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Effects of Hypoxia on Swimming and Sensing in a Weakly Electric Fish

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RESEARCH ARTICLE

Effects of hypoxia on swimming and sensing in a weakly electric fish

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ABSTRACT

Low dissolved oxygen (hypoxia) can severely limit fish performance, especially aerobically expensive behaviours including swimming and acquisition of sensory information. Fishes can reduce oxygen requirements by altering these behaviours under hypoxia, but the underlying mechanisms can be difficult to quantify. We used a weakly electric fish as a model system to explore potential effects of hypoxia on swim performance and sensory information acquisition, which enabled us to non-invasively record electric signalling activity used for active acquisition of sensory information during swimming. To quantify potential effects of hypoxia, we measured critical swim speed (U_{crit}) and concurrent electric signalling activity under high- and low-dissolved oxygen concentrations in a hypoxia-tolerant African mormyrid fish, *Marcusenius victoriae*. Fish were maintained under normoxia for 6 months prior to experimental treatments, and then acclimated for 8 weeks to normoxia or hypoxia and tested under both conditions (acute: 4 h exposure). Acute hypoxia exposure resulted in a significant reduction in both U_{crit} and electric signalling activity in fish not acclimated to hypoxia. However, individuals acclimated to chronic hypoxia were characterized by a higher U_{crit} under both hypoxia and normoxia than fish acclimated to normoxia. Following a 6 month re-introduction to normoxia, hypoxia-acclimated individuals still showed increased performance under acute hypoxic test conditions, but not under normoxia. Our results highlight the detrimental effects of hypoxia on aerobic swim performance and sensory information acquisition, and the ability of fish to heighten aerobic performance through acclimation processes that can still influence performance even months after initial exposure.

KEY WORDS: Acclimation, Dissolved oxygen, Critical swim speed, Mormyridae, Aerobic performance, Electrosensation

INTRODUCTION

Dissolved oxygen (DO) concentration is a key factor limiting the growth, reproduction and survival of non-air-breathing fishes. Low DO concentration (hypoxia) occurs when the demand for DO surpasses that available within the water and has been defined as DO concentrations <2 mg O_2 l^{-1}, or approximately 30% air saturation (Pollock et al., 2007; Levin et al., 2009). As human activities are increasing the occurrence and duration of hypoxia in regions that have historically never experienced such low DO levels (Sabo et al., 1999; Diaz, 2001; Schmidtko et al., 2017), hypoxia is now considered to be one of the greatest threats currently facing water-breathing organisms (Pollock et al., 2007; Diaz and Rosenberg, 2008). Fishes have evolved a variety of strategies for dealing with hypoxic stress (e.g. physiological adjustments that decrease metabolic demand or increase the efficiency of oxygen uptake), but these strategies are not always sufficient to mitigate the metabolic constraints of severe hypoxia (Pollock et al., 2007; Chapman and McKenzie, 2009; Richards, 2009, 2011) and may drive trade-offs between aerobically expensive behaviours.

Fish can lessen oxygen requirements under acute hypoxic stress by reducing locomotion (Wu, 2002; Chapman and McKenzie, 2009). Sustained swimming in fishes primarily utilizes aerobic muscle fibres and requires increased oxygen consumption with increased swimming speed, and therefore taxes an individual metabolically (Smit, 1965; Webb, 1971; Jayne and Lauder, 1994). Critical swim speed (U_{crit}) is a useful measure of an individual's prolonged swimming capacity (Farrell et al., 1998; Plaut, 2001), primarily measuring aerobic performance, with anaerobic contribution at the highest swimming speeds as fast-twitch white muscle is activated (see review by Domenici et al., 2013). Previous studies have highlighted significant decreases in U_{crit} and changes in swimming patterns under acute exposure to hypoxia in fishes acclimated to chronic normoxia (high DO concentration), which could potentially impact their survival in the wild (e.g. Herbert and Steffensen, 2005; Dutil et al., 2007).

In contrast to acute hypoxia exposure, chronic exposure to non-lethal levels of hypoxic stress can result in morphological, biochemical and/or physiological changes that can increase oxygen uptake capabilities, hypoxia tolerance and swimming performance in some fish species (e.g. Bushnell et al., 1984; Petersen and Gamperl, 2010). As a result, chronic exposure to non-lethal hypoxia can benefit an individual's physiological performance and ability to cope with hypoxia. Nevertheless, when challenged with hypoxia, the high metabolic cost of swim performance may still trade off with other aerobically expensive traits, including (but not limited to) specific dynamic action, reproduction, growth and sensory information acquisition (see review by Domenici et al., 2013). However, among these traits, the capacity to acquire sensory information can be exceptionally difficult to quantify because most sensory systems do not offer easily accessible proxies of acquisition performance. Here, we used weakly electric fish as a model to assess changes in locomotion and sensory information acquisition under hypoxic stress because they have an active electrosensory system that lends itself to quantifying a proxy of sensory information acquisition non-invasively in free-swimming fishes.

Weakly electric fishes, which include two phylogenetically distant lineages, the Gymnotiformes of South and Central America and the Mormyroidea of Africa, produce and utilize electric signals for both communication and prey detection (e.g. Lissman, 1958; Moller, 1995; Lavoué et al., 2012). They emit electric organ

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discharges (EODs) to sense nearby objects that cause perturbations in their electric field (Moller, 1995; Hopkins, 1999). Modulation of the rate of electric signalling translates directly to the rate at which the animals sample information about their environment (Moller, 1995). Electric signalling activity is aerobically powered in electric fishes, and there is an expected energetic cost to the individual depending on the amplitude of the EOD pulses as well as their rate of production (Carlson, 2002; Julian et al., 2003; Salazar and Stoddard, 2008; Salazar et al., 2013; Lewis et al., 2014; Moulton, 2016). It seems likely that physiologically challenging conditions, such as hypoxia, could drive changes in the relationship between locomotion and sensory information acquisition, as the two are among the competing metabolic demands an individual experiences. While there has been some work on the effects of hypoxia on weakly electric fish behaviour and performance (e.g. Nilsson, 1996; Crampton, 1998; Reardon et al., 2011; Sukhum et al., 2016), the relationship between sustained swimming performance and electric signalling activity and the effects of hypoxia on both are unknown.

The Mormyroidea consists of ca. 200 species of mormyrids and the single-species family of the Gymnarchidae (Alves-Gomes and Hopkins, 1997; Lavoué et al., 2000). Although mormyrids are generally hypoxia tolerant (Nilsson, 1996; Sukhum et al., 2016), some species have been found to persist in extremely hypoxic swamp habitats (Chapman et al., 1996, 2002; Chapman and Hulen, 2001). One of these species, *Marcusenius victoriae*, occurs across a broad range of DO conditions from the open and ecotonal waters of lakes where DO is relatively high to the dense interior of hypoxic swamps (Chapman and Hulen, 2001; Chapman et al., 2002; Moulton, 2016). Swamp-dwelling *M. victoriae* are characterized by a larger gill surface area than open-water conspecifics, which may increase oxygen uptake capacity and allow them to maintain a stable metabolic rate down to a very low critical oxygen tension (Chapman et al., 2002; Moulton, 2016). In addition, the electrosense of this swamp-dwelling population has been estimated to cost up to 20% of resting metabolic rate (RMR; T. Moulton, R. Krahe and L.J.C., unpublished observations), which suggests a high metabolic demand of EOD production and associated sensory processing in these fish. In the present study, we used specimens of *M. victoriae* collected from the Lwamunda Swamp surrounding Lake Nabugabo, Uganda, where DO levels are chronically hypoxic throughout the year in the swamp, but do fluctuate on both a diel and a seasonal basis (Reardon and Chapman, 2008).

Given the chronic hypoxia experienced by *M. victoriae*, this species provides an excellent opportunity to explore the relationship between aerobically powered traits and the degree to which phenotypic flexibility may mitigate costs of hypoxia. Our experimental design focused on identifying the relationship between swim performance and electric signal production under acute and chronic exposure to normoxic and hypoxic conditions. As individual fish would have experienced different oxygen regimes during their life as a result of the time of capture and age of individuals, we first acclimated all individuals to chronic normoxic conditions for a 6 month period in the lab before separating them into two treatment groups. Acclimation to normoxia has been used in other studies of hypoxia-tolerant fishes prior to exposure to acute, graded or chronic hypoxia (e.g. Muusze et al., 1998; Chipari-Gomez et al., 2005; Almeida-Val et al., 2011); such conditioning should minimize inter-individual variation in plastic traits and allow us to then evaluate plastic responses to controlled chronic and acute hypoxia exposure. One treatment group then underwent an 8 week acclimation to chronic hypoxia, while the other group stayed under chronic normoxia for an additional 8 week period. Following these 8 week treatments, we quantified sustained swimming (U_{crit}) and sensory information acquisition (electric signalling activity) under normoxic and hypoxic test conditions (acute exposure). Following these trials, all individuals were returned to chronic normoxia for an additional 6 month period, after which we repeated the U_{crit} trials under normoxic and hypoxic test conditions (Fig. 1). This repeated-measures approach exposed all individuals to both chronic and acute conditions, which allowed us to focus on within-subject effects of these acclimations. Overall, our goal was to answer three major questions. (1) How does hypoxia affect U_{crit}? (2) How does hypoxia affect electric signalling activity? (3) What is the relationship between swim speed and electric signalling activity?

We hypothesized that hypoxia would affect both U_{crit} performance and electric signalling activity, as they both come at an aerobic cost to the individual. We predicted a decrease in both U_{crit} and electric signalling activity under hypoxia in all treatment groups, but that fish acclimated to chronic hypoxia would not be as severely affected by hypoxic test conditions when compared with individuals acclimated to chronic normoxia. We also predicted residual effects of previous hypoxia acclimation, i.e. higher performance under hypoxic test conditions.

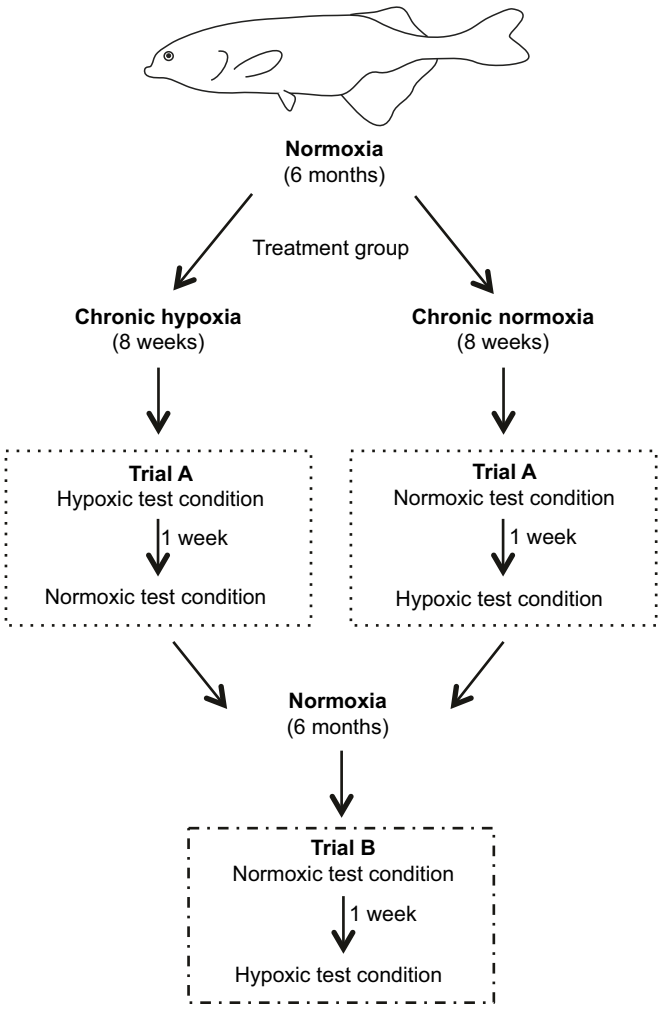

Fig. 1. Experimental design. Timeline of chronic normoxia and hypoxia acclimation and critical swim speed (U_{crit}) swim trials under normoxic and hypoxic test conditions. Details of acclimation (n=5/condition), hypoxia/normoxia exposure and trial protocols are outlined in Materials and Methods.

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Additionally, we hypothesized that individuals would alter electric signalling activity with increasing water velocity. Increased electric signalling activity should provide an individual with sensory information at a higher rate, but could also simultaneously increase their metabolic demand. Therefore, we predicted that (a) electric signalling activity would decrease when swimming at speeds approaching U_{crit}, when the metabolic demand of sustained swimming is also highest, and (b) that this would be heightened under hypoxic conditions, but could potentially be offset by chronic exposure to hypoxia.

MATERIALS AND METHODS

Study species

Marcusenius victoriae (Worthington 1929) (reported in some earlier studies as *Gnathonemus victoriae*, an old binomen; Seegers et al., 2003) is a medium-sized mormyrid [adult total length (TL): 13–22 cm] found in the Nile River basin of East Africa. Populations have been reported from lakes Victoria and Kyoga, and other small satellite lakes in the region including Lake Nabugabo (Greenwood, 1966; Ogutu-Ohwayo, 1993; Chapman et al., 1996; Namulemo and Mbabazi, 2005). This hypoxia-tolerant species occurs across a broad range of habitats, and the individuals used in this study were collected from the Lwamunda Swamp surrounding Lake Nabugabo, Uganda, at a site located 3.2 km from the main lake at −0°19′07.18″N, 31°56′47.80″E. DO levels in the swamp are chronically low, but do fluctuate on both a diel and a seasonal basis. Reardon and Chapman (2008) reported an average DO concentration of 1.06 mg O_2 l^{-1} (14% saturation) in the morning (range 0.37–1.61 mg O_2 l^{-1} over 1 year of monthly samples, June 2004 to April 2005) and 2.63 mg O_2 l^{-1} (36% DO saturation) in the afternoon (range 1.69–3.99 mg O_2 l^{-1}). Moulton (2016) reported a mean diurnal range of 9.9–11.8% DO saturation during June to August 2014.

Fish collection and housing

Marcusenius victoriae were captured for this study in the Lwamunda Swamp in July 2013 (n=7) and July 2014 (n=3). As it is difficult to catch and transport high numbers of individuals of this population, this experiment was performed separately on the individuals caught in 2013 and 2014, with each group undergoing the exact same protocols, timelines and experimental conditions using the same equipment. The same experimenter also conducted all measures, fish handling and performance trials with both groups.

Chapman et al. (2002) reported an average critical oxygen tension (P_{crit}) for this *M. victoriae* population of 0.7 mg O_2 l^{-1}, and recent work by Moulton (2016) on this population reported a P_{crit} of 0.8 mg O_2 l^{-1}. All DO concentrations used in the present study were maintained above that critical physiological point (Ultsch et al., 1978), and hypoxic DO levels were within the natural range of the population (Chapman and Chapman, 1998; Chapman et al., 2002; Moulton, 2016). Fish were transported from the field to McGill University and housed individually in 57 l aquaria at a conductivity of 115±15 µS cm^{-1}, DO concentration of 8.0±0.5 mg O_2 l^{-1} (fully aerated) and pH of 7.0±0.3 under a 12 h:12 h light:dark photoperiod at 23±1°C. Fish were fed frozen bloodworms (Hikari, Hayward, CA, USA) *ad libitum* daily. All experimental protocols were approved by the McGill University Animal Care Committee (protocol no. 5029).

Acclimation to chronic hypoxia or normoxia

All fish [TL: mean 12.5 cm, range 11.5–14 cm; standard length (SL): mean 11.24 cm, range 10.5–13 cm; body depth (BD): 2–3 cm; mass: mean 16.24 g, range 12–24 g] were introduced to normoxic DO concentrations (8.0±0.5 mg O_2 l^{-1}, 23±1°C) upon arrival in the lab and were maintained in normoxia for 6 months. Following this 6 month period, fish were randomly divided between two treatment groups. Half of the fish (n=5) were re-acclimated to chronic hypoxia and half of the fish (n=5) remained under chronic normoxia (Fig. 1). Male and female fish, easily distinguished by the shape of their anal fin, were evenly distributed between acclimation treatments (2 males, 3 females per treatment), and data were pooled across sexes within treatments because of the limited availability of individuals. Biometric details (TL/SL/mass) for each acclimation group are detailed in Table 1.

Chronic hypoxia acclimation group

DO concentrations in chronic hypoxia tanks were lowered by 1 mg O_2 l^{-1} day^{-1} from normoxia to hypoxia (1.5±0.3 mg O_2 l^{-1}, 23±1°C). Once hypoxic, the target DO was maintained for 8 weeks using a Point Four oxygen-controlling system (Pentair Aquatic Eco-systems, Langley, BC, Canada), which gently bubbled nitrogen through a diffuser when DO concentrations rose above the target. In each tank, a gentle, steady water flow (<1 cm s^{-1}) over the Galvanic O_2 sensor was maintained throughout each tank for proper mixing for accurate DO readings. Two layers of bubble wrap covered the surface of all tanks to prevent oxygen exchange and maintain water temperature (e.g. Rees et al., 2009).

Chronic normoxia group

Chronic normoxia control fish were maintained in identical conditions to those of the chronic hypoxia acclimation treatment group, except DO concentrations were maintained at normoxic levels via a steady flow of air bubbled through diffusers.

U_{crit} trial structure

Trial A: 8 week acclimation

Immediately following the chronic 8 week hypoxia or normoxia acclimation described above, each fish underwent two U_{crit} trials, one each under normoxic (8.0±0.5 mg O_2 l^{-1}, 23±1°C) and hypoxic (1.5±0.3 mg O_2 l^{-1}, 23±1°C) test conditions (Fig. 1). All individuals underwent their treatment-condition trial first (e.g. chronic hypoxia acclimation fish under hypoxia), and were given a 1 week recovery period at their acclimation condition DO before undergoing their acute exposure trial (e.g. chronic hypoxia acclimation fish under normoxia).

Acute normoxia and hypoxia exposures occurred over a 4 h period in a 38 l tank matched (pH, conductivity, temperature, initial DO concentration) to the individual's home tank. For transfer to the acclimation tank, fish were caught in a net (with no chasing) and placed in a bucket filled with water from their home tank covered in two layers of bubble wrap. Fish were then transferred from the bucket to the acclimation tank using the same net. Travel time in the bucket was approximately 2 min and air exposure during transfer was <10 s. Following a 15 min adjustment period, nitrogen or air was slowly

Table 1. Biometrics of individual *Marcusenius victoriae* in the two acclimation groups

	Normoxia group	Hypoxia group
TL (cm)	12.7 (12.0–14.0)	12.3 (11.3–13.0)
SL (cm)	11.4 (10.5–12.5)	11.1 (10.0–11.5)
Mass (g)	17.8 (12–24)	14.7 (12–16)

Data are means and ranges for total length (TL), standard length (SL) and mass of individuals in each acclimation group.

bubbled into the tank to lower or raise the DO concentration, which was measured manually every 15 min using an OxyGuard Handy Polaris 2 hand-held meter (OxyGuard International A/S, Farum, Denmark) calibrated to room temperature (24±1°C). Gas flow was controlled manually based on DO concentration, and adjusted to achieve an average change of 1.5 mg O_2 l^{-1} h^{-1}. Two layers of bubble wrap covered the surface of the tank to prevent fluctuations in DO concentration during the acute exposure period. Individuals were transferred to the U_{crit} experimental tank once target DO levels were reached (described below).

Trial B: 6 month residual effects study

Following the 8 week acclimation period and trial A, the chronic hypoxia acclimation treatment individuals were gradually returned to normoxia (Fig. 1). The DO concentration was raised 1 mg O_2 l^{-1} day^{-1} in the holding tanks from hypoxia to normoxia. All fish (including the chronic normoxia control group) were then maintained at normoxia for an additional 6 month period, before repeating U_{crit} trials under normoxic and hypoxic test conditions (Fig. 1). Individuals repeated normoxia trials first, had a 1 week recovery period, and then repeated hypoxia trials. All trials and acute hypoxia exposures were conducted as described above.

U_{crit} experimental protocol

Experimental tank

The U_{crit} of each individual was determined via an incremental velocity test in a swim tunnel. The experimental tank consisted of a clear Plexiglas flume (length 70 cm, diameter 8 cm) encircled by black mesh (length 24 cm) to provide some shielding of the fish from external visual stimuli while permitting observation by the experimenter. The inner diameter of the flume walls was over 4 cm larger than the BD of the largest fish tested in this study to maintain microturbulent flow (described below) and prevent turbulent flow as a result of boundary layer effects (Liao, 2007). The head of the flume was fitted tightly with straws, and water was pumped through a rheostat-controlled Leader Ecovort 520A pump (Leader Pumps Inc., Ladson, SC, USA) in order to create a uniform microturbulent flow (Bell and Terhune, 1970). Flow speed was calculated using a Höntzsch vane wheel flow meter (Höntzsch, Waiblingen, Germany) at the experimental temperature (23±1°C). To do this, water velocity was measured directly at the back of the swim tunnel in the centre of the tunnel aperture across 14 rheostat settings. Water velocity was measured three times at each rheostat setting at a constant water temperature for consistency. The three measures were averaged for each rheostat setting and regression analyses were used to determine the appropriate rheostat setting necessary for each relevant swim speed used in trials. Water flow speeds were confirmed before and after each trial (when the fish was no longer in the experimental set-up) using the Höntzsch vane wheel flow meter. Swim tunnel water conditions (pH, conductivity, temperature, applicable DO concentration) matched those of each fish's holding tank and the acute exposure tank. Two layers of bubble wrap covered the surface of the tank to prevent oxygen exchange. Water temperature was monitored closely throughout the trial and adjusted by gently securing bags of ice to the tank under the bubble wrap outside of the workspace and the field of view of the fish.

EOD recordings

One 5 cm carbon rod electrode was fixed at the front and one at the back of the flume out of the way of the water flow to record EOD rate and amplitude before, during and after the trial. The signal was amplified (AC/DC Differential Amplifier, Model 3000, A-M Systems, Carlsborg, WA, USA) and digitized (PCIe-6259, National Instruments, Austin, TX, USA) using MATLAB 2009a (The MathWorks, Natick, MA, USA). Recordings were taken as 1 min long consecutive files that were divided into 50 s long recordings digitized at a sampling rate of 40 kHz and 10 s long recordings digitized at a sampling rate of 100 kHz. The 10 s long, 100 kHz recordings were not intended for this study, and were not included in the subsequent analyses. EOD variables were extracted for analysis using a custom-written MATLAB script for median, minimum and maximum EOD rate (pulses s^{-1}) and coefficient of variation (CV) of pulse interval (i.e. variability in the time between successive EOD pulses). To obtain an average of each variable per 10 min speed increment of the U_{crit} trial, the 10, 50 s long, 40 kHz recordings for each speed increment were averaged across each completed swim speed (i.e. each completed 10 min, 5 cm s^{-1} increment during a U_{crit} swim trial) for analyses.

In principle, the amplitude of the EOD pulses is an interesting parameter that could be affected by DO (Reardon et al., 2011). However, EOD amplitude recordings are affected by the fish's distance from and orientation with respect to the recording electrodes (Hopkins, 1986; Franchina et al., 2001). Because of the length and diameter of the swim tunnel used in this study, the changes to EOD amplitude during the trials probably do not accurately reflect true changes in the amplitude of an individual's EOD, but rather the fish's movement throughout the swim tunnel relative to the fixed electrodes. We therefore excluded analyses of EOD amplitude from the present study.

Trial procedure

Fish were caught in a net (with no chasing) and transferred from the acute exposure tank to the U_{crit} tank, with air exposure during transfer being shorter than 10 s. Fish were given a 1 h recovery period in the flume at a water speed of <2 cm s^{-1} to minimize any potential reversible effects of acclimation (as per Fu et al., 2011). Following the 1 h recovery period, water speed was increased by 5 cm s^{-1} every 10 min (Koumoundouros et al., 2002; Plaut, 2002). Behaviour of the individual, number of turns and semi-turns, and position and swimming direction in the flume were recorded throughout each trial. Fish resting against the black mesh at the end of the flume were gently tapped to induce swimming. An individual was deemed exhausted when they no longer responded to three consecutive gentle taps, at which time the trial was immediately ended. U_{crit} was calculated as:

$$U_{crit} = U_i + U_{ii} \times (T_i/T_{ii}), \quad (1)$$

in which U_i is the last velocity at which the fish swam for the entire 10 min duration of this velocity step, U_{ii} is the velocity increment, T_i is the amount of time (of the highest velocity step) the individual swam before reaching fatigue and T_{ii} is the time interval for each increase in velocity (10 min in our case) (Brett, 1964; Plaut, 2001).

Individuals were measured post-trial for TL, SL, BD and mass, moved back into the acute exposure tank, and returned to their acclimation DO concentration (e.g. chronic hypoxia-acclimated fish to hypoxia) over a 4 h period before being returned to their home tanks. Fish were closely monitored throughout acute hypoxia and normoxia exposure for signs of stress (e.g. aquatic surface respiration; Chapman and Chapman, 1998), which no fish exhibited at any time. All individuals were starved for 24 h before the trials, and the water of the acute exposure tank was changed between individuals.

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Table 2. Effects of normoxia and hypoxia on U_{crit} in *M. victoriae*

Trial	Factor	*F*-value	Partial η^2	*P*-value
A	Swim condition	$F_{1,8}$=103.144	0.928	**<0.0001**
	Acclimation	$F_{1,8}$=8.296	0.509	**0.021**
	Swim condition×acclimation	$F_{1,8}$=1.181	0.129	0.309
B	Swim condition	$F_{1,8}$=54.362	0.872	**<0.0001**
	Acclimation	$F_{1,8}$=10.614	0.6	**0.012**
	Swim condition×acclimation	$F_{1,8}$=7.108	0.470	**0.029**

Repeated-measures two-way ANOVA results for effects of normoxic and hypoxic test conditions on critical swim speed (U_{crit}) between chronic hypoxia- and normoxia-acclimated individuals. Trial A: fish were acclimated to chronic normoxia or hypoxia for 8 weeks before U_{crit} was quantified in both acclimation groups under normoxic and hypoxic test conditions. Trial B: following an 8 week chronic acclimation, all fish were returned to chronic normoxia and held for 6 months before U_{crit} was quantified under normoxic and hypoxic test conditions. Experimental design details are outlined in Fig. 1. Bold values are statistically significant (α=0.05).

Statistical analyses

All analyses were run using SPSS Version 22 (SPSS Inc., Armonk, NY, USA) with α=0.05. All numeric data reported are means±s.e.m. All data met the assumptions of the tests performed, unless stated otherwise. This study aimed to answer the following three major questions regarding U_{crit} performance, electric signalling activity and hypoxia in this species.

How does hypoxia affect U_{crit}?

Repeated-measures (RM) two-way analysis of covariance (ANCOVA) was used to detect differences in U_{crit} between hypoxic and normoxic test conditions (within-subject factor) for fish acclimated to chronic hypoxia or normoxia (between-subject factor), and the effect of TL (covariate) on performance. However, there was no significant interaction between TL (cm) and U_{crit} (cm s^{-1}) within individuals (trial A: $F_{1,7}$=0.702; partial η^2=0.091; *P*=0.430; trial B: $F_{1,7}$=1.101; partial η^2=0.136; *P*=0.329) and no significant effect of TL (cm) on U_{crit} (cm s^{-1}) between treatments (trial A: $F_{1,7}$=4.773; partial η^2=0.405; *P*=0.065; trial B: $F_{1,7}$=0.218; partial η^2=0.030; *P*=0.654). Therefore, TL was removed to simplify the model and RM two-way analysis of variance (ANOVA) was performed. As there were only two groups being tested and *post hoc* Tukey tests were not suitable, subsequent independent-sample *t*-tests were used to detect significant differences between chronic normoxia and hypoxia acclimation within test conditions if there was a significant interaction effect. Trial B (6 month residual effects study) values were $\log_{10}$ transformed before analyses to meet the assumptions of the tests.

How does hypoxia affect electric signalling activity?

RM two-way ANOVA was performed to assess the effects of hypoxia on electric signalling activity as described above. Each EOD variable (minimum, median and maximum EOD rate; CV of pulse interval) was analysed at two swim speeds: the 'baseline' swim speed (the first swim speed: 5 cm s^{-1}) and a 'challenge' swim speed (the highest completed swim speed interval the individual achieved under each test condition). EOD performance during trial A (8 week acclimation) and trial B (6 month residual effects study) was analysed separately. Chronic normoxia-acclimated fish were excluded from the challenge swim speed analyses for trial B as only one of the five individuals tested achieved a speed above the baseline speed. Instead, paired-sample *t*-tests were performed among chronic hypoxia-acclimated fish to compare each EOD variable between normoxic and hypoxic test conditions.

What is the relationship between swim speed and electric signalling activity?

Median, minimum and maximum EOD rates and CV of pulse interval values at baseline and challenge speeds were analysed to determine differences in electric signalling activity at the baseline and challenge swim speeds between trials under normoxic and hypoxic test

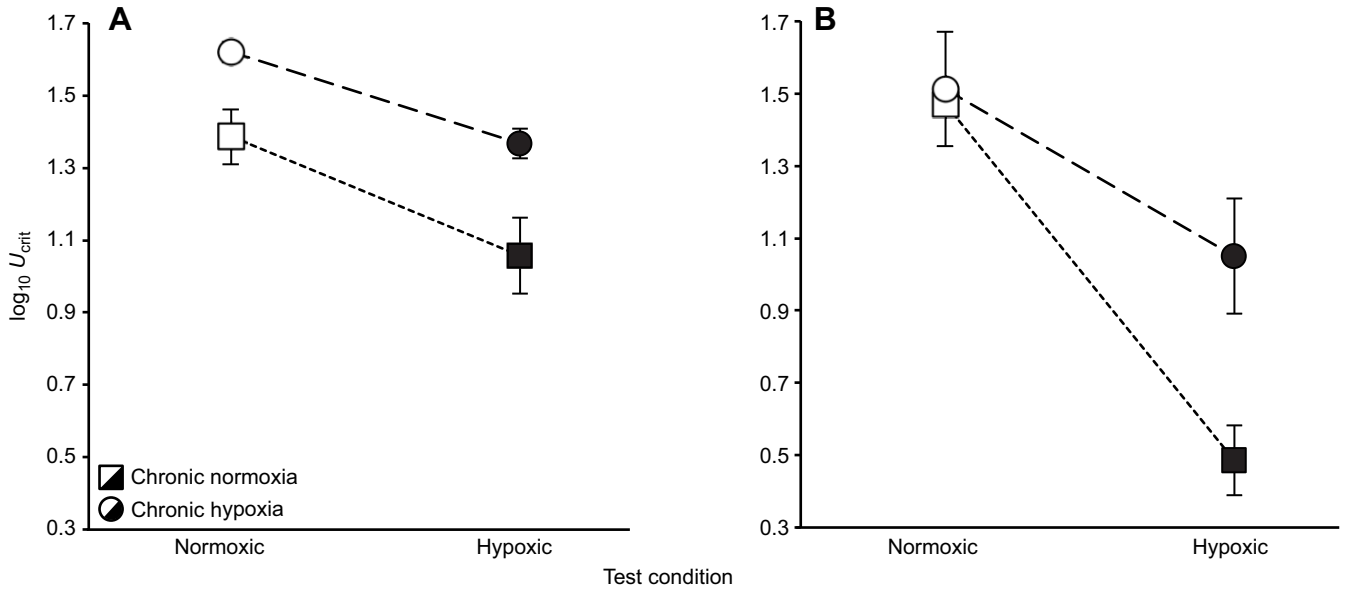

Fig. 2. Effects of chronic normoxia and hypoxia on U_{crit} in *Marcusenius victoriae*. (A) Trial A: U_{crit} results under normoxic and hypoxic test conditions following 8 weeks of acclimation to chronic normoxia or hypoxia. (B) Trial B: U_{crit} results between test conditions after all fish were returned to chronic normoxia and held for 6 months following trial A. Symbols and error bars represent mean±s.e.m. $\log_{10}$ U_{crit} values (cm s^{-1}) for individuals of the indicated acclimation group (*n*=5/condition/acclimation) under normoxic (open symbols) and hypoxic (filled symbols) conditions. $\log_{10}$ values were not used for analysis for trial A, but values were transformed for the figure to facilitate comparisons between trials. Statistical outputs from repeated-measures two-way ANOVA are detailed in Table 2 and Results.

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conditions. Paired-sample *t*-tests were performed to compare each EOD variable at the baseline swim speed and the challenge swim speed among individuals within acclimations for each swim trial. Chronic normoxia-acclimated fish were again excluded from these analyses for the hypoxic test condition of trial B.

RESULTS

How does hypoxia affect U_{crit}?

Trial A: 8 week acclimation

Hypoxic test conditions significantly affected U_{crit} of chronic normoxia- and hypoxia-acclimated individuals, but there was no significant interaction between test condition (hypoxia versus normoxia) and acclimation condition (Table 2, Fig. 2A). All individuals achieved lower U_{crit} under hypoxic test conditions [normoxia acclimated: 13.7±3.3 cm s^{-1}, 1.3±0.3 body lengths (BL) s^{-1}; hypoxia acclimated: 23.4±2.2 cm s^{-1}, 1.9±0.2 BL s^{-1}] compared with normoxic test conditions (normoxia acclimated: 28.3±3.8 cm s^{-1}, 2.3±0.3 BL s^{-1}; hypoxia acclimated: 41.5 ±2.5 cm s^{-1}, 3.5±0.3 BL s^{-1}).

Trial B: 6 month residual effects study

After being returned to normoxic holding conditions for 6 months, fish from both original 8 week acclimation treatments showed a significant effect on U_{crit} of hypoxic test conditions, and there was a significant interaction between test condition and previous acclimation treatment (Table 2, Fig. 2B). U_{crit} of previously normoxia-acclimated fish was lower than that of hypoxia-acclimated fish when tested under hypoxia (d.f.=8, t=−3.046, P=0.016; normoxia acclimated: 3.4±0.8 cm s^{-1}, 0.3±0.1 BL s^{-1}; hypoxia acclimated: 14.2±4.3 cm s^{-1}, 1.1±0.4 BL s^{-1}), but not when tested under normoxia (d.f.=8, t=−0.888, P=0.400; normoxia acclimated: 29.6±0.9 cm s^{-1}, 2.3±0.1 BL s^{-1}; hypoxia acclimated: 33.2±3.3 cm s^{-1}, 2.6±0.2 BL s^{-1}).

How does hypoxia affect electric signalling activity?

Trial A: 8 week acclimation

Test condition significantly affected median EOD rates at both the baseline and challenge swim speeds, but there were no significant effects of acclimation and nor was there an interaction effect (Table 3, Fig. 3A,B). All individuals showed significantly reduced EOD rates under hypoxic test conditions compared with normoxic conditions at both swim speeds (Fig. 3A,B). Test condition significantly affected minimum EOD rates at the challenge swim speed but not at the baseline swim speed (Table 3). All individuals showed reduced minimum EOD rates under hypoxic test conditions compared with normoxic ones (chronic normoxia acclimated: normoxic test condition: 3.3±0.4 pulses s^{-1}; hypoxic test condition: 2.01±0.03 pulses s^{-1}; chronic hypoxia acclimated: normoxic test condition: 5.3±0.7 pulses s^{-1}; hypoxic test condition: 3.2±0.7 pulses s^{-1}). Test condition significantly affected maximum EOD production at both the baseline and challenge speeds, but there were no significant effects of acclimation and nor was there an interaction effect (Table 3, Fig. 3C,D). All individuals showed significantly reduced maximum EOD rates under hypoxic compared with normoxic test conditions at both swim speeds (Fig. 3C,D). There were no significant differences in CV of the pulse interval at the baseline or challenge swim speeds between acclimations or test conditions (Table 3).

Trial B: 6 month residual effect study

Chronic normoxia-acclimated fish were excluded from the challenge swim speed analyses because only one of the five

Table 3. Effects of hypoxia on electric signalling activity in *M. victoriae*

		Trial A						Trial B					
		Baseline swim speed			Challenge swim speed			Baseline swim speed			Challenge swim speed		
Variable	Factor	*F*-value	Partial η^2	*P*-value	*F*-value	Partial η^2	*P*-value	*F*-value	Partial η^2	*P*-value	*t*-value	d.f.	*P*-value
Median EOD rate	Test condition	$F_{1,7}$=15.581	0.690	**0.006**	$F_{1,7}$=24.967	0.781	**0.002**	$F_{1,8}$=35.908	0.818	**<0.0001**	*t*=3.353	3	**0.044**
	Acclimation	$F_{1,7}$=0.001	0.000	0.972	$F_{1,7}$=1.703	0.196	0.233	$F_{1,8}$=1.487	0.156	0.259	–	–	–
	Test condition×acclimation	$F_{1,7}$=0.423	0.057	0.536	$F_{1,7}$=0.480	0.064	0.511	$F_{1,8}$=0.325	0.039	0.584	–	–	–
Minimum EOD rate	Test condition	$F_{1,7}$=4.822	0.408	0.064	$F_{1,7}$=19.211	0.733	**0.003**	$F_{1,8}$=0.250	0.030	0.630	*t*=1.652	3	0.197
	Acclimation	$F_{1,7}$=0.426	0.057	0.535	$F_{1,7}$=4.037	0.366	0.084	$F_{1,8}$=4.428	0.356	0.069	–	–	–
	Test condition×acclimation	$F_{1,7}$=0.459	0.061	0.520	$F_{1,7}$=0.945	0.119	0.363	$F_{1,8}$=0.001	0.000	0.973	–	–	–
Maximum EOD rate	Test condition	$F_{1,7}$=11.418	0.620	**0.012**	$F_{1,7}$=21.124	0.751	**0.002**	$F_{1,8}$=10.027	0.556	**0.013**	*t*=−0.208	3	0.848
	Acclimation	$F_{1,7}$=1.036	0.129	0.343	$F_{1,7}$=0.826	0.106	0.394	$F_{1,8}$=0.191	0.191	0.673	–	–	–
	Test condition×acclimation	$F_{1,7}$=3.645	0.342	0.098	$F_{1,7}$=0.934	0.118	0.366	$F_{1,8}$=3.739	0.319	0.089	–	–	–
CV of pulse interval	Test condition	$F_{1,7}$=0.777	0.100	0.407	$F_{1,7}$=1.984	0.218	0.205	$F_{1,8}$=0.264	0.032	0.621	*t*=−1.385	3	0.260
	Acclimation	$F_{1,7}$=0.027	0.004	0.875	$F_{1,7}$=1.254	0.152	0.300	$F_{1,8}$=4.731	0.372	0.061	–	–	–
	Test condition×acclimation	$F_{1,7}$=0.813	0.104	0.397	$F_{1,7}$=0.152	0.021	0.708	$F_{1,8}$=0.706	0.081	0.425	–	–	–

Repeated-measures two-way ANOVA and paired *t*-test results for effects of normoxic and hypoxic test conditions on electric signalling activity [electric organ discharge (EOD) rate and coefficient of variation (CV) of pulse interval] between chronic hypoxia- and normoxia-acclimated individuals during trials A and B. The hypoxic test condition for trial B for the chronic normoxia acclimation group was excluded from these analyses because only one of the five individuals achieved more than one swim speed during those trials. Experimental design details are outlined in Fig. 1. CV, Bold values are statistically significant (α=0.05).

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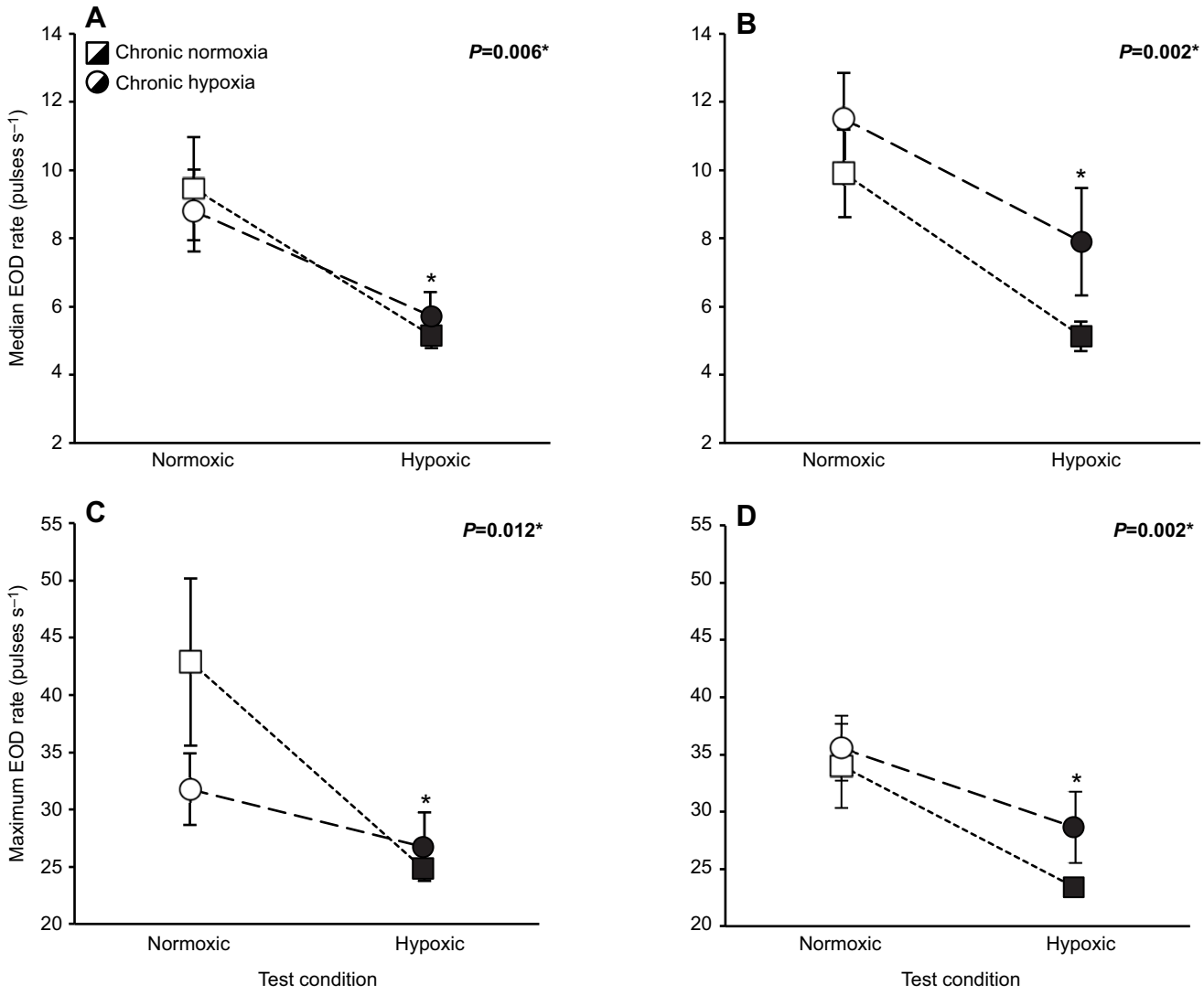

Fig. 3. Average median and maximum electric organ discharge (EOD) rates of *M. victoriae* at the baseline and challenge swim speeds during trial A. Fish (n=5/condition/treatment) were acclimated to chronic normoxia or hypoxia for 8 weeks before electric signalling activity was quantified during U_{crit} in both acclimation groups under normoxic and hypoxic test conditions. Symbols and error bars represent mean±s.e.m. values of median EOD rates at the baseline (A) and challenge (B) swim speeds and maximum EOD rates at the baseline (C) and challenge (D) swim speeds for individuals of the indicated acclimation group under normoxic (open symbols) and hypoxic (filled symbols) test conditions. The baseline swim speed was the first swim speed (5 cm s^{-1}) and the challenge swim speed was the highest completed swim speed interval the individual achieved under each test condition. Statistical outputs from repeated-measures two-way ANOVA are shown here and in Table 3. Bold values represent statistically significant differences; asterisks signify where those significant differences occurred.

individuals completed a challenge swim speed. There was a significant effect of test condition on median EOD rates at both swim speeds, but no effect of previous acclimation or any interaction (Table 3, Fig. 4A,B). All individuals showed reduced median EOD rates under hypoxic versus normoxic test conditions (Fig. 4A,B). There were no significant differences in minimum EOD rates at the baseline or challenge swim speeds between acclimations or test conditions (Table 3). There was a significant effect of test condition on maximum EOD rates at the baseline speed, but there was no significant difference in hypoxia-acclimated fish at the challenge speed (Table 3, Fig. 4C,D). Fish had reduced maximum EOD rates under hypoxic compared with normoxic test conditions (Fig. 4C,D). There were no significant differences in CV of pulse interval at either the baseline or challenge swim speeds between acclimations or test conditions (Table 3).

What is the relationship between swim speed and electric signalling activity?

Trial A: 8 week acclimation

Fish acclimated to chronic hypoxia tested under normoxia had significantly higher median and minimum EOD rates at the challenge swim speed compared with those at the baseline swim speed, while median and maximum EOD rates in chronic normoxia fish did not differ between the two swim speeds when tested under normoxia (Table 4, Fig. 5A,C,E). Chronic normoxia-acclimated fish had significantly reduced maximum EOD rates at the challenge swim speed compared with those at the baseline speed under normoxic test conditions; maximum EOD did not differ between swim speeds in chronic hypoxia-acclimated fish when tested under normoxia (Table 4, Fig. 5E). Neither acclimation group showed significant differences in CV of pulse interval between baseline and challenge speeds under normoxic test conditions (Table 4). There

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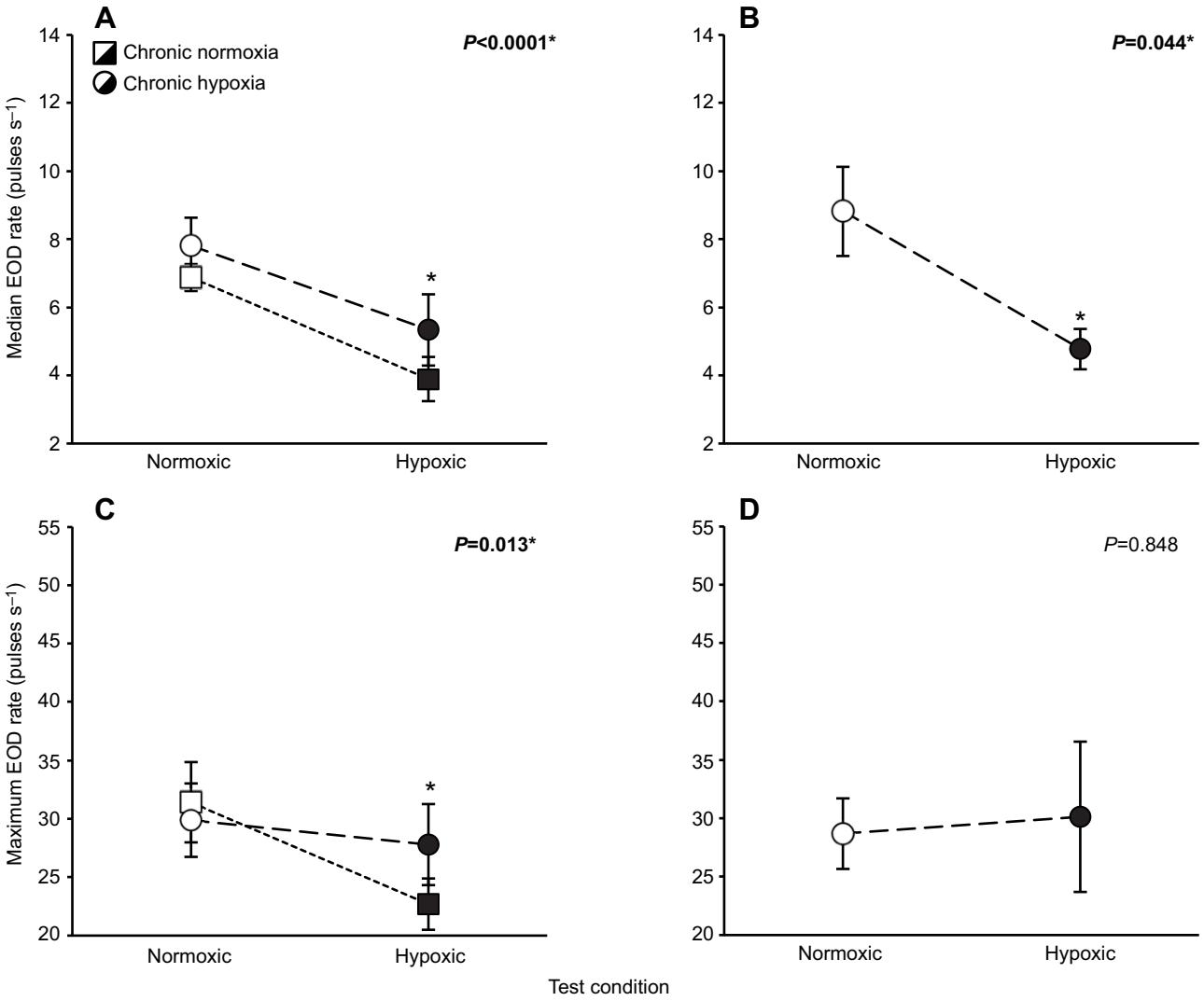

Fig. 4. Average median and maximum EOD rates of *M. victoriae* at the baseline and challenge swim speeds during trial B. All fish (*n*=5/condition/treatment) were returned to chronic normoxia for 6 months following trial A before U_{crit} was again quantified in both acclimation groups under normoxic and hypoxic test conditions. Symbols and error bars represent mean±s.e.m. values of median EOD rates at the baseline (A) and challenge (B) swim speeds and maximum EOD rates at the baseline (C) and challenge (D) swim speeds for individuals of the indicated acclimation group under normoxic (open symbols) and hypoxic (filled symbols) test conditions. Chronic normoxia-acclimated fish were excluded from the challenge speed analyses for trial B as only one of the five individuals tested completed a speed interval above the baseline speed. Statistical outputs from repeated-measures two-way ANOVA and paired-sample *t*-tests are shown here and in Table 3. Bold values represent statistically significant differences; asterisks signify where those significant differences occurred.

were no significant differences in the EOD rate variables (median, minimum, maximum) or CV of pulse interval between the baseline and challenge swim speeds under hypoxic test conditions in either acclimation group (Table 4, Fig. 5B,D,F).

Trial B: 6 month residual effects study

Chronic normoxia-acclimated fish were excluded from the hypoxic test condition analyses because only one of the five individuals completed a challenge swim speed under hypoxic test conditions. There were no significant differences in EOD rates (median, minimum, maximum) or CV of pulse interval between baseline and challenge swim speeds in fish previously acclimated to hypoxia and tested after 6 months under normoxic or hypoxic test conditions (Table 4, Fig. 6A–F). Median and maximum EOD rates also did not differ between swim speeds in fish previously acclimated to normoxia and tested after 6 months under normoxic test conditions, but these individuals did show a significant increase in minimum EOD rate at the challenge swim speed compared with the baseline speed (Table 4, Fig. 6A,C,E). The CV of pulse intervals was also significantly higher at the baseline swim speed than at the challenge swim speed in fish previously acclimated to normoxia (baseline swim speed: mean 1.5±0.3; challenge swim speed: mean 0.7±0.2), but not in fish formerly acclimated to hypoxia (baseline swim speed mean 0.8±0.1; challenge swim speed: mean 0.5±0.04) under normoxic test conditions (Table 4).

DISCUSSION

The overall goal of this study was to characterize the relationship between sustained swimming and the sampling of sensory information in a swamp-dwelling African pulse-type weakly electric fish, *M. victoriae*, under acute and chronic exposure to normoxia and hypoxia. Our results suggest high tolerance to hypoxia in this population of *M. victoriae*, which is consistent with their natural habitat. These fish also showed plasticity in swim performance and

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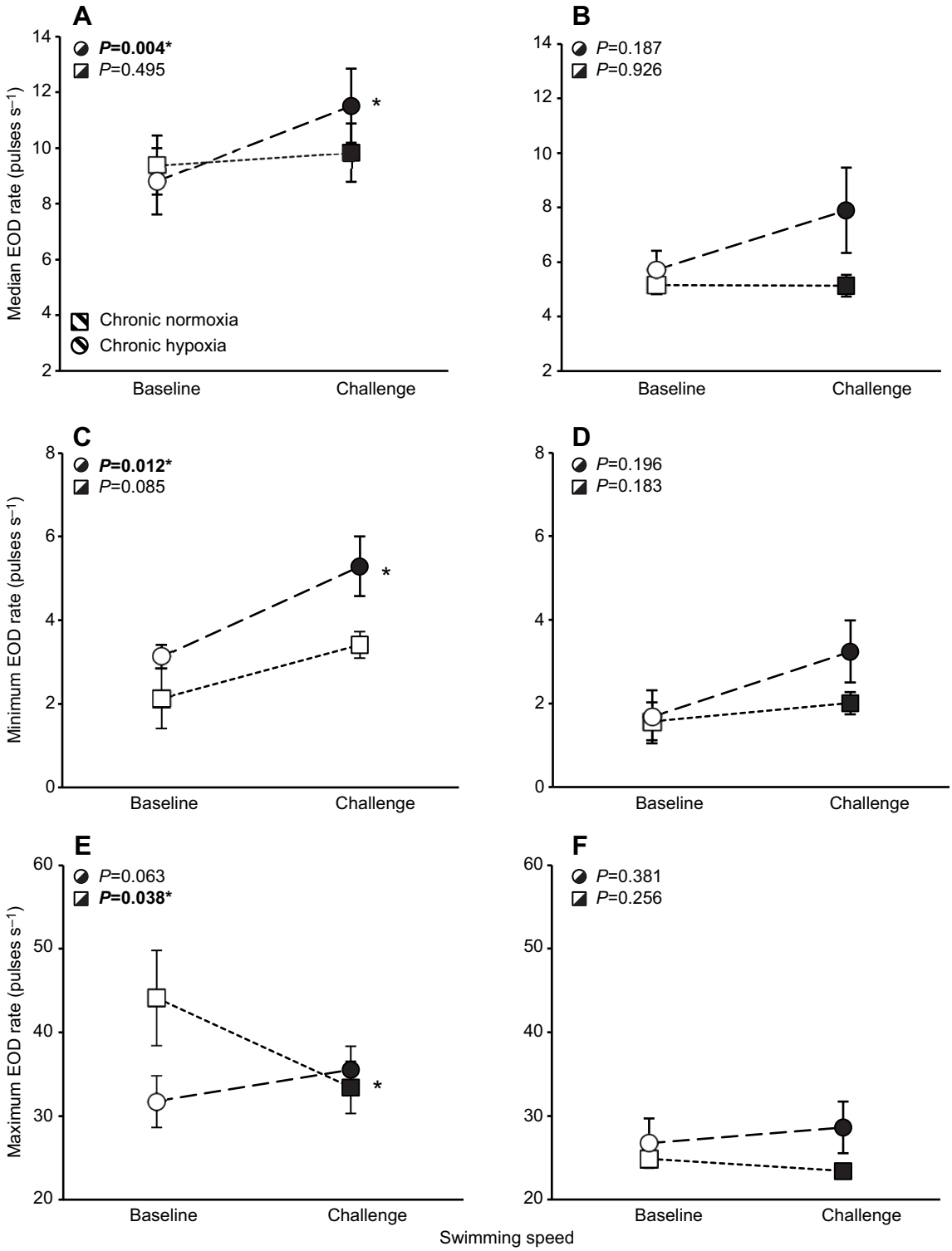

Fig. 5. Relationship between swimming speed and electric signalling activity of *M. victoriae* during trial A. Mean±s.e.m. median (A,B), minimum (C,D) and maximum (E,F) EOD rates between baseline and challenge swim speeds under normoxic (A,C,E) and hypoxic (B,D,F) test conditions during trial A (n=5/condition/treatment). The baseline swim speed was the first swim speed (5 cm s^{-1}) and the challenge swim speed was the highest completed swim speed interval the individual achieved under each test condition. Open symbols indicate the 8 week normoxic acclimation condition; filled symbols indicate the 8 week hypoxic acclimation condition. Statistical outputs from repeated-measures two-way ANOVA are shown here and in Table 4. Bold values represent statistically significant differences; asterisks signify where those significant differences occurred.

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Table 4. Relationships between swim speed and electric signalling activity during U_{crit} performance in *M. victoriae*

	Normoxic test condition						Hypoxic test condition					
	Normoxia acclimated			Hypoxia acclimated			Normoxia acclimated			Hypoxia acclimated		
Response variable	*t*-value	d.f.	*P*-value	*t*-value	d.f.	*P*-value	*t*-value	d.f.	*P*-value	*t*-value	d.f.	*P*-value
Trial A												
Median EOD rate	–0.749	4	0.495	–6.127	4	**0.004**	0.100	3	0.926	–1.589	4	0.187
Minimum EOD rate	–2.274	4	0.085	–4.379	4	**0.012**	–1.726	3	0.183	–1.551	4	0.196
Maximum EOD rate	3.053	4	**0.038**	–2.561	4	0.063	1.400	3	0.256	–0.984	4	0.381
CV of pulse interval	1.918	4	0.128	2.602	4	0.060	1.661	3	0.195	1.073	4	0.344
Trial B												
Median EOD rate	–2.719	4	0.053	–1.951	4	0.123	–	–	–	0.183	3	0.867
Minimum EOD rate	–5.204	4	**0.006**	–2.639	4	0.058	–	–	–	0.510	3	0.645
Maximum EOD rate	0.482	4	0.655	–1.035	4	0.359	–	–	–	–1.024	3	0.381
CV of pulse interval	4.841	4	**0.008**	2.614	4	0.059	–	–	–	–0.997	3	0.392

Paired *t*-test results for electric signalling activity quantified during the baseline and challenge swim speeds during U_{crit} trials for trials A and B. The hypoxic test condition for trial B for the chronic normoxia acclimation group was excluded from these analyses because only one of the five individuals achieved more than one swim speed during those trials. Experimental design details are outlined in Fig. 1. Bold values are statistically significant (α=0.05).

electric signalling activity depending on their acclimation to chronic hypoxia versus normoxia.

How does hypoxia affect U_{crit}?

As predicted, we found that hypoxic test conditions limited sustained swim performance of *M. victoriae*, a pattern consistent with findings in other non-electric fish species including rainbow trout (*Salmo gairdneri*; Jones, 1971), Atlantic cod (*Gadus morhua*; Herbert and Steffensen, 2005), goldfish (*Carassius auratus*; Smit, 1965; Fu et al., 2011), silver perch (*Bairdiella chrysoura*; Hanke and Smith, 2011) and mulloway (*Argyrosomus japonicus*; Fitzgibbon et al., 2007). All of these species, including *M. victoriae*, show significantly reduced swimming abilities under hypoxic conditions, presumably because of the high metabolic cost of aerobically powered swimming and the physiological challenge of hypoxia. However, we also found that *M. victoriae* acclimated to an 8 week period of chronic hypoxia achieved significantly higher U_{crit} under both normoxic and hypoxic test conditions than fish acclimated to chronic normoxia. Differences in U_{crit} were limited to hypoxic test conditions following 6 months re-acclimation to normoxia. Our results partially support our prediction that chronic exposure to hypoxia offsets some of the limiting effects of hypoxia on swimming. These data also show residual benefits of chronic hypoxia acclimation for future hypoxia exposure, but that it can be altered by long-term re-acclimation to normoxia.

The differences in performance and hypoxia tolerance we observed are likely to reflect plastic morphological, physiological and/or biochemical changes that occurred over the 8 week chronic hypoxia acclimation period, some of which are maintained for several months after the exposure. Previous studies have shown increased hypoxia tolerance and swimming performance in fishes exposed to chronic hypoxia as a result of changes such as transient gill remodelling, increased haematocrit, haemoglobin with higher O_2 binding affinity, increased anaerobic capacity and increased cardiac output (e.g. Chipari-Gomes et al., 2005; Petersen and Gamperl, 2010; Fu et al., 2011). Our data do not offer specific insights into the mechanisms underlying the observed responses; however, in a comparison of *M. victoriae* from the Lwamunda Swamp (hypoxic) and nearby Lake Kayanja (normoxic), Chapman and Hulen (2001) found larger gill surface area in swamp-dwelling fish, which could be a mechanism to compensate for the physiological limitations of hypoxia, though the source of the inter-populational variation observed in that study (plastic versus genetic) was not addressed.

Changes to the anaerobic metabolic capabilities between normoxia- and hypoxia-acclimated *M. victoriae* may also have contributed to the differences seen in U_{crit} performance. As U_{crit} performance encompasses both aerobic and anaerobic metabolism (e.g. Wilson and Egginton, 1994), an individual's anaerobic metabolism can significantly influence performance, especially at swimming speeds approaching U_{crit} when there is typically more anaerobic contribution to power performance (e.g. Svendsen et al., 2010). Anaerobic activity can be estimated using the non-invasive measure of excess post-exercise oxygen consumption (EPOC), which measures an individual's rate of oxygen consumption in the time frame during which excess waste (e.g. lactate) resulting from anaerobic metabolism is cleared from the body (e.g. Peake and Farrell, 2004; Svendsen et al., 2010). As individuals need more oxygen to recover from anaerobic activity, recovery from U_{crit} can take significantly longer under hypoxic conditions (e.g. Svendsen et al., 2011). We did not measure the rate of oxygen consumption during our swim performance trials; however, future studies should include EPOC measures to determine whether there is a difference in the use of anaerobic metabolism that may account for the higher U_{crit} capabilities of hypoxia-acclimated fish under normoxic test conditions and to determine how acclimation condition impacts recovery from exhaustive exercise.

Oxidative stress may also play an important role in determining the performance of individuals following normoxia exposure, although previous research suggests that hypoxia-tolerant fish may have mechanisms that lessen oxidative stress following normoxia exposure (e.g. Lushchak et al., 2001). In addition, long-term exposure to hypoxia may also result in lower levels of oxidative stress compared with normoxic conditions (e.g. Joyner-Matos et al., 2011; Joyner-Matos and Chapman, 2013). Although we did not quantify oxidative stress in this study, our results do not suggest that the individuals measured here experienced detrimental oxidative stress, as fish acclimated to chronic hypoxia achieved the highest performance under acute normoxia. Additionally, if these individuals did experience oxidative stress that negatively affected performance under normoxia, we might be underestimating the aerobic performance of these individuals.

How does hypoxia affect electric signalling activity?

In this population of *M. victoriae*, initial estimates showed that the electrosense can cost up to 20% of RMR, which suggests that oxygen availability could limit EOD production (T. M. Moulton, Krahe and L.J.C., unpublished observations). A sizable metabolic

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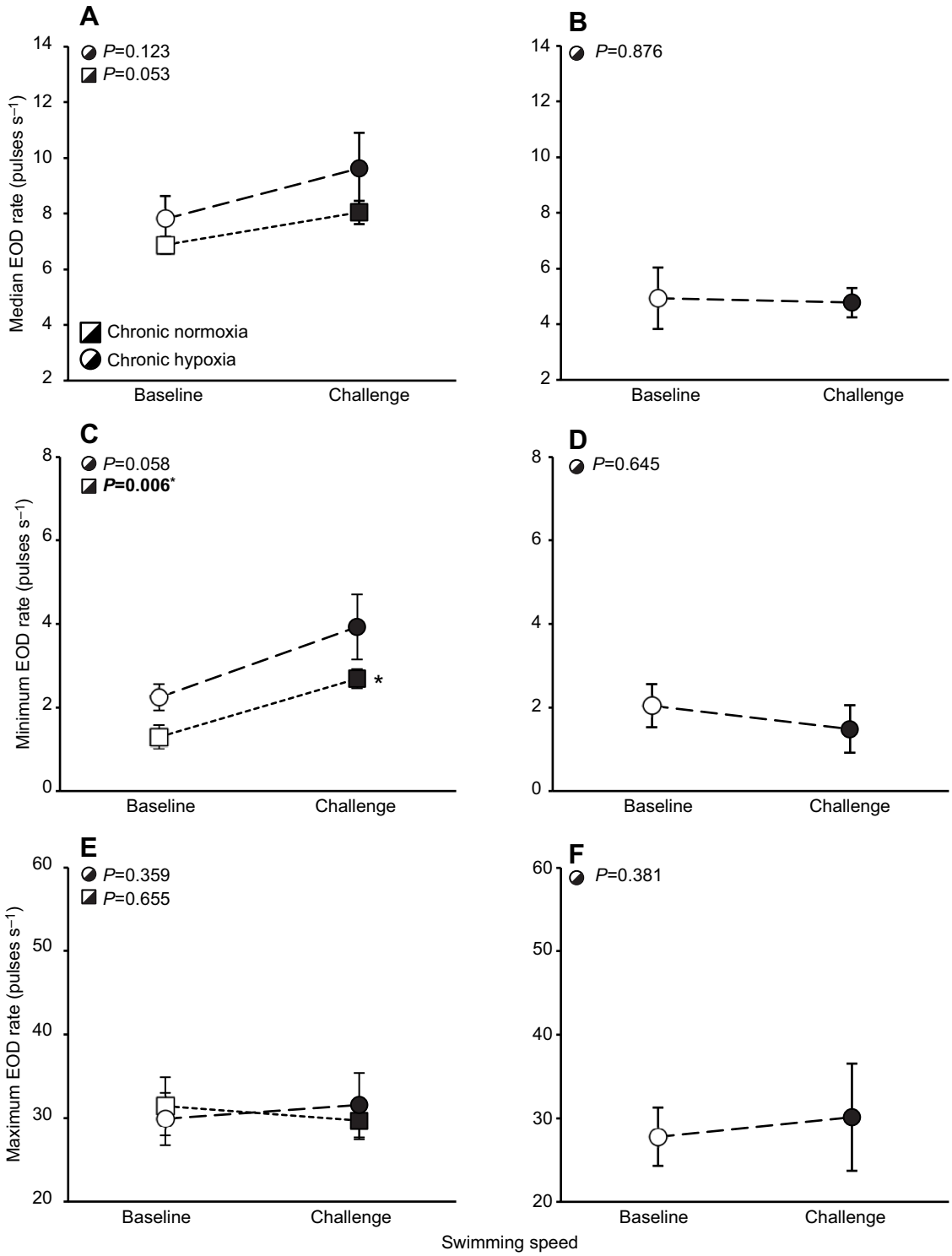

Fig. 6. Relationship between swimming speed and electric signalling activity of *M. victoriae* during trial B. Mean±s.e.m. median (A,B), minimum (C,D) and maximum (E,F) EOD rates between baseline and challenge swim speeds under normoxic (A,C,E) and hypoxic (B,D,F) test conditions during trial B (n=5/condition/treatment). Chronic normoxia-acclimated fish were excluded from the challenge speed analyses for trial B as only one of the five individuals tested completed a speed interval above the baseline speed. Open symbols indicate the 8 week normoxic acclimation condition; filled symbols indicate the 8 week hypoxic acclimation condition. Statistical outputs from repeated-measures two-way ANOVA and paired t-tests are shown here and in Table 4. Bold values represent statistically significant differences; asterisks signify where those significant differences occurred.

cost of EOD production has also been demonstrated for South American pulse-type weakly electric fish, a cost that increases with the amplitude of the EOD pulses and the rate at which they are generated (Salazar and Stoddard, 2008). Our results showed significant decreases in median and maximum EOD rates under hypoxic test conditions, regardless of acclimation. These results support our prediction that electric signalling activity would be reduced under hypoxic conditions; however, we did not find that chronic hypoxia acclimation mitigated the negative effects of acute exposure to hypoxia on signalling activity. All fish, regardless of acclimation, showed similarly reduced electric signalling activity under hypoxic test conditions at both the baseline and challenge swim speeds.

Other studies on weakly electric fishes have also reported effects of hypoxia on electric signalling activity. Reardon et al. (2011) found reductions in EOD amplitude in two wave-type South American weakly electric fishes (*Apteronotus leptorhynchus* and *Eigenmannia virescens*) under acute hypoxic stress, which could be a strategy to reduce energetic costs. Reardon et al. (2011) also found that the DO level at which the EOD amplitude decreased was related to the hypoxia tolerance of the species, such that the more hypoxia-tolerant *E. virescens* started to lower EOD amplitude at DO levels well above their P_{crit}, whereas the EOD amplitude of the less hypoxia-tolerant *A. leptorhynchus* dropped abruptly around their P_{crit}, suggesting that responses to hypoxic stress are species dependent. Sukhum et al. (2016) found that reduction of EOD rates under hypoxia is a common mechanism used among mormyrids to temporarily lower the energetic cost of the electrosensory system and showed that the DO level at which mormyrids lowered their EOD rates in response to hypoxic conditions was reflective of each species' hypoxia tolerance.

Reduction of sensory performance under severe hypoxia is not unique to weakly electric fishes, and has also been shown in non-electric fish species including crucian carp (*Carassius carassius*) and snapper (*Pagrus auratus*) (Johansson et al., 1997; Robinson et al., 2013). For example, Robinson et al. (2013) showed reduced visual acuity (ability to distinguish fine details) in snapper at DO levels approaching the species' P_{crit}. While these results suggest impaired vision in snapper under severe hypoxia, more direct quantification of visual system energetics in free-swimming fishes under hypoxia is exceptionally difficult.

Although our EOD rate reduction results are consistent with previous findings in other species, we did not see any significant differences in EOD rates under hypoxic test conditions between acclimation treatments. While a reduction in EOD rates could temporarily reduce an individual's energetic costs under hypoxic conditions, it comes at the cost of reduced sensory sampling of the environment and might affect the detection of food or approaching predators. Thus, it is conceivable that the reduced EOD rates under hypoxic test conditions approach a limit at which the loss of information becomes overly costly.

What is the relationship between swim speed and electric signalling activity?

In addition to determining how hypoxia affected EOD production in *M. victoriae*, we were also interested in investigating the relationship between swimming speed and electric signalling activity, as the metabolic cost of electrosensory information acquisition could compete with the metabolic cost of swimming, especially at high speeds and at low DO levels. These relationships are difficult to quantify, as most fishes rely on sensory systems that do not allow for non-invasive quantification of the capacity for the acquisition of sensory information. Therefore, our study is among the first to quantify effects of hypoxia on concurrent sensory information acquisition and sustained swimming performance.

A previous study in *Apteronotus albifrons*, a South American wave-type species, investigated trade-offs between swimming and sensing when individuals are hunting for prey items (MacIver et al., 2010). MacIver et al. (2010) showed that *A. albifrons* holds the body at an angle with a higher energetic cost of swimming when trying to catch prey and suggest that the added energy expenditure for swimming is offset by increased prey capture resulting from an optimized use of their electric field. In addition, previous work on *Gymnotus carapo*, a South American pulse-type weakly electric fish, showed consistently increased EOD rates during swimming compared with resting (Black-Cleworth, 1970), but the energetic relationship between swimming and increased EOD production in pulse-type fishes has not been investigated to our knowledge. Therefore, as pulse-type weakly electric fish, including *M. victoriae*, actively produce and modulate the rate of their electric signals to collect information about their environments, we predicted that fish would increase EOD rates with increasing water speeds to adjust for the expected faster relative movement of prey items. However, at swimming speeds approaching U_{crit}, correspondingly high EOD rates might become energetically too expensive, especially under hypoxia, leading to a levelling-off of EOD rate.

Our results showed variation in responses of EOD rates to swimming speeds in *M. victoriae*, which were limited to normoxic test conditions and differed between acclimations. Immediately following acclimation, individuals acclimated to chronic hypoxia had significantly increased median and minimum EOD rates at the challenge swim speed compared with the baseline swim speed under normoxic test conditions. However, this significant increase was not seen in chronically normoxia-acclimated fish, which exhibited a reduction in maximum EOD rates under normoxic test conditions. Under hypoxic test conditions, all individuals produced fewer pulses per second compared with normoxic test conditions and did not significantly increase or decrease EOD rates across swimming speeds. There were also no significant changes to pulse production regularity (CV of pulse intervals) under either test condition, which did not support our prediction that pulse production could become more regular with increased speeds.

Following the 6 month return to chronic normoxia, all fish (regardless of previous acclimation condition) achieved the same U_{crit} under normoxic test conditions. All fish also showed slight increases in minimum and median EOD rates at the challenge swim speed compared with the baseline swim speed and unchanged maximum EOD rates between swim speeds under normoxic test conditions. Interestingly, normoxia-acclimated fish had a higher CV of pulse interval (i.e. a more irregular pulse rate) at the baseline speed under normoxic test conditions in this set of trials, which was not seen in any individuals in post-acclimation trials. These results support our original prediction that pulse rate would become more regular with increased swimming speed. However, as this more irregular pulse rate at the baseline swim speed was highly variable and inconsistent among individuals and between trials, further work is needed to assess the causes and significance of these findings.

Although we were unable to compare EOD rates between acclimation treatments under hypoxic test conditions after the 6 month re-exposure to normoxia, fish previously acclimated to chronic hypoxia still showed greater hypoxia tolerance and achieved significantly higher U_{crit} under hypoxic test conditions when compared with previously normoxia-acclimated fish. However, previously hypoxia-acclimated fish showed an overall

reduced EOD rate under hypoxic test conditions that was similar across all swimming speeds.

Overall, our results suggest a possible trade-off between maintaining swimming and EOD rates when DO concentrations are limiting, even in individuals with increased aerobic capabilities. While the residual effects of the morphological or physiological changes from the 8 week hypoxia acclimation still resulted in increased hypoxia tolerance among these individuals, they no longer had the aerobic performance to support increased EOD rates at swimming speeds approaching U_{crit} under normoxic or hypoxic test conditions. These results suggest that the energetic cost of fast sensory sampling of the environment imposed by a fast flow regime may be a constraining factor for habitat choice.

The flexibility of hypoxia tolerance and aerobic performance

As previously stated, the *M. victoriae* population used in this study lives under extreme hypoxia in the wild and is typically found at DO concentrations ∼1.0 mg O_2 l^{-1} (Chapman and Chapman, 1998; Chapman and Hulen, 2001; Moulton, 2016). As a result, this population is naturally hypoxia tolerant and is expected to maintain aerobic functions even at low DO levels (Chapman and Chapman, 1998). However, our findings highlight a significant decrease in hypoxia tolerance and performance capabilities in this population after transfer to chronic normoxia. We also demonstrate that re-exposure to chronic hypoxia increased an individual's ability to cope with future periods of hypoxia. As hypoxia is occurring more frequently and for longer periods in both freshwater and saltwater throughout the world (Beadle and Lind, 1960; Sabo et al., 1999; Diaz, 2001), understanding the flexibility of hypoxia tolerance in these fishes may be relevant in predicting mechanisms for adaptation in response to this pervasive aquatic stressor.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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