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Metabolic rate and hypoxia tolerance in *Girardinichthys multiradiatus* (Pisces: Goodeidae), an endemic fish at high altitude in tropical Mexico[☆]

Monica Vanessa Garduño Paz[a,*], José Fernando Méndez Sánchez[a], Warren Burggren[b], José Luis Antonio García Martínez[a]

[a] *Laboratorio de Ecofisiología Animal, Facultad de Ciencias, Universidad Autónoma del Estado de México, El Cerrillo Piedras Blancas carretera Toluca-Ixtlahuaca km 14.5, CP 50200 Toluca, Estado de México, Mexico*
[b] *Developmental Physiology Lab, Department of Biological Sciences, University of North Texas, 1155 Union Circle #311277, Denton, TX 76203-5017, United States of America*

ABSTRACT

The darkedged splitfin (Amarillo fish), *Girardinichthys multiradiatus* is a vulnerable endemic fish species inhabiting central Mexico's high altitude Upper Lerma Basin, where aquatic hypoxia is exacerbated by low barometric pressures (lower P_{O_2}s), large aquatic oxygen changes, poor aquatic systems management and urban, agricultural and industrial pollution. The respiratory physiology of *G. multiradiatus* under such challenging conditions is unknown - therefore the main goal of the present study was to determine metabolic rates and hypoxia tolerance to elucidate possible physiological adaptations allowing this fish to survive high altitude and increasingly eutrophic conditions. Fish came from two artificial reservoirs – San Elías and Ex Hacienda - considered refuges for this species. Both reservoirs showed high dial P_{O_2} variation, with hypoxic conditions before midday and after 20:00 h, ~4 h of normoxia (15 kPa) from 16:00–20:00, and ~4 h of hyperoxia (16–33 kPa) from 12:00–16:00. Standard metabolic rate at 20 ± 0.5 °C of larvae from Ex Hacienda was significantly higher than those from San Elías, but these differences disappeared in juveniles and adults. Metabolic rate at 20 ± 0.5 °C for adults was 9.8 ± 0.1 SEM μmol O_2/g/h. The metabolic scaling exponent for adults was 0.58 for San Elías fish and 0.83 for Ex Hacienda fish, indicating possible ecological effects on this variable. Post-larval fish in Ex Hacienda and all stages in San Elias site showed considerable hypoxia tolerance, with P_{Crit} mean values ranging from 1.9–3.1 kPa, lower than those of many tropical fish at comparable temperatures. Collectively, these data indicate that *G. multiradiatus* is well adapted for the hypoxia associated with their high-altitude habitat.

1. Introduction

Fish in hypoxic environments have numerous physiological mechanisms to sustain oxygen extraction for aerobic ATP production. These include changes in ventilation, cardiac activity and hemoglobin–O_2 binding (Richards et al., 2009; Mandic et al., 2009; Verberk et al., 2011; Mendez-Sanchez and Burggren, 2017; Mendez-Sanchez and Burggren, 2019a, 2019b). These mechanisms allow a stable oxygen uptake across a range of environmental P_{O_2}s (oxyregulation). When oxygen uptake declines with declining environmental P_{O_2}, fish eventually become oxyconformers below the transition point, known as the critical P_{O_2}, (P_{crit}), at which the oxygen supply cannot be sustained even with minimal levels of aerobic activity (Rogers et al., 2016). While the notion of P_{crit} has recently come under challenge (see

Wood, 2018), most accept that the specific P_{crit} value is an indicator of the degree of hypoxia tolerance in fishes and a measure of whole animal oxygen extraction capacity (for an entry into the literature see Rogers et al., 2016; Chabot et al., 2016; Claireaux and Chabot, 2016; Burggren, Mendez-Sanchez et al., 2019a, Burggren et al., 2019b). P_{crit} values vary also with environmental temperature. Thus, P_{crit} averages tropical fish are 4.92 ± 0.19 kPa (mean ± SEM), with corresponding values of 5.74 ± 0.24 kPa for temperate fishes (Rogers et al., 2016).

The oxygen demand of aquatic organisms is strongly affected by changes in temperature and dissolved solids, which diminish the oxygen solubility and generate hypoxia in the environment (Moyes and Schulte, 2007; Richards et al., 2009). Fish are more vulnerable in hypoxic environments, especially at higher temperatures. In the European perch (*Perca fluviatilis*), for example, routine $\dot{M}O_2$ increased much more

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when fish were acutely warmed from 16 to 18 °C to 22–23 °C. Perch chronically exposed to higher temperatures (13-22 °C) showed higher MO_2, exhibiting an elevated basal energy requirement accompanied by elevated resting heart rate and reduced heart rate scope (Sandbloom et al., 2016). Increased environmental temperature may be a particular threat to many species living at high altitude. For example, the oxygen partial pressure (P_{O_2}) of even air-saturated water at > 2400 m above sea level is only ~75% of that at sea level. This low P_{O_2} for high altitude organisms is, in fact, "normoxia" - *i.e.* the natural environmental oxygen levels to which organisms have adapted. However, aquatic habitats at high altitude can also quickly become hypoxic because of the additional factors of drastic temperature changes and high evaporation rates.

The effects of temperature and hypoxia are especially problematic for species that live in aquatic systems influenced by human management. For example, in the Upper Lerma Basin (ULB) of central Mexico, land management is mainly based on maize agriculture (Hernández Santana et al., 2012). Since agriculture depends on seasonal rain, most of the landowners divert water *via* channels from the creeks or rivers to fill water reservoirs. The human management of these reservoirs allows fish species to migrate through the channels and quickly colonize new environments. Most of these reservoirs have water year-round, but some are emptied every spring. The ULB experiences periodic droughts, flooding, forest fires, frosts and hailstorms (Mastachi et al., 2016). Under these circumstances, fish species suffer drastic changes in water volume that lead to lower water transparency (turbidity), increases in suspended solids, nitrites and phosphates, variation in pH and hardness and, most importantly, changes in temperature. Also, due to their high altitude, Upper Lerma Basin aquatic ecosystems are subject to drastic changes of temperature and oxygen during the day, with lower values at dawn and higher values during midday. In the case of the Upper Lerma Basin, rapid eutrophic conditions (nutrient enrichment) provoked by poor aquatic systems management and urban, agriculture and industrial pollution may decrease aquatic P_{O_2}s even more (Diaz and Breitburg, 2009; Méndez-Sánchez et al., 2002; Nilsson and Östlund-Nilsson, 2008; Rogers et al., 2016).

Despite the challenges of high altitude, many endemic species of fish inhabit these fragile environments. *Girardinichthys multiradiatus*, also known as Mexclapique, darkedged splitfin and amarillo fish, is a small vulnerable native species inhabiting central Mexico's Upper Lerma Basin (Domínguez-Domínguez et al., 2008; De la Vega-Salazar, 2006; Gutiérrez-Yurrita and Morales Ortiz, 2004; IUCN, 2019). This species, which evolved at high altitude (2600–2800 m above sea level, masl), exhibits both sexual dimorphism and viviparity. The habitat of *G. multiradiatus* has been fragmented for the last century (Domínguez-Domínguez and Pérez de Léon, 2007), and this species now dwells mostly in reservoirs where their environment is highly altered and suffers anthropogenic management effects. In contrast to *Girardinichthys ireneae*, which is listed as critically endangered (Vega-Salazar, 2006), *G. multiradiatus* is a hardy species that can be found through the Upper Lerma Basin, despite habitat damage and pollution leading to hypoxia. In the present study we measured the routine metabolic rate and hypoxia tolerance based on the critical P_{O_2} of *G. multiradiatus* in two reservoirs within the ULB. The goal was to describe, for the first time, the metabolic physiology of this species and begin to investigate the possible physiological adaptations that allow *G. multiradiatus* to survive the relatively harsh conditions of this high-altitude environment.

2. Methods

2.1. Site selection

Two water reservoirs in the Upper Lerma Basin of Central Mexico – Ex Hacienda and San Elías - were chosen after initial scouting visits to several reservoirs in the ULB. These two reservoirs were chosen for three main reasons. First, the presence of *G. multiradiatus*, a species that

is now more common in artificial systems than in natural systems, was a driving factor. Second, we are interested in understanding how this species copes with artificial conditions manipulated by humans (occurring in both of these reservoirs). Finally, the two reservoirs present different kinds of management.

Ex Hacienda reservoir (N 19° 24′ 59.70″, W 99°41′49.51″) sits at an altitude of 2612 m. The vegetation structure is heterogenous and diverse, and includes several aquatic, semi-aquatic and submerged plant species of the families *Cyperaceae, Asteraceae, Lemnaceae, Poaceae, Apiaceae, Polygonaceae, Thyphaceae, Araliaceae* and *Nymphaeaceae,* all part of the microhabitat used by *G. multiradiatus*. Vegetation covers approximately 60% of the water surface. Ex Hacienda reservoir receives discharge only from corn fields and only occasionally are there are cattle in proximity.

The San Elías reservoir, (N 19° 24′ 25.03″ W 99° 41′ 11.15″) sits at an altitude of 2620 m. In contrast to the Ex Hacienda reservoir, most of the vegetation is submerged and restricted to very few littoral areas (covering 30% of the water surface), and includes families *Polygonaceae, Menyanthaceae*, Apiaceae and *Cyperaceae*. San Elías receives discharge from corn crops, other water reservoirs, four small houses, and has frequent cattle grazing at its edges.

2.2. Abiotic factors measurements in the field

To determine the environmental conditions in both reservoirs, the temperature and dissolved oxygen dial variations were measured for one 24 h period at the end of the dry season (May 27th–28th, 2015). Data were recorded using a Hobo U 26–001 with data logger. Abiotic data were then graphed using the software Hoboware Pro 3.5. To generate a broader view of the reservoir system, three different sampling sites (~ 200 m distant from each other) were chosen within each reservoir. For each site, measurements were made at 1 m from the shore and at a depth of 20 cm approximately. For one day, *in situ* measurements of water pH, total dissolved solids (TDS, ppm) and conductivity (CND, μs/cm), recorded using a multi-parametric sonde (HANNA HI 98129). Dissolved oxygen (DO, mg/l), oxygen saturation (%), temperature (°C) and salinity (ppm) were measured with a YSI sonde. Depth and transparency were measured with a Secchi disc and a graduated rope. Oxygen partial pressure (P_{O_2}, *kPa*) in the field was calculated from dissolved oxygen and barometric pressure. Measurements were carried out between 10:00–13:00 h.

2.3. Fish capture

Collection of a total of 50 *Girardinichthys multiradiatus* was carried out at Ex Hacienda and San Elías reservoirs. From each reservoir we made a one-time collection of 5 non pregnant adult females, 5 adult males, 5 juvenile females, 5 juvenile males and 5 larvae. These collections were made using a spoon net (1 m^2) during May within 10 days of the measurement of the abiotic factors described above. The capture was carried out in one littoral area site in each reservoir. These sites were chosen due to previously observed presence of fish and for proximity to the water sampling sites. After capture, fish were transported to the Animal Ecophysiology Laboratory facilities at the Faculty of Science, UAEM in Toluca, Mexico. Fish were acclimated for 72 h, at 20–21 °C in 10 l glass-tanks with constant aeration, 12 h light/12 h dark and pH 8–8.7. They were fed *ad libitum* once a day with TETRAmin PRO flakes.

2.4. Experimental apparatus for metabolic measurements

After the 72 h of general acclimation, fish were fasted for 24 h before metabolic measurements initiated while maintained in clean, filtered, and UV treated water. Size, age and sex were recorded for each measured fish. Sex was not determined in larvae, but juveniles and adults were sexed based on the shape, size and color of the fins and

body. Females have a more rounded head, the distance between the insertion of the dorsal fin and the tip of the snout is longer than in males, and the caudal peduncle is longer and thinner in females. The difference between juveniles and adults is based on their coloration. Juvenile females are pale, while adult females gain a yellowish with a marked black spot in the lateral anal area, with prominent body ventral part. In contrast, adult males show bright yellow body and fins, specially the dorsal fin and the caudal fins, each of which has a dark edge.

Routine oxygen consumption rates (μmol O_2/g/h) were measured using closed respirometry in a four-chamber Loligo respirometry system (DAQ-PAC-WF4). Oxygen sensors were calibrated daily at 0% (5% sodium sulphite solution) and 100% (total aeration) O_2 saturation. Fish were placed in sealed glass respirometer chambers (mean volume 41.5 ml) maintained at $20 \pm 0.5\,°C$, from which water was continuously sampled for measurements of dissolved oxygen. Water was continuously re-circulated within the chamber to ensure adequate water mixing. Details of this methodology are provided in Burggren et al. (2019).

To determine the critical P_{O_2}, or P_{crit}, individual fish were sealed inside each chamber and left to stabilize for 30 min before readings were taken, starting at a P_{O_2} 15 kPa. Measurements were taken until respirometer chamber P_{O_2} stopped decreasing or reached $\sim 1\,kPa$. A two-phase linear regression model (TP) fitted a pair of regressions to the data, with multiple iterations to determine the best fit of two intersecting lines, with the intersection point being indicating the P_{Crit}. The best fit was determined with the least sum of squares and the greatest difference in the residual variance for each regression. The greatest difference in residual variance was chosen to determine the intersection between the regression (P_{crit}) lines (Mueller and Seymour, 2011). Additional details of P_{crit} determination are provided by Burggren et al. (2019).

All animal experiments were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 with certificate number SAB/SCT.W06/088 issued by October 2006.

2.5. Statistical analysis

A Kolmogorov-Smirnov test of normality was first applied to corroborate normality of the data. Also, a Pearson's bivariate correlation was applied to physicochemical parameters to identify highly correlated parameters, followed by a Principal Component Analysis performed to define water quality variation patterns. Then a multifactorial MANOVA under the squared sum Model I was carried out, using population, sex and developmental stage as fixed factors, and P_{crit} and routine $\dot{M}O_2$ as dependent variables. A Montecarlo randomization test was applied for *p* value precision and a Least Significant Difference *post-hoc* test was applied for p corrections and sample size effect. All data are reported as means $\pm$ 1 standard error (SE).

3. Results

3.1. Environmental parameters measured in the field

Temperature (°C), pH, conductivity (CND), salinity (ppt), transparency (cm), oxygen partial pressure (kPa), depth (cm) and total dissolved solids (TDS) for water samples from Ex Hacienda and San Elías are reported in Table 1. A Principal Component Analysis was carried out in all parameters to determine a panorama of the variation in water quality within and between reservoirs (Fig. 1). Although San Elías presented a higher variance (5.28) compared to Ex Hacienda (1.13), no significant differences were found between means of all variables from the two reservoirs ($t = -0.089$, with $p > .05$).

Dial variation of P_{O_2} and temperature was recorded in both reservoirs. Temperature was very stable in both reservoirs, but San Elias was warmer at $18.9 \pm 0.01\,°C$ (Fig. 2A) compared to Ex Hacienda which was $17.7 \pm 0.03\,°C$ (Fig. 2B). P_{O_2} increased significantly after

Table 1
Mean, standard error, minimum and maximum daytime (9–13:00 h) values of physicochemical parameters of the two reservoirs from which *Girardinichthys multiradiatus* were collected.

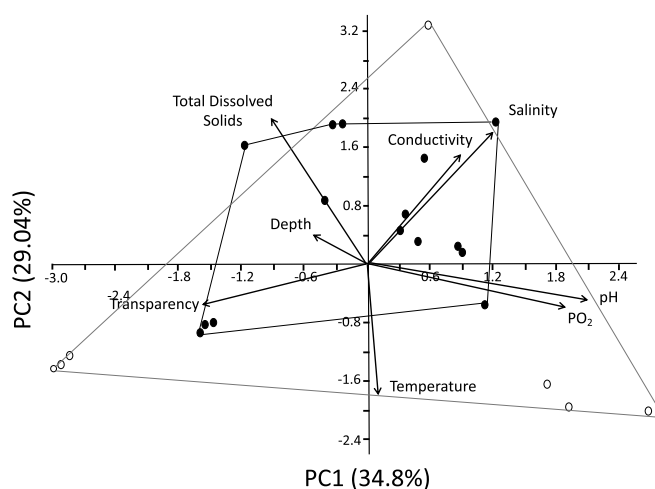|	Ex Hacienda				San Elías			
	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max
T°	17.4	0.6	14	20.9	20.1	0.7	17.2	22.9
CND (μS/cm)	276.5	5.06	250	303.8	305.6	27.3	230	527
pH	8.1	0.1	7.5	8.8	8.7	0.5	7	9.8
TDS (ppm)	0.3	0.00	0.3	0.3	0.3	0.01	0.3	0.4
Transparency (cm)	14.5	0.7	10	20	25.9	6.0	10	45
Depth (cm)	48.8	5.8	25	110	52.7	7.9	20	70
Salinity (ppt)	0.2	0.01	0.1	0.3	0.1	0.01	0.1	0.2
DO (mg/l)	4.8	0.02	4.2	6.3	4.9	0.02	3.9	7
P_{O_2} (kPa)	10.8	0.08	9.1	14.6	10.7	0.06	8.5	15.6

Fig. 1. Spatial and seasonal variation of the habitat of *G. multiradiatus*, indicated by a scatter and variable loads biplot of a principal component analysis. Dot color indicates the location - black dots are for Ex Hacienda and grey dots for San Elías. Arrow length shows the magnitude load and direction of each indicated physicochemical variable. The percentage of explained variation for the first and second component is in parenthesis.

12:00 h in Ex Hacienda, peaking at midday: P_{O_2} (0:00h-10:55h) $= 4.12 \pm 0.24$ kPa; P_{O_2} (11:00h-16:00h) $= 18.6 \pm 0.8$ kPa and P_{O_2} (16:00h-23:55h) $= 8.1 \pm 0.2$ kPa (ANOVA values, F = 321.5, d.f. = 2, 287, $p < .001$). A significant increase in P_{O_2} was also observed during the day in San Elías, $P_{O_2(0:00h-10:55h)} = 5.12 \pm 0.22$ kPa; P_{O_2} (11:00h-16:00h) $= 18.02 \pm 0.11$ kPa and PO_2 (16:00h-23:55h) $= 14.5 \pm 0.05$ kPa (ANOVA values, F = 231.7, d.f. = 2, 287, $p < .001$). Although significant increases in PO_2 occurred during the day in each reservoir, no significant differences were found between reservoirs (F = 3.146, d.f. = 1, 781, $p = .076$), with a mean of P_{O_2} of 10.9 ± 0.1 kPa in Ex Hacienda and 10.7 ± 0.1 kPA in San Elías.

Pearson's bivariate correlations were applied to physical and chemical variables. Strong significant relationships were observed between P_{O_2} and pH in both reservoirs. However, only in San Elías were significant correlations found between P_{O_2} and TDS and Transparency (See Table 2). Normoxia at 2650 masl is 15 kPa. However, as evident from these graphs, both reservoirs show hypoxic conditions except from 13:00 h to 16:00 h.

3.2. Oxygen consumption and body mass

Routine $\dot{M}O_2$ of 32 adult *G. multiradiatus* ranged from 5.3 to 11.9 μmol O_2/g/h in Ex Hacienda reservoir. The adult fish from San Elías showed more variability, with values ranging from 4.6 to
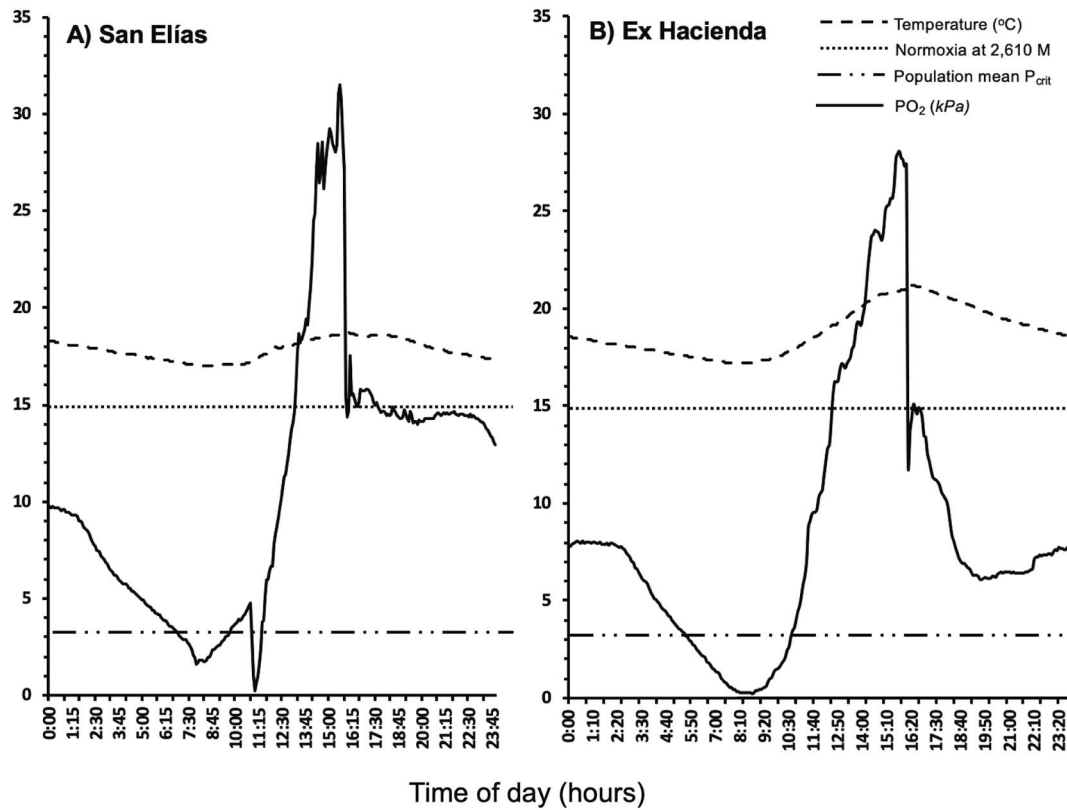
Fig. 2. Dial variation in water surface P_{O_2} and temperature measured during the hottest day in the shallowest water in early summer.

Table 2
Bivariate Pearson's correlations of physicochemical parameters in Ex Hacienda and San Elías reservoir. Values below the diagonal line are Pearson's correlation coefficients and above the diagonal are the *p* values for each correlation. Values in bold italics are significant.

Ex Hacienda Reservoir

		T°	CND	pH	TDS	Transparency (cm)	Depth (cm)	Salinity (ppm)	*kPa*
						P value			
T°		—	0.49	0.38	0.40	0.95	0.60	0.15	0.50
CND		-0.21		***0.01***	0.10	0.62	0.13	***0.00***	***0.00***
pH		-0.27	***0.70***		0.36	0.47	0.94	***0.02***	***0.00***
TDS		0.30	0.55	-0.32		1.00	0.28	0.23	0.59
Transparency (cm)	Pearson's coefficient	0.02	0.15	-0.22	0.00		0.34	0.92	0.54
Depth (cm)		0.16	0.45	0.02	0.38	0.29		0.05	0.30
Salinity (ppm)		-0.43	***0.81***	***0.62***	0.42	0.03	0.55		***0.01***
PO₂ (kPa)		-0.21	***0.78***	***0.75***	-0.19	0.19	0.31	***0.67***	

San Elías Reservoir

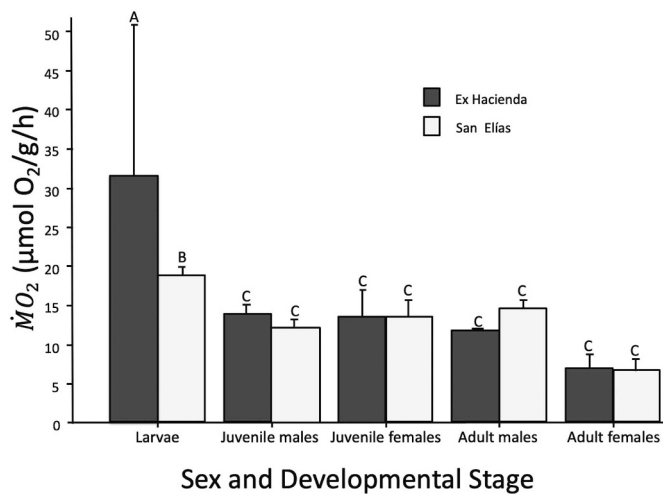|		T°	CND	pH	TDS	Transparency (cm)	Depth (cm)	Salinity (ppm)	*kPa*
						P value			
T°		—	0.12	0.09	***0.00***	0.09	0.90	***0.07***	***0.04***
CND		-0.52		***0.00***	0.14	***0.00***	0.36	***0.01***	0.98
pH		0.68	***0.96***		0.27	***0.00***	0.25	***0.00***	***0.00***
TDS		***-0.92***	0.62	-0.73		0.48	0.36	***0.00***	***0.00***
Transparency (cm)	Pearson's coefficient	-0.69	***-0.95***	***-0.93***	0.52		0.14	***0.00***	***0.00***
Depth (cm)		-0.06	-0.41	-0.50	0.64	0.62		0.30	0.27
Salinity (ppm)		***-0.59***	***0.76***	***0.99***	***0.99***	***-0.96***	-0.46		0.72
PO₂ (kPa)		***0.66***	0.01	***0.96***	***-0.95***	***-0.93***	-0.49	0.13	

Fig. 3. Comparisons of routine $\dot{M}O_2$ by population, sex and developmental stage in *G. multiradiatus* in Ex Hacienda and San Elías reservoirs. Means ± S.E are plotted. Letters indicate statistical differences between populations.

Table 3
Routine oxygen consumption of *Girardinichthys multiradiatus* by population and ontogenetic stages/sex. Mean ± SE are provided.

Site	Stage/Sex	n	Body Mass (g)	$\dot{M}O_2$ (mg O_2 h^{-1})	$\dot{M}O_2$ (µmol O_2/g/h)
Ex Hacienda	Larvae	3	0.09 ± 0.02	0.04 ± 0.01	31.55 ± 16.5
	Juvenile male	3	0.23 ± 0.02	0.06 ± 0.00	14.00 ± 1.00
	Juvenile female	3	0.43 ± 0.06	0.08 ± 0.00	13.60 ± 2.8
	Adult male	3	1.09 ± 0.09	0.09 ± 0.02	11.82 ± 0.11
	Adult female	3	1.70 ± 0.24	0.18 ± 0.02	6.98 ± 1.4
San Elías	Larvae	3	0.14 ± 0.03	0.05 ± 0.003	18.94 ± 0.40
	Juvenile male	4	0.29 ± 0.07	0.17 ± 0.04	12.21 ± 0.91
	Juvenile female	3	0.39 ± 0.26	0.08 ± 0.03	13.55 ± 1.86
	Adult male	3	0.40 ± 0.06	0.08 ± 0.03	14.60 ± 0.93
	Adult female	4	1.74 ± 0.17	0.16 ± 0.02	6.73 ± 1.42

15.5 µmol O_2/g/h. An ANOVA Post-Hoc Test based on LSD showed significant differences ($p < .001$) in routine $\dot{M}O_2$ of larvae from Ex Hacienda Reservoir compared to San Elías larvae, and from juveniles and adults from both reservoirs. There were no significant differences between sexes for Ex Hacienda (juveniles $p = .9$ and adults $p = .26$) and for San Elías (juveniles $p = .7$ and adults $p = .059$) - see Fig. 3. Larvae showed higher mean routine $\dot{M}O_2$ in both populations (Table 3).

To analyze the effects of body size, routine $\dot{M}O_2$ was plotted against body mass (g) and fitted to the allometric model equation $\dot{M}O_2 = aW^b$. The model was fitted by Ordinary Least Squares method after logarithmic transformation of both variables. The relationship between body size and routine $\dot{M}O_2$ was highly significant for fish populations from both San Elías (routine $\dot{M}O_2 = -0.86\,W^{0.6}$, $r = 0.96$, $r^2 = 0.93$, $n = 17$, $p < .001$;) and Ex Hacienda (routine $\dot{M}O_2 = -0.81\,W^{0.6}$, $r = 0.93$, $r^2 = 0.87$, $p < .01$, $n = 12$). However, no significant differences were found between population regression lines ($F = 0.013$, $p = .90$) (Fig. 4A).

When the statistical model was fitted only for post-larval *G. multiradiatus* (juveniles and adults), the pattern changed slightly - San Elías (routine $\dot{M}O_2 = -0.86\,W^{0.58}$, $r = 0.94$, $r^2 = 0.89$, $n = 14$, $p < .01$) and Ex Hacienda (routine $\dot{M}O_2 = -0.81\,W^{0.83}$, $r = 0.92$, $r^2 = 0.85$, $n = 12$, $p < .001$). When eliminating larvae from the analysis, significant differences became evident between population regression lines ($F = 4.52$, $p = .04$) (Fig. 4B).

3.3. Hypoxia tolerance and P_{Crit}

Hypoxia tolerance, as assessed by the critical PO_2 (P_{Crit}), ranged from 1.8 to 3.2 kPa in Ex Hacienda adults and 1.8 to 2.9 kPa in San Elías adults. P_{Crit}s were not significantly different (ANOVA, $F = 0.99$, g.l. = 8, $p = .47$) for any developmental stage or either reservoir. However, LDS *post hoc* tests indicated significant differences between larvae from San Elías compared with juvenile males from San Elías ($p = .04$) and juvenile females from Ex Hacienda ($p = .03$) (Fig. 5). Larvae showed the highest P_{crit} mean value for both populations, with larvae from Ex Hacienda showing the higher values of the two (Table 4). The raw data of the response of both San Elías and Ex Hacienda fish to decreasing partial pressure of oxygen in water are plotted in Fig. 6.

3.4. Relationship of P_{crit} and body mass

P_{crit} was not significantly correlated with body mass in *G. multiradiatus* from the San Elías reservoir ($r = -0.15$, $r^2 = 0.025$, $p = .54$, $b = -0.04$, $a = 0.34$) or the Ex Hacienda reservoir ($r = -0.18$, $r^2 = 0.033$, $p = .56$ $b = -0.04$ $a = 0.32$). No significant differences exist between regression lines for the two reservoirs. Individual time-P_{O_2} profiles at 20 °C of larval fish are shown in Fig. 7. At a specific P_{O_2} below P_{crit}, all larvae from San Elias reservoir died. In contrast, larvae from Ex Hacienda reservoir died before reaching any apparent P_{crit}, not surviving > 50 min in the respirometer chamber, and so were not considered in the statistical analysis. All larvae maintained equilibrium until immediately before death.

4. Discussion

4.1. Abiotic field measurements

Physical and chemical parameters of both Ex Hacienda and San Elías reservoirs in the Lerma Basin showed similar mean values to those measured for the Pánuco Basin of east-central Mexico, which are inhabited by many freshwater fishes, including viviparous fish from the Goodeidae family (Lyons et al., 2000; Torres-Olvera et al., 2018). Although the water quality of the two sampled reservoirs relative to water conditions before anthropogenic disturbance is unknown, it is clear that both reservoirs present similar abiotic conditions. Both have similar dial variation of temperature and P_{O_2} and have a rise of P_{O_2} just after midday, mostly the result of increased algal photosynthetic activity and changes in biological oxygen demand, a characteristic of eutrophic systems (Zang et al., 2011). Water P_{O_2} correlates with pH according to Table 2. Several authors have reported significant positive correlations between dissolved oxygen and pH (Luis et al., 2010; Zhang, 2009; Luo, 2002) and parallel diurnal variation for both parameters in natural and experimental systems (Zang et al., 2011). Continuous 24 h monitoring of pH and DO in natural aquatic systems often shows that variation for both parameters reaches their maximum values at ~16:00 h and its minimum at ~6:00 h (Zhang and Sun, 2004), similar to the findings of the present study for reservoirs in the Upper Lerma Basin. Although aquatic respiration consumes oxygen during the day, even greater amounts of O_2 are produced by photosynthesis, since the concentration of Chlorophyll *a* is highly correlated to DO (Zhang et al., 2009; Zhang et al., 2011). Algae and other aquatic organisms start to consume oxygen through respiration at night, and the accumulated carbon dioxide promotes HCO_3^- and H^+ formation, the latter promoting a decrease in pH. Although, DO and pH variation are similar, pH values can be altered by nitrogen and phosphorus input especially to eutrophic systems, and its positive correlation could even become inverted. A high pH might inhibit algal photosynthesis, limiting DO, which can be a limiting factor for aquatic physiological metabolism (Dai, 2009).
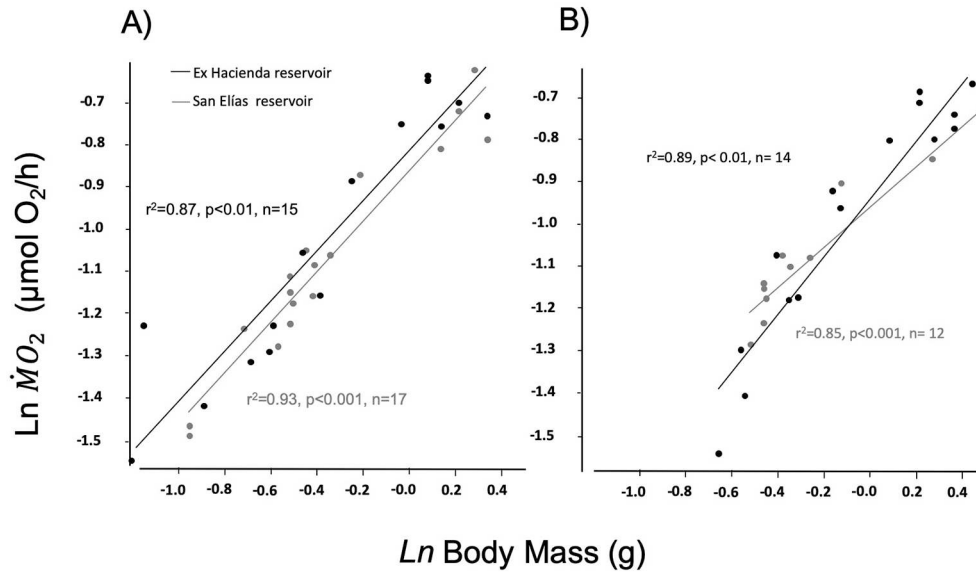
Fig. 4. Log-log plot of the relationship between body mass and routine $\dot{M}O_2$ for populations of *G. multiradiatus* in Ex Hacienda and San Elías reservoirs. A) All individuals in each population, B) Non-larval fish only.
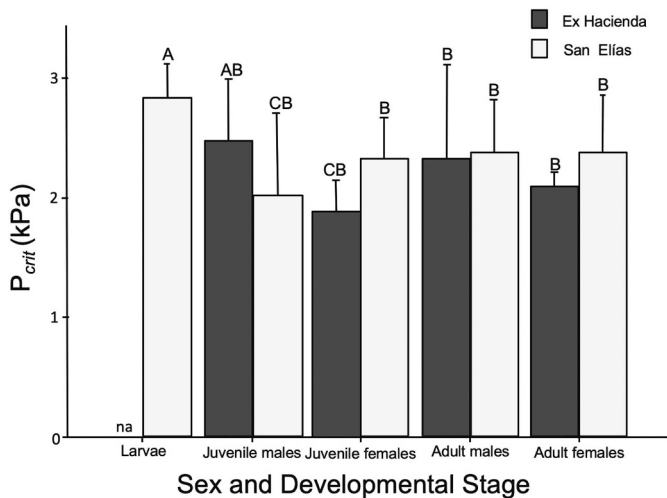

Fig. 5. P_{crit} comparisons by population, sex and developmental stage in *G. multiradiatus*. Means ± S.E are plotted. Letters indicate statistical differences between populations. na indicates data unavailable.

Table 4
Critical P_{O_2} (P_{crit}) of *Girardinichthys multiradiatus* by population and ontogenetic stages/sex. Means ± SE are provided.

Site	Stage/sex	n	P_{Crit} (kPa)
Ex Hacienda	Larvae		–
	Juvenile male	3	2.46 ± 0.05
	Juvenile female	3	1.88 ± 0.02
	Adult male	3	2.34 ± 0.08
	Adult female	3	2.10 ± 0.01
San Elías	Larvae	3	2.84 ± 0.03
	Juvenile male	4	1.99 ± 0.04
	Juvenile female	3	2.32 ± 0.04
	Adult male	3	2.35 ± 0.05
	Adult female	4	2.35 ± 0.03

4.2. Routine oxygen consumption and hypoxia tolerance of G. multiradiatus

Under normoxic conditions at an altitude of 2600 masl (15 kPa), routine $\dot{M}O_2$ of *Girardinichthys multiradiatus* from both populations showed lower values (0.10 ± 0.014 mg/O_2/h) than those for other Cyprinodontid genera in freshwater at 20 °C, such *Cyprinodon* (0.26 mg/O_2/h) and *Fundulus* (0.65 mg/O_2/h) and other livebearers like *Poecilia* (0.2 mg/O_2/h) and *Xiphophorus* (0.25 mg/O_2/h) (Nordlie, 2014). However, an $\dot{M}O_2$ of 0.13 mg O_2 h^{-1} in *Gambusia* is quite similar to those measured in the present study for *G. multiradiatus*. Although, the families Cyprinodontidae, Fundulidae and Poecillidae contain species that are tolerant to salinity or dwell in salt marshes or sea water, the family Goodeidae has nearly 40 species and all of them are strictly freshwater species with a narrow distribution within the Mexican Plateau (Miller, 2009). Another tropical fish, *Pseudocrenilabrus multicolor victoriae,* also has similar values to the obtained here, those with lake origin have a $\dot{M}O_2$ of ~4.8 µmol O_2/g/h and those with swamp origin show ~3.5 mg O_2 g^{-1} h^{-1} at a temperature of 26 °C (McDonell and Chapman, 2016).

The ecological implications of $\dot{M}O_2$ and P_{O_2} variation in North American fish across populations have been analyzed (Chown, 2012). In such studies, P_{crit}, an indicator of hypoxia tolerance, is useful for incorporation into trait-based approaches to the conservation physiology of fishes (Frimpong and Angermeier, 2009) (note, however, that the limitations of P_{Crit} and its interpretations have recently been questioned – Wood, 2018). These traits are considered plastic, where in the sailfin molly (*Poecilia latipinna*), for example, fish acclimated to hypoxia showed low P_{crit} and were more hypoxia tolerant than normoxic acclimated fish. However, no differences were found in oxygen uptake (Timmerman and Chapman, 2004). Also, in *Pagrus auratus* hypoxia acclimation increases tolerances to hypoxia, with individuals exposed to moderate hypoxia exhibiting a P_{Crit} of 3.3 ± 0.7 kPa (Cook et al., 2013). In the present study, P_{Crit} values in *G. multiradiatus* appear to be lower than the average reported for tropical fish (4.92 ± 0.19 kPa) (Rogers et al., 0.2016). This indicates that the *G. multiradiatus* in both reservoirs have an ability to survive in hypoxic conditions, that could derive from modification of lamellae surface, behavioral avoidance strategies or even aquatic surface respiration like *Poecilia latipinna* (Timmerman and Chapman, 2004). However, these factors were not measured in the present study (Rogers, 2016).
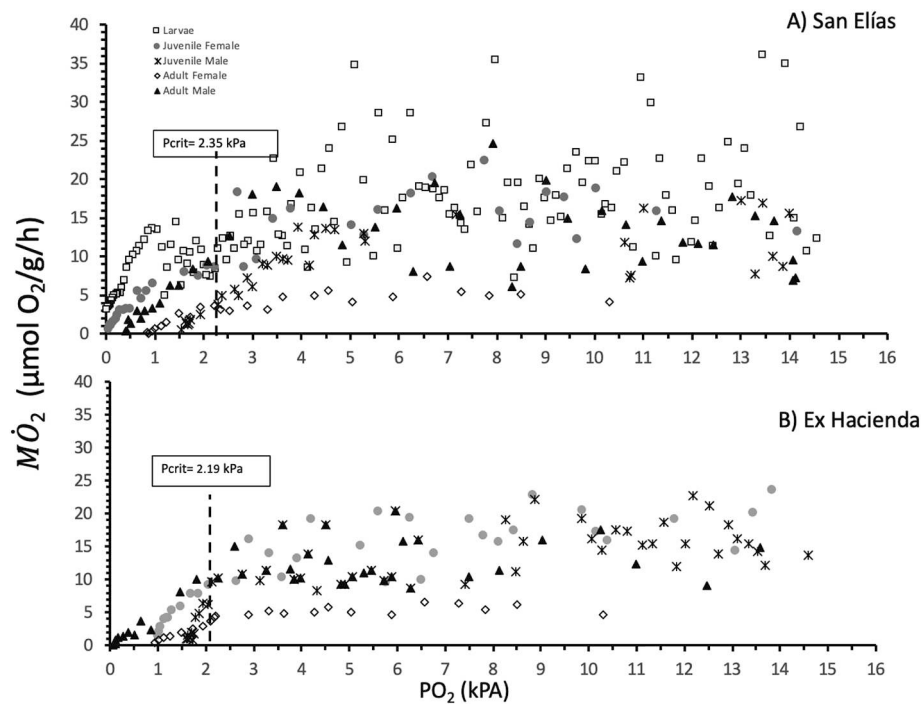
Fig. 6. Representative traces of routine $\dot{M}O_2$ with declining PO_2 in *Girardinichthys multiradiatus* from San Elías reservoir and Ex Hacienda Reservoir. Different symbols represent different stages and sexes, and each individual symbol represents a single measurement of $\dot{M}O_2$ at the indicated PO_2.

Girardinichthys multiradiatus in both reservoirs lives under hypoxic conditions during night and part of the day, except during midday and few hours in the afternoon (Fig. 2). This species shows high levels of hypoxia tolerance and lower metabolic rates compared to many other tropical fishes. Mandic and Regan (2018) analyzed species that live at high altitude and indicated that metabolic rates depend on the nature of the hypoxia exposure. According to their analysis, if organisms have access to chronic exposure, then the metabolic rate is compensated but if there is access to oxygen only seasonally then metabolic rate is depressed. If hypoxia is long and severe but organisms have higher oxygen access then metabolic rates can be compensated, otherwise they are

depressed. But, if organisms have access to acute night bouts of hypoxia exposure, like *G. multiradiatus* in San Elias and Ex Hacienda reservoirs then it might employ an aerobic response with some contribution of anaerobic metabolism (Mandic and Regan, 2018). Like in the estuarine killifish (Borowiec et al., 2015; Borowiec et al., 2018), the intermittent diel cycles with short time exposure to hypoxia and moderated P_{O_2} in these reservoirs might help fish maintain sufficient O_2 transport to support the routine demands by anaerobic lactate production without any apparent metabolic acidosis or glycogen depletion, and the oxygen debt paid off the next day when oxygen is readily available.

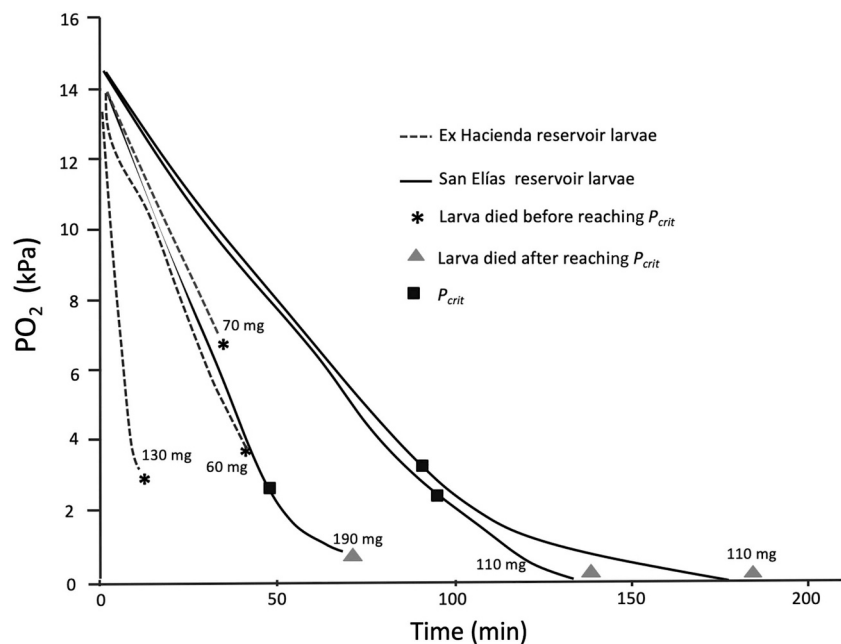*G. multiradiatus* could have several adaptations enabling its

Fig. 7. Selected time courses of P_{O_2} decreases in the closed respirometers used to measure routine $\dot{M}O_2$ and P_{Crit} at 20 °C in *G. multiradiatus* larvae.

considerable hypoxia tolerance. The Crucian carp shows regression of the interlamellar cell mass that surrounds lamellae under hypoxic conditions (Nilsson, 2007), increasing total respiratory surface area. Such adaptations are possible, though not yet explored, in *G. multiradiatus*. Also, *G. multiradiatus* may have particular mechanisms for enhancing routine O_2 uptake during hypoxia, or the ability to recruit alternate biochemical pathways for energy production. Other possible adaptations to hypoxia could include modifications in hemoglobin (Hb)-O_2 binding characteristics - *e.g.* a leftward shift in the oxygen-hemoglobin dissociation curve (Hopkins and Powell, 2001; Mandic et al., 2009; Mendez-Sanchez and Burggren, 2019a, 2019b). Additional responses for hypoxic survival could include behavioral adjustments, such as employing surface respiration (Burggren and West, 1982; Richards, 2011; Martin, 2014).

4.3. Sex and body size effects

Differences in routine $\dot{M}O_2$ between females and males have been reported in Teleost fishes. For example, males show higher routine $\dot{M}O_2$ than females in pink salmon (Makiguchi et al., 2016) and sockeye salmon Clark et al., 2009. However, in the American plaice (*Hippoglossoides platessoides*) there are no sex-based differences in routine $\dot{M}O_2$, even though females are larger (MacKinnon, 1973). In the present study, although females are larger than males on *G. multiradiatus*, no sex-based differences between males and females were found, especially between adults. Females in the experiments were not pregnant, but their reproductive state was unknown. That is, there was no record of the number of reproductive events of each fish or the specific age, since stages were determined only by coloration and shape.

As in most ectothermic freshwater fish, a strong relationship between body mass and metabolic rate was evident in post-larval *G. multiradiatus*. The mass scaling exponent *b* in fish from the San Elías Reservoir was 0.58 compared to 0.83 for fish from the Ex Hacienda reservoir. A mean exponent b of 0.79 ± 0.11 was calculated for 69 species of post-larval fish, including the orders Pleuronectiformes, Salmoniformes, Perciformes, Gadiformes, Anguiliformes and Cypriniformes (Clarke and Johnston, 1999). Winberg (1960) calculated two allometric equations for routine metabolic rate in post-larval fish, one for marine species ($MO_2 = 0.459W^{0.79}$) and another for freshwater species ($\dot{M}O_2 = 0.439W^{0.81}$). Winberg (1960) calculated a third equation for Cyprinodontiformes ($\dot{M}O_2 0.289W^{0.73}$), specifically the live-bearing family, Poeciilidae. Nordlie (2014) reported the allometric equations for several families, with all $\dot{M}O_2$s adjusted to 20 °C, for species within the families Fundulidae, ancestors of Goodeidae family ($\dot{M}O_2 = 0.399W^{0.638}$, body mass 0.58–13.3 g), Cyprinodontidae ($\dot{M}O_2 = aW^{0.68}$, mass = 0.2–9.33 g) and Poeciilidae ($\dot{M}O_2 = 0.248W^{0.696}$, body mass 0.01–2.6 g). In the present study *G. multiradiatus* in both sampling sites had a scaling exponent within the expected range for freshwater fish (exponent b = 0.66–1.0). This relationship varies with regard to lifestyle, swimming mode and ambient temperature, and is inversely related to the metabolic level (Killen, 2010). Fish from San Elías reservoir have an allometric exponent near the lower limit of the range reported. Consequently, it can be hypothesized these active predators could have a pelagic ecology within a microhabitat with a simple physical structure. On the other hand, fish from Ex Hacienda reservoir show *b* values nearer to 1, which hypothetically implies this fish's ecology involves a more benthic microhabitat that is more complex, and enables ambush foraging (Killen, 2010). In general, for teleost fish, lower mean values of *b* are related to the highest mean value for mass-specific metabolic rate estimated at the body mass corresponding to the midpoint of the allometric relationship. *G. multiradiatus* from San Elías have higher metabolic rates at the midpoint of their body mass range than fish from Ex Hacienda, this could be a consequence of the higher temperature that exists in San Elias Reservoir.

Interestingly, the inclusion of larval fish into the scaling relationship analysis eliminates the differences between reservoirs in the allometric exponent b (see Fig. 4 A), creating an overall value of 0.6. This value may indicate that, due to the feeding needs of the larvae of *G. multiradiatus*, they may occupy a pelagic trophic position (Arhonditsis et al., 2018). Also, the lack of a significant allometric correlation between body mass or size and P_{crit} might indicate that either being large or small does not give the fish any advantage on hypoxic environments. However, it is noteworthy that many aspects of *intra*-generational scaling are problematic. A basic assumption of development is that physiological state changes from the larval to adult transition, while it is widely held that allometry should be made of animals in the same physiological state (Burggren, 2005). Consequently, it is more robust to exclude larvae from the analysis rather than include them.

From our results we conclude that *G. multiradiatus* lives under hypoxic conditions most of the time with shorter periods of hyperoxia and only minimal normoxia exposure. This environmental exposure induces a high hypoxia tolerance evident from its P_{Crit} and its low metabolic rate in comparison with other Cyprinodontid and tropical fish species. This reflects evolved physiological adaptations to high altitude conditions and dial oxygen variation. Both populations present similar abiotic conditions and variation, with no significant differences between them in P_{Crit} or $\dot{M}O_2$. However, mass scaling is quite different, which according to some literature could be related with the trophic status or other ecological factors associated to the species. It is important to continue studies on larval *G. multiradiatus*, since in the present study larvae showed the highest $\dot{M}O_2$ and the less hypoxia tolerance, making this stage the most vulnerable to environmental drastic changes.

Declaration of Competing Interest

None.

Acknowledgements

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