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HYPOXIA TOLERANCE OF TWO CLOSELY RELATED *HAPLOCHROMIS* SPECIES (PISCES: CICHLIDAE): *HAPLOCHROMIS ELEGANS* TREWAVAS, 1933 AND *H. ANGUSTIFRONS* BOULENGER, 1914

FRIETSON GALIS and H. SMIT
Zoological Laboratory, University of Leiden, The Netherlands

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Abstract—1. In their natural habitat *Haplochromis elegans* and *H. angustifrons* take their food (insect larvae) mainly from oxygen-rich deposits and oxygen-poor muds respectively.

2. To investigate their hypoxia tolerance specimens of these Haplochromids were forced to swim at several constant speeds at declining oxygen tensions.

3. When oxygen tension decreased during steady swimming, both oxygen consumption rate and tailbeat frequency decreased until a critical oxygen value was reached. *H. angustifrons* was found to have critical oxygen values about 0.5 ppm lower than *H. elegans*.

4. This difference in hypoxia tolerance may be related to the difference in feeding habits.

INTRODUCTION

Haplochromis elegans and *H. angustifrons* belong to a group of closely related species, occurring in Lake George, Lake Edward and the Kazinga channel (East Africa) (Greenwood, 1973). *H. elegans* is a dweller of shallow water with sandy bottom and *Papyrus* vegetation; it feeds mainly on *Chironomus* larvae (Gwahaba, 1973; Dunn, 1975). *H. angustifrons* dwells in all parts of the lake over sandy bottoms as well as muddy deposits and feeds mainly on *Chaoborus* larvae (Moriarty *et al.*, 1973), which lie buried in the soft anoxic mud so that *H. angustifrons* has to penetrate this mud to get at the larvae. Therefore, *H. angustifrons* has to temporarily tolerate severe hypoxia during feeding.

In this study the hypoxia tolerance of both *Haplochromis* species is measured and compared in view of their feeding habits.

MATERIALS AND METHODS

The fish used in the experiments were the progeny of specimens of *Haplochromis elegans* and *H. angustifrons* caught from Lake George (Uganda) in 1972. The *H. elegans* specimens varied from 8.5 to 9.5 g of bodyweight; the weight of the *H. angustifrons* specimens ranged from 8.2 to 9 g. The fish were acclimated to 21–25°C; experiments were performed at 25°C. Two Blazka respirometers were used in which the fish were forced to swim against a water current of adjustable velocity created by a motor-driven propeller. This Blazka-type respirometer has been described by Smit *et al.* (1971). The dimensions of the respirometers used in this study are: water volume 1.8 l, lengths of inner cylinder 23 cm, cross sectional area of inner cylinder 24 cm^2. Each experimental fish stayed in the apparatus for an uninterrupted period of 7 days; they were not fed during this period. The first 3 days allowed the fish to get accustomed to the experimental conditions. During each of the other 4 days one or two experiments were performed in which the fish was forced to swim at various speeds, and oxygen consumption rates, tailbeat frequencies and critical oxygen concentrations were measured. The transition from still water to the desired water current velocity was slow and gradual and took about 1 hr. At the same time the oxygen content of the water was reduced by bubbling through with nitrogen gas until 3 ± 0.4 ppm O_2. Then the apparatus was air-tight closed, so that the oxygen concentration gradually dropped as the result of the fish's consumption of oxygen. When the critical oxygen value was reached, the fish failed to maintain the imposed speed of swimming. This was either the end of the experiment or, when it was repeated, the respirometer was flushed with air-saturated water for a few minutes until the oxygen content again reached the initial value of approx 3 ppm, after which the fish swam until its second failure. The oxygen concentration at failure is considered to be the critical value matching a particular level of locomotory activity at 25°C. After a stay of 1 week in the respirometer, the fish was replaced by another one, and returned to the apparatus one or more weeks later.

Critical oxygen values at each swimming speed were compared for *H. elegans* and *H. angustifrons* with the aid of the Mann–Whitney U-test. Tailbeat frequencies at each speed of swimming of both *Haplochromis* species were also compared with the Mann–Whitney U-test. Wilcoxon's matched-pairs test was performed to compare critical oxygen values and time to failure in sets of two successive experiments. Correlations were computed between oxygen content and tailbeat frequency and between oxygen consumption rate and tailbeat frequency.

RESULTS AND DISCUSSION

Critical oxygen concentrations were measured in 19 experiments on 4 specimens of *H. angustifrons* and 20 experiments on 5 specimens of *H. elegans* at four different swimming speeds: 16, 19, 22 and 25 cm/sec. Table 1 and Fig. 1 show that all critical oxygen concentrations for *H. elegans* at any speed are higher than the critical values for *H. angustifrons*. Further, it appears that the critical value rises at increasing swimming speed. This observation is in accordance with former findings from which a positive correlation was established between critical oxygen concentration and rate of oxygen uptake (Smit *et al.*, 1971

Table 1. Critical oxygen concentrations (ppm O_2) at four different swimming speeds (cm/sec) for *Haplochromis elegans* (upper part) and *H. angustifrons* (lower part)

	16 cm/sec	19 cm/sec	22 cm/sec	25 cm/sec
H. elegans	1.59	1.82	1.82 (1.82)	2.28
	1.49 (1.51)	1.59 (1.70)	2.03 (1.99)	1.92 (1.92)
	1.49 (1.51)	1.73 (1.75)	1.77 (1.77)	2.07 (2.09)
	1.58 (1.59)	1.52 (1.55)		1.92 (1.91)
	1.34	1.90 (2.49)		2.51
		1.69 (1.72)		1.89 (1.87)
H. angustifrons	1.25	1.38	1.47	1.58
	1.25	1.10 (1.10)	1.24 (1.28)	1.44 (1.44)
	1.00 (0.95)	1.30	1.40 (1.41)	1.50 (1.59)
	1.14 (1.14)	1.19 (1.19)	1.31 (1.30)	1.60
	1.09 (1.10)		1.37 (1.42)	
	1.11 (1.12)			

Numbers followed by bracketed numbers form sets of two successive experiments, an example of which is depicted in Fig. 2.

their Fig. 4). The critical oxygen concentration, as measured in this way, represents a reproducible value. Alterations in the sequence of the enforced speeds do not change the critical values. When the experiment is repeated immediately after a first failure, the second failure occurred at approximately the same critical oxygen concentration, i.e. there is no significant difference between the two critical values obtained in successive experiments. (Repetitions appear in parentheses in Table 1.) Since consecutive experiments yield equal critical values, there is no evidence for the repayment of an oxygen debt conceivably contracted during the first experiment. When declining oxygen content approaches the critical value there is a concomitant decrease in the rate of oxygen uptake during steady swimming. (An example is given in Fig. 2.) Since this is not followed by any oxygen debt, two explanations would be appropriate: (a) the fish generates part of its energy anaerobically without contraction of an oxygen debt and (b) the fish increases its swimming efficiency. As far as the latter point is concerned, during steady swimming a high positive correlation exists between tailbeat frequency and oxygen content ($r = 0.9$) and between tailbeat frequency and rate of oxygen uptake ($r = 0.9$). Apparently the fish changes its mode of swimming at hypoxia: the amplitude of its tailbeat increases and the use of the pectoral fins decrease at decreasing oxygen tension. By changing its mode of swimming the hypoxic fish possibly makes more intensive use of its white propulsion muscles than the normoxic fish. If the white muscles of the trunk are able to anaerobically generate energy without incurring an oxygen debt, the fish would be able to lessen its rate of oxygen uptake at a steady level of external work by switching from the use of the red muscles of trunk and pectoral fins to using its white lateral musculature. Such a system is known to exist in goldfish, which is able to diminish the increase of its oxygen uptake rate in case of increasing swimming speed without contracting an oxygen debt (Smit *et al.*, 1971). Another possibility, that does not exclude the former, is that during hypoxia swimming becomes hydrodynamically more efficient, so that the rate of oxygen consumed decreases as a result of the decreasing power required for swimming at a particular speed. During swimming at normoxia at various speeds *H. elegans* and *H. angustifrons* exhibit equal tailbeat frequencies, except at the highest speed employed (25 cm/sec), at which *H. elegans* shows the higher frequency. Preliminary measurements of the oxygen consumption rates during steady swimming at normoxia show that *H. elegans* has a consistently higher rate (approx 10%) than *H. angustifrons* at equal swimming speeds. This in itself justifies the expectation that *H. elegans* will have higher critical oxygen concentration values than *H. angustifrons*, as has been found factually. The difference in hypoxia tolerance between specimens of both *Haplochromis* species may be partly based on differences in the extent of the gill surface area and oxygen transporting capacity (thickness of the respiratory membrane, hematocrit, oxygen affinity of hemoglobin, magnitude of Bohr-effect, heart capacity, etc.). Preliminary measurements of the gill surface area indicate that *H. angustifrons*

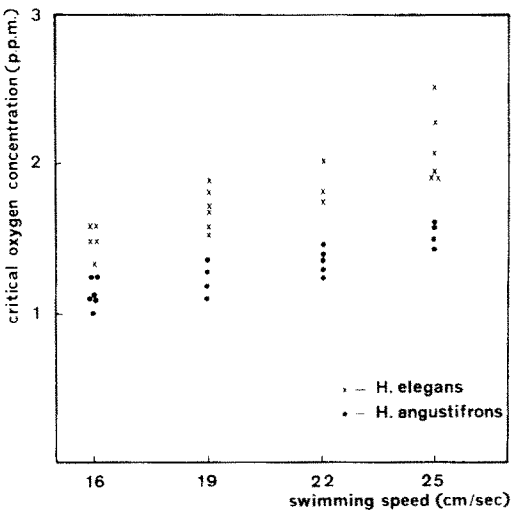

Fig. 1. Critical oxygen concentration (ppm O_2) in relation to swimming speed (cm/sec). Crosses: *H. elegans*. Dots: *H. angustifrons*.

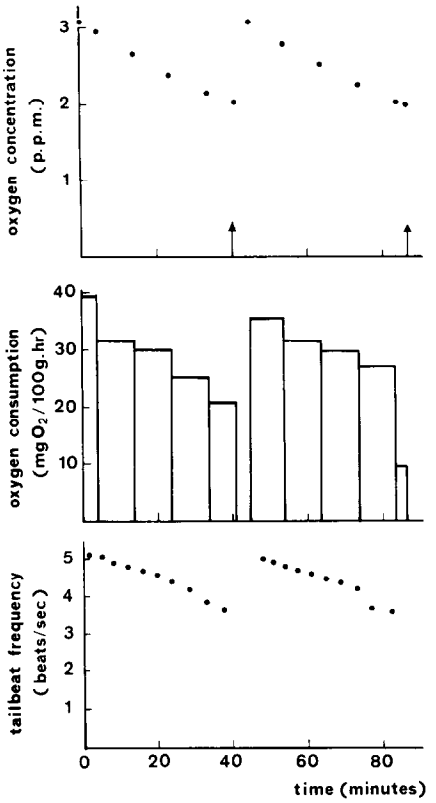

Fig. 2. Oxygen consumption rates and tailbeat frequencies of swimming *H. elegans* ♀ (weight 9.5 g) under hypoxia during two successive experiments of about 40 min each, separated by a 5 min interval. The fish swam at a constant speed of 22 cm/sec until collapse when the critical oxygen concentration was reached (arrow in top graph). The experiment started at 3.10 ppm O_2 and ran to 2.03 ppm O_2 (= critical value). Then the oxygen content was increased again to 3.10 ppm after which it declined to 1.99 ppm (= critical value). Note that the two experiments show similar results.

has a significantly larger total gill surface than *H. elegans* (Galis *et al.*, in preparation). Thus the different critical oxygen levels may be the result of differences in anaerobic capacity of the energy metabolism, hydrodynamic efficiency of swimming, resistance of the CNS to hypoxia, oxygen extracting capability, or a combination of these possibilities.

Under natural circumstances it is probably only *H. angustifrons* which regularly and temporarily encounters severe hypoxia. The greater hypoxia tolerance of *H. angustifrons* appearing from this study can therefore be considered to have adaptive significance. Future research will have to provide insight into the mechanisms by which this tolerance is achieved.

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