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Factors limiting the spread of middle- and low-altitude fishes to the Qinghai-Tibet plateau

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The distribution pattern of species is determined by the environment and their adaptability to the environment. Qinghai-Tibet Plateau has become a natural laboratory for studying adaptive evolution due to its extreme environmental characteristics such as low temperature, low oxygen, high salinity and high ultraviolet radiation (UVR). Fish are sensitive to the environmental stress, so they are ideal materials for studying high-altitude adaptation of animals. Previous studies have mainly focused on the adaptability of plateau species, but the reasons why plain species cannot spread to the plateau have been ignored. In this study, stress experiments and histological experiments were used to compare the tolerance of six Barbini fishes (family: Cyprinidae) distributed at different altitudes and regions to low temperature, low oxygen, salinity and UVR. Results showed that the tolerance of fishes to high-altitude environmental stress factors was closely related to the environmental stress of their main habitats. The high-altitude fish *Gymnocypris eckloni* had strong tolerance to all stress factors, while the other five fishes from middle and low altitudes could not adapt to single or multiple stress factors, with significant interspecific differences. Among these factors, middle- and low-altitude fishes showed common low tolerance to UVR, suggesting that high UVR, the factor lacking at low altitude areas, plays an important role. Moreover, during the uplift of the Qinghai-Tibet Plateau, *Schizothorax* fish disappeared from the middle of the plateau. We speculate that this was caused by its intolerance to the increasingly extreme plateau environment, especially salinity.

KEYWORDS

plateau fish, Schizothoracinae, environmental stress, high-altitude adaptation, fishes distribution

1 Introduction

Adaptation refers to the process of survival and reproduction in which the morphological structure and physiological function of organisms are suitable for certain environmental conditions (Brandon, 1978). In nature, the environment is constantly changing, and organisms respond to various environmental pressures (Bijlsma and Loeschcke, 2012). For individuals, they can regulate their phenotypes through phenotypic plasticity, thereby increasing their tolerance to environmental stress (West-Eberhard, 2003; Pigliucci, 2005; Ghahambor et al., 2015); for populations, specific environmental pressures will accelerate the corresponding genetic variation (Rosenberg, 2001; Galhardo et al., 2007; Martincorena et al., 2012), and will also play a selective role in the genetic variation of populations (Haldane, 1932; Charlesworth et al., 2017). These processes together constitute the adaptive evolution of organisms (Bijlsma and Loeschcke, 2012), and the distribution pattern of species is gradually formed in these processes (Zhao Z. et al., 2022).

Qinghai-Tibet Plateau, with an average altitude of more than 4500 meters, is known as the “roof of the world” and the “third pole” (Anand et al., 1986; Qiu, 2008). It is the highest plateau in the world and a hot spot for studying adaptive evolution (Qu et al., 2013; Mao et al., 2021; Li et al., 2022). Under the collision and extrusion of Indian Ocean plate and Eurasian plate, the plateau gradually uplifted during the Neogene (23 ~ 2.6 Ma) (Tapponnier et al., 2001; Su et al., 2019; Spicer et al., 2021), and the rising altitude brought severe cold, low oxygen, strong UVR and drought (Machta and Hughes, 1970; Li and Fang, 1999; Zhou and Zhang, 2005; Liu W. et al., 2009; Liu Y. et al., 2009). Plateau animals formed their own unique adaptation mechanisms to resist the extreme environment, and the distinct species distribution pattern of the Qinghai-Tibet Plateau and its surroundings was also formed (Wang X. et al., 2015).

As poikilothermal animals living in water, fish are very sensitive to water temperature, dissolved oxygen and salinity (Scholander et al., 1953; Lindsey, 1966; Kültz, 2015). Moreover, the lack of hair on the surface of fish causes direct effect of light on the skin, making fish more vulnerable to UVR (Hunter et al., 1979; Hunter et al., 1981). Therefore, fish is ideal material for studying high-altitude adaptation of animals. There are unique fish groups distributed on the Qinghai-Tibet Plateau, among which the Schizothoracinae fishes (family: Cyprinidae) are the most representative. For example, *Gymnocypris eckloni* lives in the waters with an altitude of more than 3000 m. These fish experience cold and hypoxic water, meanwhile, high-altitude clear rivers and lakes have low UVR attenuation (Bullock, 1988; Liu et al., 2021), and fish need to face more intense UVR. In addition, drought leads to a large amount of salt deposition in the water bodies, which makes the water on the Qinghai-Tibet Plateau more salty (Liu W. et al., 2009). It can be seen that the stress faced by plateau fish is multifaceted, and single or multiple plateau stress factors may cause plain fish to fail to adapt to the plateau environment.

Previous studies have mainly focused on the high-altitude adaptability of plateau species, but little is known about the reasons why plain species cannot adapt to the plateau, because it is not clear which plateau stress factors play a major role (Cao et al.,

2008; Yang et al., 2015; Xu et al., 2016; Chen et al., 2020). Under such unclear conditions, it may cause some studies to blindly select plain control species. For example, *Cyprinus carpio* with strong adaptability has strong tolerance to low temperature and low oxygen, for some plateau stress factors also exist in the plain areas. If it is selected as a control to study the adaptability of plateau fish to low temperature or low oxygen, it may cause the failure of experimental design. In addition, previous studies on the tolerance of plateau fish often focused on a single stress factor, and mostly used genetic analysis methods (Xu et al., 2016; Yang et al., 2020; Jin et al., 2022), lacking of stress tolerance experiments to verify. Therefore, the comparison of multi-group and multi-factor stress experiments can help us better understand the adaptation process of plateau fish and the causes of distribution pattern, so as to answer “why middle- and low-altitude fish cannot reach the plateau”.

In our study, we compared the tolerance of six Barbini fishes (family: Cyprinidae) distributed at different altitudes and regions (Figure 1) to low temperature, low oxygen, salinity and UVR. Through experiments, we want to answer two scientific questions: (1) What are the differences in the tolerance of fish at different altitudes to different high-altitude environmental stress factors? (2) What factors limit the spread of middle- and low-altitude fish to the plateau? Single factor or multiple factors?

2 Materials and Methods

2.1 Ethics statement

All the animal procedures were conducted with the approval of the Committee of Laboratory Animal Experimentation at Southwest University, Chongqing, China and were in full compliance with the Committee’s guidelines [protocol number (2014)25].

2.2 Experimental animals

Gymnocypris eckloni (subfamily: Schizothoracinae) is mainly distributed in the upper reaches of the Yellow River and Qaidam Basin (average altitude > 3000 m) on the Qinghai-Tibet Plateau. *Schizothorax davidi* (subfamily: Schizothoracinae) and *Percocypris pingi* (subfamily: Barbinae) live in the middle altitude (average altitude: 1000 ~ 2000 m) of the upper reaches of the Yangtze River. *Spinibarbus sinensis* (subfamily: Barbinae), *Luciobarbus capito* (subfamily: Barbinae) and *Cyprinus carpio* (subfamily: Cyprininae) come from low altitude plains (average altitude < 1000 m) (Ding, 1994). These six tetraploid fishes with close phylogenetic relationships experience different types and intensities of environmental stress in their respective habitats, which is an ideal model to explore the relationship between fish habitat characteristics and their stress tolerance (Wang et al., 2016; Yang et al., 2022). The experimental fishes with body length of 10 ± 2 cm are artificially bred offspring of the wild population and are in the juvenile stage to exclude the effects of gender (Júnior et al., 2012). They were

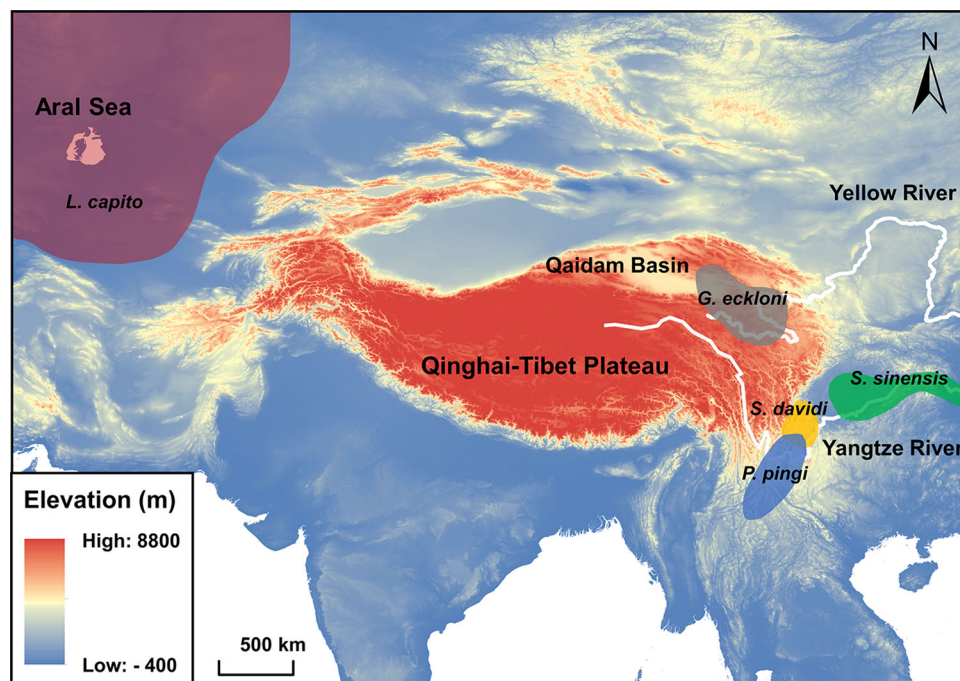


FIGURE 1

Main distribution of the wild populations of experimental fishes. Grey area: *Gymnocypris eckloni*. Orange area: *Schizothorax davidi*. Blue area: *Percocypris pingi*. Green area: *Spinibarbus sinensis*. Red area: *Luciobarbus capito*. *Cyprinus carpio* is widely distributed in the wild, and its distribution is not shown in the figure.

domesticated in the laboratory circulating aquariums (60×32×28 cm) holding 50 L of water each at $14 \pm 1^\circ\text{C}$ on a 12 L: 12 D photoperiod where they were fed twice a day. Before experiments, the experimental fishes were fasted for 24 h (Figuerola et al., 2000).

2.3 Stress experiments

The response of fish to chronic stress and acute stress is different (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Barcellos et al., 2006), so for the factors of low temperature, low oxygen and salinity, chronic stress experiments and acute stress experiments were set up to more accurately reflect the tolerance of fishes to high-altitude environmental stress. In these experiments, the loss of balance of fish was used as a sign of reaching the tolerance limit (Belanger et al., 1986), the unbalanced fish were kept in normal water and no longer participated in subsequent experiments. For the UVR exposure experiment, we set the UVR intensity according to the actual UVR intensity on the Qinghai-Tibet Plateau. All experiments were carried out in Chongqing, China (250 m above sea level), the low temperature, low oxygen and salinity experiments were carried out from November to December 2020, and the UVR experiments were carried out from March to April 2021.

2.3.1 Low temperature

2.3.1.1 Chronic low temperature experiment

Two 65 L white breeding buckets (with a bottom diameter of 34 cm) were filled with about 50 L dechlorinated tap water and put into

the refrigerator (Haier BC/BD-519HCM), and the water temperature was reduced to 12°C as the initial temperature. The oxygenating heads were added to provide sufficient oxygen. Then, we put 10 fish of each species into 2 buckets (5 fish per bucket) and closed the door of the refrigerator so that the water temperature gradually decreased at a certain rate (decreased by about 2°C per minute in the first 5 minutes, about 1°C in 5-6 minutes, and finally stabilized at $1 \pm 0.1^\circ\text{C}$), and the change of water temperature was monitored with a thermometer (Figure 2A). During the experiment, we opened the refrigerator door every 10 min to observe, recorded water temperature and fished out the unbalanced fish, then quickly closed the refrigerator door.

2.3.1.2 Acute low temperature experiment

The fish tank (25×15×20 cm) was filled with 5 L of dechlorinated ice-water mixture maintained at $1 \pm 0.1^\circ\text{C}$, and an oxygenating head was added to provide sufficient oxygen (Figure 2B). We put one fish at a time, recorded the time when the fish lost balance, and fished it out. The water was replaced and the water temperature was adjusted each time.

2.3.2 Low oxygen

2.3.2.1 Chronic hypoxia experiment

Eight transparent PE plastic bags were packed into 5 L dechlorinated tap water at $19 \pm 1^\circ\text{C}$ (between the water temperature of warm water fish and cold water fish). We put one fish of each species into each plastic bag, inserted a rubber tube clamped with a clip. Then, we discharged the air in the plastic bags to keep the same volume of each bag, sealed the bags, and observed the activity of fish. When the

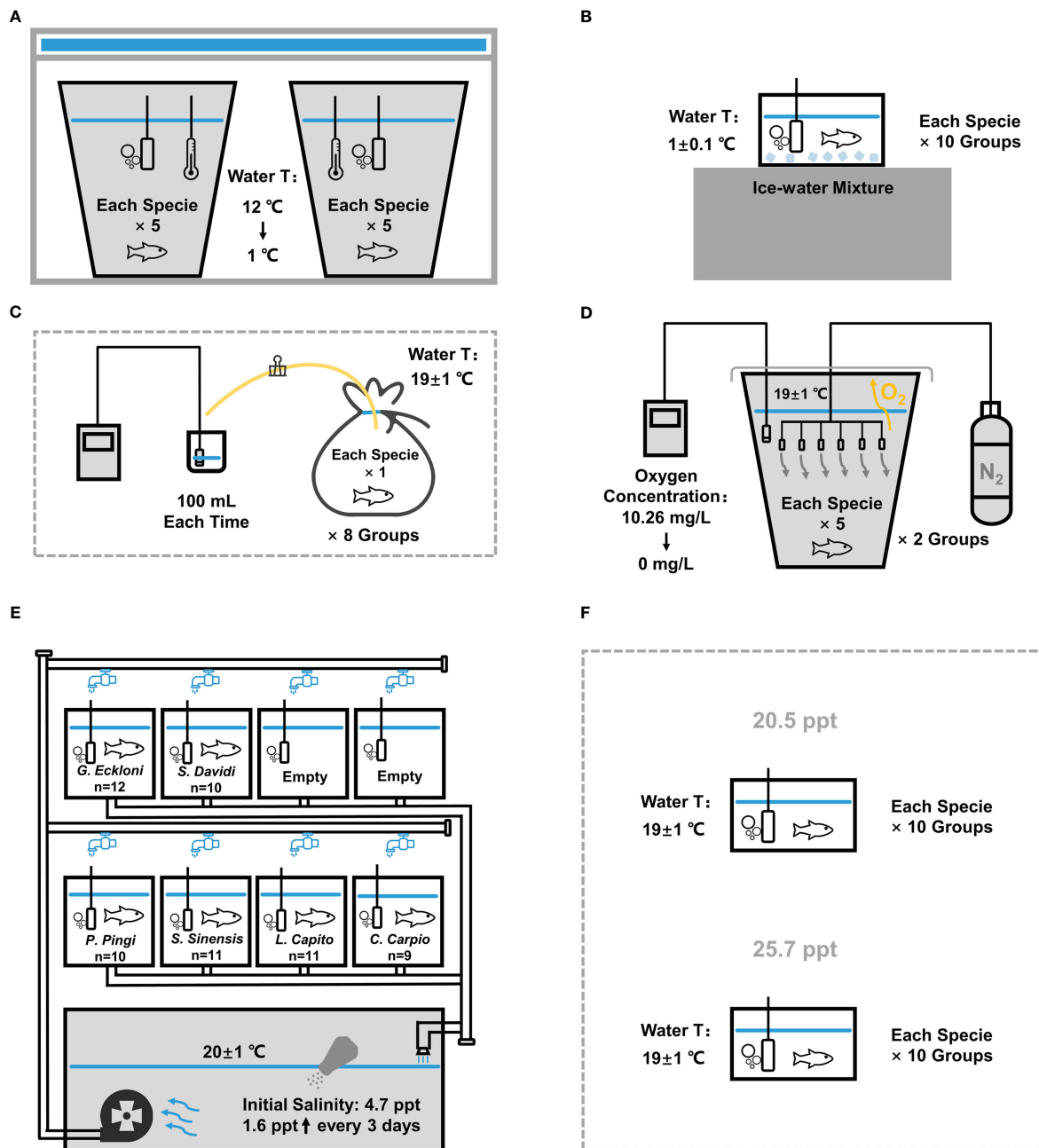


FIGURE 2 Experimental device diagram of stress experiments. (A) Chronic low temperature experiment. (B) Acute low temperature experiment. (C) Chronic hypoxia experiment. (D) Acute hypoxia experiment. (E) Chronic salinity experiment. (F) Acute salinity experiment. The experimental device of the UVR exposure experiment is shown in our previous study.

fish was out of balance, we opened the clamp on the rubber tube, and extruded 100 mL water from the plastic bag for measurement. The dissolved oxygen concentration was measured with a dissolved oxygen meter (HACH HQd, HACH, America) (Figure 2C), and the fish number and oxygen concentration were recorded.

2.3.2.2 Acute hypoxia experiment

A 65 L white breeding bucket was filled with about 50 L dechlorinated tap water at $19 \pm 1^\circ\text{C}$. The experiment was carried

out in two times, with 5 fish of each specie each time. After 1 h adaptation, the fish were treated with low oxygen by nitrogen replacement method (Zhang et al., 2014): six aeration heads connected to the nitrogen tank were evenly put into the bucket to introduce nitrogen, the dissolved oxygen meter probe was put into the water, and the bucket was covered with plastic film to reduce the contact between water and air (Figure 2D). In the first hour, the oxygen concentration was reduced from 10.26 mg/L to 2.5 mg/L; after half an hour, it gradually decreased to 1.5 mg/L; subsequently,

it decreased by 0.1 mg/L every 10 min; after reaching 0.5 mg/L, it decreased by 0.1 mg/L every 20 min until it decreased to 0. During the process, the corresponding oxygen concentration of each fish was recorded when it lost balance, and the unbalanced fish was fished out.

2.3.3 Salinity

2.3.3.1 Chronic salinity experiment

The circulating water system (with 8 aquariums (60×32×28 cm) holding 50 L of water each and a circulating flume with 230 L capacity) was filled with about 630 L dechlorinated tap water at $20 \pm 1^\circ\text{C}$, and each aquarium was equipped with an oxygenating head. Six species were placed in six aquariums of the system to adapt for 2 days. After that, we added 3 kg NaCl to the system with a salinometer (HACH HQd, HACH, America) measured a salinity of 4.7 ppt, and 1 kg NaCl was added every 3 days to increase the salinity of water in the system by about 1.6 ppt each time (Figure 2E). During the experiment, we checked the fish every day, fished out the unbalanced fish and recorded the salinity at that time.

2.3.3.2 Acute salinity experiment

According to the result of chronic experiment, the acute experiment was divided into two higher salinity levels of 20.5 ppt and 25.7 ppt, which also met the salinity range set in previous studies. (Sun et al., 1995; Karsiotis et al., 2012; Ghosh et al., 2019). Two fish tanks (25×15×20 cm) equipped with oxygenating heads were filled with 5 L of dechlorinated tap water at $19 \pm 1^\circ\text{C}$, and salt was added to make the salinity in the two tanks reach 20.5 ppt and 25.7 ppt (Figure 2F). We put one fish into each of the two fish tanks at a time, recorded the tolerance time, and then fished it out. Before each species replacement, we replaced the water, and re-adjusted the salinity and temperature.

2.3.4 UVR

In our previous studies, we have compared the UVR tolerance of *Gymnocypris eckloni*, *Schizothorax davidi*, *Spinibarbus sinensis*, *Luciobarbus capito* and *Cyprinus carpio* (Gu et al., 2022a). In this study, we supplemented the experiment of *Percocypris pingi* with the same experimental method, and the specific method can refer to our previous research. The brief method is as follows:

2.3.4.1 UVR exposure

Twelve *Percocypris pingi* with body length of 10 ± 2 cm were selected and divided into experimental group (n=6) and control group (n=6), which were placed in two 65 L white breeding buckets respectively. The water kept flowing and updated, and the water temperature was controlled at $16 \pm 1^\circ\text{C}$. The bucket of the experimental group was placed under a 275 W full-spectrum solar lamp (Sparkzoo, China), and the UVR intensity at the center of the water surface was about 0.108 MJ/m²/h (UVB/UVA=0.09). The experimental group was exposed to the solar lamp for 12 hours every day for 7 days from 7:00 to 19:00, and the UVA and UVB daily dose was approximately 1.30×10^3 kJ/m²/d. In contrast, the bucket of the control group was covered with a transparent acrylic sheet, which resulted in nearly undetectable UVR.

2.3.4.2 Histology

The experimental fishes were euthanized with an overdose of MS-222 (tricaine methane sulfonate, 500 mg/L) on the 7th night. The lateral skin tissue was collected, fixed in 4% paraformaldehyde for approximately 12 hours at 4°C and then transferred to a 70% alcohol solution for preservation. Traditional paraffin sections (6 μm) were obtained using a microtome (Leica 2245, Leica, Germany), and were stained with an AB-PAS staining kit (DG1014-6×100 mL, Dingguo). All sections were observed using a microscope (Nikon SMZ25, Japan), a complete and representative section was selected for each sample, and three random images of this section were photographed (100×) for measurement.

2.3.4.3 Damage levels

The damage caused by UVR exposure was classified into 0-5 levels (Bullock, 1988; Gu et al., 2022a).

2.4 Phylogenetic analysis

The complete mitochondrial genome sequences of six experimental fishes was downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>), and the DNA sequences were then aligned using MAFFT Online Service (Kato et al., 2019). The maximum likelihood phylogenetic tree was constructed using the default setting of IQ-TREE with default models (Trifinopoulos et al., 2016).

2.5 Statistical analyses

Data was analyzed by One-Way ANOVA and expressed as the mean \pm standard deviation (SD) with GraphPad prism 9.0 software (GraphPad, USA). The Duncan's range test was used to analyze the differences in tolerance among species with IBM SPSS Statistics (version 27.0, Armonk, New York, United States), and significant differences ($P < 0.05$) were expressed by different lowercase letters. The correlation between fish habitat stress levels and their stress tolerance was analyzed using Spearman rank correlation with IBM SPSS Statistics. All graphs were generated with GraphPad prism 9.0 software. The images of sections were measured with Photoshop 2020 (Adobe, San Jose, CA, USA).

3 Results

3.1 Low temperature

3.1.1 Chronic low temperature experiment

Spinibarbus sinensis was the first to lose balance when the water temperature was reduced to 3.3°C at 4.33 h. At 4.75 h (2.1°C), all individuals reached the tolerance limit, and the average tolerance time was $4.54 (\pm 0.16)$ h. The tolerance of *Percocypris pingi* is similar to that of *Spinibarbus sinensis*, they successively lost their balance during the 4.5 h (2.7°C) to 5.17 h (1.9°C) of the experiment, and the average tolerance time was $4.72 (\pm 0.25)$ h. After 5.5 h of the experiment, the water temperature remained stable at $1 \pm 0.1^\circ\text{C}$. At 6 h, intolerance began to

appear in *Schizothorax davidi* and *Luciobarbus capito*, individuals of the two fishes gradually lost their balance in the following 6 h, with an average tolerance time of 6.27 (± 0.24) h and 7.01 (± 1.91) h, respectively. When the experiment continued to 13 h, all individuals of *Gymnocypris eckloni* and *Cyprinus carpio* could still swim normally and did not reach the tolerance limit (Figure 3A). Compared with the first four species, they showed stronger tolerance to chronic low temperature.

3.1.2 Acute low temperature experiment

Compared with the chronic experiment, the tolerance time of all fishes was significantly shortened ($P < 0.01$). The acute low temperature tolerance of *Gymnocypris eckloni* (7.91 ± 2.85 min) was significantly stronger than that of *Cyprinus carpio* (2.69 ± 1.25 min), *Schizothorax davidi* (1.52 ± 0.36 min), *Percocypris pingi* (1.50 ± 0.31 min), *Spinibarbus sinensis* (0.96 ± 0.21 min) and *Luciobarbus capito* (0.89 ± 0.24 min) (Figure 3B, $P < 0.05$), and the tolerance time of some *Gymnocypris eckloni* individuals even exceeded 10 min.

3.2 Low oxygen

3.2.1 Chronic hypoxia experiment

Percocypris pingi first began to lose balance when the dissolved oxygen in the water was reduced to 1.86 mg/L, and the average dissolved oxygen concentration they tolerated was 1.71 (± 0.11) mg/L. The chronic hypoxia tolerance of *Spinibarbus sinensis* (1.23 ± 0.21 mg/L), *Schizothorax davidi* (1.11 ± 0.17 mg/L) and *Luciobarbus capito* (1.09 ± 0.14 mg/L) was similar, and slightly stronger than that of *Percocypris pingi*. The tolerance of *Gymnocypris eckloni* (0.85 ± 0.12 mg/L) and *Cyprinus carpio* (0.78 ± 0.07 mg/L) was similar, which was significantly stronger than that of the other four species (Figure 3C, $P < 0.05$).

3.2.2 Acute hypoxia experiment

Percocypris pingi (0.37 ± 0.05 mg/L), *Schizothorax davidi* (0.33 ± 0.08 mg/L) and *Spinibarbus sinensis* (0.33 ± 0.07 mg/L) had weak acute hypoxia tolerance. The adaptability of *Gymnocypris eckloni* (0.15 ± 0.02 mg/L) to acute hypoxia was similar to that of *Luciobarbus capito* (0.17 ± 0.02 mg/L), while the tolerance of *Cyprinus carpio* (0.04 ± 0.01 mg/L) to acute hypoxia stress was significantly stronger than that of the two (Figure 3D, $P < 0.05$).

3.3 Salinity

3.3.1 Chronic salinity experiment

Salinity has acute toxicological effects on freshwater fish, and there are great differences in salinity tolerance among different species (Kefford et al., 2004; Varsamos et al., 2005; Bachman and Rand, 2008). Under the chronic salinity stress, *Schizothorax davidi* (15.06 ± 0.83 mg/L), *Percocypris pingi* (15.38 ± 0.67 mg/L) and *Spinibarbus sinensis* (15.91 ± 0.85 mg/L) quickly reached the tolerance limit, and the tolerance of the three was slightly different. The chronic salinity tolerance of *Gymnocypris eckloni* (16.77 ± 0.72 mg/L) was significantly stronger than that of the first three fishes, but weaker than that of *Luciobarbus capito* (17.50 ± 0.57 mg/L) and *Cyprinus carpio* (17.61 ± 0.58 mg/L) (Figure 3E, $P < 0.05$).

3.3.2 Acute salinity experiment

At the salinity level of 20.5 ppt, *Schizothorax davidi* (1.43 ± 0.16 h), *Gymnocypris eckloni* (1.75 ± 0.22 h), *Percocypris pingi* (1.82 ± 0.30 h), *Spinibarbus sinensis* (2.46 ± 0.25 h) and *Cyprinus carpio* (2.91 ± 1.07 h) were out of balance at about 2 h. The tolerance time of *Luciobarbus capito* (4.96 ± 0.88 h) to acute salinity stress at 20.5 ppt level was significantly longer than that of the other five species (Figure 3F, $P < 0.05$). At the salinity level of 25.7 ppt, all the six species reached the tolerance limit in a very short time, especially *Schizothorax davidi* (0.40 ± 0.05 h) showed intolerance at 0.33 h of the experiment (Figure 3G). The acute experiment showed different results compared with the chronic experiment, it was speculated that 20.5 ppt exceeded the short-term salt tolerance limit of five species except *Luciobarbus capito*, while 25.7 ppt exceeded the limit of all species in the experiment. Therefore, fishes with strong tolerance in chronic salinity experiment did not perform better in acute experiment.

3.4 UVR

UVR can cause a series of acute damage to fish, especially the direct damage to skin (Bullock and Coutts, 1985). High-intensity UVR can lead to severe degeneration of fish epidermis, loss of scales, increase of epidermis thickness, rupture of club cells, and reduction, necrosis or shedding of mucous cells (Osman and Harabawy, 2010). In previous experiments, *Spinibarbus sinensis* (5), *Cyprinus carpio* (4) and *Luciobarbus capito* (2.67 ± 0.82) had weak UVR tolerance, *Schizothorax davidi* (1.17 ± 0.41) was slightly stronger, and *Gymnocypris eckloni* (0) had the strongest UVR tolerance. In this experiment, all the *Percocypris pingi* individuals in the experimental group died in the last three days of exposure, and the damage level was 5 (Figures 3H, 4A–L). Only the skin of control group was taken and made into paraffin sections.

4 Discussion

4.1 Factors limiting the spread of low-altitude fishes to the plateau

In the long evolutionary process, the tolerance of animals to environmental stress is compatible with the environment in which they live (Wu et al., 2014; Witt and Huerta-Sánchez, 2019). Qinghai-Tibet Plateau has extreme environmental conditions that are not available in low-altitude plain areas, plateau fish have acquired the ability to resist high-altitude environmental stress during long-term adaptive evolution (Zhuang et al., 2012; Little and Seebacher, 2013; Guan et al., 2014; Kültz, 2015; Mustafa et al., 2015; Sushchik et al., 2018; Nobrega et al., 2019; Evans and Kültz, 2020; Yuan et al., 2020). In this study, the stress experiments of low temperature, low oxygen, salinity and UVR were carried out. As a high-altitude fish, *Gymnocypris eckloni* showed high tolerance in all stress experiments, including salinity experiments. During the “Gonghe Movement” (0.15 Ma), Qinghai Lake was separated from Yellow River, and *Gymnocypris przewalskii* (living in the saltwater lake Qinghai Lake) and *Gymnocypris eckloni* were two

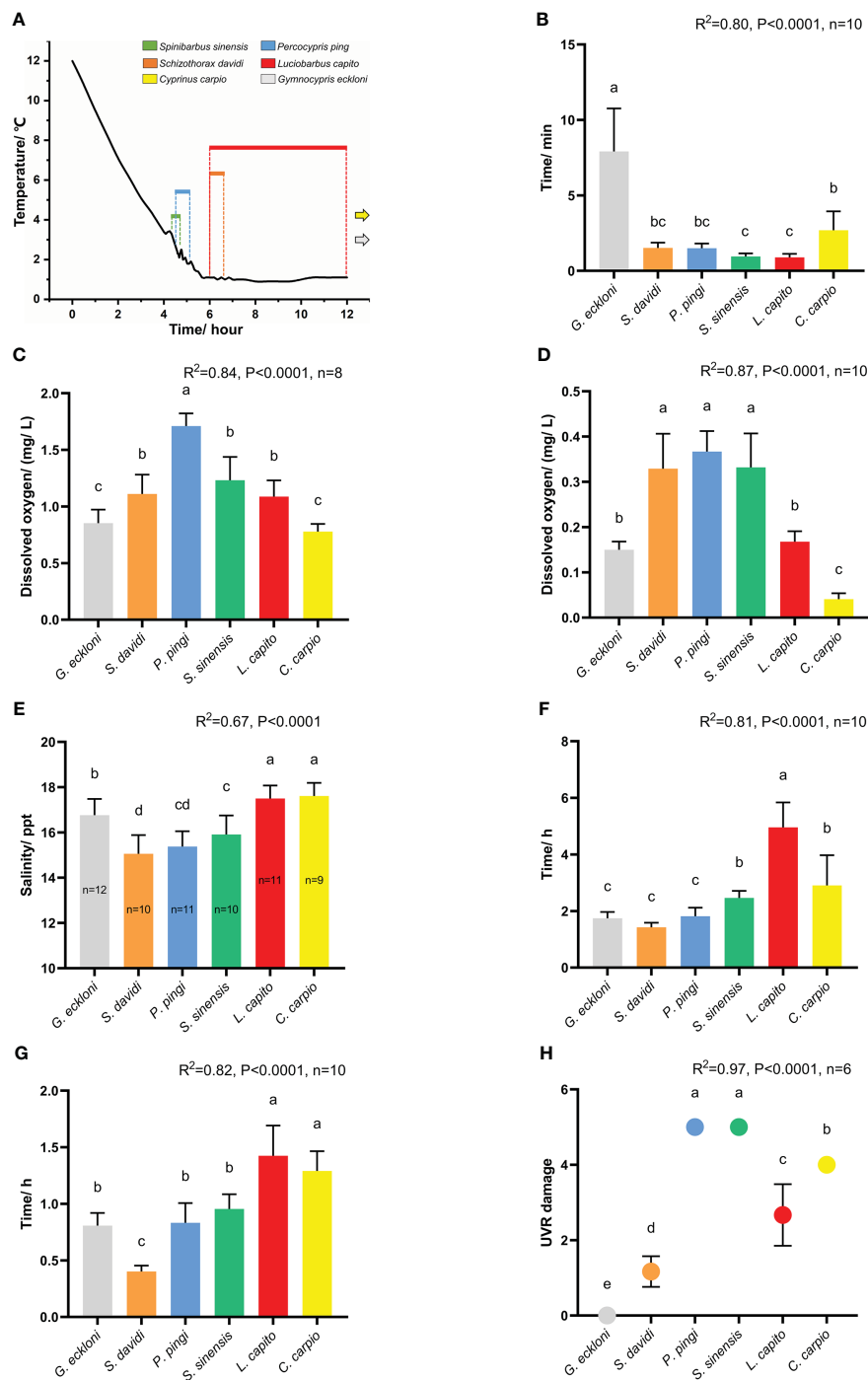


FIGURE 3

The tolerance of experimental fishes to stress factors in stress experiments. (A) Chronic low temperature experiment (n=10). The black curve indicates temperature change; the colored lines indicate that the experimental fishes lost their balance at the corresponding time; the grey and yellow arrows indicate that *Gymnocypris eckloni* and *Cyprinus carpio* are not out of balance in the experiment. (B) Acute low temperature experiment. (C) Chronic hypoxia experiment. (D) Acute hypoxia experiment. (E) Chronic salinity experiment. (F) Acute salinity experiment (20.5 ppt). (G) Acute salinity experiment (25.7 ppt). (H) UVR exposure experiment. The height and whiskers indicate the mean \pm SD. Different lowercase letters (a–d) indicate significant differences in Duncan’s range test ($\alpha=0.05$).

groups that differentiated after this event (Zhao et al., 2005; Zhao et al., 2007; Qi et al., 2013). Due to the short differentiation time, most of the genetic similarities between the two are still retained, and some other *Gymnocypris* fishes are distributed in saltwater lakes of the central Qinghai-Tibet Plateau (e.g., *Gymnocypris namensis*)

(Yuan et al., 2002), which explains the reason why *Gymnocypris eckloni* living in fresh water has strong salinity tolerance. The experiment results of it also proved that this experimental method can effectively reflect the actual adaptability of fish to plateau stress factors. Interestingly, fishes at middle and low

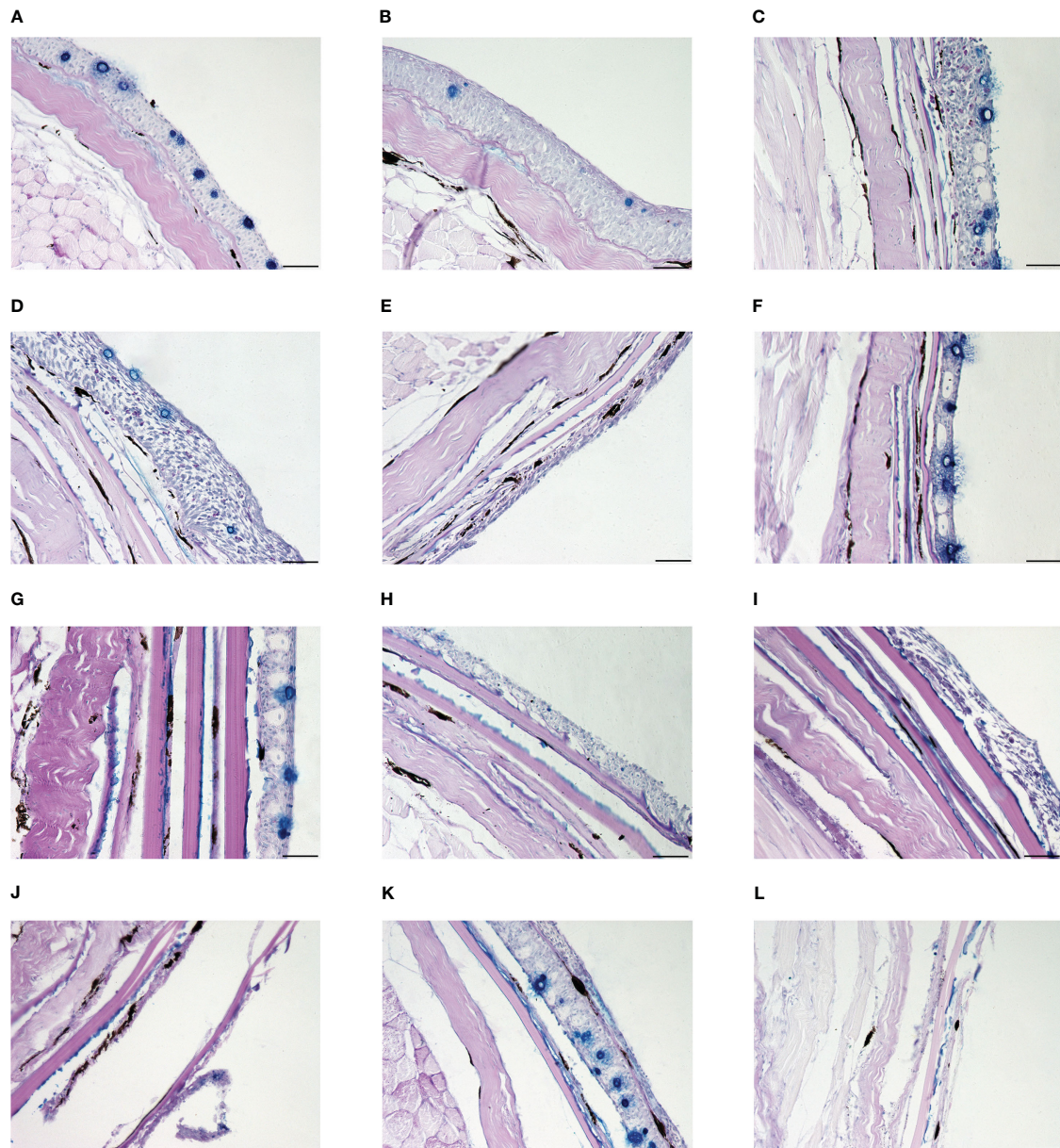


FIGURE 4

The skin histological structure of experimental fishes in UVR exposure experiment. The scale is 50 μm . (A) Control group of *Gymnocypris eckloni*. (B) Experimental group of *Gymnocypris eckloni* (0 level damage). (C) Control group of *Schizothorax davidi*. (D) Experimental group of *Schizothorax davidi* (1 level damage). (E) Experimental group of *Schizothorax davidi* (2 level damage). (F) Control group of *Percocypris pingi*. (G) Control group of *Spinibarbus sinensis*. (H) Control group of *Luciobarbus capito*. (I) Experimental group of *Luciobarbus capito* (2 level damage). (J) Experimental group of *Luciobarbus capito* (3 level damage). (K) Control group of *Cyprinus carpio*. (L) Experimental group of *Cyprinus carpio* (4 level damage).

altitudes were not intolerant to all high-altitude environmental stress factors, but were related to their own habitat environment (Supplementary Table, $P < 0.05$), with large interspecific differences. *Luciobarbus capito* is an euryhaline fish native to salt lakes in Central Asia, the salinity of the environment in which its wild population lives is the highest among the six Barbini species. Long-term adaptation resulted in its strong resistance to salinity, and it can rapidly regulate its osmotic pressure balance by $\text{Na}^+/\text{K}^+/\text{ATPase}$ under salinity stress (Geng et al., 2016). However, its tolerance to low temperature, low oxygen and UVR was weak, which limited its diffusion to the plateau. The tolerance of

Schizothorax davidi to low temperature and UVR was slightly stronger than that of *Spinibarbus sinensis*, but its salinity tolerance was the weakest among the six species. As a carnivorous fish, *Percocypris pingi* has a higher oxygen consumption rate under emergency conditions (Bau and Parent, 2001), and histological sections showed that the density of mucous cells and club cells in its epidermis was higher, while the epithelial cells that play a major role in resisting UVR were thinner (Gu et al., 2022a; Gu et al., 2022b). Therefore, its tolerance to low oxygen and UVR was slightly lower than that of *Schizothorax davidi* with similar altitude distribution. *Cyprinus carpio* is a well-known freshwater fish with

strong adaptability, it is widely distributed and there are a variety of environmental stress factors in its habitat. They sometimes feed on the larvae of Diptera insects, which are usually produced in anoxic, high-salt polluted water. Furthermore, *Cyprinus carpio* are also found in the cold waters of northern Eurasia, and they can maintain membrane fluidity by increasing the synthesis of long-chain unsaturated fatty acids (Tiku et al., 1996; Zhao R. et al., 2022). It has acquired the ability to resist low temperature, low oxygen and salinity stress during long-term adaptation, but UVR is also a limiting condition for its diffusion to high altitude (Figure 5).

For low-altitude fishes, any stress conditions can be fatal, and there are always one or several stress factors that limit their access to the plateau. It is worth noting that in this study, fishes at middle and low altitudes showed common low tolerance to UVR, while *Gymnocypris eckloni* showed strong UVR tolerance. This suggests that among these high-altitude environmental stress factors, UVR, which is generally lacking in low altitude areas, may play a decisive role.

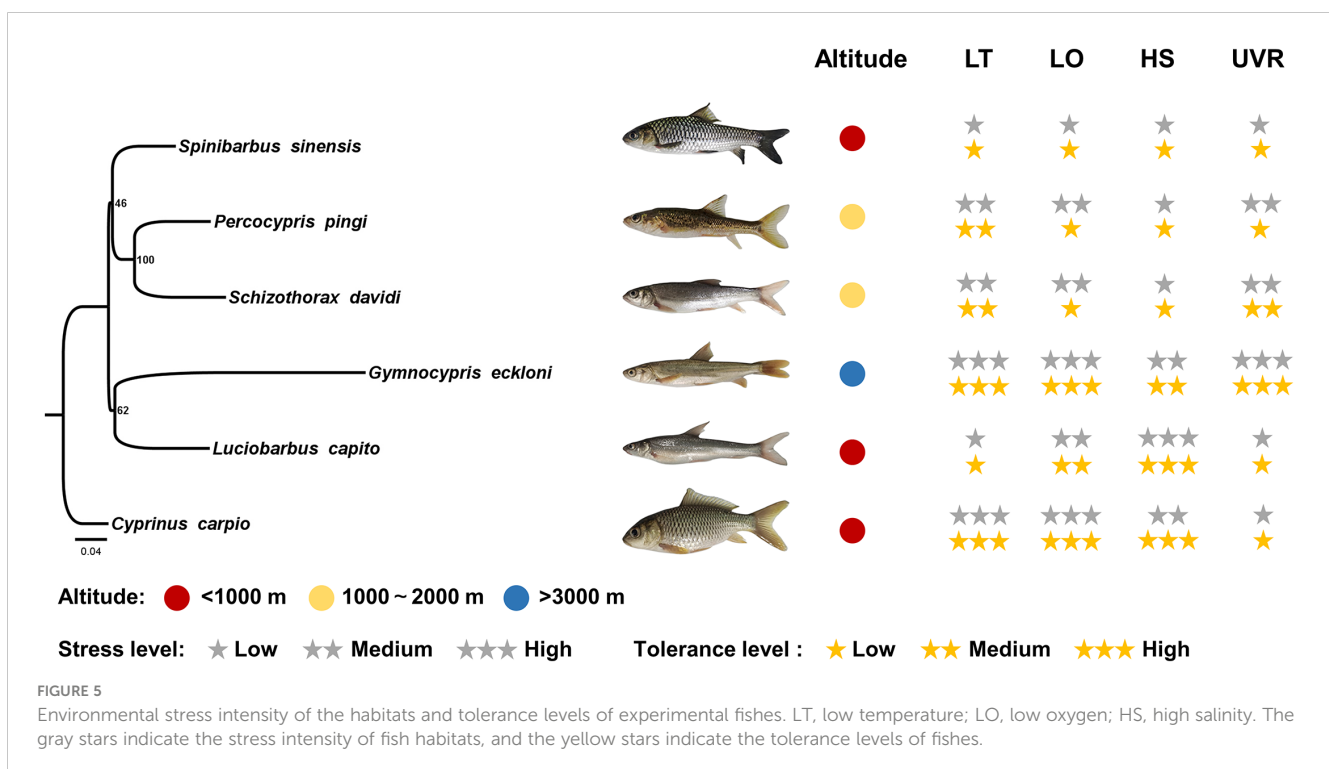
Low temperature, low oxygen and high salinity stress also exist in plain areas, and low-altitude fishes will also acquire adaptive evolutionary characteristics in the process of resisting to environmental stresses. Therefore, when selecting the plain control species for the studies of fish high-altitude adaptability, in addition to paying attention to its altitude distribution, the adaptability of the control species itself should also be considered.

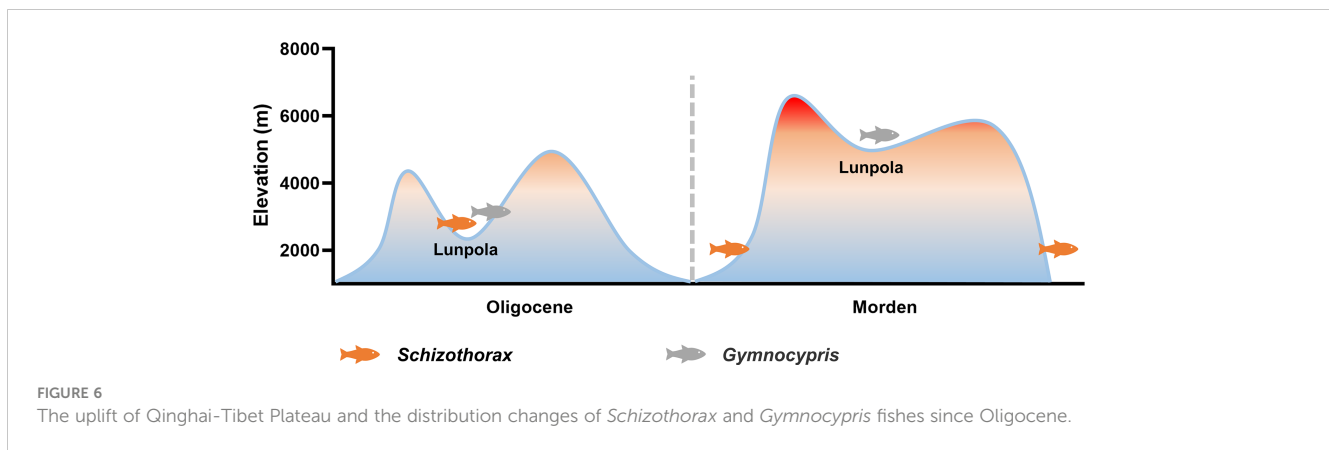
4.2 Uplift of Qinghai-Tibet Plateau and distribution change of *Schizothorax* fish

Qinghai-Tibet Plateau has experienced an uplift process of millions of years (Li and Fang, 1999; Tapponnier et al., 2001). In the Paleogene

(66 ~ 23 Ma), the central part of the plateau was a deep valley with an average altitude of less than 2300 m, surrounded by a mountain system with an altitude of more than 4000 m (Su et al., 2019). Fossil evidence indicates that during that period, the organisms adapted to the tropical climate lived in the Lunpola Basin in the central Tibetan Plateau (Cyr et al., 2005; Deng et al., 2012; Sun et al., 2014; Su et al., 2019). In the Neogene (23 ~ 2.6 Ma), the central part of the Tibetan Plateau gradually uplifted to about 4600 m above sea level, forming the present Tibetan Plateau (Spicer et al., 2021) (Figure 6).

Schizothoracinae fish is a group mainly distributed in the Qinghai-Tibet Plateau and its surrounding areas. Studies have shown that Schizothoracinae fish evolved from Barbinae (family: Cyprinidae) fish in Tertiary (66 ~ 2.6 Ma). According to the modifications of scales, pharyngeal teeth and barbels, the subfamily Schizothoracinae was divided into three grades: primitive [e.g., *Schizothorax davidi* (genus: *Schizothorax*)], specialized, and highly specialized [e.g., *Gymnocypris eckloni* (genus: *Gymnocypris*)] schizothoracine fishes (Cao et al., 1981). The groups with higher specialization grade are more adaptable to the extreme environment of the plateau. Fossil evidence indicates that the extant *Schizothorax* fish originated from the Qaidam Basin (3500 m above sea level) before Oligocene (34 ~ 23 Ma) (Yang et al., 2021). Before the Eocene, Qaidam Basin was an inland sea (Wu et al., 2013; Wang Y. et al., 2015). Since the Eocene, it has rapidly dried up, forming some scattered salt lakes, and the water salinity has gradually increased (Gao et al., 2019; Ye et al., 2020; Tang et al., 2021). *Schizothorax* fish should theoretically have strong salinity tolerance. But strangely, in our experiments, the salt tolerance of *Schizothorax davidi* was the weakest among six species, which seemed to be inconsistent with the environmental characteristics of its origin. We propose two possible scenarios: (1) the salinity of lakes in the ancient Qaidam Basin is low; (2) the primitive *Schizothorax* fish lived in the freshwater system outside the





basin. This may provide new insights into the reconstruction of the paleoenvironment of ancient Qaidam and the origin of *Schizothorax* fish.

In the 1970s, Miocene (23 ~ 5.3 Ma) *Schizothorax* fish fossils were unearthed from the Lunpola Basin (4500 m above sea level) (Wu and Chen, 1980). This indicates that during the Oligocene-Miocene period, the primitive *Schizothorax* fish was distributed in the central part of the Qinghai-Tibet Plateau, while the distribution of existing *Schizothorax* fish was limited to the eastern, southern and western marginal areas of the Qinghai-Tibet Plateau (Cao et al., 1981), suggesting that its distribution has changed since the Neogene (Figure 6). During the uplift of the central Tibetan Plateau in the Neogene, the climate here gradually became extreme. Meanwhile, geological changes of the plateau have also changed the distribution of water system, rivers were blocked and lakes were divided, the original fishes here were extinct or migrated (Wu and Tan, 1991). We speculate that the intolerance of *Schizothorax* fish to low temperature, low oxygen, and especially salinity led to its extinction in the central Tibetan Plateau. This also suggests that the adaptation to salinity is also an important feature of fish high-altitude adaptation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

All the animal procedures were conducted with the approval of the Committee of Laboratory Animal Experimentation at Southwest University, Chongqing, China and were in full compliance with the Committee's guidelines [protocol number (2014)25].

Author contributions

SL, HG and ZW conceived the ideas and designed this study. SL wrote the original manuscript and completed data analysis. HG and

YW completed stress experiments. HG and SL completed histology experiments. SL, HG and ZW participated in the discussion of the experimental results and the improvement of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1193699/full#supplementary-material>

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