Supplementary Information for

Normoxia exposure reduces hemoglobin and gill size in a hypoxia-tolerant weakly electric fish

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The Lake Nabugabo system

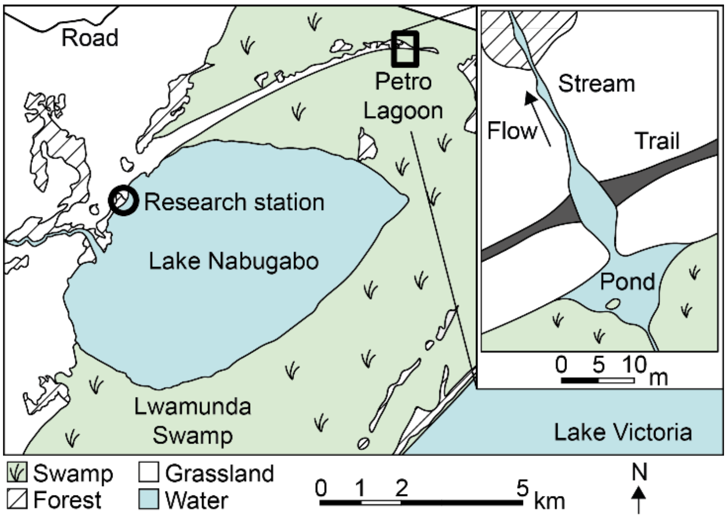

Fig. A1 Map of Lake Nabugabo and Petro Lagoon, Uganda. Lake Nabugabo is separated from Lake Victoria by the extensive Lwamunda Swamp wetland, which surrounds Lake Nabugabo to the north, east and south. To the west, Lake Nabugabo is bordered by forest and grassland. At Petro Lagoon (figure inlay), water from the swamp collects in the pond area and flows outwards from the swamp to seasonally inundated grassland. The outflow is intersected by a trail that crosses through a shallow section of the stream. *Petrocephalus degeni* were collected from the pond area of the lagoon (asterisk). The position of the Lake Nabugabo field research station on the shore of Lake Nabugabo is marked by a black circle. Modified from Mucha et al. 2022

Long-term measurement of background respiration

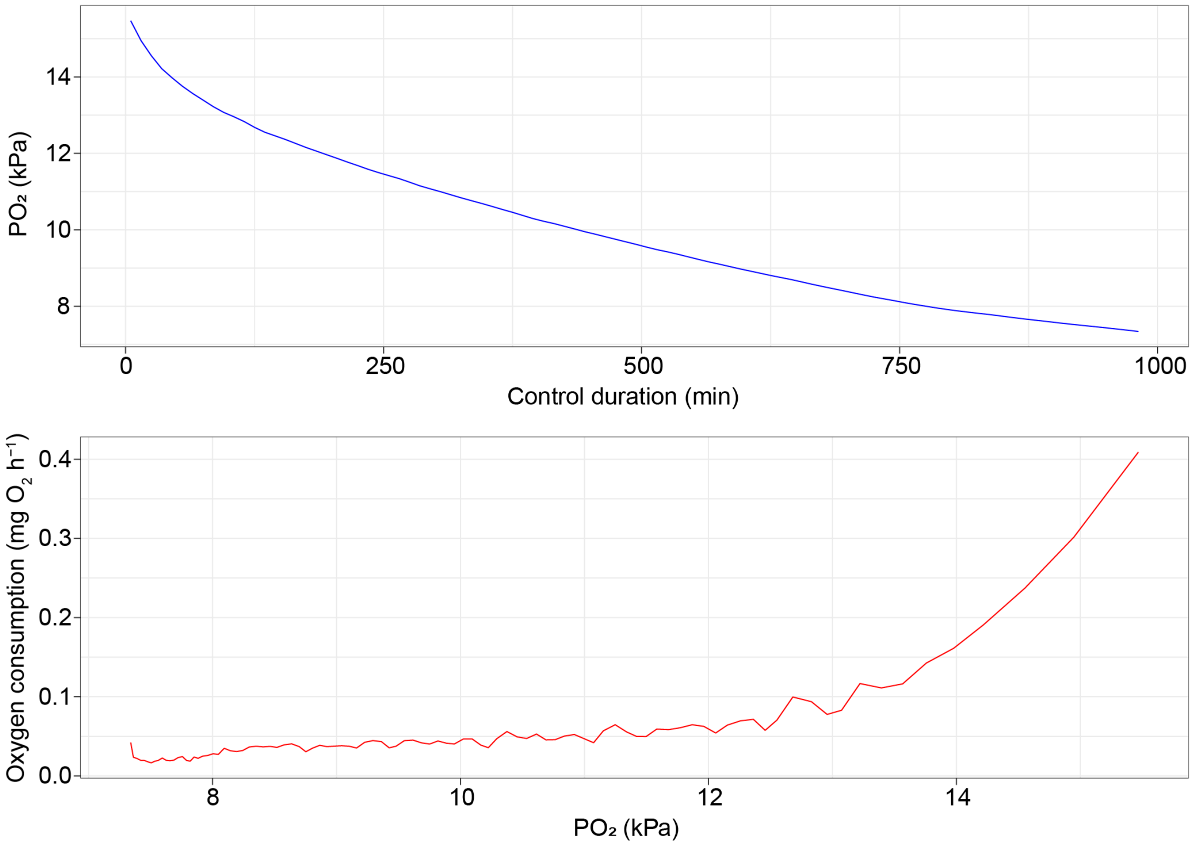

Fig. A2 Long-term measurement of background respiration. Data from one overnight dissolved oxygen measurements in the respirometer without a fish present. Top: oxygen partial pressure (PO_2) over measurement time. Bottom: oxygen consumption rate over PO_2 Note the elevated background respiration at $PO_2 > 12$ kPa

Comparison of methods for P_{crit} estimation

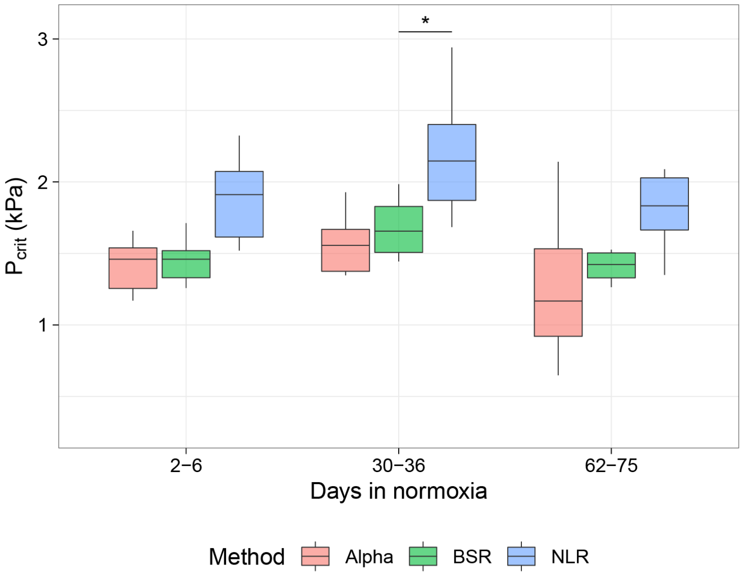

Fig. A3 - Comparison of methods for P_{crit} estimation. Shown are P_{crit} values that were derived from the same respirometry trial data via P_{crit}-Alpha (Alpha), broken-stick regression (BSR) and non-linear regression (NLR). See Table A1 for statistical test outcomes ($P < 0.05$*).

There has been considerable debate about the most appropriate methodological approach to quantify P_{crit}. We tested three different approaches that have been suggested by previous studies: broken-stick regression (BSR, Yeager and Ultsch 1989), non-linear regression (NLR, Marshall et al. 2013), and the P_{crit} - Alpha method (Seibel et al. 2021). Other approaches, such as the low-linear PO_2 method (Reemeyer and Rees 2019) and the sub-prediction interval metric (Birk et al. 2019) were excluded because we did not quantify SMR (low-linear PO_2) or because the method results in a systematic underestimation of P_{crit} (sub-prediction interval). Non-linear regression tended to produce

Table A1 - Effect of computation method on P_{crit} values. Alpha, P_{crit}-Alpha method; BSR, broken-stick regression; DF, degrees of freedom; NLR, non-linear regression P_{crit}, critical oxygen tension; η^2_g, generalized eta squared for effect size. Bold numbers indicate statistically significant outcomes.

Test	Trait	Effect	F	DF	P	η^2_g
ANCOVA	P_{crit}	**Method**	**7.938**	**2,45**	**0.001**	**0.261**
		Acclimation	2.258	2,45	0.116	0.091
		Method x Acclimation	0.510	2,45	0.728	0.043
		Acclimation	**Comparison**	**P**		
Tukey	P_{crit}	2-6	Alpha - BSR	0.989		
		2-6	Alpha - NLR	0.584		
		2-6	BSR - NLR	0.668		
		30-36	Alpha - BSR	0.642		
		30-36	**Alpha - NLR**	**0.010**		
		30-36	BSR - NLR	0.060		
		62-75	Alpha - BSR	0.705		
		62-75	Alpha - NLR	0.058		
		62-75	BSR - NLR	0.232		

erroneous results when $\dot{M}O_2$ increased towards P_{crit} and yielded the highest P_{crit} values of the three approaches (Table A1, Fig. A3).

Correlation of gill morphometrics

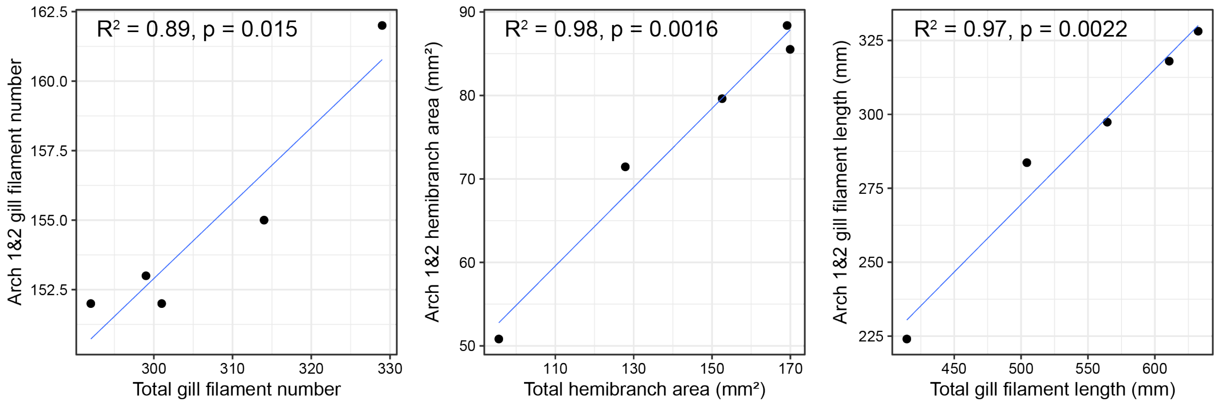

Fig. A4 - Correlation of gill morphometrics across gill arches. Shown are measurements of gill filament number, gill hemibranch area from the first two gill arches vs. measurements from all four gill arches. R^2 and p-values show the results of Pearson correlation analysis.

Correlations among measured variables

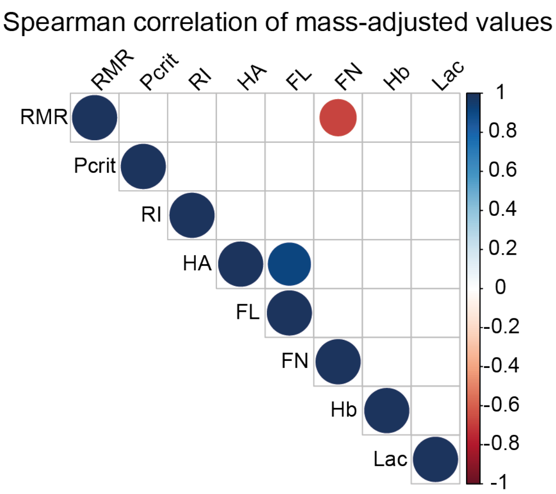

Fig. A5 - Correlations between measured traits (n = 11 fish). Routine metabolic rate (RMR), critical oxygen tension (Pcrit), regulation index (RI), gill hemibranch area (HA), gill filament length (FL), gill filament number (FN), blood hemoglobin (Hb) and blood lactate concentration (Lac) were tested for correlations using Spearman's rank correlation. Colors indicate the sign of the correlation, hue and size of dots indicate the strength of the correlation. Fields with non-significant correlations (P >0.05) are left blank

Effect of normoxia acclimation on respiratory traits

Table A2 – Effect of normoxia acclimation on quantified traits. DF, degrees of freedom; FL, gill filament length; FN, gill filament number; HA, gill hemibranch area; Hb, hemoglobin concentration; Lac, lactate concentration; P_{crit}, critical oxygen tension; RI, regulation index; RMR, routine metabolic rate; η^2_g, generalized eta squared for effect size. Significant outcomes of ANOVA and Kruskal-Wallis were followed by Tukey's post-hoc test (ANOVA) or Wilcoxon test with Bonferroni correction of p-values (Kruskal-Wallis) to identify significant differences between groups. Bold numbers indicate statistically significant outcomes. [a] blood measurements from fish after respirometry; [b] blood measurements from fish after undisturbed normoxia acclimation.

Test	Effect	Trait	F	DF	P	η^2_g
ANOVA	Acclimation group	RMR	1.257	2, 15	0.313	0.144
		P_{crit}	0.346	2, 15	0.713	0.044
		RI	2.407	2, 15	0.124	0.243
		FL	**6.848**	**2, 17**	**0.007**	**0.446**
		FN	1.45	2, 17	0.262	0.146
		Hb[a]	2.57	2, 11	0.121	0.318
		Lac[a]	1.51	2, 10	0.267	0.232
			H	**DF**	**P**	
Kruskal-Wallis	Acclimation group	HA	**9.83**	**2**	**0.007**	
			F	**DF**	**p**	**R^2**
Linear regression	Days in normoxia	Hb[b]	**21.77**	**1, 30**	**< 0.001**	**0.421**
		Lac[b]	0.002	1, 47	0.961	0

References

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