



Metabolic physiology of the Mayan cichlid fish (*Mayaheros urophthalmus*): Re-examination of classification as an oxyconformer

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ABSTRACT

The Mayan cichlid (*Mayaheros urophthalmus*) is a freshwater fish inhabiting warm, potentially hypoxic and/or brackish waters, in Mexico and Central America. Despite its description as highly hypoxia tolerant, *M. urophthalmus* has been classified physiologically as an 'oxyconformer', which would place it in a very small (and shrinking) category of fishes that purportedly cannot maintain oxygen consumption ($\dot{M}O_2$) as ambient PO_2 falls. However, hypoxia tolerance is often associated with strong oxyregulation, not oxyconformation as described for *M. urophthalmus*. To resolve these inconsistencies, we measured $\dot{M}O_2$, the ambient PO_2 at which $\dot{M}O_2$ begins to decline as PO_2 falls (P_{crit}), and gill ventilation rate (f_G) in the Mayan cichlid. Variables were measured at 23°, 28° and 33 °C and temperature sensitivity (Q_{10}) calculated for each function. $\dot{M}O_2$ at air saturation was 2.9 ± 0.2 , 4.3 ± 0.4 , and $5.9 \pm 0.3 \mu\text{mol O}_2/\text{g/h}$ at 23°, 28° and 33 °C, respectively. P_{crit} s were low at 2.6 ± 0.8 kPa, 3.2 ± 0.8 kPa and 4.7 ± 0.9 kPa at 23°, 28° and 33 °C, respectively. Q_{10} values for $\dot{M}O_2$ were 2.56 ± 0.21 (23–28 °C), 1.89 ± 0.15 (28–33 °C) and 2.2 ± 0.1 (full temperature range of 23–33 °C), suggesting overall Q_{10} s typical for tropical freshwater fish. f_G was 39 ± 3 , 45 ± 4 , and 53 ± 6 breaths/min at 23°, 28° and 33 °C, respectively, and increase 2–3 fold in severe hypoxia at each temperature. Experiments employing hyperoxia up to 35 kPa indicate a strong 'hypoxic drive' for gill ventilation. Collectively, these data show that, in contrast to a previous characterization, the Mayan cichlid is a strong oxyregulator exhibiting attributes (e.g. very low P_{crit}) typical of very hypoxia-tolerant fishes.

1. Introduction

Many Central and South American fishes have been categorized as hypoxia resistant or tolerant (Almeida-Val et al., 1995; Baptista et al., 2016; Chávez-Lopez et al., 2005; Tian et al., 2017). Among these is the Mayan cichlid, *Mayaheros urophthalmus* (formerly *Cichlasoma urophthalmus*). While preferring well oxygenated oligohaline water (Chávez-Lopez et al., 2005), this species (also known as the Mexican Mojarra) can live and reproduce in a wide variety of freshwater to slightly brackish habitats (Martínez-Palacios and Ross, 1986; Nico et al., 2007; Schofield et al., 2010; Stauffer and Boltz, 1994). Additionally, the Mayan cichlid shows considerable hypoxia tolerance, as evident from the very low water PO_2 thresholds (~ 20 mmHg at 30 °C) for both aquatic surface respiration (ASR) and buccal air bubble holding (Schofield et al., 2009). This species is also tolerant of high environmental temperatures up to 39 °C, but has limited cold tolerance (Schofield et al., 2010). Perhaps because of its tolerance to a wide

variety of environmental conditions, the Mayan cichlid has significant importance to aquaculture in Central America (Martínez-Palacios et al., 1993; Martínez-Palacios and Ross, 2004; Miller et al., 2005). However, this species has also been studied because of its highly invasive nature, expanding into more northerly habitats in the Everglades of Florida, USA and waters in Thailand, for example (Fauce and Lorenz, 2000; Loftus, 1987; Nico et al., 2007; Paperno et al., 2008; Schofield et al., 2009).

Hypoxia tolerance in fishes is often linked to their ability to maintain oxygen consumption even as ambient PO_2 falls. An oxyconformer, then is an animal that cannot (or does not) maintain oxygen consumption as ambient PO_2 decreases – e.g. (Burggren and Randall, 1978; Rogers et al., 2016; Steffensen, 2007). Conversely an oxyregulator maintains oxygen consumption at or near normoxic levels even as ambient PO_2 falls toward the critical PO_2 (P_{crit}), at which point oxygen consumption finally begins to sharply decline. In this context, despite the considerable information available for the Mayan cichlid, a major

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anomaly persists in the literature. Studying invasive Mayan cichlids from southern Florida, Martínez-Palacios and Ross (1986) described the Mayan cichlid as relatively hypoxia tolerant freshwater fish, as other investigators have before and after. Yet, these authors documented a surprisingly high P_{Crit} of approximately 9.3 kPa (70 mmHg). Further, these authors commented quite unequivocally that "...these fish are oxygen conformers and no attempt is made to regulate oxygen uptake". This single reported value of P_{Crit} reported for the Mayan cichlid is far higher than values reported for many other tropical freshwater fishes (Rogers et al., 2016). In fact, Rogers et al. (2016) comprehensive meta-analysis of P_{Crit} in fishes, < 10% of 72 observations of 33 species freshwater fish tested at temperatures of 20–36 °C had P_{Crit} values higher than that reported for the Mayan cichlid by Martínez-Palacios and Ross (1986).

A high P_{Crit} for Mayan cichlids would seemingly be in stark contrast to the view that hypoxic resistance in fishes is generally associated with a lower value for P_{Crit} (Burggren et al., 2019; Mandic et al., 2009; Reardon and Chapman, 2010; Rogers et al., 2016). Indeed, Stephensen (2007), in discussing the likelihood that any fish is an oxyconformer, comments "Overall, most fish species behave as perfect oxygen regulators". It could be proposed that the Mayan cichlid's hypoxic resistance derives from the ability to tolerate tissue hypoxia as ambient PO_2 falls - i.e. oxyconform - rather than resisting hypoxia by increasing gill ventilation and oxygen extraction to maintain arterial oxygen transport. Yet, if Mayan cichlids are indeed "oxyconformers", it would place this species in an extremely small category of vertebrates that are putative oxyconformers.

The rather surprising finding of a high P_{Crit} for the Mayan cichlid reported in just a single study by Martínez-Palacios and Ross (1986), and their subsequent conclusion that this fish is an "oxyconformer", has gone unchallenged for > 30 years, surprising given the general acceptance of hypoxia tolerance by the Mayan cichlid,.. Moreover, the metabolic physiology of Mayan cichlids has not been extensively investigated over a range of temperature and oxygen levels. Consequently, we have investigated the relationship between oxygen consumption, gill ventilation frequency and ambient water PO_2 at three different temperatures for *M. urophthalmus*. We hypothesized that, despite a previous report, the Mayan cichlid is indeed representative of typical Central and South American cichlids and indeed many tropical fishes, acting as an oxyregulator maintaining oxygen consumption until experiencing low ambient PO_2 s.

2. Methods

2.1. Fish acquisition and maintenance

Young adult Mayan cichlids were captured in the wild in Tabasco State, and transported to Toluca, Estado de México, Mexico, where they were maintained for three months at 27.5 °C in filtered, air-saturated water (pH 8.0–8.1, hardness 240–280 mg CaCO_3/l , $[\text{NH}_3] < 0.5 \text{ mg/l}$, total dissolved solids 0.8–0.29 ppt). Fish were kept in a 240 l tank at a stocking density of < 1.4 g/l. All fish were exposed to a natural daylight cycle, approximately 13:11 light:dark during the season in which these experiments were performed. Fish were fed ad libitum twice daily with a diet of commercial fish food, and their length and weight measured weekly. Average body mass increase during this 3 month period was $10.7 \pm 3.6 \text{ g}$ ($20.8 \pm 4.6\%$). No deaths (except as a result of cannibalism) occurred during the three-month acclimation period used in metabolic experiments.

2.2. Oxygen consumption measurements

Routine mass-specific oxygen consumption ($\dot{M}\text{O}_2$, $\mu\text{mol O}_2/\text{g/h}$) was measured using closed respirometry in young, sexually mature *M. urophthalmus* (body mass $55.4 \pm 8.1 \text{ g}$, $n = 6$) that had been fasting for 12–16 h, to minimize any influence of specific dynamic action. Acrylic cylindrical containers with 2–3 mm thick transparent walls were used

as respirometers. Two sizes were used (volumes 880 ml or 1376 ml), depending upon fish mass. The opaque lid of each respirometer contained a valved inlet and outlet. Respirometers were submerged in a 120 l glass tank, maintained at the desired experimental temperature of 23°, 28° or 33 °C. The tank containing the respirometers was wrapped with opaque material to shield the investigators' movements. Respirometers were oriented so that the opaque lid prevented fish from observing each other while in the respirometers.

Each fish was placed in a respirometer and then allowed to acclimate to respirometer conditions for ~2 h. During this acclimation period, the respirometer was continuously flushed with air-equilibrated water pumped from a small submersible water pump at a rate of 450–600 ml/min, thus turning over the volume of the respirometer every 2–3 min. Special care was taken to ensure that no air bubbles were trapped within the respirometer. Following respirometer acclimation, the valves on the inlet and outlet of the respirometer lid were carefully closed without disturbing the fish, which began the metabolic measurements. Importantly, recorded values of $\dot{M}\text{O}_2$ in the first minutes of after measurements began were very similar to those taken 1–2 h into the respirometry run, as long as the PO_2 was above P_{Crit} . This indicates that the initial 2 h acclimation period was sufficient for the fish to return to resting conditions after handling for initial placement in the respirometer. The decline in PO_2 within each sealed respirometer containing a fish, and the associated instantaneously calculated $\dot{M}\text{O}_2$, was measured until $\dot{M}\text{O}_2$ values, which had previously been more or less stable, began to sharply decline with further decrease in PO_2 . This inflection point represented the arrival at the fish's P_{Crit} , which was subsequently calculated after the measurement was completed (see below). P_{Crit} was typically reached in 2–6 h depending on the fish's body mass, temperature and rate of O_2 consumption. $\dot{M}\text{O}_2$ measurements were continued for a maximum of 15 min after the P_{Crit} inflection, after which the valves on the respirometers containing the fish were then opened to allow continuous flushing with air-saturated water.

PO_2 decline in each respirometer due to the fish's respiration was measured using temperature-compensated fiber-optic planar sensors (PreSens) connected to a Witrox 4 m (Loligo Systems ApS). Each respirometer had an optode sensor attached to the inside of the respirometer wall. A fiber optic laser probe both causing and capturing fluorescence in the internal optode sensor was attached perpendicularly to the outside of the respirometer immediately opposite the internal optode sensor. Fluorescence signals from the optode sensors were captured with a computer running AutoResp software (Loligo Systems ApS). Mass-specific oxygen consumption was calculated from the rate of O_2 decline in each chamber over time, the chamber volume, the elapsed time, and the mass of the fish, after (Burggren et al., 2019). Blank respirometers were run to determine microbial respiration. There was no stirring in these blanks, which has been suggested to disproportionately affect the accuracy of the amount of microbial respiration subtracted from the total $\dot{M}\text{O}_2$ (Rodgers et al., 2016). However, our blank measurements revealed no detectable oxygen consumption due to microbial respiration, so there was no need to apply a correction to the metabolic data for the fish.

No active stirring was provided within the sealed respirometers. Preliminary direct observations of fishes in the respirometers were made through a small observation port in the covering of the holding tank. These preliminary observations revealed significant water stirring within the respirometer, evident from the movements of small particulate matter in the water in these initial experiments. Stirring and mixture of water in the respirometer was caused by the fish, itself, as a result of pectoral, pelvic and tail fin movements to maintain position, as well as occasional exploratory behavior including occasional turning around in the respirometer. Importantly, this considerable stirring and water mixing was occurring in the area where the PreSens sensor was located, preventing any build-up of boundary layers adjacent to the respirometer wall.

In addition to visual verification of stirring, evidence of adequate

continuous stirring came from the measurements, themselves. The Loligo software was set to sample PO_2 in each respirometer every second and from that calculate an instantaneous $\dot{M}O_2$. The software also calculated a r^2 value for the regression through the preceding 60 $\dot{M}O_2$ determinations to indicate PO_2 measurement variability. A high r^2 would indicate the desired low sample to sample variability across multiple $\dot{M}O_2$ measurements, whereas a low r^2 would indicate a high sample to sample variability, which could arise from insufficient or irregular stirring within the respirometers. R^2 values in these measurements were typically in the range of 0.96–0.99, indicating very little measurement-to-measurement variability in sampled PO_2 .

Given that fish during the actual P_{Crit} runs were assumed to be exhibiting similar movements (and producing stirring within the respirometer), we thus classified the metabolic measurements as “routine” oxygen consumption. (See Discussion for additional comments on methodology).

Finally, because the laboratory in which fish were acclimated and measured is located at an altitude of ~2650 m in Toluca, “normoxia” is actually a PO_2 of ~15 kPa, compared to ~19.6 kPa at sea level. To approximate acute conditions of sea level, compressed O_2 gas was injected into the water flushing the respirometers to raise its PO_2 to ~20 kPa, which served as the starting point for the P_{Crit} runs.

2.3. Critical PO_2 calculation

P_{Crit} is the PO_2 below which oxygen consumption cannot be maintained as ambient PO_2 falls. Assessment of P_{Crit} is typically determined by examining the entire relationship between ambient PO_2 and $\dot{M}O_2$, and then either visually or statistically determining the break point in this relationship at which $\dot{M}O_2$ rapidly falls with further decreases in ambient PO_2 . In this study we determined P_{Crit} by using a modified “broken stick” statistical regression methodology (Yeager and Ulstch, 1989). This procedure identifies the P_{Crit} from the intersection point of two separate lines that best fit all $\dot{M}O_2$ data points above and all below the PO_2 approximating P_{Crit} , evident from the sharp inflection in the relationship between ambient PO_2 and $\dot{M}O_2$. Essentially, this process starts by determining the $\dot{M}O_2$ values above the inflection that result in a r^2 of 0 – that is, indicating that there is no relationship between $\dot{M}O_2$ and decreasing PO_2 (e.g. oxyregulation). An accompanying r^2 is then calculated for a second regression line created from the remaining points below the apparent P_{Crit} . Next, a data point for $\dot{M}O_2$ formerly contributing to the regression above the P_{Crit} line for higher PO_2 s is moved to the pool of values contributing to the lower regression line, and the effect on the resulting r^2 value of that regression is determined. This process is repeated for with multiple successive regressions, repeatedly moving data points from one regression line to the other, until the r^2 of the upper regression remains a 0 while the r^2 for the lower regression line reaches its maximum value, indicating the best overall fit of the two regressions for the $\dot{M}O_2$ data. The intersection of these final two regressions thus identifies the P_{Crit} . Typically, the r^2 for the lower regression line below the P_{Crit} ended at a value of > 0.90.

2.4. Experimental protocols for $\dot{M}O_2$ measurements

2.4.1. Effects of multiple successive $\dot{M}O_2$ measurements

An initial set of experiments was carried at 28 °C to document the recovery in normoxia or near-normoxia of the fish following a P_{Crit} respirometer run, and to determine any apparent effects (e.g. generation of an O_2 debt) created by exposure to the low PO_2 s necessary to determine P_{Crit} (Fig. 1). Thus, a first respirometry run was conducted as described in Section 2.2., continuously measuring $\dot{M}O_2$ as PO_2 progressively fell to P_{Crit} and slightly below. After the first measurement taken while the respirometer was near-normoxia (far left $\dot{M}O_2$ value in Fig. 1), the P_{Crit} run was completed, and the valves in the respirometer lids were then opened to flush the interior of the respirometer with air-saturated water at ~20 kPa for a standard ~2 h acclimation period

(hours 2–4 in Fig. 1). Then, three rapid successive “recovery” $\dot{M}O_2$ measurements were made during which the PO_2 in the respirometers was not allowed to fall to lower than 14–16 kPa (i.e. near normoxia) during each recovery run, with an intervening 10 min period of respirometer flushing with air-saturated water occurring between runs. These much more rapid $\dot{M}O_2$ measurements in normoxia typically required only 15–45 min. The averaged value for these three replicates is indicated as the second bar from the left in Fig. 1. After these three replicate measurements, the respirometer valves were opened and the respirometer continuously flushed overnight with air saturated water. The next morning, ~14 h later, the respirometers were sealed and third set of $\dot{M}O_2$ measurements were made (2nd bar from right, Fig. 1), following the same protocol as previous measurements. Finally, to assess the possibility of any O_2 limitation to oxygen consumption at the ambient air-saturated PO_2 of Toluca (~17 kPa), or as a result of accumulation of possible unpaid O_2 debt from the previous $\dot{M}O_2$ measurements, the respirometers were flushed with hyperoxic water (PO_2 ~23 kPa, 28 °C), generated by bubbling water with pure O_2 gas. A final series of three rapid, successive $\dot{M}O_2$ measurements were made in hyperoxia (right hand-most bars in Fig. 1).

2.4.2. Effects of acute temperature change on $\dot{M}O_2$

The next set of experiments were conducted two days after the first set described above. The cichlids, which had been held at 28 °C since arriving in the laboratory, were placed into the respirometers being flushed with air saturated water at 28 °C and allowed to acclimate in the respirometers for 2 h. The respirometers were then sealed and a P_{Crit} run employing multiple successive $\dot{M}O_2$ measurements was made at 28 °C. After these initial measurements 28 °C, taking ~3–5 h depending upon the particular fish, the fish were removed from the respirometers, fed during a 2 h period at 28 °C, and then returned to the respirometers, which were constantly flushed with air saturated water. The temperature of the water entering the respirometers was then lowered over a 2 h period to 23 °C (rate of temperature change ~ 2.5 °C/h), and the fish allowed to acclimate overnight to this lower temperature for ~12–14 h. The $\dot{M}O_2$ and P_{Crit} measurement process was then repeated, after which fish were once again fed over a 2 h period and then returned to the respirometer. The temperature was then raised to 33 °C over a four hour period (rate of temperature change ~ 2.5 °C/h) and the fish acclimated overnight at this new higher temperature. The following day, $\dot{M}O_2$ and P_{Crit} measurements were made at 33 °C. The following day, $\dot{M}O_2$ and P_{Crit} measurements were made at 23 °C.

2.5. Gill ventilation frequency measurements

In a final set of experiments conducted over a three day period following the $\dot{M}O_2$ measurements had been completed, Mayan cichlids were placed individually in respirometers for the standard ~2 h acclimation period in water at 28 °C at a PO_2 of ~20 kPa. Gill ventilation frequency, f_G (breaths/min), was determined by direct visual observation of opercular movements through a small observation port in the cover of the tank holding the respirometers, taking care to avoid disturbing the fish within the respirometers. Two to three successive measurements of f_G were made over a 60 s period, and the values averaged to create the mean f_G at the measurement PO_2 . Two initial f_G measurements over a 30 min period were made, and the respirometers then closed and the internal water PO_2 allowed to diminish over several hours to below the P_{Crit} level, recording f_G at numerous times as PO_2 progressively fell. These experiments were repeated at 33 °C and 23 °C over two successive days, following the same protocol as described for the $\dot{M}O_2$ measurements at multiple temperatures.

Experiments were also conducted to assess if there was an ‘hypoxic drive’ to gill ventilation in the Mayan cichlid, and whether there were any differences between ‘normoxic’ ventilation rates at sea level and the elevation of Toluca. To determine this, ambient PO_2 of water flushing

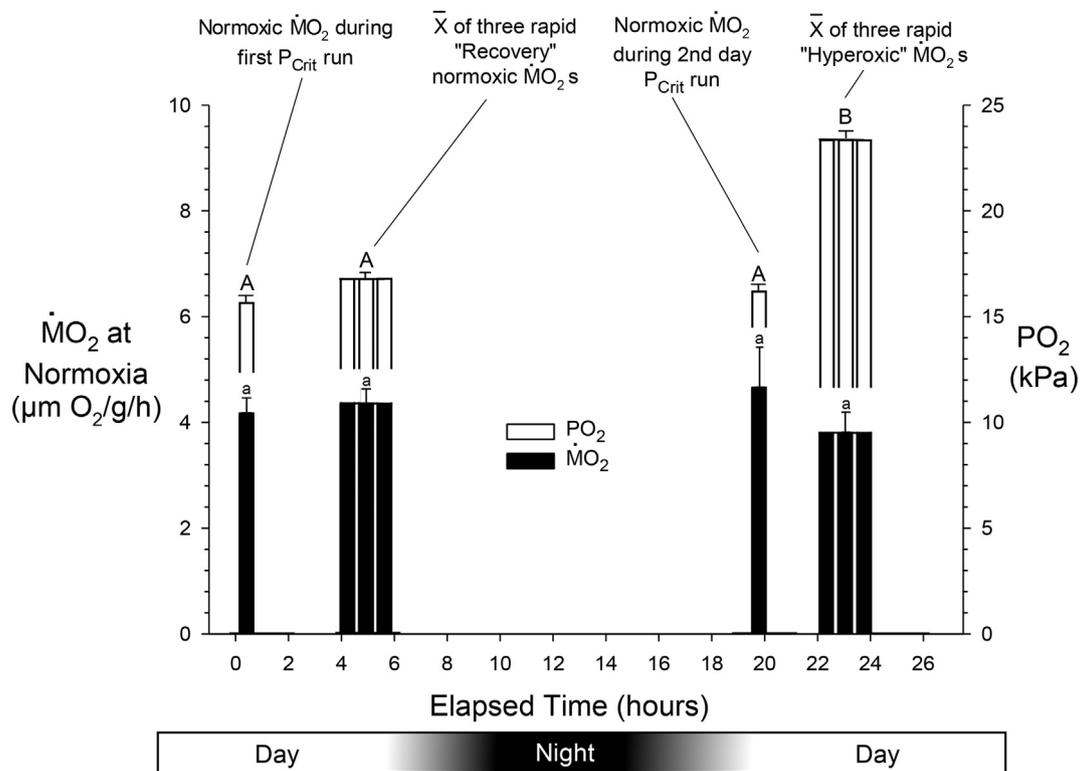


Fig. 1. Oxygen consumption at 28 °C in Mayan cichlids (*Mayaheros urophthalmus*) measured at distinct, successive periods over ~24 h to assess the effects of repeated P_{Crit} measurements and of hyperoxia. The plotted $\dot{M}O_2$ (left Y-axis) was measured at the associated PO_2 (right Y-axis). The initial left-most $\dot{M}O_2$ was the value measured in normoxia (~15.5 kPa) at the beginning of the first full P_{Crit} run. The next plotted $\dot{M}O_2$ value to the right of that first run represents the mean of three replicates in normoxia completed in rapid succession over a two hour period, ~3 h after the first P_{Crit} run. Then, after a ~14 h overnight period, a second full P_{Crit} run beginning with normoxia was conducted, and the initial normoxic $\dot{M}O_2$ value from that run plotted. Finally, to determine the effects, if any, of hyperoxia on $\dot{M}O_2$, a second set of three replicates completed in rapid succession over a two hour period were conducted in hyperoxia (~23 kPa). For all plots, upper and lower case letters indicate significance of differences between mean PO_2 s and mean $\dot{M}O_2$ s assessed by One-way ANOVA. Values presented are means \pm se. $n = 6$ fish for all measurements. See text for additional details.

the respirometers was raised by injected O_2 gas into the water flushing the respirometers to achieve a ~35 kPa, nearly twice the level of oxygen at which the fish were acclimated. The respirometers were then closed, and f_G measured at 28 °C as described above until PO_2 fell to ~15 kPa, levels slightly below normoxia in Toluca. This procedure for measuring gill ventilation was repeated over two successive days at 33 °C and 23 °C.

2.6. Statistics

One-way repeated measures ANOVAs were performed for each temperature to test significant differences in $\dot{M}O_2$ and P_{Crit} . Two-way ANOVA (i.e. temperature and PO_2 as covariables for the $\dot{M}O_2$ and f_G determinations) was considered but then rejected as a statistical approach, since the temperature measurements were performed on separate days with an intervening period ½ day period in which the fish were removed from the respirometer, fed and then replaced in the respirometer, indicating that each respirometry run was an independent event rather than a repeated measure under the same conditions.

Following one-way repeated measures, Holm-Sidak multiple comparison procedures were then used to determine significant differences between $\dot{M}O_2$ s and f_G s at different ambient PO_2 levels and at different temperatures.

A significance level of 0.05 was employed for all statistical tests to accept significant differences between experimental groups.

3. Results

3.1. Oxygen consumption

3.1.1. Assessment of experimental protocol effects

To determine if there was any effect on $\dot{M}O_2$ of sequential exposure to PO_2 s below the Mayan cichlid's P_{Crit} , we compared $\dot{M}O_2$ in normoxia before the first P_{Crit} run, then after a 4 h recovery, and again after ~12–14 h. A final measurement was made in hyperoxia (~23 kPa) to determine whether $\dot{M}O_2$ might be oxygen-limited by the ambient PO_2 s of Toluca or previous P_{Crit} measurement runs. There were no significant differences ($P > .10$) between $\dot{M}O_2$ values measured under any of the normoxic conditions described above and depicted in Fig. 1, including the final P_{Crit} run in hyperoxia. Nor was there a significant difference ($P > .05$) between the P_{Crit} s determined in the first P_{Crit} run at time = 0 to 3 h and the second run at time = 19–22 h in Fig. 1.

3.1.2. Acclimation temperature, ambient PO_2 and $\dot{M}O_2$

Oxygen consumption at the ambient PO_2 of Toluca (~15 kPa) was 2.9 ± 0.2 , 4.3 ± 0.4 , and $5.9 \pm 0.3 \mu\text{mol O}_2/\text{g/h}$ at 23°, 28° and 33 °C, respectively (Fig. 2). These values translated into Q_{10} values for $\dot{M}O_2$ of 2.56 ± 0.21 for the temperature range of 23–28 °C and 1.89 ± 0.15 for the temperature range of 28–33 °C (Fig. 3). Q_{10} for $\dot{M}O_2$ over the total temperature range of 23–33 °C was 2.18 ± 0.10 .

Ambient PO_2 significantly ($P < .001$) affected $\dot{M}O_2$ for all three measurement temperatures (Fig. 3). P_{Crit} values calculated from the decline in $\dot{M}O_2$ at low ambient PO_2 s were 2.60 ± 0.76 , 3.17 ± 0.79 and 4.68 ± 0.91 kPa at 23°, 28° and 33 °C, respectively. These comparably low P_{Crit} values compared to other cichlids and most other fishes indicates that the Mayan cichlid is a strong oxyregulator.

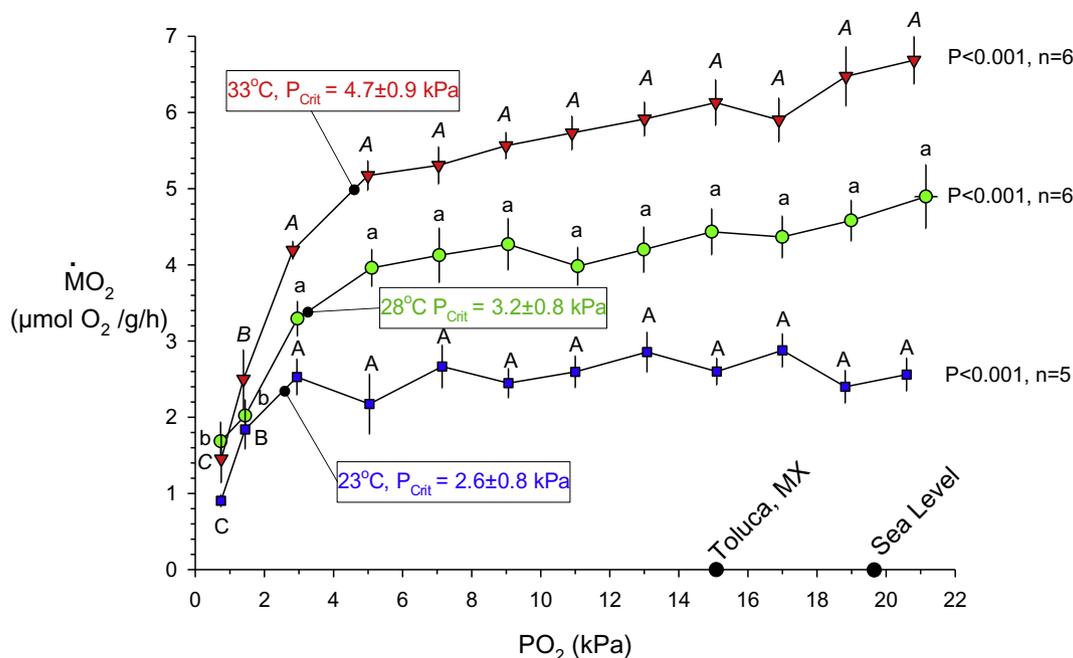


Fig. 2. Relationship between oxygen consumption ($\dot{M}O_2$) and ambient PO_2 in the Mayan cichlid (*Mayaheros urophthalmus*) measured after 12–14 h acclimation at 28°, then 33° and finally 23°C. PO_2 s at important oxygen levels are indicated on the x-axis. Lower case, upper case and upper case italic letters indicate significance of differences between individual means. ANOVAS and n values are indicated to right of each data plot. Values presented are means \pm se.

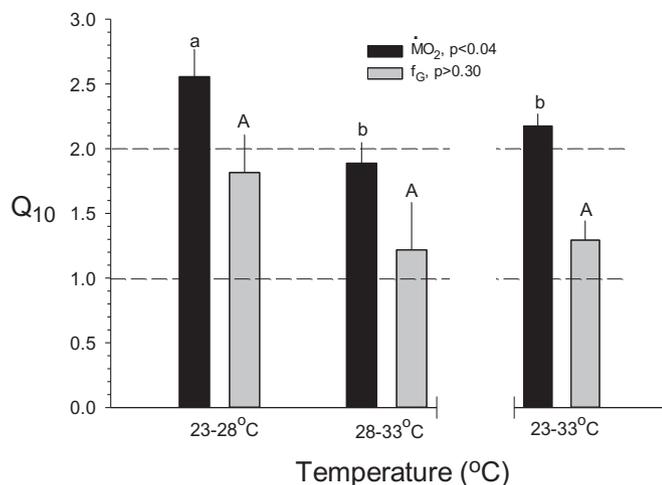


Fig. 3. Temperature sensitivities, expressed as Q_{10} , for oxygen consumption ($\dot{M}O_2$, black bars) and gill ventilation rate (f_G , gray bars) in the Mayan cichlid (*Mayaheros urophthalmus*). Q_{10} s for each variable measured at air-saturation levels were calculated over the 5°C intervals of 23–28° and 28–33°C (two sets of bars on left of figure) and over the 10°C interval 23–33°C (single set of bars after the X-axis break on the right). Horizontal dashed lines indicate Q_{10} values of 1.0 and 2.0. Lower case letters, applying to $\dot{M}O_2$, and upper case letters, applying to f_G , indicate significant differences between Q_{10} means for each variable, assessed by One-way Repeated Measures ANOVA. Values presented are means \pm se. $n = 5$ for temperature intervals 23–28°C and 23–33°C and $n = 6$ for interval 28–33°C.

Further indication of a high tolerance to hypoxia is that all fish recovered from brief exposure to ambient PO_2 s 1–1.5 kPa below their P_{Crit} .

3.2. Gill ventilation frequency

3.2.1. Acclimation temperature, ambient PO_2 and f_G

As anticipated, gill ventilation increased with increasing

temperature and declining ambient PO_2 (Fig. 4) f_G at (16–17 kPa was 39 ± 3 , 45 ± 4 , and 53 ± 6 breaths/min at 23°, 28° and 33°C, respectively. These values translated into Q_{10} values for f_G of 2.56 ± 0.21 for the temperature range of 23–28°C and 1.23 ± 0.35 for the temperature range of 28–33°C (Fig. 3). Q_{10} for f_G over the total temperature range of 23–33°C was 1.30 ± 0.12 .

3.2.2. Hyperoxia and the “hypoxic drive” for Gill ventilation

Gill ventilation was also measured at PO_2 s acutely above normoxia. PO_2 over the range of ~15–35 kPa significantly ($P < .001$) altered f_G at all three temperatures (Fig. 4B). At 33°C, for example, f_G decreased from ~53 breaths/min down to only 29 breaths/min at 35 kPa and just 23 breaths/min at 35 kPa. Similar patterns were evident at the other two measurement temperatures (Fig. 4B), collectively indicating a considerable hypoxic ventilatory drive in the Mayan cichlid.

4. Discussion

4.1. Critique of the methodology

An assumed lack of stirring associated with closed respirometry has been suggested as a confounding factor affecting $\dot{M}O_2$ and P_{Crit} measurements in fishes (Rodgers et al., 2016; Rogers et al., 2016; Steffensen, 2007). Small, relatively lethargic fishes may not provide sufficient stirring when placed in large respirometers. As noted in the Methods section above, while no experimental stirring was provided by the experimental apparatus in the current experiments, relatively thorough stirring within the apparatus provided by the movements of the fish was documented (see Methods Section 2.2). Moreover, values of $\dot{M}O_2$ for the Mayan cichlid were very comparable with similar fishes at similar temperatures measured using a variety of techniques (Table 1).

Regarding P_{Crit} determination, there has been a long-standing debate about the merits of closed vs. open respirometry, with three recent studies in particular thoroughly summarizing the arguments – and coming to quite different conclusions (Regan et al., 2017; Rogers et al., 2016; Snyder et al., 2016).

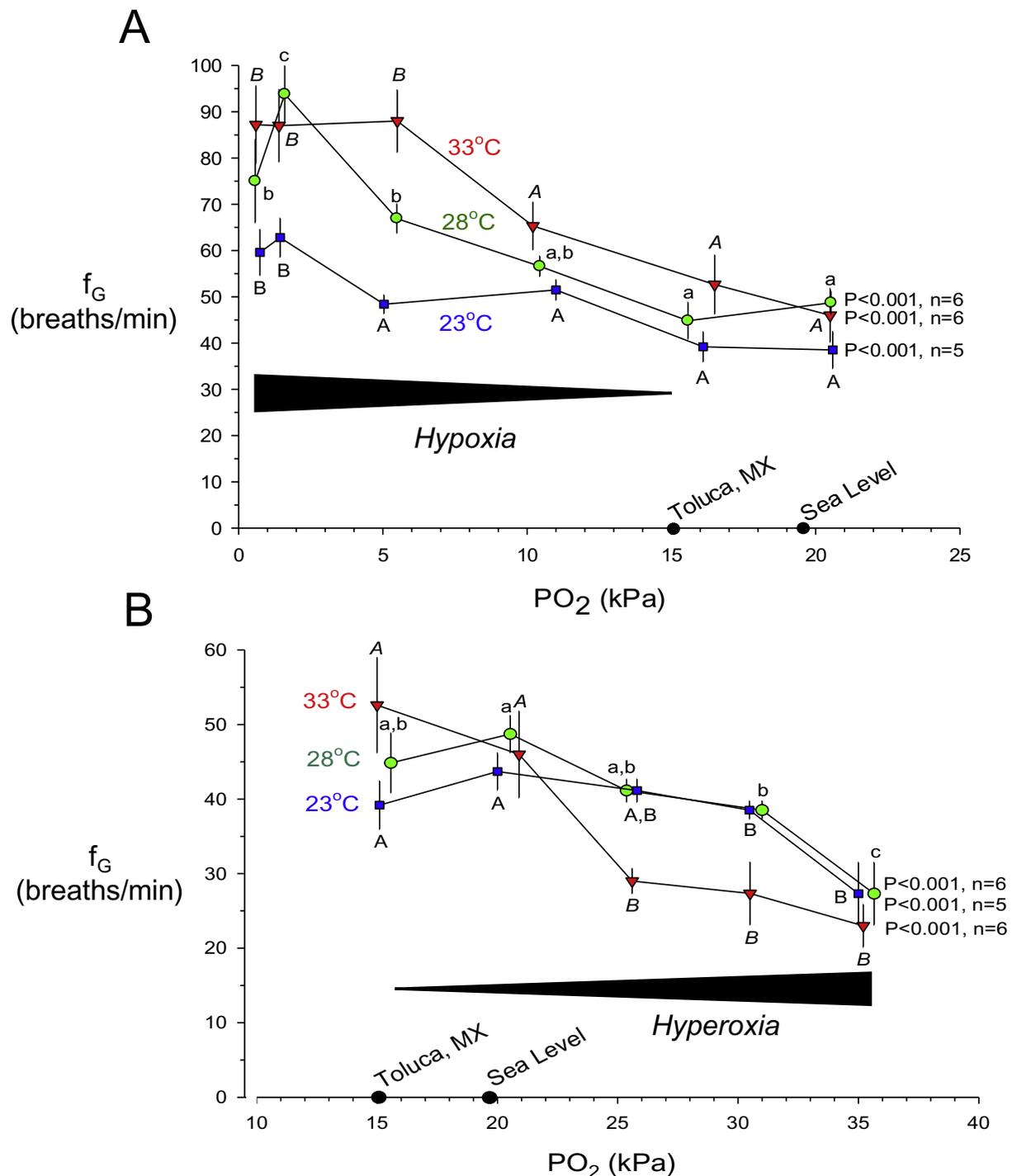


Fig. 4. Relationship between ambient PO₂ and gill ventilation rate (f_G) in the Mayan cichlid (*Mayaheros urophthalmus*) measured after 12–14 h acclimation at 23°, 28° and 33 °C. Panel A shows the effects of PO₂ declining below ambient levels (hypoxia), while Panel B shows the effects of PO₂s above ambient (hyperoxia). PO₂s at important oxygen levels are indicated on the x-axis. Upper case and lower case italic letters indicate significance of differences between individual means for 28°, 23° and 33 °C data, respectively. ANOVAS and n values are indicated to right of each data plot. Values presented are means \pm se.

Contributing to the debate, a careful set of experiments has compared closed and open respirometry for measuring MO_2 and P_{crit} in the goldfish *Carassius auratus auratus* at 17 °C (Regan and Richards, 2017). Importantly, this study additionally used calorimetry, suggested to be the “gold standard” for metabolic measurements, to calibrate and standardize their findings. Their results for the goldfish indicated no significant differences between respirometry techniques in either MO_2 or P_{crit} measured over a wide range of PO₂s. Moreover, there was no buildup of metabolic end-products during 4 h of deep hypoxia. Build-up

of metabolic products – especially CO₂ – has been suggested as a possible influence on P_{crit} , but assessment of CO₂ effects have often used ambient PCO₂s much above anything that would be expected to normally occur during a few hours in a respirometer.

Adding another voice to the debate, the whole concept of P_{crit} has recently been savaged (Wood, 2018). He also suggests that the method of measurement can influence the value for P_{crit} , but goes beyond to suggest “there is no good justification for the concept” and that “it is not a reliable index of hypoxia tolerance”.

Table 1

Examples of routine oxygen consumption measured in air-saturated water of cichlid fishes, including those similarly sized to the Mayan cichlids used in the present study.

Species	Body Mass (g)	Temperature (°C)	Routine $\dot{M}O_2$ ($\mu\text{mol O}_2/\text{g/h}$)	Reference
Mayan cichlid (<i>Mayaheros urophthalmus</i>)	55.4	23° 28° 33°	2.5 4.5 6.0	Present Study
Convict cichlid (<i>Cichlosoma nigrofasciatum</i>)	6.0	25°	16	
Tabasco strain of Nile Tilapia (<i>Oreochromis niloticus</i>)	8	25° 30° 35°	5.0 10.1 21.9	
Nile Tilapia (<i>Oreochromis niloticus</i>)	220–260	25°	2.4	(Zeraik et al., 2013)
Lake Magadi tilapia (<i>Alcolapia grahami</i>)	3	25° 32° 39°	7 13 29	(Wood et al., 2016)
Oscar (<i>Astronotus ocellatus</i>)	98	28°	4.1	(De Boeck et al., 2013)
Oscar (<i>Astronotus ocellatus</i>)	156–255	28°	4.3	(Lewis et al., 2007)
Oscar (<i>Astronotus ocellatus</i>)	115–470	28°	2.6	(Scott et al., 2008)
Mouthbreeding Cichlid (<i>Pseudocrenilabrus multicolor</i>)	0.8–4.8	25°	7.8	(Reardon and Chapman, 2010)
Dwarf Victorian Mouthbreeding Cichlid (<i>Pseudocrenilabrus multicolor victoriae</i>)	4.4–8.7	24° 28° 32°	3.4 3.1 6.9	(McDonnell and Chapman, 2016)

Against this backdrop, the purpose of the current study is not to refute or support the various positions on respirometry and P_{Crit} outlined above. Rather, the more focused intent of our study is to correct the record for metabolic physiology of the Mayan cichlid using conventional approaches, as we will now elaborate upon.

4.2. Routine oxygen consumption of the Mayan cichlid

The routine $\dot{M}O_2$ of the Mayan cichlid measured in the present study is well within the range of those values determined for other warm-water cichlid fishes (Table 1). Presumably mass-specific $\dot{M}O_2$ of the Mayan cichlid will decrease with increasing body mass, as it does in other cichlids and other aquatic and marine fish generally, although this variable was not measured in the present study.

Not surprisingly, routine $\dot{M}O_2$ of the Mayan cichlid increased with acute exposure to increasing temperature at air saturation, from $\sim 2.5 \mu\text{mol O}_2/\text{g/h}$ at 23 °C to $6.0 \mu\text{mol O}_2/\text{g/h}$ at 33 °C. Q_{10} for routine $\dot{M}O_2$ calculated over this temperature range was ~ 2.2 , again well within the range of metabolic temperature sensitivity for tropical freshwater fishes.

4.3. The Mayan cichlid as oxyregulator

The higher the rate of tissue oxygen consumption, the more compelling is the need to maintain oxygen consumption despite either internal increase in metabolic demand, or external limitations to oxygen availability. As a consequence, almost every vertebrate that has been examined is a so-called “oxygen regulator” that will evoke physiological adjustments in both perfusion and ventilation of its gas exchange organ (s) (Rogers et al., 2016; Steffensen, 2007). Certainly, the P_{Crit} varies greatly in fishes, even within a specific grouping such as freshwater fish

between 20° and 36 °C (Rogers et al., 2016). Nonetheless, P_{Crit} s are typically well below air-saturation water PO_2 s. Indeed, only a few fish species have ever been claimed to be oxygen conformers, whereby their rate of oxygen consumption begins to fall when environmental oxygen begins to fall. Examples are the toadfish *Opsanus tao* (Hall, 1929); the white sturgeon *Acipenser transmontanus* (Burggren and Randall, 1978), the plaice *Pleuronectes platessa* (Steffensen et al., 1982) and the swamp eel *Synbranchus marmoratus* (Graham and Baird, 1984). As a result of these older experiments being repeated with new and or multiple simultaneous oxygen consumption measurement techniques, the number of fish species truly viewed as oxyconformer is rapidly shrinking (Rogers et al., 2016; Steffensen, 2007; Ultsch et al., 1981).

The present study continues the trend of reclassifying oxyconforming fish as oxyregulators by clearly refuting the earlier study by Martínez-Palacios and Ross (1986). We have demonstrated that the Mayan cichlid not only is a true oxyregulator, maintaining $\dot{M}O_2$ as ambient PO_2 falls until quite severe hypoxia is reached, but is a very competent one at that, with P_{Crit} s of 2.60, 3.17 and 4.68 at 23°, 28° and 33 °C, respectively (Fig. 2). In fact, these P_{Crit} values for the Mayan cichlid are among the lowest 10–15% of P_{Crit} values for freshwater fishes in the range of 26–33 °C (Rogers et al., 2016). Low P_{Crit} s are typically associated with hypoxia tolerance (although the universality of this association has recently been called into question - (Wood, 2018)). The observation of very low P_{Crit} s fits well with the general observation that the Mayan cichlid can inhabit hypoxic waters and tolerate hypoxic experimental conditions.

Temperature increases the P_{Crit} of almost all fishes that have been examined – see (Rogers et al., 2016). Temperature also affected the P_{Crit} of the Mayan cichlid (Fig. 2), although the differences were not large.

4.4. Gill ventilatory response to temperature, hypoxia and Hyperoxia

As anticipated, f_G of Mayan cichlids in air-saturated water increased with acute exposure to increasing temperature (Fig. 4). However, as evident from a Q_{10} of only ~ 1.3 over the range of 23°–33 °C, gill ventilation is not particularly temperature sensitive.

Gill ventilation increased markedly with advancing hypoxia at all three measurement temperatures (Fig. 4) reflecting attempts to increase the convective supply of oxygen in the water to the branchial lamellae. However, f_G plateaued or actually decreased at all three temperatures when fish reached severe hypoxia at or below P_{Crit} levels. Interestingly, it appeared that even high temperatures and severe advancing hypoxia could not drive gill ventilation rates above 80–90 breaths/min at either 28° or 33 °C, suggesting a possible upper limit to gill ventilation rates under these conditions.

Many vertebrates have a so-called ‘hypoxic drive’ for ventilation demonstrated by the depression of ventilation when ambient PO_2 s are experimentally elevated well above normoxic levels (Florindo et al., 2018; Haouzi et al., 2014; Porteus et al., 2015; West et al., 1989). Gill ventilation in the Mayan cichlid appears to also have a strong hypoxic drive, with significant, considerable depression of f_G occurring when ambient PO_2 s are increased well above air-saturation levels (Fig. 4B). The hypoxic drive is generally thought to result from chemoreceptor influences on central pattern generators stimulating lung or gill ventilation (Florindo et al., 2018; Haouzi et al., 2014). Certainly the existence of both of peripheral and central chemoreceptors has been well established in both aquatic and airbreathing fishes (Florindo et al., 2018; Jonz, 2018; Jonz et al., 2015; Perry et al., 2009; Zaccone et al., 2018). While the metabolic physiology of the cichlids has been well studied, far less is known about chemoreceptor function and influences in cichlids.

4.5. Conclusions

The Mayan cichlid has been shown in this study to be a highly competent oxyregulator, maintaining routine $\dot{M}O_2$ at control rates until

PO₂ has decreased to < 2–3 kPa. This maintenance of $\dot{M}O_2$ is aided in part by a considerable branchial hyperventilation. These data refute an earlier observation that the Mayan cichlid is an oxyconformer, and support the ecological data that indicates that this species can inhabit very warm, hypoxic aquatic habitats.

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Declaration of interest

None.

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