (depending on the treatment) by two Haake DC5 thermoregulators (Thermo Electron Corporation, Karlsruhe, Germany). In both cases, water was pumped through plastic pipes by thermoregulators. The adjustment of these two sources enabled regulation of the temperature gradient. Compressed air was bubbled through a 6-mm diameter rubber tube with regularly spaced holes to ensure aeration and vertical water mixing. This device was maintained under constant natural light. For each treatment, i.e. each AT, one particular thermal gradient was adjusted (Table 2).

Each trial started with the introduction of a single fish in the thermal gradient tank, in a position with the temperature close to the fish's AT. Data collection started after a 30 min acclimation period to experimental conditions. The fish was then allowed to select its PT by steadying its position within the tank for at least 5 min. The temperatures selected were monitored with a certified digital thermometer Lutron TM-917 provided with a Pt100 thermo resistance (Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan), avoiding to disturb the fish. Trials lasted from 90 to 120 min and were all performed between 9:00 and 14:00 local time. Average PT was calculated for each individual.

Metabolic rates

In order to determine routine resting metabolic rates (VO_{2R}) of *G. platei* juveniles maintained at different AT ($n = 44$), we measured oxygen consumption rates using “stop-flow” respirometric chambers. Each chamber was made of polyvinyl chloride (PVC) pipe section of 517.4 ml with an acrylic top. The opaque respirometer removed visual stimulation for the fish while allowing spontaneous movements. These chambers were immersed in a bath of air-saturated freshwater at appropriate AT. Each chamber was equipped with a Strathkelvin 1302 Clark-type polarographic O_2 electrode, connected to a Strathkelvin 928 6-channel O_2 system (Strathkelvin Instruments Limited, North Lanarkshire, Scotland). Chambers were sequentially closed (2.75 h for 2, 4 and 10°C; 1.75 h for 16°C) and refilled (0.25 h for all treatments) with oxygen saturated freshwater. These close and refill cycles were determined to prevent oxygen saturation from falling below 80% saturation. This percentage assures normal

respiration, avoiding displays related to anoxia. A chamber without fish was used as control to account for potential bacterial oxygen consumption. In this work, fundamental principles described by Svendsen et al. (2016) were considered in the design and methodology of respirometry experiments, such as chamber size, flush rate, flush time, measurement periods and temperature control, among others.

Fish were fasted for 1 week before trials to ensure measurements were taken in a post-absorptive state. Trials started when fish were transferred to chambers. Fish were held during 72 h, a period necessary to ensure the O_2 consumption rate reached stable values. Once O_2 consumption rate reached stable values, weight-specific oxygen consumption was measured. A metabolism baseline (BL) was calculated for each AT, as the average of O_2 consumption values. All values were corrected for non-fish respiration by subtracting the value from control chamber treatment. We also quantified the effect of temperature on biological processes through the temperature quotient Q_{10} (Jobling, 1994) as:

$$Q_{10} = (BL_{t_2}/BL_{t_1})^{10/(t_2-t_1)}$$

where BL are the mean metabolic rates at temperatures t_1 (low temperature) and t_2 (high temperature), respectively.

Statistical analyses

Simple linear regression analysis (SLR) was used to test relationships between acclimation temperature and CTMax/CTMin and PT on AT. One-way Analyses of Variance (one-way ANOVA) were used to test the effect of AT on the CTMax/CTMin, PT and metabolic baselines, followed by Tukey post hoc test using $\alpha = 0.05$. Assumptions of normality and homoscedasticity were evaluated through Shapiro–Wilk and Levene tests, respectively (Zar, 2010). All statistical analysis were conducted using InfoStat statistical software (version 2011; Di Rienzo et al., 2011). Figures were made using SigmaPlot (version 11.0; Systat Software, Inc.)

Results

Thermal tolerance

Galaxias platei acclimated at 2, 4, 10 and 16°C exhibited CTMax values between 25.58 and 30.78°C

(Table 1). Regression analysis found a highly significant relationship between CTMax values and AT (SLR; $F_{1,76} = 923.772$; $P < 0.0001$), being defined by the following model:

$$CTMax = 0.385 \cdot AT + 24.498$$

The R^2 value for the model indicates that 92.4% of the variability in the critical thermal maxima is explained by changes in AT.

Values of CTMax were notably dependent on AT. They differed significantly between AT (ANOVA; $F_{3,74} = 363.6948$; $P < 0.0001$), except between 2 and 4°C acclimated fish, where statistical differences were not observed (Tukey test, $P > 0.05$). The data met the assumptions of normality ($P = 0.1127$) and homoscedasticity ($P = 0.5676$).

The CTMin values of *G. platei* could only be determined for AT = 16°C (0.13°C, Table 1). When fish acclimated to 2, 4 and 10°C were tested for CTMin values, fish movements diminished as water temperature reached 0°C, but they did not reached the LOE temperature. As the absolute lowest temperature tolerated by many fish in freshwater may be lower than the freezing point, it is usually reported as 0°C owing to difficulties in achieving sub-0°C temperatures in freshwater (Beitinger & Bennett, 2000). Taking this in account, the CTMin of *G. platei* at 2, 4 and 10°C would be referred as 0°C from now on.

However, a regression analysis was made using CT_{Min} values obtained at AT = 10 and 16°C, in order to draw a polygon and thus, estimate the thermal tolerance zone de *G. platei* (see below). The regression

analysis for CTMin data (SLR; $F_{1,36} = 6.995$; $P = 0.0120$) yielded by the following model:

$$CT_{Min} = 0.0219 \cdot AT - 0.2193$$

The R^2 value for the model indicates that 17.4% of the variability in the critical thermal minima is explained by changes in AT.

The thermal tolerance zone generated with CTM shows that *G. platei* exhibited a total polygonal area of 876.021°C² (Fig. 1). The intrinsic tolerance zone was calculated in 703.776°C². The upper and lower acquired thermal tolerance zones represented 18.50 and 1.17% of the fish thermal niche, respectively, and comprised 172.245°C² of the total polygon area.

In CTM trials, approximately 4% of fish tested did not recovered when they were returned to the AT; mortality probably occurred because they were not removed promptly after LOE was reached.

Thermal preference

Preferred temperatures for each acclimation temperature (AT) are summarized in Table 2.

Regression analysis between PT values and AT showed highly significant relationships (SLR; $F_{1,78} = 291.220$; $P < 0.0001$) and it was defined as follows:

$$PT = 1.2289 \cdot AT + 0.2742$$

The R^2 value for the model indicates that 79.10% of the variability in the preferred temperature is explained by changes in AT.

Table 1 Experimental results on Critical Thermal Maxima/Minima (CTMax/CTMin) (mean ± SD) for *G. platei* from Tierra del Fuego

AT (°C)	Cold tolerance				Heat tolerance			
	n	TL (mm)	BM (g)	CTMin (°C)	n	TL (mm)	BM (g)	CTMax (°C)
2	20	67.61 ± 4.37	2.04 ± 0.52	0	20	67.55 ± 4.27	1.91 ± 0.46	25.59 ± 0.64 ^a
4	20	71.75 ± 9.37	2.54 ± 0.90	0	20	75.21 ± 6.27	2.66 ± 0.77	25.73 ± 0.72 ^a
10	20	69.00 ± 2.79	1.79 ± 0.32	0	20	69.65 ± 5.75	2.03 ± 0.67	28.22 ± 0.39 ^b
16	19	67.85 ± 3.21	1.85 ± 0.36	0.13 ± 0.21	18	67.65 ± 2.68	1.76 ± 0.31	30.78 ± 0.39 ^c

Acclimation temperature (AT), total length (TL ± SD) and body mass (BM ± SD) of fish are also indicated. Values of CTMin = 0: survived freezing of water

Means labelled with different superscript letter differed significantly in Tukey post hoc tests ($P < 0.05$). $P_{2-4} = 0.8443$; other contrasts $P < 0.0001$

Total length and body mass did not show statistical differences between AT treatments ($F_{3,153} = 2.385$, $P = 0.0714$; $F_{3,153} = 2.540$, $P = 0.0585$, respectively)

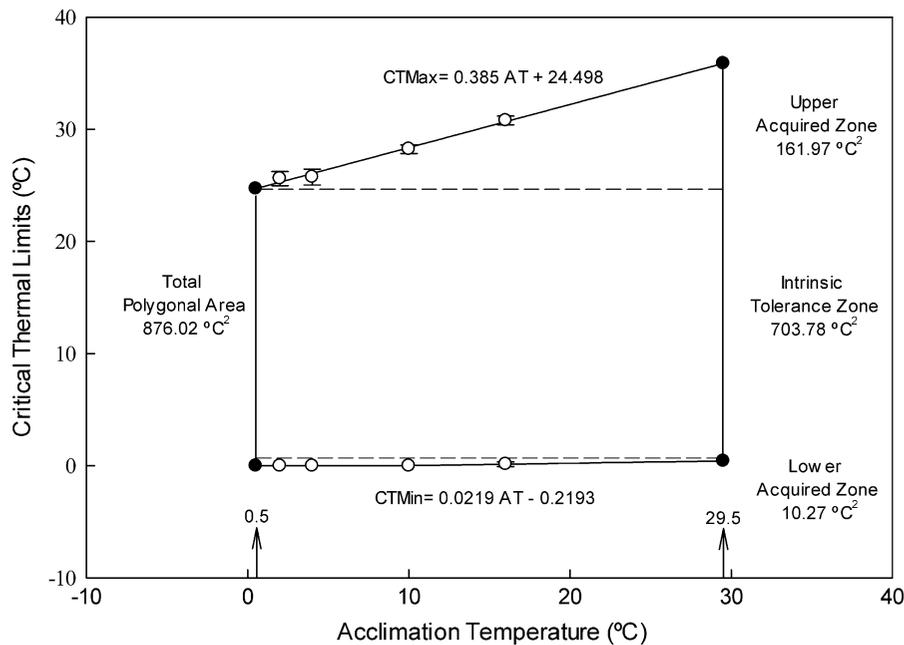


Fig. 1 Thermal tolerance polygon for *G. platei* from Tierra del Fuego. The polygon was constructed from upper/lower chronic temperatures and CTMax/CTMin values (unfilled circle) (mean \pm SD) measured across *G. platei*' acclimation range. CTMax/CTmin values at extreme acclimation temperatures

(filled circle) extrapolated from regression analyses of CTMax/CTMin values on AT are also indicated. The total polygonal area (TPA) is divided into an intrinsic tolerance zone (ITZ) and upper and lower acquired tolerance zones (UAZ, LAZ). Arrows indicate the total acclimation range for *G. platei*

PT values showed significant differences between AT (ANOVA; $F_{3,75} = 126.160$; $P < 0.0001$) and Tukey's post hoc comparison also revealed significant differences between treatments (Tukey test, $P < 0.05$), except for 2 and 4°C-acclimated *G. platei* (Tukey test, $P > 0.05$). For the rest of treatments, an increase in AT shifted thermal preference upward (Fig. 2). The data met the assumptions of normality ($P = 0.0640$) and homocedasticity ($P = 0.9999$).

Metabolic rates

Galaxias platei coupled its routine metabolic rates, measured as oxygen consumption, with AT. The oxygen consumption significantly increased with increasing temperatures (ANOVA; $F_{3,40} = 25.157$; $P < 0.0001$). Pairwise multiple comparison procedures showed that routine metabolic rates significantly differed between AT (Tukey test; $P < 0.05$) except for 10 and 16°C-acclimated *G. platei* (Fig. 3). The data met the assumptions of normality ($P = 0.1797$) and homocedasticity ($P = 0.0517$).

In addition, Q_{10} calculated as the change of VO_{2R} with temperature, showed a peak of 9.751 between 2 and 4°C, falling to 1.834 and 1.298 between 4–10 and 10–16°C, respectively (Table 3).

All measured variables during metabolic rates trials are summarized in Table 3.

Discussion

In this work, we provide novel information about thermal responses of *G. platei*. Results showed that *G. platei* from Tierra del Fuego has good abilities to acclimate to different temperatures, unlike what was found by Milano (2003). CTMax mean values for *G. platei* juveniles ranged from 25.59 to 30.78°C. These values were well above the temperatures registered in Lake Udaeta. This is in accordance with the existing literature, where it is shown that the upper temperature tolerances of most fish are higher than typical temperatures of their natural habitats (Mundahl, 1990). Information about CTMax of other galaxiid species is scarce; nevertheless available literature

Table 2 Experimental results on preferred temperature (PT) (mean \pm SD) for *G. platei* from Tierra del Fuego

AT (°C)	n	Gradient (°C)	TL (mm)	BM (g)	PT (°C)
2	19	1.20–11.40	67.84 \pm 4.53	2.02 \pm 0.46	2.64 \pm 1.32 ^a
4	20	1.90–16.90	71.45 \pm 8.77	2.42 \pm 0.76	4.01 \pm 1.35 ^a
10	20	2.50–24.20	69.89 \pm 2.52	2.07 \pm 0.35	15.13 \pm 5.20 ^b
16	20	3.50–28.00	67.98 \pm 3.40	2.10 \pm 0.31	18.57 \pm 2.95 ^c

Acclimation temperature (AT), total length (TL \pm SD) and body mass (BM \pm SD) of fish are also indicated

Means labelled with different superscript letter differed significantly in Tukey post hoc tests ($P < 0.05$). $P_{2-4} = 0.5126$; $P_{10-16} = 0.0054$; other contrasts $P < 0.0001$

Total length and body mass did not show statistical differences between AT treatments ($F_{3,75} = 1.91$, $P = 0.1354$; $F_{3,75} = 2.14$, $P = 0.1027$, respectively)

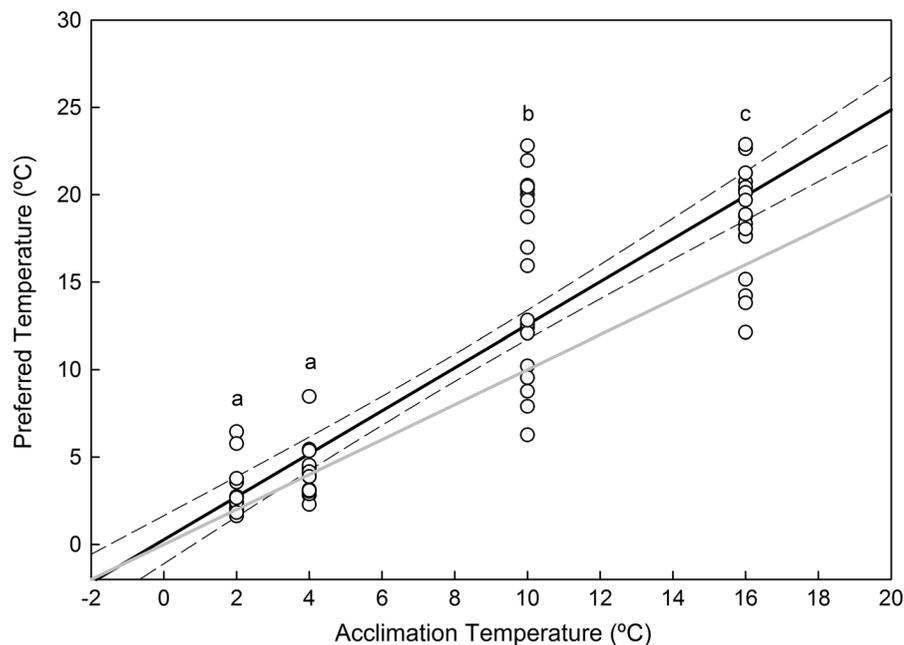


Fig. 2 Preferred temperatures for *G. platei* from Tierra del Fuego, as a function of acclimation temperatures. Different letters denote significant differences between PTs (Tukey post

hoc test, $\alpha < 0.05$). Dashed lines indicate 95% confidence intervals and PT = AT line (grey) is also indicated

reports high tolerances between 28.0 and 31.7°C for five New Zealand species acclimated at 16°C (Richardson et al., 1994). Present results for *G. platei* acclimated at 16°C (30.70°C) are consistent with those of Richardson et al. (1994). On the other hand, CTMin values were only estimated for *G. platei* acclimated to 16°C, since those acclimated to lower temperatures resisted 0°C, and they remained active at the freezing point of water. This lack of LOE in *G. platei* acclimated at the lowest AT, suggests that their CTMin would be below 0°C, which allows them to survive temperatures in waters

that are ice covered (Currie et al., 1998). Not surprisingly, *G. platei* is one of the few native freshwater species found in Tierra del Fuego, where small lakes freeze in winter and/or the water temperature remains just above 0°C (Cussac et al., 2004). In many freshwater species the CTMin and the ultimate lower lethal is indeterminate since fish exhibit activity at 0°C (Fry, 1971). The thermal tolerance scope (CTMax–CTMin) of *G. platei* juveniles ranged from 25.59 to 30.65°C, suggesting that juveniles of *G. platei* can cope with a wide range of environmental temperatures (Shultz et al., 2016).

Fig. 3 Oxygen consumption rates (mean \pm SD) of *G. platei* from Tierra del Fuego. Different letters indicate significant differences (Tukey post hoc test, $\alpha < 0.05$). Numbers in parenthesis indicate Q_{10} s between different AT

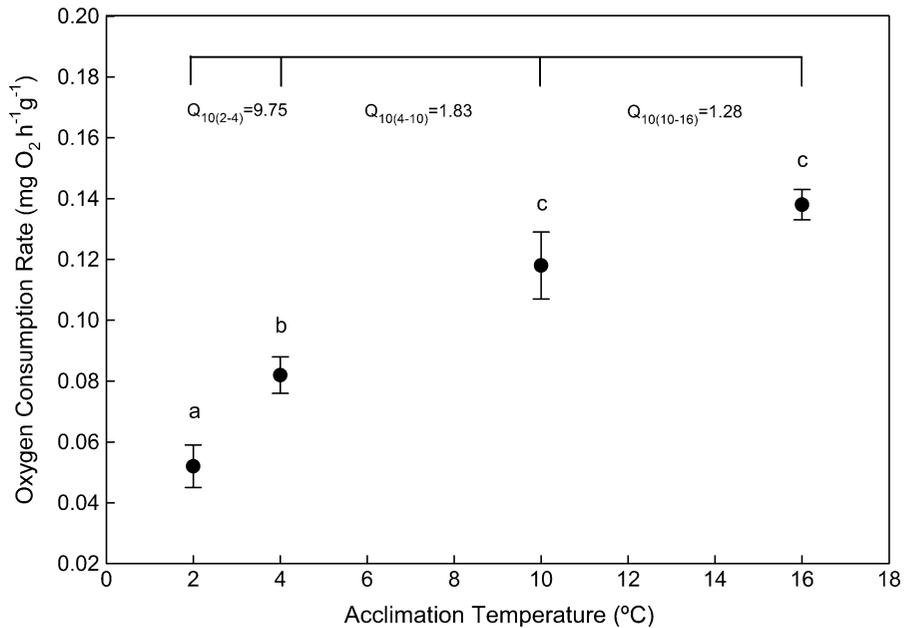


Table 3 Experimental results on routine metabolic rates (VO_{2R}) (mean \pm SD) at different acclimation temperatures for *G. platei* from Tierra del Fuego

AT (°C)	n	TL (mm)	BM (g)	VO_{2R} (mg O ₂ h ⁻¹ g ⁻¹)	Temperature interval (°C)	Q_{10}
2	11	88.49 \pm 5.66	5.03 \pm 1.30	0.052 \pm 0.023 ^a		
4	10	88.80 \pm 4.35	3.80 \pm 0.59	0.082 \pm 0.018 ^b	2–4	9.751
10	12	88.83 \pm 3.75	4.06 \pm 0.63	0.118 \pm 0.037 ^c	4–10	1.834
16	11	87.71 \pm 5.89	4.26 \pm 1.96	0.138 \pm 0.015 ^c	10–16	1.298

Acclimation temperature (AT), total length (TL \pm SD) and body mass (BM \pm SD) of fish, and Q_{10} are also indicated

Means labelled with different superscript letter differed significantly in Tukey post hoc tests ($P < 0.05$). $P_{2-4} = 0.0426$; $P_{4-10} = 0.0094$; $P_{10-16} = 0.2725$; other contrasts $P < 0.0001$

Total length and body mass did not show statistical differences between AT treatments ($F_{3,40} = 0.121$, $P = 0.9473$; $F_{3,40} = 2.826$, $P = 0.0507$, respectively)

The relative size, position and shape, among others features, of a fish' ecological thermal tolerance polygon recapitulates specific adaptive tactics necessary for survival in its thermal environment (Fangue & Bennett, 2003; Eme & Bennett, 2009a). In this study, *G. platei* exhibited an intermediate to large thermal tolerance polygon (876.02°C²), which suggests that it is a eurythermic fish. Furthermore, this species had a large intrinsic tolerance (80.33%) and a moderate ability to acquire additional heat or cold tolerance through acclimation (19.67%). In particular, higher acclimation-dependent areas were nearly 16 times greater than lower acclimation-dependent areas, which could indicate that acclimation plays a major

role in high rather than in low thermal tolerance. Also, this relationship indicates that these fish maintain consistently high levels of cold tolerance. Freshwater fish have a variety of polygons sizes (Brett, 1956; Fangue & Bennett, 2003; Elliot, 2010), reaching 1470°C² in the sheepshead minnow *Cyprinodon variegatus* Lacépède, 1803 (Bennett & Beiting, 1997) and 1600°C in Amargosa pupfish (*Cyprinodon nevadensis amargosae* Eigenmann & Eigenmann, 1889) (Feldmeth et al., 1974). In addition, salmonids have small to intermediate polygons, display low and left-shifted, i.e. they are heat intolerant fish (Fangue & Bennett, 2003), including Chinook salmon (*Oncorhynchus tshawytscha* Walbaum, 1792) with

529°C², charr (*Salvelinus fontinalis* Mitchell, 1814) with 625°C² (Brett, 1956) and brown trout (*Salmo trutta* L. 1758) with 583°C² (Elliot, 1981), all present in Tierra del Fuego. An ecological polygon is unavailable for rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), but thermal scope data from Currie et al. (1998) suggests an ecological thermal area similar in shape and position to *G. platei*. Thus, under a global warming scenario, invasive species would be more sensitive to climate change than *G. platei*, except rainbow trout, which has the potential to be its most apt competitor.

Galaxias platei also exhibited a positive relationship between preferred and acclimation temperatures. Johnson and Kelsch (1998) developed a model that predicts the temperature-preference relationships on the basis of environmental temperature variation experienced by a species through its recent evolutionary history. As the model predicts, the temperature preference relationship is a positive function of acclimation, which could be due to *G. platei* experiencing an annual cycle of relatively high amplitude (more than 5°C). According to the model, this relationship is typical of eurythermic fish. A positive relationship between PT and AT was also described by Aigo et al. (2014) for native perca (*Percichthys trucha* Valenciennes, 1833) and the invasive rainbow trout (*O. mykiss*) from northern Patagonia. As in general, rainbow trout displayed lower PT and a narrower range of PT as perca, they suggested that the invasive salmonids could be more sensitive to warming than perca. With this case in mind, a community level study should be desirable to analyse possible effects of water warming on *G. platei* in Tierra del Fuego.

In poikilothermic animals, the metabolic responses quantified in terms of oxygen consumption show a linear correlation to temperature due to its direct effect on the kinetics of the enzyme reactions involved (Hochachka & Somero, 1971; Hazel & Prosser, 1974; Dalvi et al., 2009). Routine metabolic rates of *G. platei* juveniles were directly related to AT, i.e., the oxygen consumption increased with temperature. However, no significant change in the VO_{2R} was observed between 10 and 16°C. Previous works have suggested that the temperature at which the oxygen consumption rate is gradually lessened could be related with the final preferred temperature (Kita et al., 1996). This phenomenon can be explained with the optimal

temperature at which enzymes work. When ambient temperature exceeds such an optimum, then a drop in the rate of one enzyme in a reaction chain would signify a slowing of the metabolic rate (Hazel & Prosser, 1974; Kita et al., 1996). Unlike our present results, Boy et al. (2017) found that *G. maculatus* has similar values of VO_{2R} in winter (0.12 mg O₂ h⁻¹g⁻¹ at 4°C) and summer (0.11 mg O₂ h⁻¹g⁻¹ at 10°C) experimental conditions. This difference could be explained by the fact that *G. maculatus* has the ability to maintain basal metabolism, locomotion and feeding activity during both winter and summer, and that both are highly energy demanding seasons (Boy et al., 2017). Therefore, *G. maculatus* could be subjected to extreme cold temperatures during winter, close to its tolerance limit, resulting in an energy expenditure higher than expected theoretically to maintain homeostasis (Pörtner, 2010).

In general, Q₁₀ values in ectotherms fall with increasing temperature (Prosser, 1986; Sollid et al., 2005). Many authors have also suggested that the decrease in Q₁₀ indicates that the metabolic rate of the fish has decreased and that more energy is potentially available for growth (Das et al., 2004; Díaz et al., 2007; Dalvi et al., 2009), suggesting that the Final Temperature Preferendum (FTP) may be estimated indirectly based on the relationship between oxygen consumption and acclimation temperature. The FPT gives a good indication of the optimum temperature for growth (Jobling, 1981). As expected, the Q₁₀ values calculated from the oxygen consumption rates of *G. platei* juveniles decreased as temperature increased. The lowest Q₁₀ value was observed between 10 and 16°C (1.298), which indicates that, within the temperatures range of Tierra del Fuego *G. platei* would be well suited at maintaining homeostasis at these temperatures, i.e. that FPT would be between 10 and 16°C. Thus, it can be hypothesized that *G. platei* could have a higher growth rate during warmer seasons. The highest Q₁₀ value (9.75) was calculated between 2 and 4°C and was far beyond respiratory Q₁₀ values of 2–3, which are characteristic for freshwater fish within their normal temperature range (Chau-Berlinck et al., 2002). In this case, this high value could be indicating a limited capacity of oxygen consumption and the approximation to the critical low temperature threshold (Hundt et al., 2015). Thus, *G. platei* could be under some degree of thermal stress, expending more energy in order to satisfy basal

metabolic needs (Crawley, 2013). However, it should be noted that as a general rule for living animals, Q_{10} values were not uniform throughout the temperature range, and usually Q_{10} rose as temperature fell (Krogh, 1916; Davenport, 2012). This pattern is linked with the temperature at which enzyme systems work, as was mentioned previously. Moreover, similar values has been calculated for the zoarcid (*Austrolycus depressiceps* Regan, 1913), a marine subantarctic species, at 2–10°C (Vanella & Calvo, 2005) and for the Antarctic eelpout (*Pachycara brachycephalum* Pappenheim, 1912) at 3–9°C (Hardewig et al., 1998). A greater effect of temperature limiting the oxygen consumption for the sluggishness in this thermal range has been suggested (Vanella & Calvo, 2005).

Conclusion

This is the first time that behavioural, physiological and biochemical responses to temperature are registered for *G. platei* juveniles from its southernmost distribution. These data are important not only to enlighten on the thermal ecology of this species but also to predict their vulnerability to climate change both in the short and long-term. Although our study organisms represent a peripheral population of this species, future climatic changes would not seem to seriously affect this population—at least directly by lethality—because of their eurythermal nature. These data may also provide useful insights into the understanding of the conservation of *G. platei* in relation to invasive species. Patagonia's freshwater fishery has traditionally focused on promoting salmonids, while little effort has been dedicated to the protection of native fish (Pascual et al., 2007; Correa & Hendry, 2012). Since impacts by exotic species appear to be dependent on temperature, future research may focus on the freshwater community taking into account native and exotic species as well as effects of different environmental factors.

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