Energetics of Hypoxia in a Mouth-Brooding Cichlid: Evidence for 
Interdemic and Developmental Effects

E. E. Reardon1,*
L. J. Chapman1,2
1Department of Biology, McGill University, 1205 Dr. Penfield Avenue, Montreal, Quebec H3A 1B1, Canada; 2Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, New York 10460

ABSTRACT

We used a common-garden rearing experiment to explore environmentally induced tolerance to hypoxia in the African mouth-brooding cichlid Pseudocrenilabrus multicolor. F1 fish originating from three field populations were grown under low or high dissolved oxygen (DO), and their resting routine metabolic rate (RMR), critical oxygen tension (Pcrit), and marginal metabolic scope (MMS) were quantified. In a second rearing experiment, we compared the RMR of brooding and nonbrooding females of low-DO origin grown under low and high DO. Fish reared under low DO had a lower Pcrit than fish reared under high DO. There was also an interaction between treatment and gender; females had a higher Pcrit than males when reared under normoxia. Variation in RMR was driven primarily by population effects, and there was an interaction between treatment and population. Regardless of population or treatment, males had a higher MMS than females. Fish reared under low DO had a higher MMS than fish reared under high DO, except for the high-DO population in which there was no treatment effect. Brooding females had a higher RMR than postbrooding females regardless of the growth treatment, indicating an energetic cost to brooding. The results suggest a strong element of developmental plasticity in Pcrit across populations and both plastic and genetic components of variation in the RMR and MMS. This study also highlights the cost of parental care in mouth-brooding fishes, which may increase the fitness of the offspring at the energetic expense of the parent, a cost that may be elevated under hypoxia.

Introduction

The increasing frequency and extent of aquatic hypoxia (low dissolved oxygen [DO]) associated with eutrophication of coastal and inland waters is now considered to be among the most pressing and critical water pollution problems. Serious effects include the production of extensive “dead zones,” such as those in the Gulf of Mexico and Lake Erie, that can affect important fisheries (Diaz 2001; Dybas 2005; Pollock et al. 2007). Hypoxia exposure has been shown to trigger both lethal and sublethal effects in fishes, including, as examples, reduced feeding, reduced reproductive capacity, reduced growth, reduced metabolic scope, and slower prey response time (reviewed in Wu 2002; Pollock et al. 2007). These effects clearly vary across species (reviewed in Wu 2002; Pollock et al. 2007; Chapman and McKenzie 2009) but also depend on the frequency, intensity, and duration of the hypoxic events (Rutjes 2006). Many experimental studies have documented effects of short-term exposure to hypoxia (hours to weeks). However, understanding long-term consequences of sublethal exposure has become increasingly important in predicting population and community-level effects.

Fishes can compensate for hypoxic conditions by decreasing their need for oxygen, increasing the amount of oxygen available, or combining both strategies. Well-documented responses to short-term hypoxia include such behavioral and physiological mechanisms as avoidance, increased gill ventilation activity, increased aquatic surface respiration (or surface skimming), reduced motor activity, reduced metabolic scope, increased hemoglobin/hematocrit concentrations, and anaerobic metabolism (summarized in Rutjes 2006; Pollock et al. 2007; Chapman and McKenzie 2009). Rees et al.’s (2001) zebrafish (Danio rerio) study on the effects of acclimation to lethal hypoxia provided evidence that severity of the effect is reduced if there is prior short-term acclimation to low-DO conditions. Development under hypoxia may provide additional advantages for compensating for hypoxia as adults (Rutjes 2006; Widmer et al. 2006). Long-term or lifelong responses to hypoxia are not well documented but include developmentally induced shifts in gill and body morphology (Chapman et al. 2000, 2008) and physiology (e.g., shifts in relative proportions of isohemoglobins, increased glycogen stores; Rutjes et al. 2007).

In field populations, responses to long-term hypoxia may reflect both environmentally induced and heritable effects. In their study of the widespread African cichlid (Pseudocrenilabrus multicolor), Chapman et al. (2000, 2008) reported strong patterns of variation in gill size of fish across populations from low- and high-DO sites. In a common-garden rearing experiment, in which fish from three field populations were raised
under low- and high-oxygen conditions, Chapman et al. (2008) found a strong element of developmental plasticity in a suite of morphophysiological traits, including gill size, body shape, and brain size, as well as evidence for genetic effects. Similarly, in their studies of the zebrafish (D. rerio) and several species of cichlids, Widmer et al. (2006) and Rutjes et al. (2007), respectively, detected developmental plasticity in a variety of different traits, including gill size, head shape, hemoglobin and hematocrit levels, swim velocities, lactate production, and glycogen stores. Rutjes et al. (2007) also reported differences in the way three closely related cichlid species adjusted physiologically to development under hypoxia, through shifts in relative proportions of isohemoglobins. These earlier studies suggest that developmental plasticity may be an important mechanism for dealing with long-term hypoxia.

Long-term response to hypoxia exposure (one or more generations) should, theoretically, increase a fish’s overall tolerance to hypoxia and minimize the degree of metabolic depression and decreased spontaneous activity observed in many studies of short-term exposure. In their review of the behavioral consequences of hypoxia, Chapman and McKenzie (2009) found that a drop in spontaneous activity levels in fishes was a response common to several species exposed to short-term hypoxia, and in some of these species, the decrease in activity was associated with either metabolic depression or a decrease in critical oxygen tension (P_{crit}). The reduction in spontaneous activity levels is assumed to be an effort to reduce energy expenditure during hypoxic episodes. One previous study has explored variation in routine metabolic rate (RMR) and activity level among three species of cichlids reared under high- and low-DO conditions and measured under both DO conditions (Rutjes 2006). In low-DO-reared fishes, no difference was found in metabolic rate or activity level measured under high and low DO. However, high-DO fishes exhibited reduced metabolic rate when measured under low DO, relative to those measured under high DO with no difference in activity level (Rutjes 2006). Rutjes’s (2006) findings support the idea that development under hypoxia may mitigate the need for metabolic depression.

In this study, we quantified the P_{crit}, resting RMR, and marginal metabolic scope (MMS) of the maternal mouth-brooding cichlid (P. multicolor) reared under low- and high-oxygen conditions, to explore the role of developmental plasticity in determining overall tolerance to hypoxia. We used fish from three populations of origin in Uganda (high oxygen, low oxygen, and fluctuating oxygen) to allow us to detect both population and treatment effects. The fishes’ P_{crit} was measured as our integrative index of hypoxia tolerance (Verheyen et al. 1994; Chapman et al. 2002a). Fishes will regulate their metabolic rate over a range of DO concentrations. The P_{crit} is the point at which a further reduction of DO causes a shift in metabolic rate from DO independent to DO dependent (Beamish 1964; Ultsch et al. 1978), and a low P_{crit} has been linked to high physiological tolerance to hypoxia (summarized in Chapman and McKenzie 2009). We quantified resting RMR to detect whether the morphophysiological response was adequate to mitigate metabolic depression. In addition, from our P_{crit} and resting-RMR data, we calculated MMS. While MMS is not a direct measure of metabolic scope, recent studies have shown a strong positive correlation between the two metrics; thus, MMS can provide an index of capacity for metabolic performance (Neill and Bryan 1991; Del Toro-Silva et al. 2008). In a second common-garden experiment, we focused specifically on the metabolic costs of brooding under hypoxia, using adult stock from one low-DO population of P. multicolor by quantifying the resting RMR of brooding and nonbrooding females grown under low and high DO. On the basis of previous studies, we predicted that P. multicolor would show a lower P_{crit} and reduced metabolic scope when grown under low DO, although the magnitude of this effect may differ among the populations and genders. We also predicted that long-term exposure to hypoxia (rearing) may minimize metabolic depression through developmental response. However, brooding females may have a higher resting RMR than nonbrooding females because of the increased cost of carrying young.

Methods

Species and Study Site

Pseudocrenilabrus multicolor victoriae is a small, mouth-brooding cichlid found throughout the Lake Victoria basin across a range of DO regimes and habitats (Chapman et al. 2000, 2002a, 2002b, 2008; Martı́nez et al. 2009; Reardon and Chapman 2009). We used a lab-rearing study to detect plasticity in resting RMR and P_{crit} in response to alternative oxygen environments and to ask whether plasticity varies among populations. The parental stock originated from three field populations that experience either low-, high-, or fluctuating-DO regimes (see Reardon and Chapman 2009 for a map of the system and a full description of environmental characters at each site). The Lwamunda Swamp is a dense, hypoxic wetland that surrounds Lake Nabugabo. With a mean DO of 1.85 mg L$^{-1}$ (17.52 mmHg), the DO at this site is well below saturation throughout the year (monthly range: 1.09–2.56 mg L$^{-1}$; Reardon and Chapman 2009). Lake Kayanja is a satellite lake of Lake Nabugabo, where P. multicolor is abundant in the grassy margins of the lake; DO averages 6.97 mg L$^{-1}$ (117.37 mmHg) over the year (monthly range: 6.46–7.40 mg L$^{-1}$; Reardon and Chapman 2009). The third site was located in the Mpanga River in western Uganda, where DO averages 3.53 mg L$^{-1}$ (72.81 mmHg) but fluctuates both seasonally because of seasonal rains and flushing of a large upstream wetland (monthly range: 2.2–5.7 mg L$^{-1}$; Reardon and Chapman 2009) and supra-annually because of flooding of adjacent woodland that can reduce DO levels below 2.0 mg L$^{-1}$. The Mpanga River site is located >150 km west of the Nabugabo system and joins the westward-flowing section of the Katonga River feeding into Lake George. Although the Mpanga and Nabugabo systems are in separate drainage basins, there is evidence that P. multicolor from both regions originated from one source population (Crispo and Chapman 2008). In addition, the swampy valley of the Katonga River may maintain a contemporary link between the two systems.
Laboratory Experiment I: Energetics of Hypoxia; Developmental and Interdemic Effects

Adult fish from the three Ugandan field populations were live-captured in metal (Standard Gee) minnow traps and live-transferred to our aquarium facility. Brooding pairs of parental stock were held in separate, normoxic aquaria until the young were completely released from the female’s mouth. When a brood was released by a female, it was divided into two groups and randomly allocated to one of 36 76-L aquaria (18 per DO treatment). Broods from six families per population (Lwamunda Swamp [low DO], Lake Kayanja [high DO], and Mpanga Kahunge [fluctuating DO]) were used in the experiment to provide family-level replication; the number of families was limited because of the time required to measure \( P_{com} \) and RMR on individual fish. We reduced broods randomly to eight young per aquarium after 3 mo because this density allows fish to grow to a size that reflects some field populations.

The DO in the experimental growth tanks was maintained by continuously pulsing nitrogen through a diffuser in each aquarium to reduce DO levels. DO measurements were recorded twice daily with a YSI analog DO meter, and nitrogen was added if necessary. DO averaged 7.6 \( \pm \) 0.023 mg L\(^{-1}\) SE (141.96 mmHg) in the normoxic tanks and 1.3 \( \pm \) 0.025 mg L\(^{-1}\) (24.69 mmHg) in the hypoxic aquaria. Underwater filtration was achieved with a Mag drive pump (with sponge biofiltration) modified by 3/4-in tubing to ensure adequate circulation. Additional water-quality characters (e.g., pH, ammonia, nitrate, conductivity) were measured biweekly. Aquaria were held at 25°C (means: normoxia, 24.9\( ^\circ \) \( \pm \) 0.029\( ^\circ \)C [SE]; hypoxia, 25.1\( ^\circ \) \( \pm \) 0.05\( ^\circ \)C) and exposed to a 12L : 12D photoperiod. We fed the fish once per day on HBH fry bites until they were 3 mo old and thereafter on Tetramin food flakes. To ensure that maturity was reached, we let the F\(_1\) fish grow for at least 10 mo before beginning respiratory measurements. To quantify RMR and \( P_{com} \), we used one of two identically constructed intermittent, flow-through respirometers, whereby \( \text{V}_{O_2} \) was estimated by oxygen decline in a closed container in response to fish respiration (following Julian et al. 2003). Throughout each experiment, a low constant water current velocity of 0.833 mL s\(^{-1}\) was maintained. Water volume of the respirometers ranged from 114.9 to 162.4 mL, depending on the size of the respiratory chamber, which was chosen on the basis of the total size of the fish (range: total length, 3.7–7.2 cm; mass, 0.81–4.75 g). To ensure a postabsorptive state, each fish was held in an acclimation tank (held at normoxia) next to the respirometer tank for 24 h before entering the experimental chamber. Early in the morning (to mimic an extended metabolism to provide family-level replication; the number of families

Laboratory Experiment II: Energetics of Brooding after Development under Divergent Oxygen Regimes

Additional adult live specimens were wild-caught from the Lwamunda Swamp population of \( P. \) multicolor in Uganda and live-transferred to our aquatic facility. Four families of the Lwamunda Swamp population were bred; \( F_1 \) broods were split, and full siblings were raised under high and low DO as part of an experiment exploring developmental variation in life-history traits (Reardon and Chapman 2009). \( F_1 \) females were observed daily for evidence of brooding (not feeding, dropped jaw, secretive). When a female was observed to be brooding, she was carefully removed from the tank and held overnight in an acclimation chamber next to the respirometer. We recorded the RMR of the brooding females in the first 48 h of brooding (prehatching egg phase; Mrowka and Schierwater 1988), following the methodology described below. After each metabolic rate recording, the eggs were removed from the female’s mouth for size measurement (Reardon and Chapman 2009). One week after the brood’s removal, the postbrooding metabolic rate of each female was recorded.

To allow a more direct comparison of total oxygen consumption (\( \text{V}_{O_2} \)) between brooding and postbrooding females, we subtracted the estimated \( \text{V}_{O_2\text{rest}} \) from the total metabolic rate of each brooding female. Mean individual egg \( \text{V}_{O_2} \) (0.512 \( \pm \) 5.18 \times 10\(^{-3} \) [SE] \mu g \text{O}_2 \text{h}^{-1}) was multiplied by the total number of eggs in each brood to estimate \( \text{V}_{O_2\text{egg}} \). We used the metabolic rate of individual eggs taken from \( P. \) multicolor females of swamp origin reared under low and high DO measured in a complementary study (Reardon 2009).

Over the course of the experiment, 16 females reproduced, and their metabolic rates were recorded during and after brooding. There was no effect of family or the interactions with family (\( P > 0.415 \)), so it was removed from the model. In some cases, we were not able to record both stages on the same female. Thus, ANOVA was used to test for an effect of treatment (high- vs. low-DO rearing environment), brooding stage (brooding with \( \text{V}_{O_2\text{rest}} \) brooding minus \( \text{V}_{O_2\text{egg}} \); Postbrooding), and their interaction on mass-adjusted RMR, with female included as a random factor in the model.

Respiratory Measurements

To quantify RMR and \( P_{com} \), we used one of two identically constructed intermittent, flow-through respirometers, whereby \( \text{V}_{O_2} \) was estimated by oxygen decline in a closed container in response to fish respiration (following Julian et al. 2003). Throughout each experiment, a low constant water current velocity of 0.833 mL s\(^{-1}\) was maintained. Water volume of the respirometers ranged from 114.9 to 162.4 mL, depending on the size of the respiratory chamber, which was chosen on the basis of the total size of the fish (range: total length, 3.7–7.2 cm; mass, 0.81–4.75 g). To ensure a postabsorptive state, each fish was held in an acclimation tank (held at normoxia) next to the respirometer tank for 24 h before entering the experimental chamber. Early in the morning (to mimic an extended metabolism to provide family-level replication; the number of families

<table>
<thead>
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<th>Family</th>
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<td>High</td>
<td>No effect</td>
<td>( P &gt; 0.415 )</td>
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night), a fish was gently placed in the metabolic chamber and acclimated to the reservoir water for 2 h before the experiment began. At the end of the acclimation period, the system was closed to the outside environment (the aerated reservoir tank that the metabolic chamber was submerged in). The fish were left in the chamber for an additional 1 h before metabolic rates were calculated. Decreases in oxygen concentration (% air saturation) were used to estimate the rate of \( \dot{V}_{O_2} \) of the fish. Oxygen and temperature data were recorded every 30 s during the trial with Ocean Optics FOXY probes and thermistors, respectively. The oxygen recordings were measured at a mean temperature of \( 25.1^\circ \pm 0.04^\circ \text{C} \) (SE) to mimic rearing-environment temperatures. The experimental chamber remained closed for an average of 6.14 ± 0.13 h (SE) and reached an averaged minimum DO of 5.69 ± 0.24 mmHg (SE). Generally, the fish became quiescent upon entry into the experimental chamber and remained relatively inactive throughout the experiment. In a separate study, we recorded metabolic rate for 24 h, starting when the fish entered the experimental chamber. The average time for the RMR to stabilize was 1.5 h (Reardon and Chapman 2010). We recognize that closed respirometry systems can affect metabolic rate, as fish may have to cope with accumulations of CO\(_2\), metabolic products, and reductions in pH. Although we did not measure CO\(_2\) in the experimental chamber, the chamber was completely flushed after the acclimation period before measurements to minimize potential effects. In addition, we estimated the pH decline for a 6-h run with an average fish-to-water volume ratio (\( n = 6 \) fish) and found only a modest decline of 0.4 units.

We used our modification of the program created by Yeager and Ultsch (1989) to convert DO data from percentage saturation to \( P_{O_2} \), (mmHg) and \( \dot{V}_{O_2} \), (mg O\(_2\) h\(^{-1}\)). The program also calculated \( \dot{P}_{O_2} \), using both the midpoint and critical-point methods described in Yeager and Ultsch (1989). Results between the midpoint and critical-point methods were similar, thus we report only the midpoint critical-tension data in this article (Fig. 1). Mass-adjusted RMR was calculated, following Ultsch et al. (1978) and Ultsch (1995), as

\[
\dot{V}_{O_2,\text{adjusted}} = (\text{mean mass of all fish used})^{b-1} \times (\text{observed mass})^{b-1} \times \text{observed } \dot{V}_{O_2},
\]

with the exponent for freshwater fish \( (b = 0.81) \) reported by Winberg (1961); all \( \dot{V}_{O_2} \) values are expressed as mg O\(_2\) g\(^{-1}\) h\(^{-1}\).

There was no effect of respiratory system on either RMR \( (P = 0.792) \) or \( \dot{P}_{O_2} \) \( (P = 0.276) \), so data from both respiratory systems were combined for analysis. MMS is the ratio of resting RMR to the limiting oxygen concentration (LOC; defined as the \( \dot{P}_{O_2} \) expressed in mg O\(_2\) L\(^{-1}\)) multiplied by 1 mg O\(_2\) L\(^{-1}\) (Neill and Bryan 1991). It was calculated using an equation from Neill and Bryan (1991): MMS = \( (\dot{V}_{O_2,\text{adjusted}} / \text{LOC}) \times (1 \text{ mg L}^{-1}) \).

Regardless of population or gender, fish grown under low DO had a lower \( P_{O_2} \) than fish grown under high DO (Table 1; Fig. 2). There was no overall gender effect (Table 1); however, there was an interaction between treatment and gender \( (P = 0.023; \text{Table 1}) \). To explore the significant interaction term, effects of gender on \( P_{O_2} \) were tested within each growth treatment. When reared under hypoxia, male and females did not differ in \( P_{O_2} \) \( (F = 0.787, P = 0.377) \); however, when reared under normoxia, male Pseudocrenilabrus multiclor males exhibited a significantly lower \( P_{O_2} \) than did females \( (F = 7.734, P = 0.007; \text{Fig. 2}). \)

For all fish (combined across treatments and populations), there was a positive relationship between mass and unadjusted RMR (linear regression: \( r = 0.806, P < 0.001; \text{Fig. 3}) \). There was a strong effect of population on mass-adjusted RMR but also a population \( \times \) treatment interaction (Table 1; Fig. 4). When grown under hypoxia, the fluctuating-DO population (Mpanga River) had a higher metabolic rate than either the high- or the low-DO population \( (F = 13.351, P < 0.001; \text{Sidak post hoc tests}) \). There was no population effect on mass-adjusted RMR when fish were grown under normoxia \( (F = 1.438, P = 0.245; \text{Fig. 4}) \). The effect of gender was weak \( (P = 0.082; \text{Table 1}) \) but suggests an overall higher RMR in females than in males (log mean RMR: females, \(-1.094 \pm 0.009\); males, \(-1.126 \pm 0.011 \text{ [SE]}) \). There was no relationship between RMR and \( P_{O_2} \) (linear regression: \( r = 0.003, P = 0.971 \)).

Figure 1. Example of changes in the rate of oxygen consumption of one high- and one low-DO (dissolved oxygen)-reared Pseudocrenilabrus multiclor in response to oxygen depletion of the respirometer.
Regardless of treatment or population, males had a higher MMS than females. Regardless of gender, fish reared under low DO had a higher metabolic scope than those reared under high DO (Table 1; Fig. 5). Although there was no overall population effect, there was a treatment × population interaction term (Table 1) driven by the high-DO population where there was no difference in MMS between treatments (P = 0.901; Fig. 5). For the fish originating from low- and fluctuating-DO populations, fish grown under low DO had a higher metabolic scope than those reared under high DO (low-DO origin: P = 0.008; fluctuating-DO origin: P < 0.001; Fig. 5).

In our second experiment, fish of swamp origin were reared under high- or low-DO conditions (Rutjes 2006) report a lower Pcrit than high-DO-reared siblings. Previous studies that have quantified interdemic or interspecific variation in Pcrit on fish from divergent oxygen environments (Verheyen et al. 1994; Chapman et al. 2002a; Melynchuk and Chapman 2002; Timmerman and Chapman 2004a) or across species reared in high- or low-DO conditions (Rutjes 2006) report a lower Pcrit, asso-

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In our second experiment, fish of swamp origin were reared under high- or low-DO conditions (Rutjes 2006) report a lower Pcrit than high-DO-reared siblings. Previous studies that have quantified interdemic or interspecific variation in Pcrit on fish from divergent oxygen environments (Verheyen et al. 1994; Chapman et al. 2002a; Melynchuk and Chapman 2002; Timmerman and Chapman 2004a) or across species reared in high- or low-DO conditions (Rutjes 2006) report a lower Pcrit, asso-

Discussion

In fishes, phenotypically plastic responses to hypoxia may be critical for species living in habitats characterized by strong spatial and temporal variation in DO. Selection for environmentally induced response to hypoxia may also increase the potential for colonization of new environments and be favorable in the long term by preadapting populations to respond to environmental change, such as increasing levels of eutrophication and associated hypoxia. In addition, strong selection pressure for hypoxia tolerance in oxygen-scarce habitats may lead to interdemic variation among populations with broad habitat ranges. This interdemic variation may derive from differences in the geographical origins of the populations or the environmental histories of the individuals within the populations. The results of this study suggest a strong element of developmental plasticity in the Pcrit of Pseudocrenilabrus multicolor across populations and both plastic and genetic components of variation in RMR and MMS in P. multicolor that may reflect the selective environment of the parental stock. This study also highlights the cost of parental care in mouth-brooding fishes, which may increase the fitness of the offspring at the energetic expense of the parent.

Pseudocrenilabrus multiclor reared under low DO exhibit a lower Pcrit than high-DO-reared siblings. Previous studies that have quantified interdemic or interspecific variation in Pcrit on fish from divergent oxygen environments (Verheyen et al. 1994; Chapman et al. 2002a; Melynchuk and Chapman 2002; Timmerman and Chapman 2004a) or across species reared in high- or low-DO conditions (Rutjes 2006) report a lower Pcrit, asso-

![Figure 2. Mean critical tension values (± SE) for F1 Pseudocrenilabrus multiclor grown under high and low DO (dissolved oxygen). There was no effect of population of origin in this analysis but significant treatment × population and treatment × gender interactions. Asterisks represent significant differences of P < 0.001 in Pcrit within populations between treatments. Within treatments, different letters represent a significant difference between genders.](image-url)
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Figure 3. Relationship between body mass and unadjusted routine metabolic rate.

Figure 4. Mean mass-adjusted resting routine metabolic rate (RMR; ± SE) for F1, Pseudocrenilabrus multicolor originating from one high-, one low-, and one fluctuating-DO (dissolved oxygen) population grown in a split-brood design under high or low DO. The asterisk represents a significant difference between growth treatments within a population. Within each treatment, different letters represent a significant difference among populations. There was both an effect of population and a significant interaction between population and treatment.
conditions, which may contribute to a difference in metabolic scope. The larger gill size in the hypoxia-reared fish may provide the fish a bigger “engine” for aerobic scope, compared with high-DO-reared fish, particularly since RMR measurement took place under high DO (Pauly 1981; Neill and Bryan 1991).

In fish of lake origin, there was no treatment effect on MMS. Low plasticity in both RMR and $P_{\text{crit}}$ may reflect Lake Kayanja’s stable high-DO environment.

In this study, we were surprised to find gender effects on $P_{\text{crit}}$, RMR, and MMS because this is seldom reported in the literature. In our experiment, there was a female-biased sex ratio that differed between treatments: 71% females in low DO and 54% females in high DO (Chapman et al. 2008). In the fish respirometry literature, we found only five studies (three in the same system; one measured females only) that indicated the gender of the specimens measured for estimates of $P_{\text{crit}}$ or RMR, and only four of the six studies actually tested for gender effects (Mrowka and Shierwater 1988; Timmerman and Chapman 2003, 2004a, 2004b; Östlund-Nilsson and Nilsson 2004; Salazar and Stoddard 2008). Although the gender effect varied across species, the clutch-carrying gender generally had an increased metabolic rate relative to the other gender. In many cases, estimates of RMR, $P_{\text{crit}}$, and MMS are generated from a small sample size without taking gender or potential population differences into account, to create a mean for a species (summary in Brett and Groves 1979; Chapman and McKenzie 2009). Yet in $P$. multicolor, males are aggressive and territorial, while females bear the energetic load of producing and mouth-brooding offspring. Thus, it makes sense that energetic requirements, metabolic capacity, and hypoxia tolerance differ between genders. Evidence for this difference is provided by the reduced aerobic capacity (MMS) in females relative to males, regardless of the rearing DO. In addition, regardless of treatment, brooding females of swamp origin exhibited a higher RMR than did postbrooding females, indicating an energetic cost to mouth brooding. This finding supports those of a similar study that quantified the basal metabolic rate of brooders and postbrooders in wild-caught females from the Lwamunda Swamp that were acclimated to high or low DO and measured under their acclimation DO (Reardon and Chapman 2010). Reardon and Chapman (2010) also found a reduced brooding period in females acclimated to low DO (mean: 14 d) relative to high-DO-acclimated females (mean: 20 d), suggesting that females brooding under hypoxia offset the energetic cost by holding the young in the mouth for a shorter time rather than by increasing oxygen uptake. In their study of the live-bearing sailfin molly, Timmerman and Chapman (2003) reported an elevated metabolic rate for females in late-stage gestation, which supports the existence of an energetic cost of bearing young. In contrast, in their study on two species of paternal mouth-brooding cardinalfish ($Apogon fragilis$ and $Apogon leptacanthus$), Östlund-Nilsson and Nilsson (2004) reported that any elevation in metabolic rate of brooding cardinalfish was due entirely to the metabolic rate of the developing brood. They suggested that the direct energetic costs are insignificant for the parent, supporting an earlier study on $P$. multicolor (Mrowka and Shierwater 1988). However, they also reported that mouth-brooding males spat out their broods under hypoxia and that they exhibited reduced aerobic capacities and reduced ability

### Table 2: ANOVA results testing for an effect of growth treatment (high or low dissolved oxygen), brooding stage (day 1 brooding [with and without the metabolic rate of the brood] and postbrooding), and their interactions on mean mass-adjusted routine metabolic rate (RMR)

<table>
<thead>
<tr>
<th>Trait</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>3.880</td>
<td>.064</td>
</tr>
<tr>
<td>Brooding stage</td>
<td>2</td>
<td>4.590</td>
<td>.022</td>
</tr>
<tr>
<td>Female (treatment)</td>
<td>15</td>
<td>3.232</td>
<td>.007</td>
</tr>
<tr>
<td>Treatment × stage</td>
<td>2</td>
<td>1.282</td>
<td>.298</td>
</tr>
</tbody>
</table>

Note. Female included as a random factor.
measurements were made over a range of DO above the critical speed. We should point out that the low-DO treatment was above the \( P_{\text{crit}} \) reported for this species either reared under low DO (this study) or in field populations (Chapman et al. 2002a).

While many other haplochromine cichlids have become extinct (Kaufman et al. 1997; Balirwa et al. 2003), \emph{P. multicolor}'s flexibility in tolerance to hypoxia may account for its eurytopic distribution in the Nile basin and for its persistence in the face of massive ecological changes in the Lake Victoria basin. For \emph{P. multicolor}, the ability to exploit hypoxic refugia (dense wetlands) has clearly contributed to its survival (Chapman et al. 1996a, 1996b, 2002a; Rosenberger and Chapman 2000), and its level of phenotypic plasticity in morphological (Chapman et al. 2008), biochemical (Martínez et al. 2009), and physiological traits may facilitate recolonization of habitats should conditions change or revert to an earlier state.

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Literature Cited


Chapman L.J., J. Albert, and F. Galis. 2008. Developmental to increase ventilatory frequencies under hypoxia relative to nonbrooding males and females. Although responses varied between species, their results clearly indicate some cost to mouth-brooding offspring (Östlund-Nilsson and Nilsson 2004). Given the number of studies that have explored the RMR and \( P_{\text{crit}} \) of bearing fishes and the contrasting patterns among the few species studied, it is clear that gender effects should be taken into account in fish respirometry studies.

Although the results from both of the experiments in this study seem physiologically relevant, the ecological relevance must be interpreted in light of the experimental design. RMR measurements were made over a range of DO above the \( P_{\text{crit}} \). Thus, fish reared under low or high DO were measured in a novel DO situation in which oxygen was progressively depleted from the respirometer through consumption by the fish. It would, perhaps, be more ecologically relevant if the metabolic rates for fish reared under both low and high DO were measured under stable low DO and stable high DO, respectively. This would indicate the metabolic performance of the fish in the environment in which it was raised. This was done for wild-caught brooding and nonbrooding female \emph{P. multicolor} acclimated to high and low DO with similar results as the brooding experiment reported in this study (Reardon and Chapman 2010). An important step in understanding the maintenance of divergent phenotypes of \emph{P. multicolor} in the field is also to see whether the phenotype that performs optimally in its own environment performs suboptimally in the alternative environment (reviewed in Dewitt and Scheiner 2004). Thus, it would be interesting to measure RMR under both high- and low-DO conditions. Fish reared under high DO may suppress RMR if measured under low-DO conditions, whereas we might expect, from the findings of Rutjes (2006) and Widmer et al. (2006), no difference in RMR of low-DO-reared fish measured under both DO treatments. In the future, it would also be useful to directly measure the metabolic scope of fish reared under low and high DO to indicate how fish respond when aerobically challenged by swimming at flow rates below their critical swimming speed.

Figure 6. Mean mass-adjusted resting routine metabolic rate (RMR; ±SE) for day 1 brooding (with and without the metabolic rate of the brood) and postbrooding \emph{Pseudocrenilabrus multicolor} females originating from a low-DO (dissolved oxygen) swamp population grown in a split-brood design under high or low DO.
Timmerman C.M. and L.J. Chapman. 2003. The effect of gestational state on oxygen consumption and response to hyp-


