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BRIEF COMMUNICATION

Hypoxia and male behaviour in an African cichlid *Pseudocrenilabrus multicolor victoriae*

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This study tested the prediction that hypoxia may reduce the frequency of energetically expensive behaviours by quantifying male mating and aggressive displays in the cichlid *Pseudocrenilabrus multicolor victoriae* after long-term acclimation (5 months) to either high dissolved oxygen (DO) or low DO. Regardless of DO treatment, males engaged in more aggressive displays than mating displays; however, males acclimated to low DO reduced their total number of displays compared to high DO-acclimated males.

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As limited resources are available to an organism for growth, maintenance and reproduction, energetic trade-offs may become more apparent under extreme environmental conditions (Jones & Reynolds, 1999a, b). Hypoxia (low dissolved oxygen, DO) can operate as a significant environmental stressor, particularly for water-breathing organisms, given the high costs of oxygen acquisition in water (Wootton, 1990) and has been shown to negatively affect activity, growth, survival and reproduction in some aquatic species (Wu *et al.*, 2003; Chapman & McKenzie, 2009).

Fishes show a diverse suite of responses to hypoxia including biochemical, physiological and morphological adjustments (Herbert & Steffensen, 2005; Pollock *et al.*, 2007; Martinez *et al.*, 2009; Wells, 2009; Crispo & Chapman, 2010). Behavioural shifts provide additional flexibility for dealing with low-oxygen conditions. For example, many fishes increase their gill ventilation rate and volume (Petrosky & Magnuson, 1973; Randall, 1982) or engage in aquatic surface respiration (ASR), skimming the surface film of water where diffusion can produce a micro layer

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of well-oxygenated water (Kramer, 1987; Chapman & McKenzie, 2009). These behaviours facilitate increased oxygen uptake. Another option for fishes under hypoxia is to reduce the frequency of energetically expensive behaviours associated with the acquisition of other resources (Abrahams *et al.*, 2005; Marks *et al.*, 2005). Such behaviours may include: the acquisition and defence of food or mate resources, establishment and maintenance of territories and acquisition of hierarchical positions (Baerends & Baerends-Van Roon, 1950; Fryer & Iles, 1972). Modifications of behaviours associated with food and mate resources may influence the reproductive success of males by affecting male–male aggressive encounters (intrasexual selection) or male mating displays to attract females (intersexual selection) (Andersson, 1994). Differences in the costs and benefits of these display types may alter a fish's relative investment in one display over the other.

Several hypoxia acclimation studies report negative effects of hypoxia on male behavioural displays including aggressive encounters and mating displays. For example, Abrahams *et al.* (2005) reported a reduction in opercular displays in the Siamese fighting fish *Betta splendens* Regan 1910 when exposed to their own mirror images under hypoxia *v.* exposure under normoxia. Many of these studies, however, have been short-term experiments with acclimation periods ranging from a few minutes to several hours (Sneddon & Yerbury, 2004; Abrahams *et al.*, 2005; Marks *et al.*, 2005). Only a few studies have explored long-term acclimation effects of hypoxia (*e.g.* >1 week) on fish behaviour. This is important because biochemical or physiological shifts after long-term acclimation to sublethal hypoxia may alleviate energetic constraints associated with initial exposure to hypoxia (Martinez *et al.*, 2009). For example, in their study of sailfin mollies *Poecilia latipinna* (LeSueur 1821), Timmerman & Chapman (2004a) found that the use of ASR in hypoxia-acclimated fish was an immediate response to hypoxia that decreased over the acclimation period, which they attributed to gradual physiological compensation. In a second related study, Timmerman & Chapman (2004b) quantified both aggressive and reproductive behaviours in *P. latipinna* after a 10 week acclimation period to hypoxia or normoxia. Interestingly, they found no effect of hypoxia on aggression (both sexes combined), but significantly lower reproductive activity under hypoxia, suggesting that reproductive displays may be more costly in this species. Together these studies (1) highlight the importance of documenting both intersexual and intrasexual displays and (2) suggest that hypoxia may differentially affect behavioural displays depending on energetic cost of the display and acclimation time to hypoxia.

The goal of this study was to quantify differences in male behaviour of the cichlid *Pseudocrenilabrus multicolor victoriae* Seegers 1990 acclimated long-term (5 months) to high or low DO in a competitive mating environment. The study was designed to test for an overall decline in the total number of intersexual (competition for female preference) and intrasexual (competition for access to females) displays in response to hypoxia exposure and to detect differences in the degree of decline between inter- and intrasexual displays. In a review of behavioural consequences of hypoxia in fishes, Chapman & McKenzie (2009) found that a common response of fishes exposed to short-term hypoxia was a drop in the level of activity; and, in some species, this was associated with metabolic depression. In *P. m. victoriae*, Reardon & Chapman (2010a) reported a lower standard metabolic rate in fish acclimated long-term to low DO relative to high DO. It was thus predicted that acclimation to hypoxia would reduce the frequency of behavioural displays.

Pseudocrenilabrus m. victoriae is a small mouth-brooding cichlid found across a range of oxygen regimes throughout the Lake Victoria basin in East Africa (Reardon & Chapman, 2009). Fish for this study were collected from the Lwamunda Swamp, a permanently hypoxic wetland surrounding Lake Nabugabo, Uganda (00° 45' S; 31° 45' E) with mean morning DO of 1.1 mg O₂ l⁻¹, and mean afternoon DO of 2.6 mg O₂ l⁻¹ (Reardon & Chapman, 2008). This population of *P. m. victoriae* exhibits both morphological (*e.g.* large gills) and physiological (*e.g.* high haematocrit) adaptations for low-DO conditions relative to populations of *P. m. victoriae* inhabiting high-DO water (Chapman *et al.*, 2000, 2002; Martinez *et al.*, 2009).

Fish were transferred live to aquarium facilities at McGill University (Canada). Two 208 l (55 U.S. gallon) aquaria were each divided into six chambers using mesh partitions that eliminated direct contact of fish in neighbouring chambers but did not preclude transfer of visual or chemical stimuli. One male and two females were placed in each chamber within each tank. One tank was acclimated to hypoxia (mean ± s.e. DO 1.41 ± 0.05 mg O₂ l⁻¹) and the other to normoxia (mean ± s.e. DO 7.75 ± 0.05 mg O₂ l⁻¹) for 5 months prior to the beginning of observations for this experiment as part of an ongoing acclimation study on reproductive traits in *P. m. victoriae* (Reardon & Chapman, 2010a). All individuals were sexually mature and bred intermittently throughout the acclimation period. Males were size-matched between DO treatments. Although all males grew during the experiment, mean male sizes were similar at the beginning of acclimation and end of observations [mean ± s.e., initial standard lengths (L_S): low DO, $n = 4$, L_S 53.8 ± 1.5 mm, mass 4.56 ± 0.30 g; high DO, $n = 4$, L_S 52.8 ± 1.7 mm, mass 3.98 ± 0.34 g; end: low DO, $n = 4$, L_S 58.3 ± 1.3 mm, mass 6.02 ± 0.31 g; high DO, $n = 4$, L_S 61.5 ± 1.0 mm, mass 6.65 ± 0.40 g]. Fish were fed TetraMin flakes (www.tetra-fish.com) *ad libitum* once a day. All filters and plants were placed at the rear of the chambers to increase fish visibility during observations. Both aquaria were held at mean ± s.e. temperature of 24.40 ± 0.02° C and exposed to a 12L:12D photoperiod. Other water quality variables that were monitored (*e.g.* pH and conductivity) were similar between the treatments, and thus the factor that differed most between the two acclimation tanks was DO level.

Observations, randomized across chambers, were carried out between January and March 2007. Four males in each treatment (high DO, low DO) were observed for a 5 min interval eight times over the 3 month period. Over this time, mortality was very low (two females from the hypoxia treatment, one female from normoxia), and no effects of sample period on the number of females per chamber were detected on recorded behaviours. The observer sat in front of the fish to allow them to acclimate to the observer's presence for 10 min prior to beginning observations. For each observation, the total number of times the observed male performed either a mating display or an aggressive display in a 5 min interval was recorded. Only one fish was observed per 5 min trial reducing the possibility of missing a behavioural display. In addition to the number and types of displays, a suite of other behaviours was recorded at the same time but only behaviours directed towards another fish are included in the analysis. The male behavioural displays were categorized as either aggressive (male directed, intrasexual) or reproductive (female directed, intersexual) (Baerends & Baerends-Van Roon, 1950; Barlow, 1963). Mating displays included: chasing a female that resulted in flight or wiggling their fins and body in front of a female (mating wiggle). Displays were recorded when directed towards either a female

within the chamber of the focal fish or in an adjacent chamber. Aggression displays included: open or closed mouth charges towards another male or a lateral flexion while oriented perpendicular towards another male. These displays were directed towards a male in a neighbouring chamber despite the mesh that prevented physical contact between males.

Repeated-measures ANCOVA was used to detect effects of time (observation period, eight in total), treatment (low *v.* high DO), display type (mating or aggressive) and their interactions on the number of displays per 5 min observation period. Male L_S was included as a covariate to correct for potential size effects on display frequency (Peters, 1983).

Repeated-measures ANCOVA indicated homogeneous slopes for the bilogarithmic relationship between display type and male L_S ($P > 0.05$) and between DO and male L_S ($P > 0.05$). The number of displays did not differ significantly across the eight periods (repeated-measures ANOVA, $P > 0.05$), and there was no significant interaction between observation period and display type (repeated-measures ANOVA, $P > 0.05$). Within each treatment tank and for each display type, there was no effect of chamber position or number of females on the number of displays (repeated-measures ANOVA, $P > 0.05$). The lack of a chamber effect indicates that within a tank, fish at terminal ends of the tank with one neighbour did not differ quantitatively in their behaviour compared with males with two neighbouring chambers. The olfactory cues in the water might negate the effect of the number of fish available for a given male to interact with.

Overall, males made more aggressive displays towards other males than mating displays towards females; however, the interaction between display type and DO was not significant, indicating that the frequency of aggressive displays relative to mating displays did not differ between treatments (Table I and Fig. 1). The covariate (male L_S) was also significant; larger males tended to display more often than smaller males (Table I and Fig. 1 values adjusted to mean male L_S of 63 mm). Noonan (1983) provided evidence that cichlid females generally prefer larger males. The correlation between size and number of mating displays may partially explain the preference for larger males. This suggests that energetic allocation to displays was biased towards intrasexual competition and this bias was not affected by DO availability.

While the ratio of display types was unaffected, there was still an overall decrease in the number of both aggressive and mating displays under low DO; males performed more displays (regardless of type) under high DO compared to low DO

TABLE I. Summary of repeated measures ANCOVA to detect the effects of dissolved oxygen (DO) treatment, type of display (either mating or aggressive) and their interaction on the number of behavioural displays performed by eight male *Pseudocrenilabrus multicolor victoriarie* across eight focal observations lasting 5 min each with \log_{10} male standard length (L_S) as a covariate

Variable	<i>F</i>	d.f.	<i>P</i>
Male L_S	5.591	1,11	<0.05
DO	5.348	1,11	<0.05
Display type	28.763	1,11	<0.001
Display type \times DO	0.059	1,11	>0.05

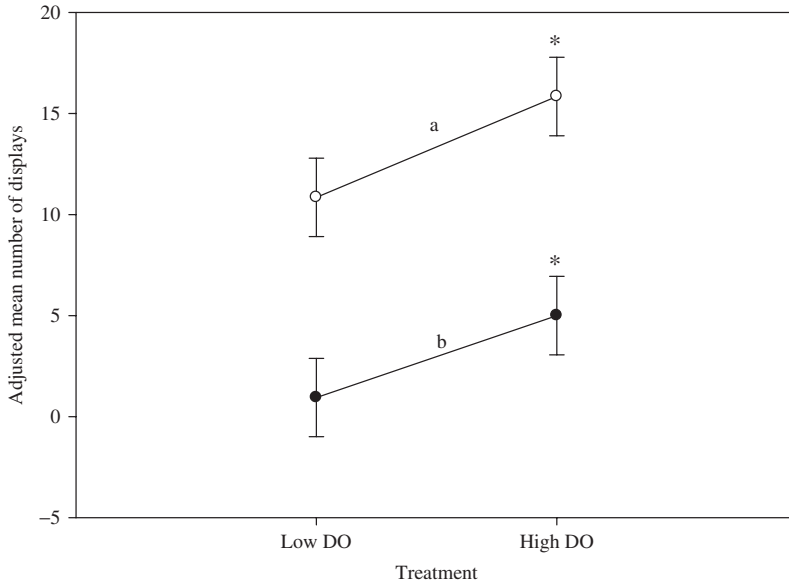


FIG. 1. The adjusted mean \pm s.e. number of mating displays (●) ($n = 8$ observations per individual on four fish per treatment) performed by male *Pseudocrenilabrus multicolor victoriae* and directed towards a female (these displays include a 'mating wiggle' and chasing the female), and the adjusted mean \pm s.e. number of aggressive displays towards other males (○) ($n = 8$ observations per individual on four fish per treatment) (these displays include a full body, lateral flexion while oriented perpendicular towards another male and open and closed mouth charging). All sample means were adjusted for mean \log_{10} male standard length (L_S) of 0.7969 (antilog = 63.0 mm) because there was an effect of L_S on the number of displays. *, a statistical difference between dissolved oxygen (DO) treatments. Lines with different lower case letters indicate a statistical difference in display type (mating *v.* aggressive).

(Table I and Fig. 1). This supports the prediction for fewer behavioural displays in males acclimated to low DO relative to those acclimated to high DO, a pattern that is consistent with many short-term studies that have demonstrated negative effects of hypoxia on male behavioural displays including aggressive encounters and mating displays (Sneddon & Yerbury, 2004; Abrahams *et al.*, 2005; Marks *et al.*, 2005; Carter & Wilson, 2006). More generally, this finding supports several reports in the literature of reduced activity levels in fishes exposed to hypoxia (Hubbs *et al.*, 1967; Schurmann & Steffensen, 1994; Chapman & McKenzie, 2009).

Given that the fish in this study were acclimated to the treatment conditions for 5 months prior to observations, observed differences in behaviours may reflect long-term response to hypoxia acclimation. Other energetically costly behaviours such as ASR were not observed and were probably mitigated by biochemical and physiological compensation associated with hypoxia acclimation well before observations commenced (Timmerman & Chapman, 2004a; Pollock *et al.*, 2007). For example, Martinez *et al.* (2009) found that F_1 offspring of *P. m. victoriae* from the Lwamunda Swamp reared under normoxia in the laboratory showed increased haematocrit and lactate dehydrogenase (LDH) activities when subsequently exposed to 4 weeks acclimation to hypoxia *v.* normoxia.

Earlier studies have demonstrated that fish collected from this low-DO population exhibit a number of adaptations to deal with low DO, such as larger gills and

increased levels of haemoglobin, haematocrit and LDH when compared to conspecifics from high-DO habitats (Chapman *et al.*, 2000; Martinez *et al.*, 2009). It is possible that because these fish may be pre-adapted to hypoxia, acclimation to high DO (the novel environment) may be the more stressful of the two treatments. If normoxia was a stressful environment, a decrease in behavioural displays in the high-DO treatment might be expected, a prediction that is not supported by these results. In contrast, their respiratory adaptations may provide these fish with a larger 'engine' for more efficient oxygen uptake within a high-DO environment (Reardon & Chapman, 2010*b*; K. M. Gotanda, E. E. Reardon, S. M. C. Murphy & L. J. Chapman, unpubl. data). If so, increased oxygen supply may facilitate an increase in behavioural displays relative to fish acclimated to their natural condition of low DO, which is consistent with the results. Interestingly, in the same population, wild *P. m. victoriae* females exhibit higher metabolic rates in response to long-term acclimation to high DO relative to low DO (Reardon & Chapman, 2010*a*), suggesting that high-DO acclimated fish may be able to invest more into energetically costly behaviours relative to a fish held under low DO. It would be useful to replicate this study on fish originating from high-DO habitats in the wild.

The results from this study indicate that long-term acclimation to different oxygen conditions may affect both intersexual and intrasexual behaviour, which may have important consequences for reproductive success. Understanding the influence of hypoxia on fish behaviour and the associated potential direct or indirect effects on fitness is of growing importance given the increased threat of hypoxia in aquatic systems around the world (Chapman & McKenzie, 2009; Diaz & Breitburg, 2009).

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