Effects of hypoxia stress on gill remodeling and apoptosis of blunt snout bream *Megalobrama amblycephala* "Pujiang No.2"

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Abstract

Blunt snout bream (*Megalobrama amblycephala*) is an important species of freshwater aquaculture, but it is also a hypoxia-sensitive fish. Since 2007, the selective breeding under hypoxia was performed on a wild blunt snout bream population from Poyang Lake, China, and the new breed "Pujiang No.2" was obtained in 2020, which showed markedly improved hypoxia-tolerance. In this study, the changes of morphology and apoptosis in blunt snout bream "Pujiang No.2" gills were studied after exposed to 4, 7 days of hypoxia. Additionally, the *Bcl-2* and *Bad* were firstly identified in blunt snout bream, and their molecular characteristics were analyzed.

Materials and Methods

Twenty juvenile fish were exposed to hypoxia for up to 7 days at 25 ℃. Five fish were taken out for sampling at 0, 4, 7 days of exposure and 7 days of recovery, respectively. Light microscopy (LM) and scanning electron microscopy (SEM) were used to observe the changes of gill morphology and structure, and morphometric analysis was carried out. TUNEL staining was used to detect the apoptosis of gills. Additionally, the *Bcl-2* and *Bad* were firstly identified in blunt snout bream, and their molecular characteristics were analyzed. qRT-PCR was used to analyze the changes of *Bcl-2* and *Bad* mRNA expression levels in gills under hypoxia.

Results

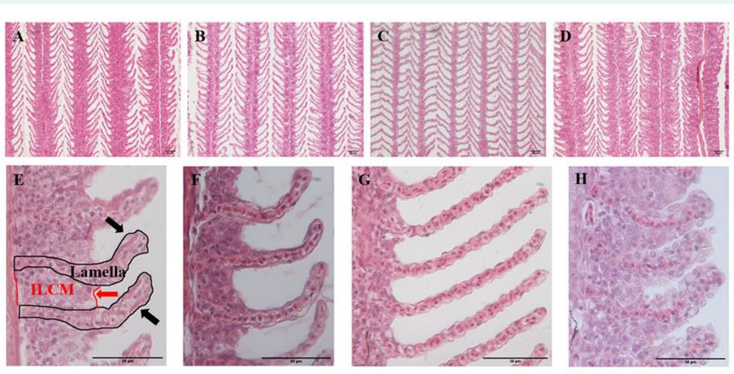


Fig. 1. Light microscope (LM) micrographs of the gill lamellae for blunt snout bream "Pujiang No.2" under hypoxia and recovery treatment. Picture series starts with normoxia (A, E), 4 days of hypoxia (B, F), 7 days of hypoxia (C, G), and 7 days of recovery in normoxic water (D, H).

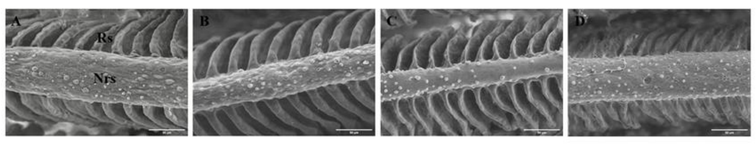


Fig. 2. Scanning electron microscopy (SEM) micrographs of the gill lamellae for blunt snout bream "Pujiang No.2" under hypoxia and reoxygenation. A-D respectively show that blunt snout bream were exposed to hypoxia for 0, 4, 7 days and returned to normoxia for 7 days.

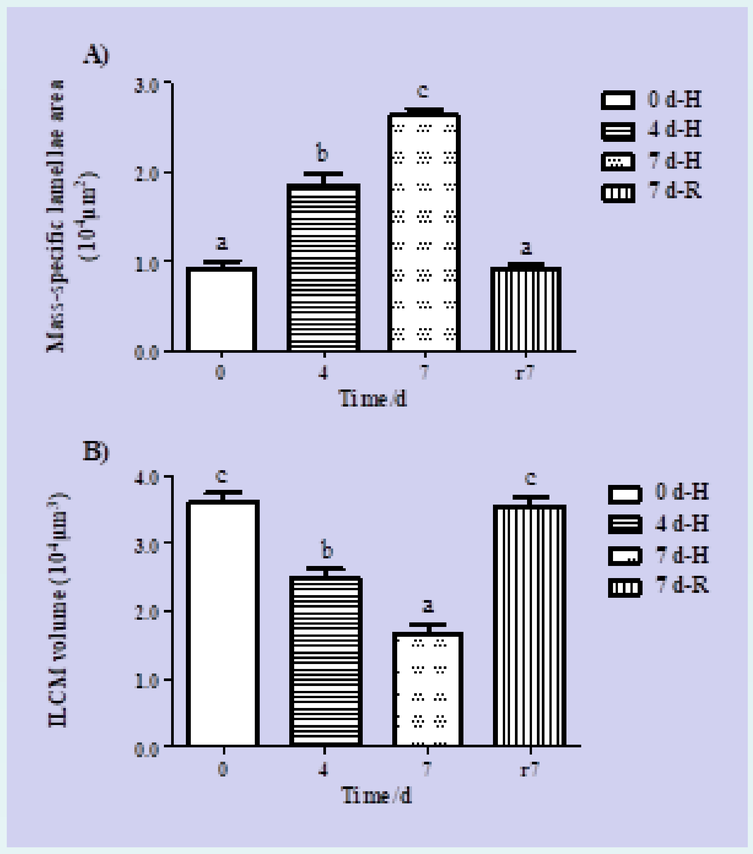


Fig. 3. Mass-specific lamellae area (A) and ILCM volume (B) change of blunt snout bream "Pujiang No.2" under hypoxia and restoration. 0 d-H: normoxia. 4 d-H: 4 days of hypoxia. 7 d-H:7 days of hypoxia. 7 d-R:7 days of recovery.

Table 2

Morphometric characteristics of blunt snout bream "Pujiang No.2" gills under hypoxia and recovery treatment.

	Gill morphometry (25°C, DO of hypoxia was kept at 2.0 mg·L^{-1})			
	Normoxia	4d of hypoxia	7d of hypoxia	7d of recovery
Protruding lamella height (μm)	48.12 ± 1.33^{a}	90.05 ± 1.69^{b}	130.87 ± 0.67^{c}	50.37 ± 1.17^{a}
Protruding lamella basal length (μm)	80.28 ± 0.54^{a}	87.09 ± 0.53^{b}	95.10 ± 0.78^{c}	81.66 ± 0.75^{a}
Protruding lamella thickness (μm)	18.77 ± 0.31^{c}	14.13 ± 0.15^{b}	9.45 ± 0.10^{a}	18.28 ± 0.26^{c}
Distance between lamellae (μm)	8.41 ± 0.26^{a}	11.28 ± 0.22^{b}	14.24 ± 0.53^{c}	8.88 ± 0.15^{a}
ILCM height (μm)	53.79 ± 1.26^{c}	26.18 ± 0.61^{b}	12.45 ± 0.19^{a}	50.47 ± 0.75^{c}

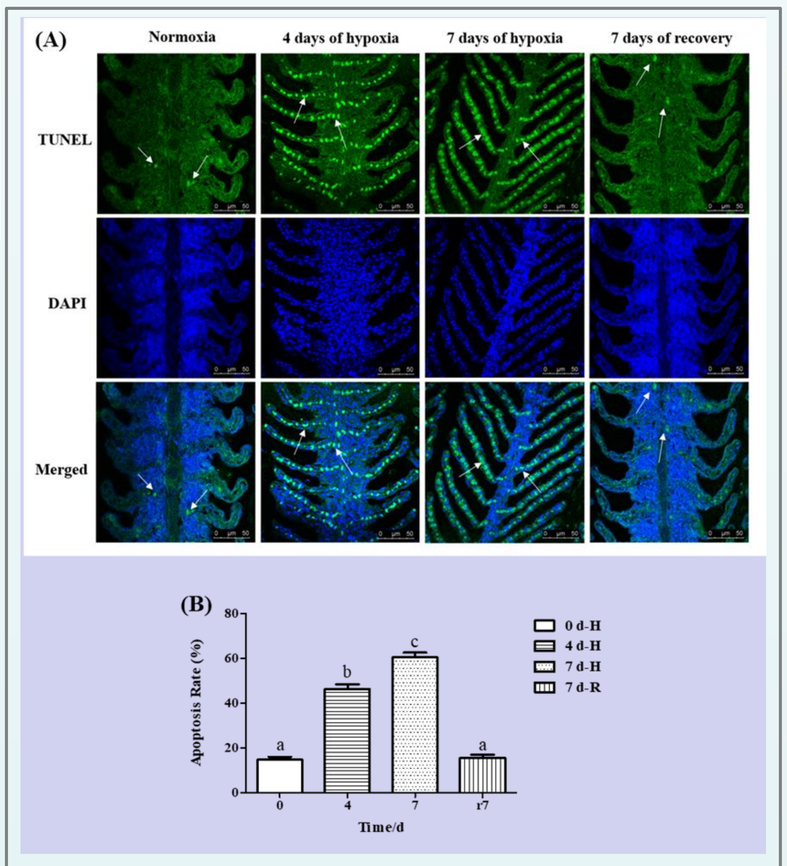
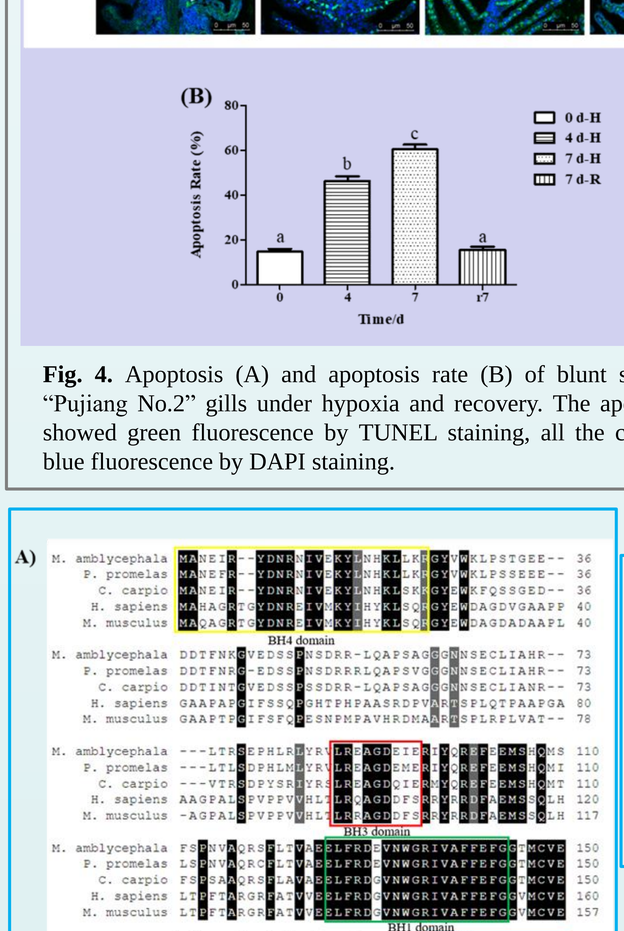
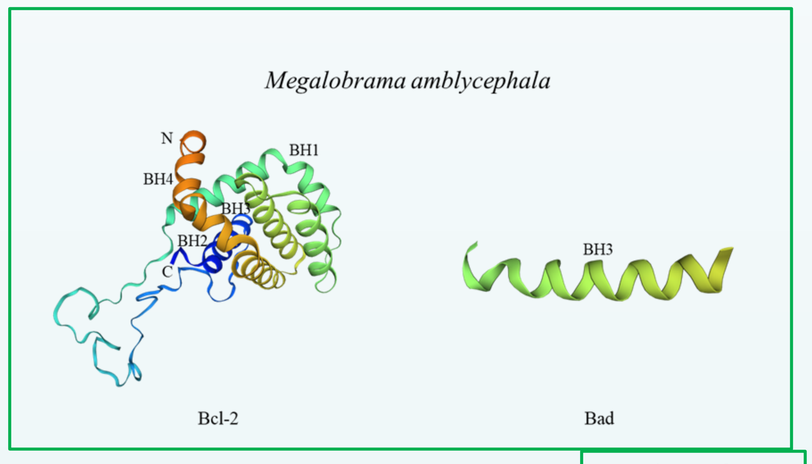


Fig. 4. Apoptosis (A) and apoptosis rate (B) of blunt snout bream "Pujiang No.2" gills under hypoxia and recovery. The apoptotic cells showed green fluorescence by TUNEL staining, all the cells showed blue fluorescence by DAPI staining.

Fig. 5. Multiple-sequence alignment of *Megalobrama amblycephala* Bcl-2 (A) and Bad (B) with other homologous species amino acid sequences. The Bcl-homology (BH) domains, BH1 domain (green box), BH2 domain (blue box), BH3 domain (red box) and BH4 domain (yellow box) are shown.





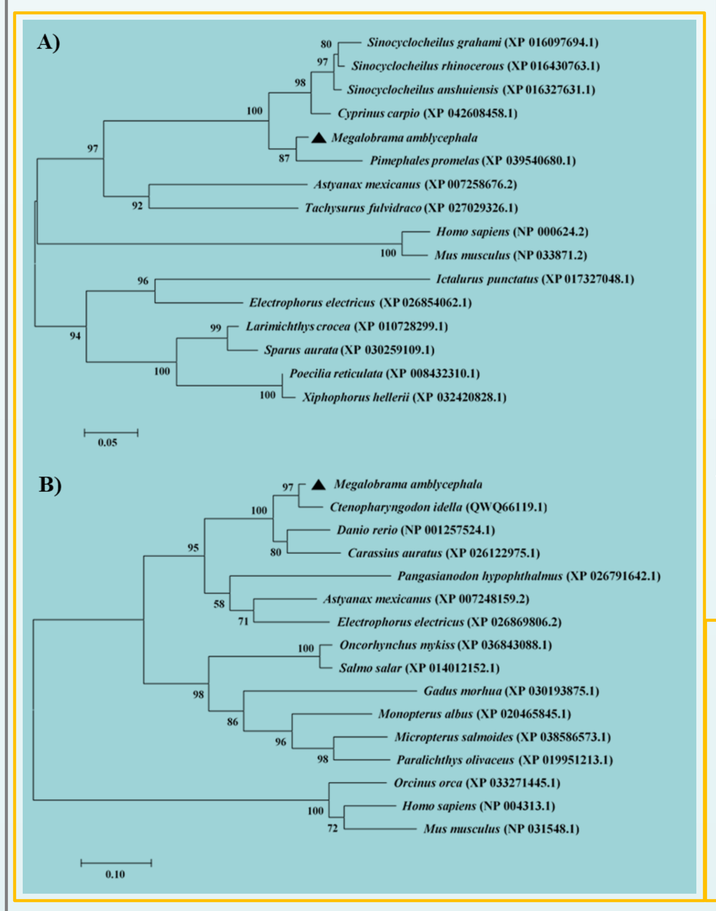


Fig. 7. The phylogenetic tree of Bcl-2 (A) and Bad (B) from Megalobrama amblycephala with other homologous species

Fig. 6. The protein three-dimensional structure of Megalobrama amblycephala Bcl-2 and Bad. There were α-helixes forming the Bcl-homology (BH) domains. BH1 (turquoise), BH2 (blue), BH3 (yellowgreen) and BH4 (brown) were illustrated.

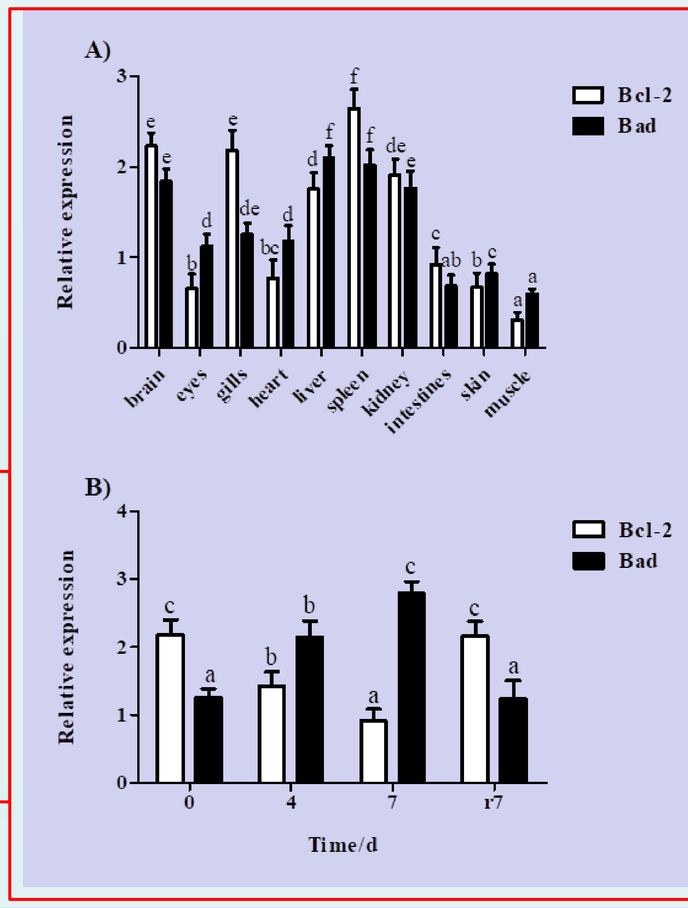


Fig. 8. Tissue distribution analysis of Bcl-2, Bad mRNA in the healthy blunt snout bream (A) and the relative expression levels of mRNA of Bcl-2, Bad in the blunt snout bream gills after 0, 4, 7 days of hypoxia and 7 days of restoration (B) were analyzed by the quantitative real-time PCR (qRT-PCR)

Concusions

Our study showed that the new variety "Pujiang No.2" of blunt snout bream have the ability of gill remodeling in response to hypoxia. The apoptosis signals were also increased in gills under hypoxia. The *Bcl-2* and *Bad* were firstly identified from blunt snout bream and were found widely present in all tissues investigated. The *Bcl-2* and *Bad* may be participated in the regulation of hypoxia-induced apoptosis. These findings indicate that the increase of apoptosis signals with the changes of *Bcl-2* and *Bad* expression levels might be contributed to the gill remodeling of blunt snout bream under hypoxia stress. Collectively, our results offer a new perspective into the cellular and molecular mechanism of hypoxia-induced gill remodeling in teleost fishes.