

Similar responses to hypoxia in two air-breathing anabantoid fishes with different gill morphology



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Introduction

As the dissolved oxygen decreases in water, oxygen absorption may not satisfy the aerobic metabolism in fish. Fish may reduce ATP demand via channel arrest such as NKA activity suppression or meet routine ATP requirement with metabolic pathway adjustment. All Anabantoid fishes are aquatic air-breathing fish. In previous studies, it is known that some species of Anabantoidei, *Helostoma temminckii* and *Macropodus opercularis*, Na⁺/K⁺-ATPase (NKA) (Fig. 1B,1D) did not differ significantly in gills but air-breathing respiration (ABR) frequency increased under hypoxia (Fig. 1A, 1C). The aim of the present study is to investigate (1) if Anabantoidei increases ABR to compensate for the oxygen debt under hypoxia and (2) if ATP is generated from metabolic pathway adjustment to supply energy for NKA under hypoxia. (3) If glycogen-rich cells (GR cells) in gills of Anabantoidei undergo glycogenolysis to maintain NKA activity in the nearby mitochondrial-rich cells (MR cells).

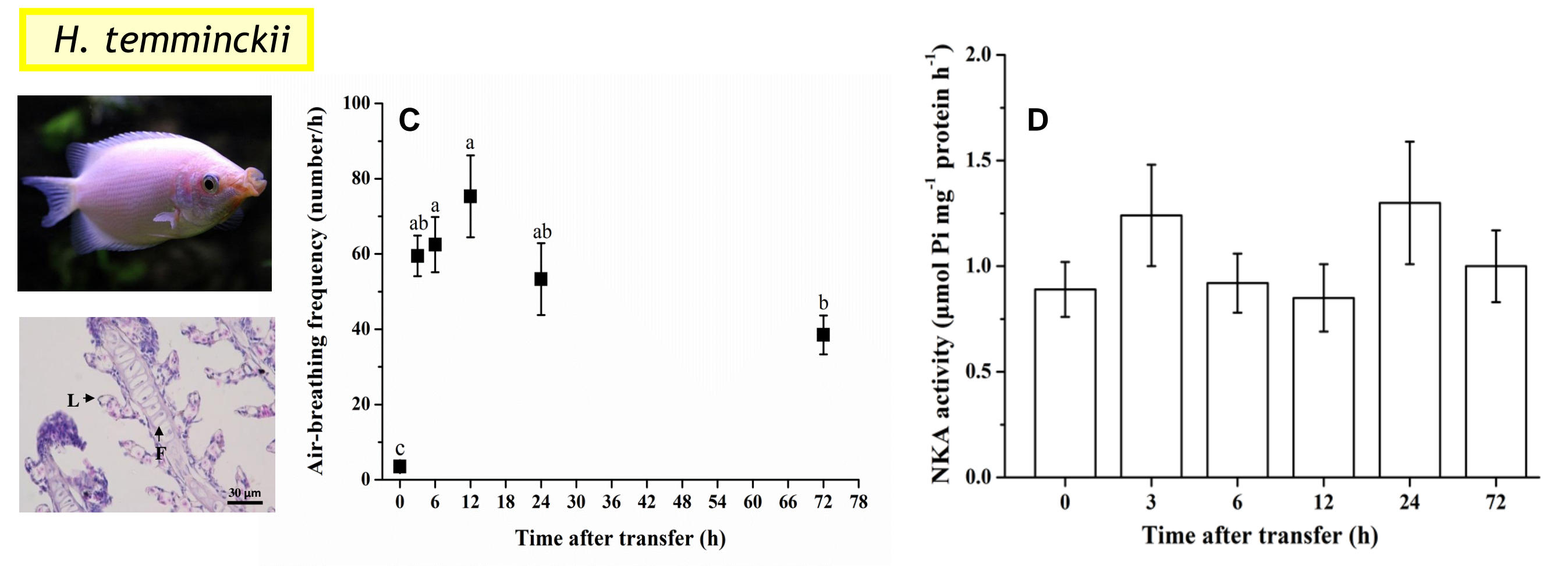
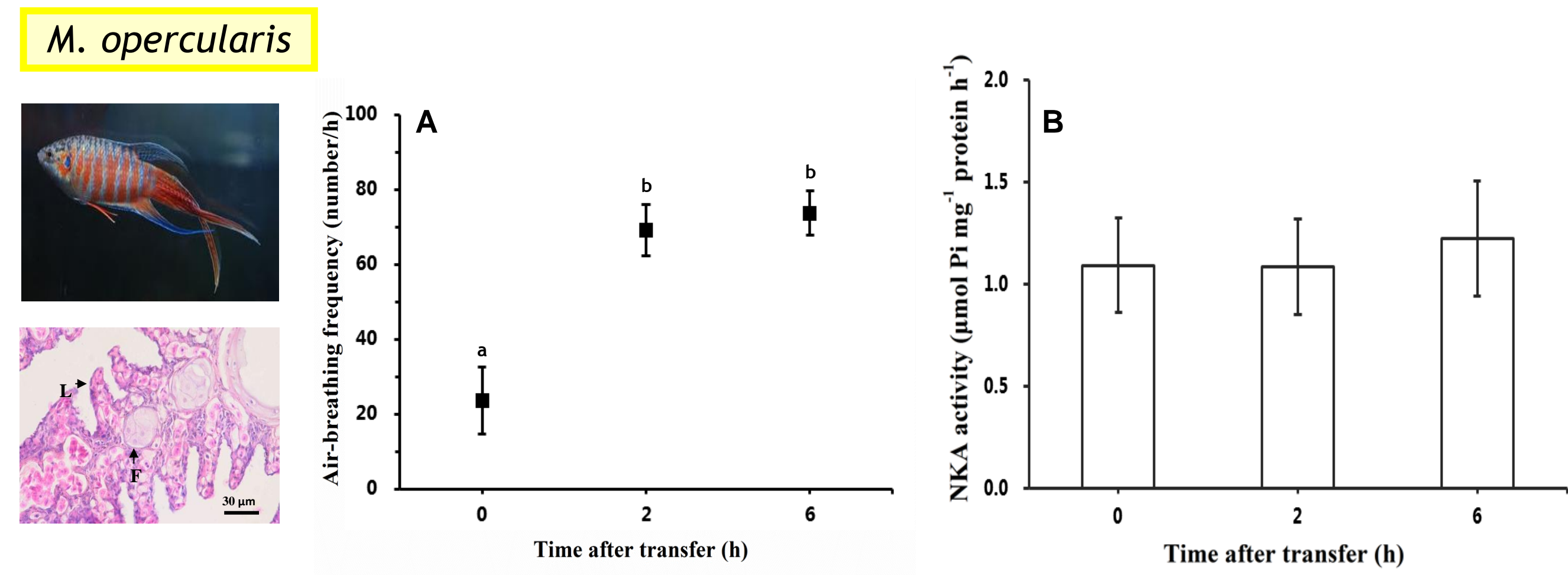


Fig.1 The ABR frequency (A) and NKA activity (B) in the gills of *M. opercularis* exposed to normoxia (0 h) and hypoxia (2, 6 h). The ABR frequency (C) and NKA activity (D) in the gills of *H. temminckii* exposed to normoxia (0 h) and hypoxia (3, 6, 12, 24, 72 h). Data are presented as mean ± S.E.M (n= 6-8). The different letters indicate a significant difference among times (One-way Anova, Dunnett's post hoc test, $p < 0.05$).

Result

M. opercularis mortality increased under hypoxia without ABR

	Normoxia	Hypoxia	Non-ABR + Normoxia	Non-ABR + hypoxia
Dissolved Oxygen (mg/L)	4.73 ± 0.54	1.20 ± 0.49	4.79 ± 0.27	1.11 ± 0.32
Mortality	0% (0/28)	0% (0/28)	1.23% (2/18)	100% (8/8)*

(*: loss balance in 15min)

Water NH₄⁺ concentration increased but lactate concentration do not change in the gills of *M. opercularis* under hypoxia

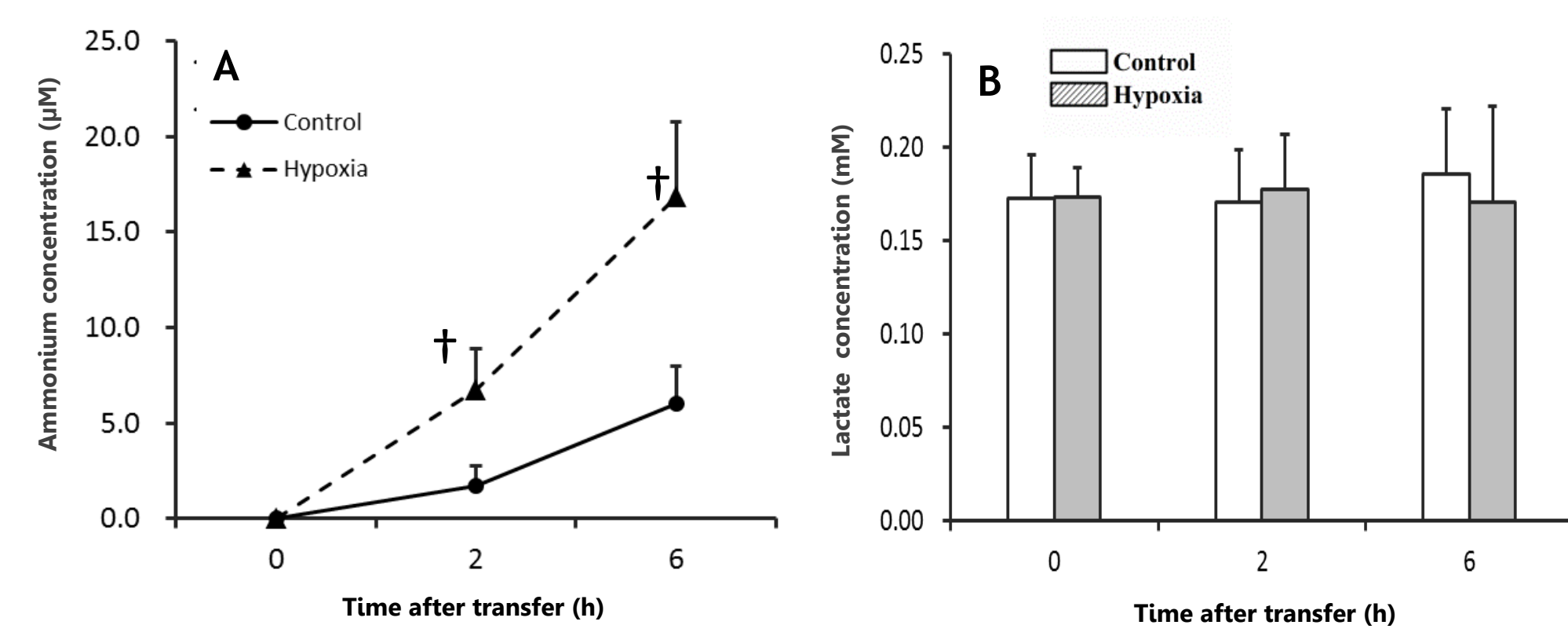


Fig.2 Ammonium concentration in water sample (A) and lactate concentration in the gills (B) under hypoxia. Data are presented as mean ± S.E.M (n= 8-10). † indicates a significant difference compared with normoxia (Two-way Anova, grouping by Tukey's HSD test, $p < 0.05$).

Plasma glucose concentration in *M. opercularis* did not change under hypoxia



Fig.3 Plasma glucose concentration under hypoxia. Data are presented as mean ± S.E.M (n= 10). Two-way ANOVA was conducted among different treatments and time points.

Glycogen content decreased under hypoxia in the gills and liver of *H. temminckii*

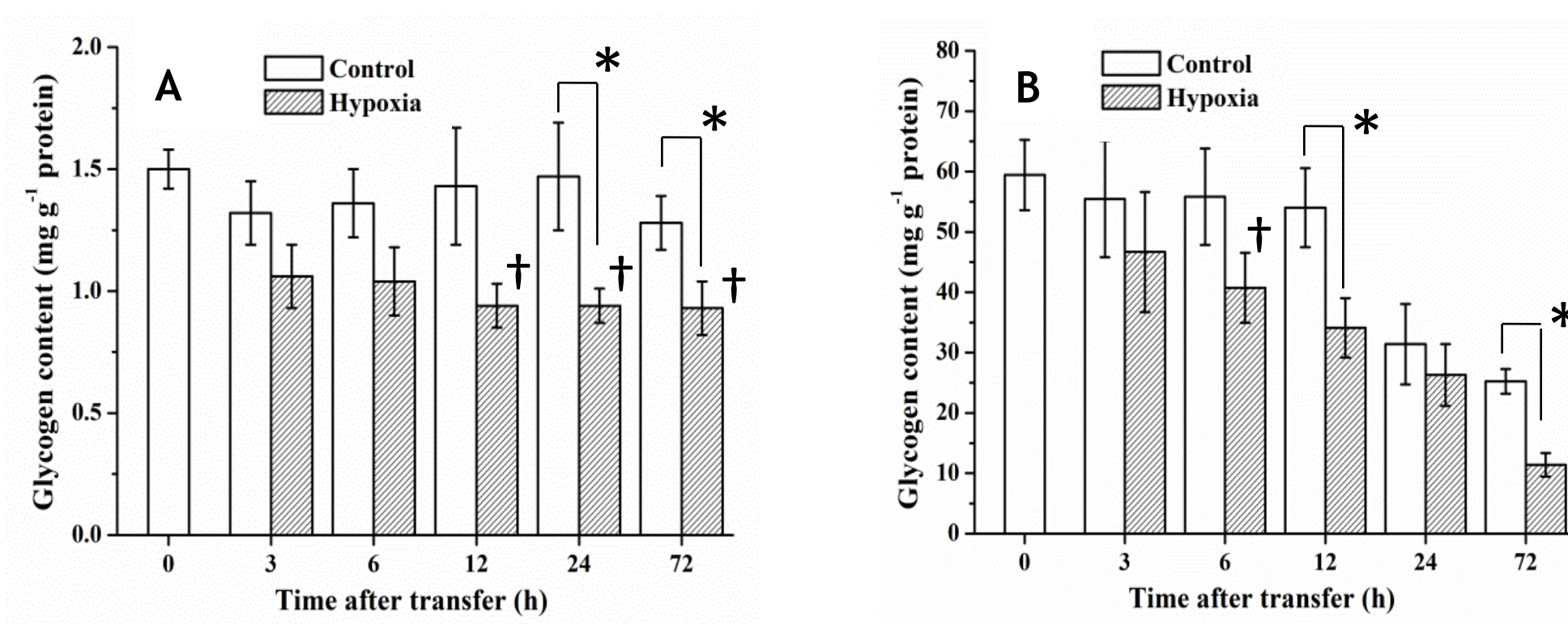


Fig.5 Glycogen content exposed to hypoxia in the gills (A) and liver (B). Data are presented as mean ± S.E.M (n= 10). * indicates a significant difference from the respective control at the same exposure time (Student's *t*-test, $p < 0.05$). † indicates a significant difference compared with control (0 h) (One-way Anova, Dunnett's post hoc test, $p < 0.05$).

Glycogen phosphorylase (GP) mRNA expression did not change but protein expression increased in the gills of *H. temminckii*

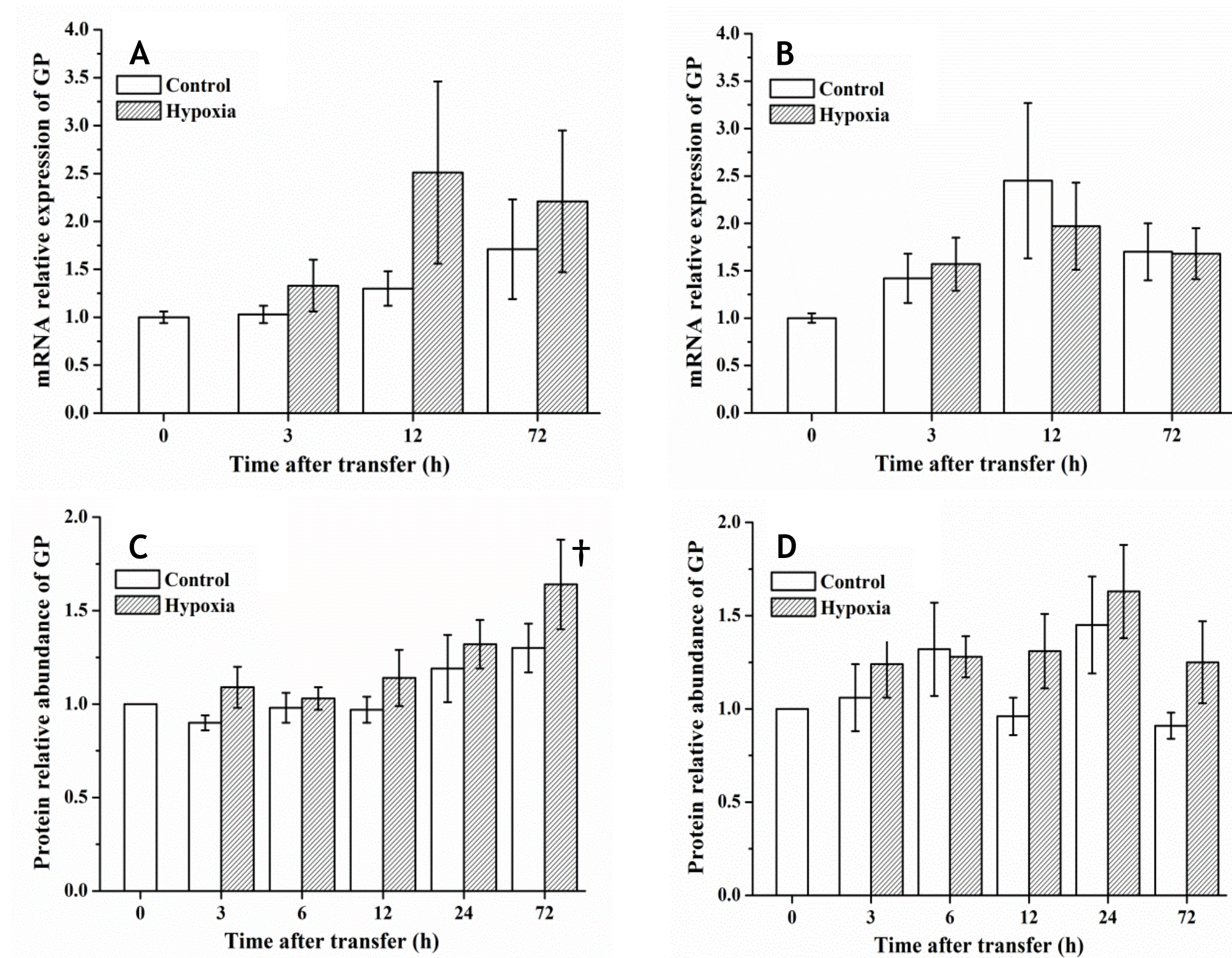


Fig.4 The relative mRNA expressions of GP in the gills (A) and liver (B) under normoxia (control, 0 h) and hypoxia. The relative protein abundance of GP in the gills (C) and liver (D) under normoxia and hypoxia. Data are presented as mean ± S.E.M (n= 8). One-way ANOVA was conducted among different treatments ($p < 0.05$). † indicates a significant difference compared with normoxia (One-way Anova, Dunnett's post hoc test, $p < 0.05$).

Discussion

ABR is an indicator of how ABR fish meet the hypoxic stress (Val *et al.*, 2006). Glycogen is a primary glucose energy source in hypoxia (Zhou *et al.*, 2000). Under hypoxia, Anabantoids increased the ABR frequency to satisfy oxygen demand from aerobic metabolism. ABR is an energy-cost behavior, and therefore, ABR frequency declines in the long-term. Furthermore, glycogen degraded to glucose as fuel to maintain NKA activity. The increase in ammonium levels in water can be an indication of enhanced protein metabolism.

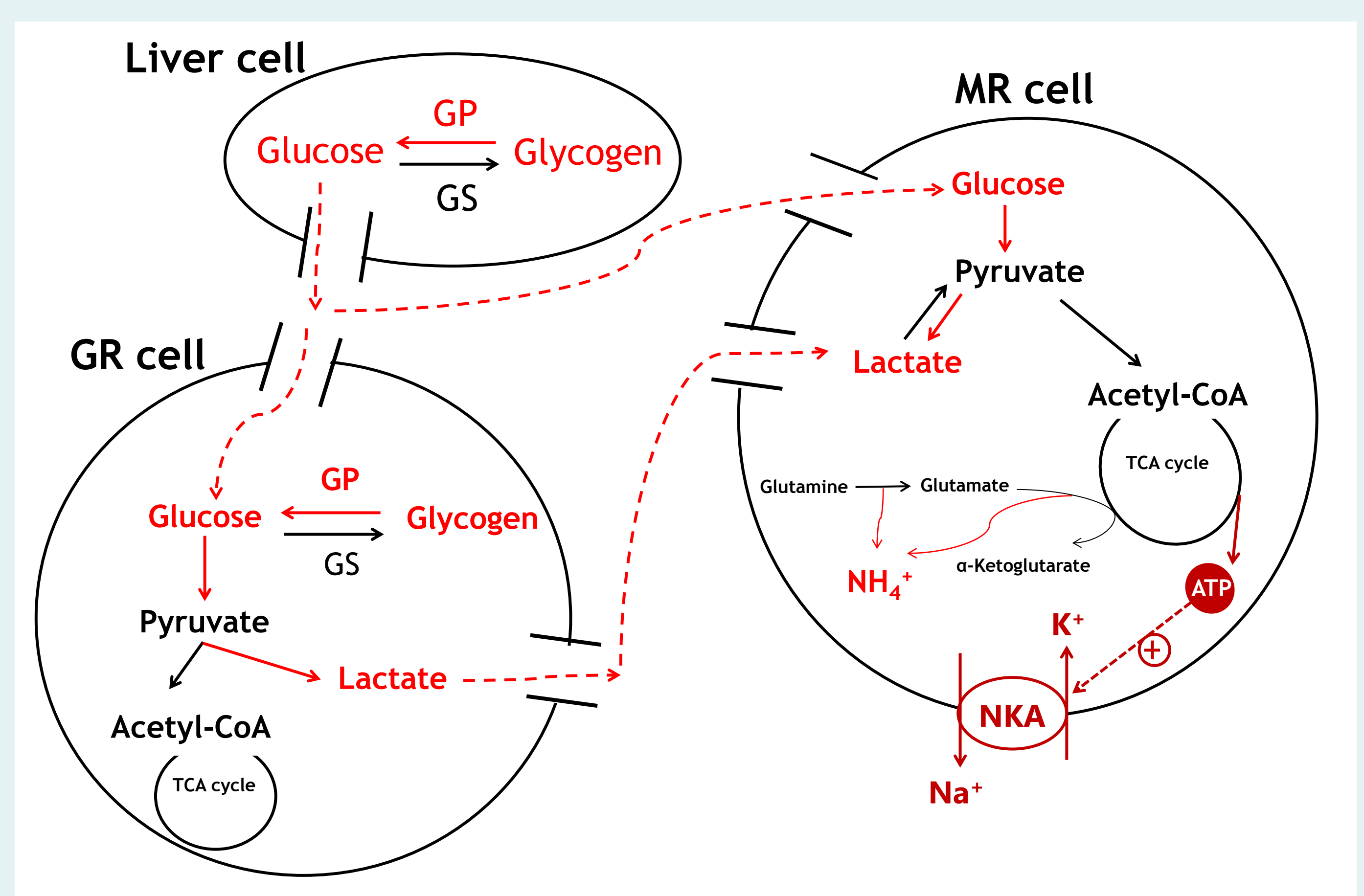
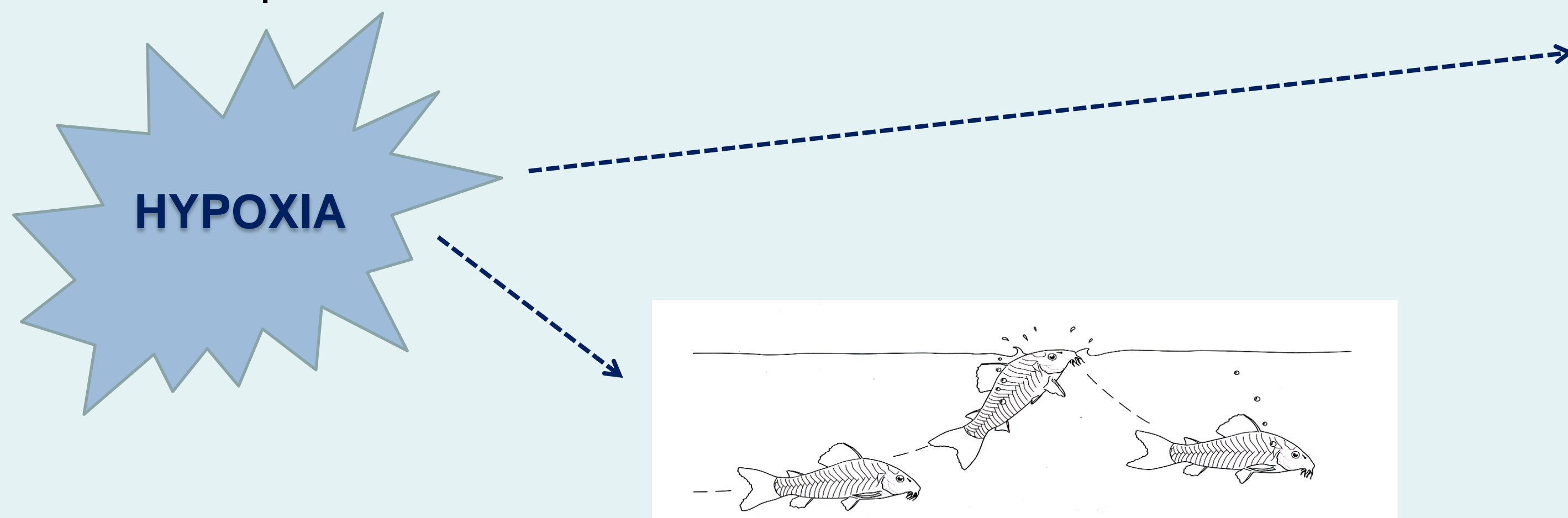


Fig.6 Proposed model for responses to hypoxia in anabantoid fishes. (modified from Graham, 1997 and Hwang, 2010)