Respiratory oxygen consumption among groups of *Pseudocrenilabrus multicolor victoriae* subjected to different oxygen concentrations during development

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The routine metabolic rate of *Pseudocrenilabrus multicolor victoriae*, laboratory-reared, F1 offspring of parents collected from a hypoxic swamp, did not differ between sibs raised under normoxia and hypoxia. In the offspring of parents originating from a river where the oxygen levels were generally high but fluctuating, the metabolic rate of fish raised under normoxia was higher than for sibs raised under extreme hypoxia. Although further experiments with a larger random sample of the parental stock will be necessary to validate the patterns, the difference between the experiments may indicate the selective environment of the parents.

Key words: metabolic rate; Cichlidae; Africa; wetlands; developmental plasticity.

INTRODUCTION

A general assumption regarding adaptations in fishes to life in oxygen-poor waters is that a low critical oxygen tension (Pc) and a low metabolic rate facilitate survival of individuals and populations of such species under conditions of extreme hypoxia. One of a multitude of African fishes with populations existing in a variety of habitats is *Pseudocrenilabrus multicolor victoriae* (Schoeller). This species is a small haplochromine cichlid found in the Nile River system and the Lake Victoria basin (Greenwood, 1965; Schierwater & Mrowka, 1987). It occupies a wide range of habitats including fast-flowing rivers, intermittent streams, ecotonal regions of lakes, and dense wetlands (Chapman et al., 1996a, b; Rosenberger & Chapman, 1999; Chapman et al., 2000). When it occurs in dense *Miscanthidium violaceum* and papyrus swamps, it is often the dominant species with respect to numbers and biomass (L. J. Chapman & C. A. Chapman, unpubl. data). Like all haplochromine cichlids, *P. multicolor victoriae* is a mouthbrooder; the eggs and developing young are protected in the mouth of the female. It feeds on a large variety of plants and animals including algae, insects and other fishes (Greenwood, 1965). In a previous study swamp-dwelling individuals of this species were found to have a relatively low routine metabolic rate, only 30% of that expected for teleosts of similar body mass using equations derived by Winberg (1955, 1961), and a low critical oxygen tension of 6.6 mm Hg (Rosenberger, 1997; Rosenberger & Chapman, 2000).

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The principal purpose in this study was an evaluation of the possibility of phenotypic plasticity in routine metabolic rates, comparing F1 laboratory-reared individuals whose parents had been collected from waters of either normoxic or hypoxic ambient dissolved oxygen concentrations. Routine metabolic rates were determined on these F1 groups, comparing the rates of individuals from a single pair of parents following development in either hypoxic or normoxic conditions.

MATERIALS AND METHODS

Parents of fish used in these experiments were obtained from two natural African populations in Uganda, one from a chronically hypoxic swamp, and the other from a river with fluctuating but generally high levels of dissolved oxygen. The first site was a dense hypoxic wetland, the Lwamunda Swamp (c. 4 km in width) that surrounds Lake Nabugabo (31°50′ E; 31°56′ S to 0°20′ E; 0°25′ S) and separates it from Lake Victoria. This wetland is dominated by the grass *M. violaceum* that forms a dense mat that is interspersed with occasional lagoons and small streams. *Pseudocrenilabrus multicolor victoriae* is found throughout this dense wetland, but is particularly abundant in the small lagoons and channels within the swamp. Dissolved oxygen concentration was measured at several sites within a small lagoon where the parental stock was collected for this study. Over a 1 year period of observation, monthly dissolved oxygen levels in the upper 20 cm of water averaged 1·5 mg l\(^{-1}\) (c. 25 mm Hg) during the early morning and 1·8 mg l\(^{-1}\) (c. 33 mm Hg) during the early afternoon (Fig. 1). Seasonal variation in dissolved oxygen was associated with seasonal rains, however, oxygen levels remained far below saturation throughout the year. Water temperature averaged 22·3°C in the morning and 23·8°C in the early afternoon, and average temperatures ranged over the year between 21·3 and 25·2°C in the early morning and between 21·3 and 27·2°C in the early afternoon. The second site was located in the Mpanga River in western Uganda, a large ever-flowing river that drains Kibale National Park and feeds into Lake George. The fish were collected from a section of open river (0°25′ N; 23°22′ E) where dissolved oxygen was generally high, but underwent seasonal fluctuations. There was little diel variation in dissolved oxygen or water temperature in this system. Monthly sampling of several microhabitats in the early afternoon indicated an average dissolved oxygen level of 4·3 mg l\(^{-1}\) (c. 72 mm Hg) over a 10 month period. Although this stretch of river is an open area of ever-flowing water, the site is heavily influenced by the flushing of a huge papyrus swamp upstream during the rainy season. In the year of study, seasonal flushing lowered oxygen concentration in the main river to 2·2 mg l\(^{-1}\) (c. 37 mm Hg) during the month of November (Fig. 1). Mean monthly water temperature at the river site averaged 20·1°C (mean monthly range=19 to 21·7°C). In addition, *P. multicolor victoriae* moves into sections of inundated *Acacia* sp. scrubland during high flooding where dissolved oxygen levels can also be quite low. In a series of measurements in the inundated scrubland taken in November of 1998, dissolved oxygen levels averaged 2·85 mg l\(^{-1}\) (c. 48 mm Hg) and may have reached lower levels when waters stagnated.

Individuals of the original parental stock were transported from Africa to the laboratory in Gainesville, FL, U.S.A. where matings were conducted. The F1 offspring were used in the experiments, subjecting some individuals from each group to normoxic conditions, and another group of equal size to hypoxic conditions during the entire developmental period. When the brood of fish was released, they were divided between two 208 l aquaria. Initially, 15 young were placed in each aquarium and then cropped to 10 when it was felt that conditions were stable in the tanks. The control tank was held at normoxia (by aeration), while the oxygen level in the experimental tank was reduced to 1·0 mg l\(^{-1}\) slowly over a 1 week period and held at this level for 5 months. A digital oxygen controller was used to maintain the oxygen level at 1·0 mg l\(^{-1}\) by monitoring the oxygen concentration and periodically pulsing necessary amounts of nitrogen into the tank through a diffuser. The tanks were held at 25°C and exposed to a 12L : 12D. The
young fish were initially fed fry bites (HBH growth formula) and then switched to a diet of flake food (Tetraflakes) and dried *Daphnia* sp. Individuals were harvested after 8 months of growth; each fish was treated as a replicate in the analysis. Body mass of fish ranged from 0.73 to 3.55 g.

Routine respiratory oxygen consumption was measured in a closed system as described by Nordlie *et al.* (1991). Oxygen pressures were measured calibrating the system for ambient air pressure and water vapour pressure. Values were converted to mass of oxygen consumed using solubility equations of Mortimer (1981). The fish to be measured were held together overnight in an open container filled with water from the tank of origin. This container was aerated overnight in a dark water bath, and the next morning the individual fish were transferred into the metabolism chambers (125 cc Erlenmeyer flasks made opaque with aluminum foil and duct tape), which remained open with an aerator in each for 2 h before the chambers were sealed and metabolic determinations

![Graph](image_url)

**Fig. 1.** Average monthly dissolved oxygen levels for (a) an open-water site of the Mpanga River in western Uganda located downstream from an extensive papyrus swamp (July 1995 to April 1996), and (b) a small lagoon in the Lwamunda Swamp, a *Miscothidium violaceum* dominated wetland that surrounds Lake Nabugabo in eastern Uganda (November 1998 to November 1999). At the Mpanga River site, dissolved oxygen concentration was measured in the upper 20 cm of water at several micro-sites over a 200 m stretch of river, and data were collected in the early afternoon (dil change in this system is minimal). At the Lwamunda Swamp site, dissolved oxygen concentration was measured at several micro-sites in a swamp lagoon (c. 300 m² in area), and data were collected in the early morning (■) and early afternoon (■). Dissolved oxygen was measured with a YSI Model 51b or Model 95 meter. X, Data not available.
were begun. This was done to minimize the amount of metabolic waste products in the metabolic chambers. Food was withheld for 24 h before metabolic determinations were made to ensure that individual fish were in a post absorptive state. Measurements were made every 15 or 30 min, depending on the relative size of the individual fish, with longer interval used for the smaller individuals. Oxygen partial pressures \((P_O_2)\) were determined using a Radiometer dissolved oxygen electrode attached to a Radiometer PHM 71 Acid-Base Analyzer.

Following each metabolic run, the flask was opened and the individual fish was removed, damp dried, and placed into a tared volume of aerated water of the same temperature as the water bath \((21 \pm 0·1^\circ C)\), and body mass was determined on an Ohaus balance \((0·00 g)\). Metabolic rate measurements were made as close to 20°C as was possible with the bath system to facilitate comparison with other studies on cichlids from the same region of East Africa. Critical oxygen tension \((P_c)\) values were determined by eye following the technique of Ultsch et al. (1978). All calculations of routine metabolic rate were of mean values over a period of time within which the final oxygen measurement showed a \(P_O_2\) value that remained equal to or higher than the \(P_c\) value. All metabolic rates are expressed in terms of mg O\(_2\) h\(^{-1}\) and at 20°C.

Analysis of covariance was used to compare total metabolic rate between the two groups of full sibs within each experiment with body mass as the covariate. Both variables were log\(_{10}\) transformed. Adjusted means (sample means adjusted for a common mean body mass and a common regression line) were calculated from the ANCOVA analysis. ANCOVA was also used to calculate adjusted means in a broader comparison of the \(P.\) multicolor victoriae groups to other studies of cichlids from the same region of East Africa.

**RESULTS**

Experiment number 1 compared routine metabolic rates of two groups, one group raised under normoxic conditions, the other under hypoxic conditions, and whose parents had been collected from waters of varying oxygen concentrations, but >2 mg l\(^{-1}\) (Mpanga River site). The range of body masses for individuals raised under normoxic conditions was 1·34 to 3·55 g \((n=8)\), and for those raised under hypoxic conditions, 1·00 to 1·95 g \((n=6)\). Results of the ANCOVA indicated that there was no significant difference between slopes of the relationship of log\(_{10}\) metabolic rate (mg O\(_2\) h\(^{-1}\)) and log\(_{10}\) body mass (g) between the normoxic and hypoxic groups \((P=0·404)\). The intercepts (representing absolute levels of metabolic rate), however, differed \((n=14, F_{1,11}=11·779, P=0·006)\). Fish raised under normoxia had a higher mean metabolic rate \((0·37 mg O_2 h^{-1})\) than fish raised under hypoxia \([0·24 mg O_2 h^{-1}]\), metabolic rate adjusted to the mean body mass of 1·68 g, Fig. 2(a)].

The second experiment compared routine metabolic rates of individuals whose parents were from the chronically hypoxic waters of the Lwamunda Swamp, again comparing offspring from a single parental pair, half of which were raised under normoxic conditions, the other half raised under chronically hypoxic conditions. In this case, the ANCOVA showed no significant difference between the slopes of the two groups \((P=0·213)\), nor between the intercepts for the two groups \((P=0·210)\). The adjusted mean values of routine metabolic rates for the two groups at the mean body mass of both \((1·33 g)\) were 0·24 and 0·26 mg O\(_2\) h\(^{-1}\) [Fig. 2(b)], respectively for those raised under normoxic conditions and hypoxic conditions [range of body masses for individuals under normoxic
conditions, 0·80 to 1·87 g (<i>n</i> = 8), and for those under hypoxic conditions, 0·73 to 2·11 g (<i>n</i> = 8).

Although it is assumed that the phenotypic difference in metabolic rate observed in experiment 1 occurred in response to oxygen availability in the tank, it could be that other factors may have contributed to the observed difference, since fish in the same population and treatment were raised in the same aquarium. Since the difference in dissolved oxygen among treatments, however, was so extreme, and differences in other water quality characters were small, it is likely that dissolved oxygen availability was a major contributor.
DISCUSSION

The experiments carried out compared routine rates of oxygen consumption using a ‘split brood’ design, with all individuals used in an experiment being the offspring of a single pair of parents. For the F1 offspring of parents from the chronically hypoxic swamp, there was no detectable difference in metabolic rates between sibs raised under normoxia and those raised under hypoxia. For offspring of parents from the Mpanga River, however, the metabolic rate of fish raised under normoxia was higher than for sibs raised under extreme hypoxia. Although further experiments with a larger random sample of the parental stock will be necessary to validate the patterns, the difference between the experiments is interesting and may reflect the selective environment of the parents. When environmental conditions fluctuate sufficiently or environments within a species range differ, selection may, in some cases, favour genetic systems that preserve phenotypic plasticity (Slatkin & Lande, 1976; Rollo, 1994; Via et al., 1995).

Pseudocrenilabrus multicolor victoriae from the Mpanga River experiences a highly fluctuating dissolved oxygen regime, although the main river never reaches levels as low as the Lwamunda Swamp. Dispersal data are not available for this species, however, it is possible that P. multicolor victoriae may disperse into the extremely hypoxic swamps that lie upstream of this river site. In addition, ‘supra-annual’ high flooding that inundates areas of Acacia sp. scrubland produces stagnant, hypoxic waters that are exploited by P. multicolor victoriae and many other species in the river (L. Chapman, unpubl. data). Thus, fish from this population experience variable oxygen regimes, and have access to nearby hypoxic waters habitually or supra-annually. If young fish disperse into hypoxic waters, developmental plasticity in metabolic rates may provide a flexible response to a new environment. In a similar split brood experiment on offspring derived from an Mpanga River stock population, Chapman et al. (2000) found that total gill surface area was 18% larger in fish raised under extreme hypoxia than in their full sibs raised under normoxia, further supporting development plasticity in respiratory characters in this population. Pseudocrenilabrus multicolor victoriae that inhabit the Lwamunda Swamp experience chronic hypoxia that is very severe in the dry season. The small lagoons where parental stock fish were collected are c. 3000 m from the open waters of the lake, and there are no other nearby well-oxygenated waters in the wetland. It is possible that such stable conditions have led to a loss of flexible response in routine metabolic rate and potentially other respiratory characters in this population. Although these results are not definitive because of the use of a single breeding pair from each site, they are consistent with the idea that the selective environment of the parents may contribute to interdemic variation in the level of phenotypic response to hypoxia.

Rosenberger & Chapman (2000) compared several respiratory characters (critical oxygen tension, metabolic rate, gill surface area and respiratory behaviour) among field populations of three species of haplochromine cichlids: P. multicolor victoriae, Astatotilapia velifer (Trewavas), and Prognathochromis venator (Greenwood). In their study, P. multicolor victoriae were collected from the hypoxic waters of the papyrus-choked Juma River (the major tributary of Lake Nabugabo). Rosenberger & Chapman (1999) reported an average
dissolved oxygen concentration of 1·6 mg l\(^{-1}\) from sites deep within the Juma River. Individuals of *A. velifer* used by Rosenberger & Chapman (2000) were collected from the ecotonal wetland where the Juma River meets the open waters of Lake Nabugabo. Schofield & Chapman (1999) reported an average dissolved oxygen concentration of 2·5 mg l\(^{-1}\) in the marginal vegetation of wetland ecotones of Lake Nabugabo and 3·4 mg l\(^{-1}\) in waters 5 m offshore of wetland ecotones. Individuals of *P. venator*, were collected from open waters of Lake Kayanja, an open water site near Lake Nabugabo with high dissolved oxygen levels (Chapman et al., 2000; Rosenberger & Chapman, 2000). Rosenberger & Chapman (2000) concluded that *P. multicolor victoriae* individuals from the Juma River were the most tolerant of hypoxic conditions among the three species based on their respiratory behaviour and critical oxygen tension. Metabolic rates were similar between *P. multicolor victoriae* and *P. venator*, but both species exhibited lower metabolic rates than *A. velifer*. Table I compares the total metabolic rates from the split brood experiments to total metabolic rates for *P. victoriae* from the Juma River, *A. velifer* and *P. venator*. ANCOVA was used to calculate sample means adjusted for a common mean body mass and a common regression line for all the groups. This analysis produces different mean values for *P. multicolor victoriae* from the split brood experiments than those reported in Fig. 2 because combining all seven groups creates a different slope and average body mass from which adjusted means were calculated. Nevertheless the trends are the same; and comparison of all four split brood groups at a common body mass shows that the rate for the hypoxic group from the Mpanga River site is similar to the rates for both groups of Lwamunda Swamp fish. Comparison of the results of the split brood groups grown under hypoxia or normoxia seem consistent with the results of Rosenberger & Chapman (2000) in that F1 individuals from the Lwamunda Swamp (raised under normoxia and hypoxia) and F1 individuals from the Mpanga River (raised under hypoxia) fall within or close to the range reported for field populations of *P. multicolor victoriae* and *P. venator* (Table I). F1 individuals of *P. multicolor victoriae* whose parents were taken from the Mpanga River and who were reared under normoxic conditions, show higher routine metabolic rates than field populations of *P. multicolor victoriae* and *P. venator*, but much lower than *A. velifer*, an active fish from waters of moderate dissolved oxygen availability.

*Pseudocrenilabrus multicolor victoriae* is one of a number of species of African fishes, whose populations inhabiting hypoxic waters have survived extinction that might have come through predation by the predatory Nile perch *Lates niloticus* (L.). The Nile perch is a large centropomid fish that was introduced into lakes of the Lake Victoria basin in the 1950s and 1960s. Expansion of Nile perch populations in Lakes Victoria, Kyoga, and Nabugabo coincided with the disappearance of several indigenous fishes (Kaufman, 1992; Witte et al., 1992a, b). Nile perch, however, cannot persist in waters of low dissolved oxygen content and therefore, hypoxic wetlands seem to serve as important faunal refugia in the Lake Victoria basin (Chapman et al., 1996a, b; Schofield & Chapman, 1999, 2000). A low aquatic surface respiration threshold, along with a low routine metabolic rate, a low critical oxygen tension and a large gill surface area (Rosenberger & Chapman, 2000; Chapman et al., 2000) suggest that *P. multicolor victoriae* is well equipped to cope with constantly low ambient oxygen
The total metabolic rate of (a) *Pseudocrenilabrus multicolor victoriae* (present study) and (b) field populations of *Pseudocrenilabrus multicolor* from the hypoxic waters of the Juma River, *Prognathochromis venator* from the open waters of Lake Kayanja and *Astatotilapia velifer* from the ecotonal wetlands (moderately high, but fluctuating oxygen) of Lake Nabugabo. Data on the field populations were derived from Rosenberger (1997) and Rosenberger & Chapman (2000, unpubl. data). All values were adjusted to a mean body mass of 2 g (using ANCOVA) and 20° C.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Treatment</th>
<th>Mass range (g)</th>
<th>Total metabolic rate (mg O₂ h⁻¹), 2 g fish</th>
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<td>(a)</td>
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<tr>
<td><em>Pseudocrenilabrus multicolor victoriae</em></td>
<td>Mpanga River</td>
<td>F₁ (normoxia)</td>
<td>1·34 to 3·55</td>
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<td></td>
<td></td>
<td>F₁ (hypoxia)</td>
<td>1·00 to 1·95</td>
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<td></td>
<td>Lwamunda Swamp</td>
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<td></td>
<td></td>
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<td>(b)</td>
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<tr>
<td><em>Pseudocrenilabrus multicolor victoriae</em></td>
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<td>Field (low oxygen)</td>
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concentrations, and this may have facilitated its survival in deep swamp refugia. Developmental plasticity in respiratory characters may have facilitated its widespread distribution in the basin and led to its colonization of dense wetlands, by preserving the possibility for response to new environments and environmental change. Future studies that explore developmental flexibility in other characters and in populations from a variety of environmental settings are needed to understand the links between the environment and the degree of environmentally-induced phenotypic plasticity in this species.

References


