

# 1 **The weakly electric fish, *Apteronotus albifrons*, avoids hypoxia before it reaches critical levels**

2 Running title: *Apteronotus albifrons* avoids hypoxia

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9 shuttle-box choice chamber

## 10 **Summary**

11 The weakly electric knifefish, *Apteronotus albifrons*, avoids hypoxia below 22% air saturation. Avoidance  
12 correlates with increased swimming activity, but not with a change in electric organ discharge frequency.

## 13 **Abstract**

14 Anthropogenic environmental degradation has led to an increase in the frequency and prevalence of  
15 aquatic hypoxia (low dissolved-oxygen concentration, DO), which may affect habitat quality for water-  
16 breathing fishes. The weakly electric black ghost knifefish, *Apteronotus albifrons*, is typically found in  
17 well-oxygenated freshwater habitats in South America. Using a shuttle-box design, we exposed juvenile  
18 *A. albifrons* to a stepwise decline in DO from normoxia (>95% air saturation) to extreme hypoxia (10% air  
19 saturation) in one compartment and chronic normoxia in the other. Below 22% air saturation, *A.*  
20 *albifrons* actively avoided the hypoxic compartment. Hypoxia avoidance was correlated with upregulated  
21 swimming activity. Following avoidance, fish regularly ventured back briefly into deep hypoxia. Hypoxia  
22 did not affect the frequency of their electric organ discharges. Our results show that *A. albifrons* is able  
23 to sense hypoxia at non-lethal levels and uses active avoidance to mitigate its adverse effects.

## 24 **1. Introduction**

25 All water-breathing fishes depend on dissolved oxygen (DO) for their long-term survival (Kramer, 1984).  
26 In many aquatic ecosystems, DO concentration fluctuates naturally and can reach critically low levels due

27 to water stratification and high temperatures as well as biological decomposition and respiration  
28 processes (Diaz, 2001; Graham, 1990; Kramer, 1984), a condition called aquatic hypoxia. Natural hypoxia  
29 is particularly widespread in tropical freshwaters where high water temperature elevates organic  
30 decomposition and reduces oxygen solubility. In addition, recent anthropogenic influences such as global  
31 climate change and eutrophication of water bodies have led to an increase of frequency and severity of  
32 hypoxic events in oceanic and coastal regions (Breitburg et al., 2018; 2018; Diaz and Rosenberg, 2008;  
33 Goldberg, 1995; Pörtner, 2001; Pörtner and Knust, 2007; Pörtner and Peck, 2010; Schmidtko et al., 2017)  
34 as well as freshwater lacustrine systems (Jenny et al., 2016a; Jenny et al., 2016b). Many fishes respond to  
35 hypoxia by migrating to better oxygenated habitats if those are available (Bell and Eggleston, 2005;  
36 Brown et al., 2015; Crampton, 1998; Pihl et al., 1991). Such avoidance behaviour can provide individuals  
37 with the flexibility to mitigate hypoxic stress without the immediate need for physiological or  
38 biochemical adjustments, though this is largely speculative. Furthermore, not all fish species show active  
39 avoidance behaviour (Cook et al., 2011), and some hypoxia-tolerant species even actively seek hypoxic  
40 zones as refuges from predators (Anjos et al., 2008; Chapman et al., 2002; Vejřík et al., 2016). To  
41 broaden our understanding of hypoxia avoidance behaviour in fish we subjected a Neotropical weakly  
42 electric fish to oxygen choice experiments. We selected a species that is reported as sensitive to hypoxia  
43 and therefore likely to exhibit active avoidance behaviour. To our knowledge, this is the first study of a  
44 weakly electric fish in a behavioural hypoxia avoidance experiment.

45 The black ghost knifefish, *Apteronotus albifrons*, belongs to the gymnotiform weakly electric fishes, a  
46 group that constitutes a major food web component in many floodplains of the Amazon and Orinoco  
47 basins (Crampton, 1996; Lundberg et al., 1987). Weakly electric fish generate an electric field around  
48 their body by discharging a specialized electric organ. *Apteronotus albifrons* produces wave-type electric  
49 organ discharges (EODs): continuous, quasi-sinusoidal EODs with frequencies between 800 and 1200 Hz  
50 (Crampton and Albert, 2006; Hopkins, 1976). By sensing perturbations of the electric field, weakly  
51 electric fish are able to navigate and locate objects in dark and turbid waters (Lissmann and Machin,  
52 1958) and communicate with conspecifics (Heiligenberg, 1989). Their EODs are easy to measure, which  
53 makes them particularly well suited to research on the energetics of sensation and communication (e.g.  
54 Julian et al., 2003; Markham et al., 2016; Moulton et al., 2020; Reardon et al., 2011; Salazar et al., 2013).  
55 As apteronotid fish naturally occur in well oxygenated habitats (Crampton, 1998), it has been suggested,  
56 that they are not able to tolerate hypoxia. The aim of our study was to find out how hypoxia affects the  
57 swimming behaviour and the active electric sense of *A. albifrons*. We quantified swimming behaviour  
58 and EOD frequency while exposing fish to progressive hypoxia in a shuttle-box choice chamber and

59 offering a normoxic refuge at all times. We hypothesized that *A. albifrons* will begin to avoid hypoxia at  
60 moderate DO levels as part of their natural respiratory strategy. Based on a study of the closely related  
61 brown ghost knifefish, *Apteronotus leptorhynchus*, which only found a small decrease in EOD frequency  
62 under hypoxic stress (Reardon et al., 2011), we hypothesized that *A. albifrons* will not modulate their  
63 EOD frequency while experiencing hypoxia.

## 64 **2. Materials and Methods**

### 65 *Experimental animals and housing conditions*

66 We used farm-bred *Apteronotus albifrons* (Linnaeus, 1766) obtained from a commercial supplier  
67 (AQUALity Tropical Fish Wholesale, Inc., Mississauga, Ontario, Canada). Experiments were performed  
68 with 16 individuals with a mean body mass of 3 g (range: 1.7 – 4.2 g), a mean standard body length (SBL)  
69 of 8.9 cm (range: 7.6 – 10.4 cm), and an electric organ discharge (EOD) frequency of 807 – 1151 Hz at  
70 26°C. Sexually mature *A. albifrons* typically have a SBL of 14 – 30 cm and a body mass of at least 20 g  
71 (Dunlap and Larkins-Ford, 2003; Nelson and MacIver, 1999; Serrano-Fernández, 2003). Thus, it is likely  
72 that most, if not all, of our experimental animals were sexually immature, and we did not distinguish fish  
73 by sex for data analysis. This was confirmed by gonadal inspection in one case. Fish were housed in tanks  
74 of 75 L in groups of 3 – 4 individuals per tank. Individual fish were separated with plastic mesh tank  
75 dividers, and each fish had access to one PVC tube as shelter. The water temperature averaged 25.7°C  
76 (range: 25.4 - 25.9°C), conductivity 200  $\mu$ S (190 - 210  $\mu$ S), and pH 7.1 (6.8 – 7.3). Normoxic air saturation  
77 levels (>95%) were maintained by bubbling air into the tanks. Fish were kept at a 12:12 h light:dark  
78 photoperiod and were fed daily a small amount of frozen bloodworms (chironomid larvae, Hikari Sales  
79 USA, Inc., Hayward, California, USA). Controlled conditions were maintained for a minimum of two  
80 weeks before the start of experiments. All procedures were approved by the McGill University Animal  
81 Care Committee (protocol # 5408).

### 82 *Hypoxia avoidance setup*

83 We used a shuttle-box dissolved oxygen choice chamber (Loligo Systems Inc., Denmark) to quantify  
84 hypoxia avoidance behaviour (Fig. 1). The choice chamber consisted of two circular compartments (each  
85 50 cm in diameter) connected by a central passage (W = 8.5 cm, L = 14 cm). A PVC tube (L = 15 cm, inner  
86 diameter 2.6 cm) was placed symmetrically in both compartments as shelter to minimize stress and to  
87 reduce arbitrary swimming activity. The two compartments received water from separate buffer tanks  
88 where the dissolved oxygen (DO) was controlled by bubbling air or nitrogen gas into the water. DO was

89 measured before the water entered the compartments with a galvanic oxygen probe (MINI-DO, Loligo  
90 Systems). Water exchange between choice chamber and buffer tanks was maintained with aquarium  
91 pumps (Universal Pumpe 1048, EHEIM GmbH & Co. KG, Germany). Water temperature was maintained  
92 via silicon rubber heating mats (OMEGA Engineering, Inc., USA) that were wrapped around the buffer  
93 tanks and controlled by a thermostat (Inkbird Tech C.L., China) with a submerged temperature sensor  
94 placed in the passage between the compartments. Circular acrylic glass lids were submerged in the  
95 choice chamber ca. 1 cm below the water surface to reduce the diffusion of atmospheric oxygen into the  
96 water and to prevent fish from accessing the surface during trials.

97 Fish position was recorded with a camera (UI-1640-SE-C-GL, IDS Imaging Development Systems GmbH,  
98 Germany) mounted above the shuttle-box. ShuttleSoft software (ver. 2.6.4, Loligo Systems) was used to  
99 log fish position and DO and to control air saturation in the buffer tanks via a DAQ-M device (Loligo  
100 Systems Inc., Viborg, Denmark), which operated solenoid valves at the gas tubing. EODs were measured  
101 via submerged carbon rod electrodes in the choice chamber. Two electrodes were placed in each  
102 compartment near the PVC tube that served as a shelter, and one grounding electrode was placed in the  
103 passage between the compartments. The choice chamber was set up in an isolated room to minimize  
104 disturbance.

#### 105 *Hypoxia avoidance trials*

106 Trials were conducted at water parameters resembling housing conditions (conductivity of 200  $\mu$ S and  
107 pH of 6.9 – 7.3) with a total water volume of 60 L. Due to varying room temperatures, water  
108 temperature at the initiation of the trials varied between 25.4 and 25.9°C. During trials, temperature  
109 decreased on average by 0.15°C (0–0.4°C) due to room ventilation.

110 Fish were fasted for 36 h prior to experiments to ensure a post-absorptive state. For each trial, one fish  
111 was introduced into the choice chamber in the afternoon and left for 16 h to acclimate overnight. The  
112 side of introduction was chosen randomly, and the fish could freely shuttle between both compartments  
113 throughout the experiment. Water was aerated until the start of trials, and measurement devices were  
114 calibrated to 100% air saturation before each trial. Trials started in the morning at 9:30 h (30 min after  
115 the onset of the light photoperiod). Both compartments were maintained at >95% air saturation for 40  
116 min to record baseline behaviour at normoxia. Each of the 16 fish exhibited a pronounced preference for  
117 one of the two compartments during baseline controls. We subsequently induced stepwise hypoxia in  
118 the compartment of the choice chamber where the fish preferred to stay while maintaining water in the  
119 non-preferred compartment at high DO levels (> 80% air saturation). DO concentration was

120 incrementally lowered to the following air saturation levels: 70%, 50%, 30%, 25%, 20%, 15%, and 10%.  
121 Each DO concentration was maintained for 10 min followed by a 10 min decrease to the next lower  
122 concentration (Fig. 2A). After the lowest DO concentration was reached, the hypoxic compartment was  
123 reoxygenated, and data acquisition was continued for 20 min. The total trial duration (baseline + hypoxia  
124 induction + reoxygenation) was 200 min. Upon completion of a trial, the fish was weighed and its SBL  
125 measured.

#### 126 *Data acquisition and processing*

127 During each trial, the fish position was tracked from above based on image contrast. X and Y coordinates,  
128 distance moved (cm), swimming velocity ( $\text{cm s}^{-1}$ ), and air saturation (%) were logged every second. The  
129 log file was processed with Microsoft Excel®2010 (Microsoft Corp., Redmond, Washington, USA) and R  
130 (ver. 3.2.5, <https://www.r-project.org>). Electrical EOD recordings were band-pass filtered (300 Hz – 5  
131 kHz) and amplified (1000x gain, A-M Systems Model 1700, USA). Signals were then digitized with a  
132 sample rate of 20 kHz (National Instruments USB-6211, USA) and saved on a computer using custom-  
133 written Matlab programs (The MathWorks, Inc., USA).

#### 134 *Statistical analyses*

135 All statistical analyses were performed with R (ver. 3.2.5, <https://www.r-project.org>). Raw data from  
136 video tracking and processed datasets used for statistical analyses are available online (uploaded to  
137 figshare repository, link will be made available upon acceptance).

#### 138 *Side preference during normoxic baseline recordings*

139 Side preference was tested with a two-sided single-sample Wilcoxon rank-sum test on residence time in  
140 the preferred compartment against the null hypothesis that fish would spend 50% of the time in each of  
141 the compartments (= no preference). As fish did not tend to rest in the passage between compartments,  
142 we ignored this possibility for this test.

#### 143 *Swimming behaviour*

144 Residence time in hypoxia (% of time spent in the hypoxic compartment), average swimming speed  
145 (body lengths per second,  $\text{BL s}^{-1}$ ), and distance moved (m) were summarized as medians over the 40 min  
146 normoxic baseline period and each following 10 min interval of the trial.

147 Residence time in the hypoxic compartment, swimming speed and distance were tested for significant  
148 changes throughout the trial using Friedman's rank-sum test with experimental time as independent  
149 variable. In case of a significant result, this was followed by pairwise Wilcoxon rank-sum tests with Holm-  
150 Bonferroni correction of p-values to identify the experimental time at which a significant deviation from  
151 normoxic baseline recordings occurred.

152 Due to water exchange between the choice chamber and buffer tanks, there was a constant circular  
153 water current in the compartments. Swimming speed and distance were not corrected for water current;  
154 rather, these metrics are used to indicate changes of swimming activity, such as stationary behaviour vs.  
155 exploration/avoidance.

### 156 *Electric organ discharges*

157 EOD frequency was extracted from recordings using custom written routines in Matlab R2017a (The  
158 MathWorks, Inc., Natick, Massachusetts). Recorded signals were Fourier-transformed, and the frequency  
159 with the highest power spectral density estimate (frequency resolution 0.076 Hz) was picked as the EOD  
160 frequency for every second of the recording. Median EOD frequency over the 40 min baseline period and  
161 each following 10 min interval of the trial was calculated for each fish. To account for individual  
162 differences in the baseline EOD frequency of each fish, values were normalised as percent change from  
163 normoxic baseline values for each of the 10 min intervals following baseline recordings. To test for an  
164 effect of hypoxia on EOD frequency, we used a random-slope linear mixed-effect model (LMM) with  
165 change of median EOD frequency as dependent variable. Based on AIC score, the best fit was achieved  
166 by including the interaction of inversed DO concentration with residence time in hypoxia (i.e. the lower  
167 the DO concentration in which the fish stayed, the higher the interaction term) and experimental time as  
168 fixed effect and fish ID as random effect. The intercept of the LMM was set to zero.

169 EOD amplitude was strongly affected by the position and orientation of the fish relative to the recording  
170 electrodes. As we could not always determine the exact fish position and orientation (e.g. when fish  
171 were in their shelters or swimming in the passage between compartments), we excluded EOD amplitude  
172 from our analysis.

### 173 *Hypoxia avoidance threshold*

174 We quantified the threshold for hypoxia avoidance by modelling the correlation between residence time  
175 in hypoxia and DO concentration with a modified version of the program for  $P_{crit}$ -determination by  
176 Yeager and Ultsch (Yeager and Ultsch, 1989). The program estimates the best fit of two linear regressions

177 to a dataset, iteratively minimizing their residual sum of squares. Two LMMs with random intercepts  
178 were calculated with residence time in the hypoxic compartment as the dependent variable. DO  
179 concentration was included as fixed effect, and fish ID was included as a random effect. The hypoxia  
180 avoidance threshold was defined as the DO concentration at which the regression lines of both LMMs  
181 intersected. Conditional and marginal  $R^2$  values of both LMMs were calculated based on the method by  
182 Nakagawa and colleagues (Nakagawa et al., 2013). T-test statistics and p-values for the null hypothesis of  
183 zero correlation between residence time in hypoxia and DO were calculated with degrees of freedom  
184 obtained through Satterthwaite approximation. The R code for these procedures was adapted from the  
185 rMR package (Moulton, 2018).

### 186 *Repeatability trials*

187 To test the repeatability of our experimental protocol and results, hypoxia avoidance trials were  
188 repeated after 4 weeks with five fish. Trials were conducted as described above, and residence time in  
189 the hypoxic compartment was tested for differences between the first trial and the repeatability trial  
190 using a two-way repeated measures ANOVA with DO concentration as between-subject effect, and  
191 experimental day as within-subject effect.

## 192 **3. Results and Discussion**

### 193 *Apteronotus albifrons show pronounced side preference and stationary behaviour at normoxia*

194 During normoxic baseline recordings, all 16 individuals showed a pronounced preference for one  
195 compartment of the shuttle-box choice chamber over the other (Wilcoxon single sample rank-sum test,  $p$   
196  $< 0.001$ ) with 10 fish spending the whole baseline period exclusively on one side and no fish spending  
197 less than 79% of the time on one side. Among all 16 fish, the two compartments were chosen 8 times  
198 each, indicating that there was no bias to either side of the choice chamber. During this period, fish  
199 predominantly rested in the PVC tubes that were provided as shelters. We subsequently induced  
200 stepwise hypoxia in the compartment of the choice chamber where the fish preferred to stay.

### 201 *Increased locomotor activity drives hypoxia avoidance at safe oxygen levels*

202 Moderate hypoxia above 20% air saturation did not significantly affect swimming behaviour. Fish  
203 predominantly rested in their shelters and showed only small deviations from normoxic baseline  
204 behaviour. Below 20% air saturation, swimming activity increased and fish spent less time in the hypoxic  
205 compartment with significant deviations from baseline recordings below 15% air saturation (Wilcoxon

206 rank-sum test with Holm-Bonferroni *post-hoc* correction,  $p < 0.05$ , Fig. 2B-D, Table S1, S2). To determine  
207 the threshold for the onset of hypoxia avoidance, we modelled the impact of hypoxia on residence time  
208 in the hypoxic compartment using two linear mixed-effect models (LMMs) with random intercepts and  
209 slopes (Fig. 3, Table S3). Based on this method, we identified the threshold for the onset of hypoxia  
210 avoidance behaviour at the intersection of both linear regressions at 22% air saturation. Above the  
211 threshold, air saturation had no significant effect on the residence time in the hypoxic compartment  
212 (adjusted marginal  $R^2 = 0.02$ ,  $p = 0.075$ ). Below the threshold, air saturation significantly affected  
213 residence in the hypoxic compartment (adjusted marginal  $R^2 = 0.307$ ,  $p < 0.001$ ). In repeatability trials,  
214 residence times in the hypoxic compartment did not differ significantly from original trials (ANOVA:  $F =$   
215  $0.114$ ,  $p = 0.753$ , Table S4).

216 These results show that *A. albifrons* use an active hypoxia avoidance strategy that follows a threshold  
217 dynamic with little or no effect of moderate hypoxia and a strong effect of deep hypoxia on swimming  
218 behaviour. The avoidance threshold lies above the threshold for aquatic surface respiration for *A.*  
219 *albifrons* ( $ASR_{50}$ , the oxygen level at which fish spend 50% of their time engaged in breathing water from  
220 the surface film), estimated as 18.3% (Vassileva, Krahe and Chapman, unpublished data). The threshold  
221 for hypoxia avoidance in *A. albifrons* also falls well above the critical oxygen tension ( $P_{crit}$ ) for the closely  
222 related species *Apteronotus leptorhynchus* and the distantly related gymnotiform *Eigenmannia virescens*.  
223  $P_{crit}$ , the oxygen partial pressure below which the oxygen consumption of the fish switches from oxygen  
224 regulation to oxygen conformation, was estimated as 10.5% air saturation for *A. leptorhynchus* and 7.1%  
225 for *E. virescens* (Reardon et al., 2011). The early onset of an avoidance reaction likely provides *A.*  
226 *albifrons* with the flexibility to seek better oxygenated areas before hypoxic stress impairs its physiology.  
227 This is consistent with anecdotal observations that wave-type gymnotiform fishes are typically found in  
228 habitats with a DO concentration of 40% - 60% air saturation and avoid swimming into hypoxic or anoxic  
229 waters (Crampton, 1998). The active response to hypoxia described here is similar to the behavioural  
230 responses of other species, such as red hake (*Urophycis chuss*, Bejda et al., 1987), tuna (*Katsuwonus*  
231 *pelamis* and *Thunnus albacares*, Bushnell and Brill, 1991), Atlantic herring (*Clupea harengus*, Domenici et  
232 al., 2000), weakfish (*Cynoscion regalis*, Brady et al., 2009), and rainbow trout (*Oncorhynchus mykiss*,  
233 Poulsen et al., 2011).

234 After leaving hypoxia, fish remained active and occasionally ventured back to the hypoxic side. This  
235 sustained change from stationary swimming to active roaming was evident in the distance travelled,  
236 which remained significantly higher than baseline values at air saturations below 15% (Wilcoxon rank-  
237 sum test with Bonferroni-Holm *post-hoc* correction,  $p < 0.01$ , Fig. 2C,D). This behaviour was somewhat



238 unexpected as a short bout of activity would have sufficed to leave the hypoxic compartment of the  
239 choice chamber while minimizing energy expenditure and predation risk. The lasting upregulation of  
240 locomotor activity could be caused by the initial displacement from their shelter. In a natural setting,  
241 hypoxic areas are likely to be more extended than in our setup and a sustained increase of locomotor  
242 activity might be necessary to reach better oxygenated areas. Excursions into hypoxia have been found  
243 in other fish species, sometimes associated with foraging behaviour (Claireaux et al., 1995; Cook and  
244 Herbert, 2012; Herbert et al., 2011; Jones, 1952; Rahel and Nutzman, 1994; Wannamaker and Rice,  
245 2000). Based on these findings, it has been hypothesized that hypoxia avoidance behaviour is not directly  
246 triggered by external DO concentration but rather relies on various physiological cues that imply  
247 “respiratory distress” (Cook et al., 2011; Jones, 1952). This indirect relationship between external DO  
248 concentrations and behavioural response allows for the integration of many additional cues into an  
249 avoidance response, thus increasing its flexibility in different environmental contexts. Although  
250 ultimately, the physiological need for oxygen is the driver of hypoxia avoidance, the onset of this  
251 behaviour could be dependent on the interaction of several relevant factors such as habitat cover (Hill,  
252 1968), presence of predators (Wolf and Kramer, 1987), availability and quality of an oxygen refuge  
253 (Herbert et al., 2011), and acclimation to different oxygen regimes (Cook et al., 2013). Although *A.*  
254 *albifrons* are known to inhabit well-oxygenated waters, they are likely to experience hypoxia occasionally  
255 in their natural habitat. Thus the ability to venture into hypoxic waters without immediate avoidance  
256 would allow them to forage or migrate and thus could provide an important fitness benefit.

257 *Electric organ discharge frequency is not a part of the hypoxia mitigation strategy of A. albifrons*

258 Median EOD frequency decreased marginally throughout hypoxia avoidance trials with the lowest values  
259 averaging to -1.02% change from normoxic baseline EOD frequency at the beginning of reoxygenation  
260 (Fig. 2E, Table S5). According to LMM estimates, this decrease resulted from a small but significant  
261 negative effect of experimental time on frequency. The most likely cause of the marginal decrease of  
262 median EOD frequency is the slight cooling of water temperature during trials, which amounted to an  
263 average decrease of 0.15°C. Assuming a  $Q_{10}$  value of 1.55 for EOD frequency (Dunlap and Ragazzi, 2015),  
264 temperature change explains a reduction of EOD frequency by 0.7%. The interaction term of residence  
265 time in hypoxia and inverted DO concentration had a negligible positive effect on frequency, indicating  
266 that fish did not reduce their EOD frequency in response to hypoxia. Although in theory, a reduction of  
267 discharge frequency might reduce the energetic cost of the electric sense and thus could be a useful  
268 means to survive hypoxia (Salazar et al., 2013), the lack of evidence that wave-type gymnotiforms  
269 employ frequency reduction as a measure to save energy suggests that wave-type gymnotiforms are

270 unable to effectively reduce their EOD frequency, even under inescapable hypoxic stress (Crampton,  
271 1998; Markham et al., 2009; Reardon et al., 2011). Whereas we can make no inferences about the  
272 capacity of *A. albifrons* to regulate EOD frequency under hypoxic stress, our results show that *A.*  
273 *albifrons* leave hypoxia before EOD frequency is affected, regardless of whether by active regulation or  
274 as a mere consequence of hypoxic stress.

275 Another parameter that contributes to the energetic cost of EODs is their amplitude (Markham et al.,  
276 2009; Salazar et al., 2013; Stoddard and Salazar, 2010). So far, reduction of EOD amplitude has only been  
277 found under inescapable hypoxic conditions approaching the respective  $P_{crit}$  in *A. leptorhynchus* and *E.*  
278 *virescens* (Reardon et al., 2011). The comparatively high hypoxia avoidance threshold of 22% air  
279 saturation suggests that *A. albifrons* avoided hypoxia well before its EOD amplitude was affected.  
280 However, as we could not reliably quantify EOD amplitude of freely swimming fish in our study,  
281 additional experiments are needed to clarify whether the DO concentrations at which fish began to leave  
282 hypoxia in our experiment have an effect on EOD amplitude.

### 283 *Conclusion and outlook*

284 We show here that *A. albifrons* use an active hypoxia avoidance strategy that is comparable to that of  
285 other fishes with active life styles. Our results suggest that active avoidance serves to mitigate negative  
286 implications of hypoxia on sensing and physiology rather than adapting to it. These results are in line  
287 with previous studies and field observations of wave-type gymnotiforms (Crampton, 1998; Reardon et  
288 al., 2011) and suggest a low tolerance of *A. albifrons* to hypoxia below 20% air saturation. With regard to  
289 the expected increased prevalence of hypoxia in the future, this proactive avoidance strategy is likely to  
290 cause habitat shifts and a reduced abundance of *A. albifrons* in affected habitats. More hypoxia-related  
291 behavioural studies are needed for us to better understand the flexibility of behaviour in different  
292 environmental contexts and the relationship between physiological and behavioural hypoxia tolerance.

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### 296 **Competing Interests**

297 The authors declare no competing interests.

### 298 **Author Contributions**

299 All authors participated in the design of this study. S.M. performed all experiments and data analyses.  
300 R.K. wrote Matlab scripts for recording EODs. S.M. drafted the manuscript and all authors took part in its  
301 revision.

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426

## 427 **Figure Legends**

428 **Fig. 1: Schematic of shuttle-box oxygen choice chamber.** Blue arrows indicate water flow in the  
429 compartments and between choice chamber and buffer tanks. Plexiglas lids, heating system and grounding  
430 electrode are not shown.

431 **Fig. 2: Behavioural responses of *A. albifrons* during hypoxia avoidance trials.** (A) DO concentrations  
432 during a control trial with no fish. Grey bars represent the target DO concentration, black circles represent  
433 control measurements. (B) Percentage of time spent in the hypoxic compartment. We induced hypoxia in  
434 the compartment where the fish preferred to stay. (C) Distance travelled in the entire choice chamber. (D)  
435 Swimming velocity in the entire choice chamber. (E) Linear regression of EOD frequency change as  
436 percentage change from baseline EOD frequency based on LMM. Circles represent median values, vertical  
437 bars represent first and third quartile, values from the first 40 min were pooled as normoxic baseline  
438 behaviour, asterisks indicate statistically significant differences from normoxic baseline behaviour  
439 (pairwise Wilcoxon rank-sum tests with Holm-Bonferroni correction of p-values,  $p < 0.05$  \*,  $p < 0.01$  \*\*,  $p$   
440  $< 0.001$  \*\*\*).

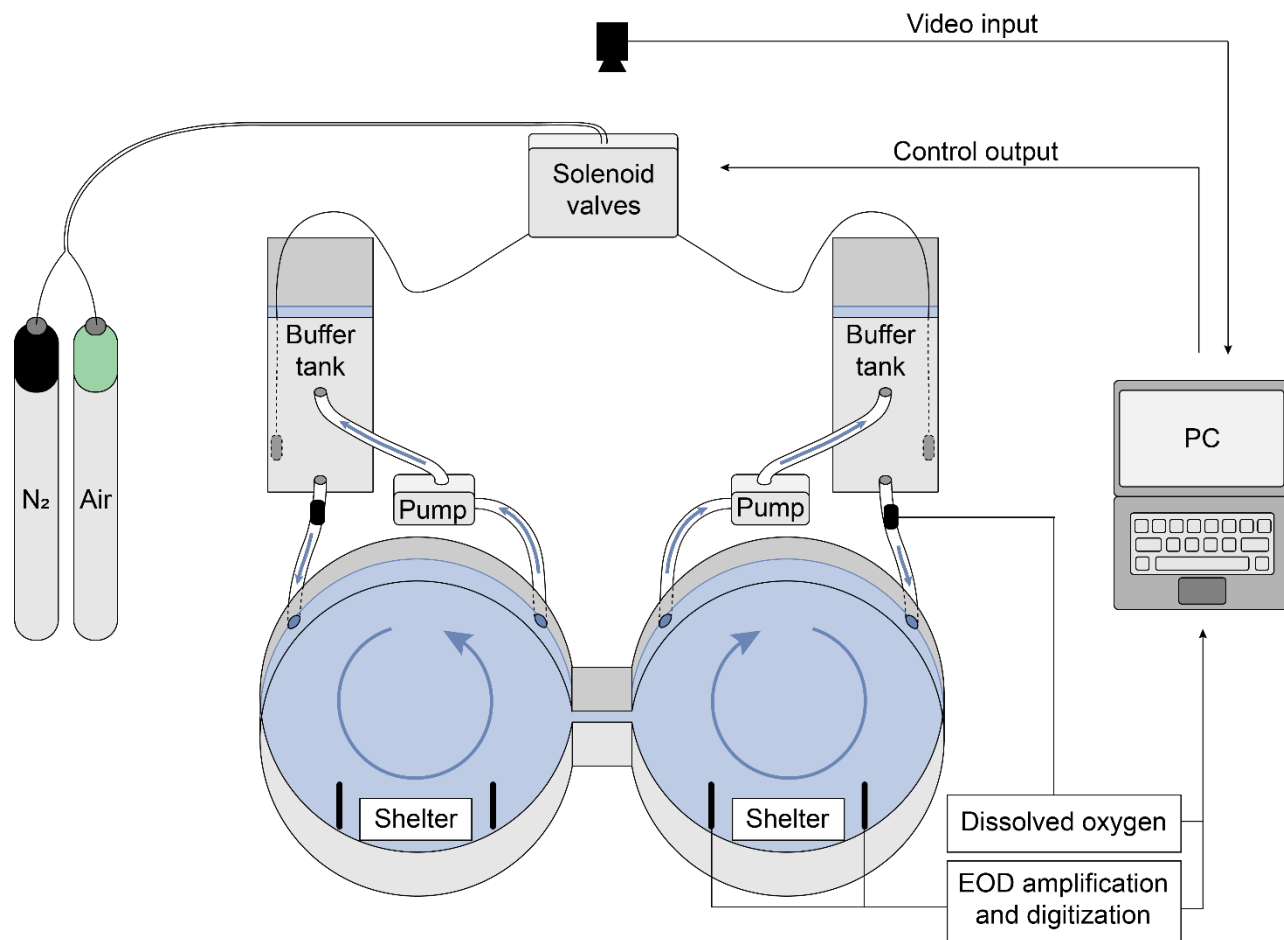
441 **Fig. 3: Residence time in the hypoxic compartment as function of the dissolved oxygen in % air**  
442 **saturation.** Grey circles represent the percentage of time that individual fish spent in the hypoxic  
443 compartment at each air saturation that was established in this compartment ( $n = 16$  fish, points are  
444 jittered along the x-axis to reveal overlapping measurements), dashed lines represent linear regressions  
445 based on LMMs, the black diamond at their intersection indicates the computed hypoxia avoidance  
446 threshold,  $R^2 =$  adjusted marginal  $R^2$ ,  $p =$  probability of zero correlation between air saturation and  
447 residence in hypoxia.

## 448 **Tables**

449 See supplementary info for tables S1-S5.

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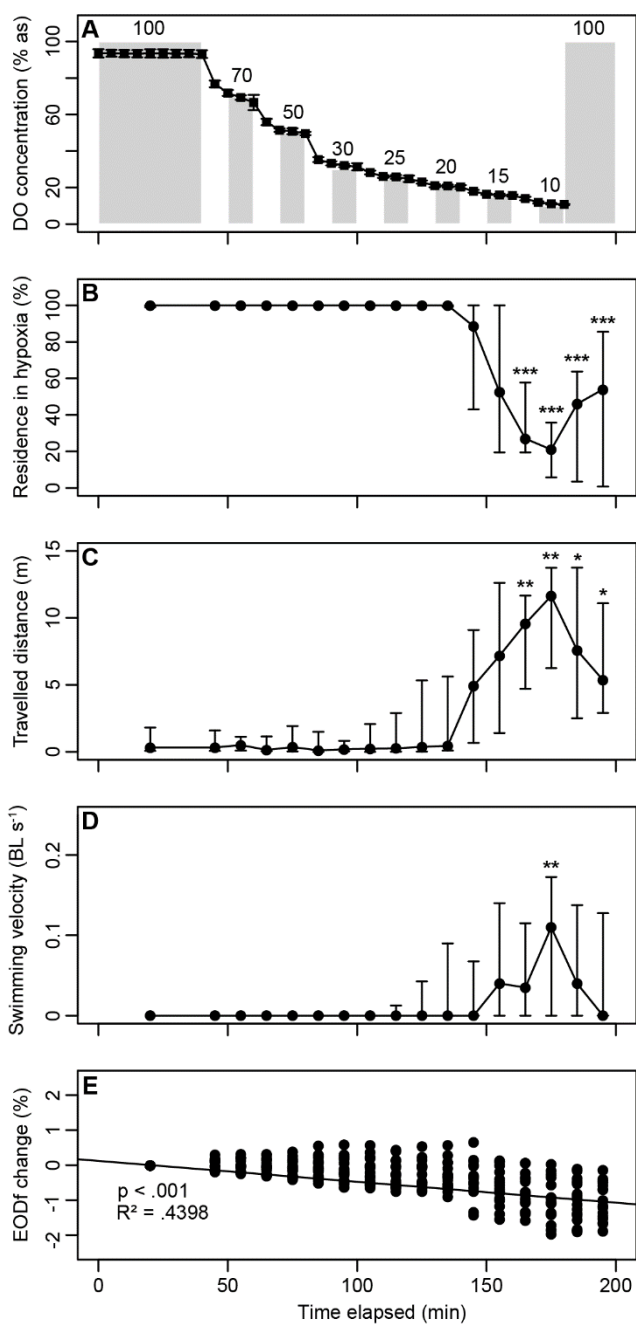
451 **Figures**



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453 **Fig. 1: Schematic of shuttle-box oxygen choice chamber.** Blue arrows indicate water flow in the compartments and between  
454 choice chamber and buffer tanks. Plexiglas lids, heating system and grounding electrode are not shown.

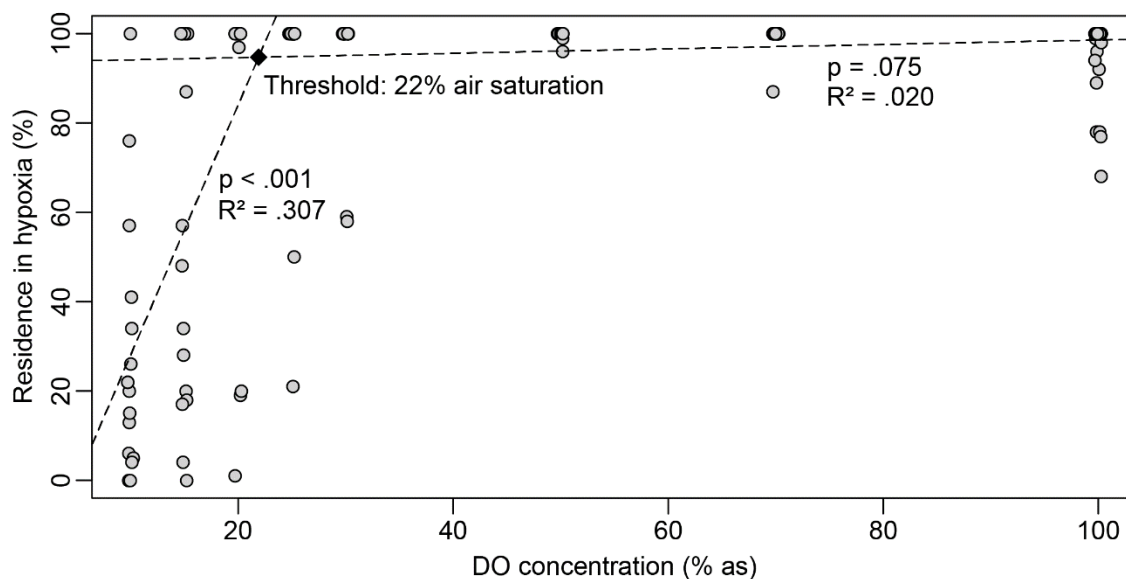
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**Fig. 2: Behavioural responses of *A. albifrons* during hypoxia avoidance trials.** (A) DO concentrations during a control trial with no fish. Grey bars represent the target DO concentration, black circles represent control measurements. (B) Percentage of time spent in the hypoxic compartment. We induced hypoxia in the compartment where the fish preferred to stay. (C) Distance travelled in the entire choice chamber. (D) Swimming velocity in the entire choice chamber. (E) Linear regression of EOD frequency change as percentage change from baseline EOD frequency based on LMM. Circles represent median values, vertical bars represent first and third quartile, values from the first 40 min were pooled as normoxic baseline behaviour, asterisks indicate statistically significant differences from normoxic baseline behaviour (pairwise Wilcoxon rank-sum tests with Holm-Bonferroni correction of p-values,  $p < 0.05$  \*,  $p < 0.01$  \*\*,  $p < 0.001$  \*\*\*).



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**Fig. 3: Residence time in the hypoxic compartment as function of the dissolved oxygen in % air saturation.** Grey circles represent the percentage of time that individual fish spent in the hypoxic compartment at each air saturation that was established in this compartment (n = 16 fish, points are jittered along the x-axis to reveal overlapping measurements), dashed lines represent linear regressions based on LMMs, the black diamond at their intersection indicates the computed hypoxia avoidance threshold,  $R^2$  = adjusted marginal  $R^2$ , p = probability of zero correlation between air saturation and residence in hypoxia.