Acute toxicity of salinity to *Sinogastromyzon Szechuanensis*

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Abstract. We set a salinity gradient, and then measure the tolerance of *Sinogastromyzon szechuanensis* to salinity at the initial stage and after a period of adaptation. The results show that the half lethal concentrations (LC₅₀) of *Sinogastromyzon szechuanensis* at 24 h, 48 h, 72 h and 96 h are 10.50 g/L, 10.15 g/L, 9.83 g/L and 9.46 g/L respectively, and the safe concentration is 2.32 g/L. In the early stage of salinity mutation, the respiratory frequency of *Sinogastromyzon szechuanensis* increases with the increase of salinity. The respiratory frequency of 4 g/L group is 177±10.42 times/min, significantly higher than that of 0 g/L group (P<0.05). After 4 h of adaptation, when the salinity is above 7 g/L, the respiratory rate increases compared with the initial stage, when the salinity is above 7 g/L, the respiratory rate increases significantly compared with the initial stage (P<0.05). The respiratory rate of 2 g/L group is 150.0±5.57 times/min, which is significantly higher than that of 0 g/L group (P<0.05), and the salinity is close to the safe concentration, 2.32 g/L. Under 0~7 g/L salinity stress, after 4 h adaptation in each salinity group, the asphyxia point of *Sinogastromyzon szechuanensis* first decreases and then increases with the increase of salinity, and the asphyxia point of 2 g/L is the lowest, 0.81±0.02 mg/L. After 96 h adaptation, the asphyxia point of *Sinogastromyzon szechuanensis* in each salinity group decreases, and the asphyxia point of 0.1 g/L is the lowest, 0.60±0.06 mg/L. Conclusion: *Sinogastromyzon szechuanensis* has a low salinity tolerance, and its asphyxiation point is higher than that of common freshwater fish. Therefore, we suggest controlling the salinity to be 0~2 g/L, which is helpful to enhance the hypoxia tolerance and survival rate of *Sinogastromyzon szechuanensis*.

1. Introduction

*Sinogastromyzon szechuanensis* belongs to the Balitoridae and the *Sinogastromyzon*, which is distributed in the upper reaches of the Yangtze River and its tributaries, and in the rivers of the southwest hills [1]. *Sinogastromyzon szechuanensis* tastes delicious, and it has rich nutritional value, rich essential amino acids and unsaturated fatty acids [2], and it also has good ornamental value [3]. However, due to overfishing and habitat damage, the population resource is greatly reduced [4]. In 1999, Chongqing municipal government put it on the list of key protected fish. In 2011, it was submitted to the International Union for Conservation of Nature (IUCN) and was listed in the Red List of Threatened Species [5]. At present, the main researches on *Sinogastromyzon szechuanensis* are focused on its resource investigation [6-7], morphometry [8-10], anaesthetic selection [11-12], reproduction development [13-14], mitochondrial genome and phylogenetic analysis [15].

Salinity, as an important indicator that influences the fish survival and growth, is widely used in aquaculture to treat diseases, but unreasonable salinity can lead to fish death. At present, studies on the tolerance of fish to salinity are as follows: Hypophysalmichthys molitrix [16], Larimichthys crocea
[17], Poecilia reticulate [18], Carassius auratus var. Pengza [19], Carassius auratus gibelio [20], Pseudorasbora parva [21], Schizothorax graham [22], Monopterus albus [23] Chalcalburnus chalcoides aralensis [24]. The reason why the study on the tolerance of Sichuan Chinese loach to salinity is carried out in this experiment is to provide theoretical and data reference for the species protection and artificial culture.

2. Materials and methods

2.1. Material

From October 2017 to November 2018, we fished Sinogastromyzon szechuanensis from the Neijiang section of Sichuan Tuojiang River system and then transported them to the aquaculture room of Jiangsu Animal Husbandry & Veterinary College to culture them for more than one year, so as to make them fully adapt to the artificial breeding environment. In December 2019, we carried out this experiment. The test water is tap water after 48 hours’ aeration. The amount of dissolved oxygen was more than 8.0 mg/L, the water temperature was 15.5±0.5 ℃, and the pH was 7.6±0.1. The selected specifications of Sinogastromyzon szechuanensis were relatively uniform, the body weight was (8.495±1.11) g, and they were healthy, disease-free and active. Before the formal experiment, let them stop eating for 24 hours.

2.2. Experimental method

Tolerance of Sinogastromyzon szechuanensis to salinity. The salinity gradient of the pre-experiment was prepared according to arithmetic progression of 1 g/L, and totally 10 groups of 6~15 g/L NaCl solution were prepared, and there was one 3L conical flask in each group, and five Sinogastromyzon szechuanensis were put into the flask, and aerate the bottles, and observe the activity of fish, and record the number of deaths within 24 hours, 48 hours, 72 hours and 96 hours. When the salinity was more than 12 g/L, all the fish died 24 hours later. When the salinity was 7 g/L or below, all the fish survived after 96 hours. According to the results of the pre-experiment, we set the salinity according to the equal logarithm space, and they were 8, 8.56, 9.16, 9.8, 10.48, 11.22 and 12 g/L, respectively with three parallels, and in each parallel (3 L conical flask), we put 10 Sinogastromyzon szechuanensis, and then inflate with air pump to supplement oxygen. Observe Sinogastromyzon szechuanensis’s behavior and activity, and correctly record the death numbers of 24, 48, 72, 96h and fish them out in time. At the end of the experiment, use linear regression method to calculate the half lethal concentration (LC50), and calculate the safe concentration.

Salinity stress has an effect on respiratory rate. The salinity in each conical flask was 0, 1, 2, 3, 4, 5, 6, 7, 8, 8.56, 9.16, 9.8, 10.48, 11.22 and 12 g/L respectively. When Sinogastromyzon szechuanensis were stable, we started the experiment and continue for 4 hours. At the beginning and end of the experiment, randomly select three Sinogastromyzon szechuanensis in each group and record the number of respiratory rate per minute.

The effect of salinity stress on asphyxiation point. Divide them into two groups and do not feed them throughout the experiment. In one group, we set salinity gradients of 0, 1, 2, 3, 4, 5, 6 and 7 g/L respectively, and in the experiment, set three parallels. In each parallel (1000 mL conical flask), we put six Sinogastromyzon szechuanensis, and then fill the flask with water and inflate it to make the fish adapt to it for 4 hours, then stop aeration and seal it with fresh-keeping film, and it could be seen that 50% of the fish were out of balance, comatose or dying, and then we take samples immediately and adopt GB 7489-87 to measure the dissolved oxygen value, and this is the asphyxia point. In the other group, let fish adapt to 0,1,2,3,4,5,6 and 7 g/L salinity gradients for 96 h, and the other steps were the same as above, and then we measure the asphyxia point.

2.3. Data processing

Safe concentration $= \frac{0.3 \times 24 \ h \ LC_{50}}{(24 \ h \ LC_{50}/48 \ h \ LC_{50})^2}$[25], and the data gotten were processed by Excel, and use SPSS 19 one-way ANOVA, and use Duncan’s multiple comparisons method for difference between
groups. When $p<0.05$, the difference is significant, and when the letters are the same, there is no significant difference ($p>0.05$). The statistical value is expressed by mean ± standard error (mean ± SE).

### 3. Results and analysis

#### 3.1. Tolerance of Sinogastromyzon szechuanensis to salinity

The mortality of *Sinogastromyzon szechuanensis* at 24 h, 48 h, 72 h and 96 h in groups with salinity gradients of 8, 8.56, 9.16, 9.8, 10.48, 11.22 and 12 g/L, and use the cumulative mortality and salinity to carry out linear regression (Tab. 1). The half lethal salinity ($LC_{50}$) of each time point (Tab. 2). The half lethal concentrations of 24 h, 48 h, 72 h and 96 h for *Sinogastromyzon szechuanensis* are 10.50 g/L, 10.15 g/L, 9.83 g/L and 9.46 g/L, respectively, and the safe concentration is 2.32 g/L.

### Table 1. Mortality of *Sinogastromyzon szechuanensis* in different salinity levels.

| Salinity (g/L) | 24 h | 48 h | 72 h | 96 h |
|---------------|------|------|------|------|
| 8             | 0    | 0    | 0    | 10   |
| 8.56          | 0    | 0    | 0    | 10   |
| 9.8           | 0    | 0    | 0    | 10   |
| 10.48         | 0    | 20   | 0    | 10   |
| 11.22         | 0    | 60   | 60   | 60   |
| 12            | 70   | 80   | 80   | 80   |

### Table 2. Half lethal salinity of *Sinogastromyzon szechuanensis* in different times.

| Processing time | Regression equation | Correlation coefficient | n | LC50 |
|-----------------|---------------------|-------------------------|---|------|
| 24 h            | $y=0.2546x-2.1743$  | 0.9205                  | 30 | 10.50|
| 48 h            | $y=0.2691x-2.2322$  | 0.9860                  | 30 | 10.15|
| 72 h            | $y=0.25x-1.9582$    | 0.9627                  | 30 | 9.83 |
| 96 h            | $y=0.2018x-1.41$    | 0.9245                  | 30 | 9.46 |

#### 3.2. Effects of salinity stress on respiration frequency of *Sinogastromyzon szechuanensis*

The salinity has a significant effect on the respiratory rate of *Sinogastromyzon szechuanensis* in 0 h and 4 h groups ($P<0.05$). The respiratory frequency increases with the increase of salinity (Tab. 3). In 0 h group, the respiratory frequency when the salinity is 4 g/L is 177±10.42 times/min, significantly higher than that of 0 g/L group ($P<0.05$). The respiratory rate when the salinity is 2 g/L is 150.0±5.57 times/min, which is significantly higher than that of 0 g/L group ($P<0.05$). In 0–7 g/L salinity range, the respiratory frequency of *Sinogastromyzon szechuanensis* in 4 h group is significantly lower than that in 0 h group ($P<0.05$). When the salinity is 8.56–12 g/L, the respiratory frequency of *Sinogastromyzon szechuanensis* in 4 h group is significantly higher than that in 0 h group ($P<0.05$).

### Table 3. Effect of salinity on the respiratory rate of *Sinogastromyzon szechuanensis*.

| Salinity (g/L) | IRR(times/min) (n=42, x± SD) 0 h group | TRR/(times/min) (n=42, x± SD) 4 h group |
|---------------|----------------------------------------|---------------------------------------|
| 0.00          | 156.33±10.97a                          | 135.33±8.39A                          |
| 1.00          | 158.67±13.65ab                         | 139.00±10.82AB                        |
| 2.00          | 166.67±4.16abc                         | 150.00±5.57BC                         |
| 3.00          | 169.67±11.72abc                        | 152.67±7.51C                         |
| 4.00          | 177.00±14.42bcd                        | 154.33±6.66C                         |
| 5.00          | 178.00±3.61cd                         | 159.67±3.51C                         |
| 6.00          | 178.33±9.07cd                         | 162.67±6.03C                         |
3.3. Effect of salinity stress on asphyxia point of Sinogastromyzon szechuanensis

The range of 0~7 g/L, the asphyxia point of Sinogastromyzon szechuanensis in each salinity group after 96 h adaptation is significantly lower than that after 4 h adaptation (P<0.05). With the increase of salinity, the asphyxia point of Sinogastromyzon szechuanensis adapts for 4 hours first decreases and then increases (Tab. 4). When the salinity is 2 g/L, the lowest asphyxia point is 0.81±0.02 mg/L, but the difference is not significant with other groups (P>0.05). When the salinity is 7 g/L, the highest asphyxia point of Sinogastromyzon szechuanensis is 1.04±0.09 mg/L, significantly higher than other groups (P<0.05). The asphyxiation point of Sinogastromyzon szechuanensis adapts for 96 hours increases with the increase of salinity, and the lowest asphyxia point appears in the 0 g/L and 1 g/L groups, which is 0.60±0.1 mg/L, and the highest value appears in the 7 g/L salinity group, which is 0.88±0.09 mg/L. When the salinity is over 3 g/L, the asphyxia point of Sinogastromyzon szechuanensis is significantly higher than that of the groups whose salinity is below 3 g/L (P<0.05).

Table 4. Effect of salinity on suffocation point of Sinogastromyzon szechuanensis.

| Salinity (g/L) | IAP(times/min) (n=42, x± SD) | TAP(times/min) (n=42, x± SD) |
|---------------|-------------------------------|-------------------------------|
|               | 4 h group                      | 96 h group                    |
| 0.00          | 0.88±0.09a                    | 0.60±0.05A                    |
| 1.00          | 0.84±0.08ab                   | 0.60±0.10A                    |
| 2.00          | 0.81±0.02ab                   | 0.67±0.01AB                   |
| 3.00          | 0.88±0.09ab                   | 0.76±0.08BC                   |
| 4.00          | 0.84±0.08ab                   | 0.76±0.08BC                   |
| 5.00          | 0.92±0.08ab                   | 0.78±0.08BC                   |
| 6.00          | 0.92±0.08ab                   | 0.80±0.13BC                   |
| 7.00          | 1.04±0.09b                    | 0.88±0.09C                    |

NOTE: Values with same letters in same index mean no significant differences (P>0.05); values with different letters in the same index mean significant differences (P<0.05), water temperature 15.5±0.5 °C.

4. Discussion

4.1. Tolerance of Sinogastromyzon szechuanensis to salinity

Salinity is often regarded as a hindrance factor, and when salinity changes, fish will change its metabolic pathway. No matter in hyperosmotic or hypotonic environment, most of energy is spent on osmosis and ion regulation, and the entering and getting out of ion mainly depends on gill filament chloride cells [26]. When the change range of salinity exceeds the tolerance range of fish, the blood biochemical index [27], the activity of non-specific hydrolytic immune enzyme, non-specific antioxidant immune enzyme and various digestive enzymes of fish will all be seriously influenced, even causing death of fish [28]. Different species of fish have different osmosis and the same species
but different specifications of fish also have different osmosis, which depends on innate evolution and acquired domestication [29].

It can be seen from table 3 that the half lethal concentration of salinity to *Sinogastromyzon szechuanensis* and several other fish’s decreases with the increase of time, which indicates that the tolerance to salinity gradually decreases with the increase of time. The order of safety concentration from large to small is: *Chalcalburnus chalcoides aralensis*, *Monopterus albus*, *Misgurnus mohotyi Dybowski*, *Paramisgurnus dabryanus*, *Chalcalburnus chalcoides aralensis* (juveniles), *Triplophysa dalaica*, *Pseudorasbora parva*, *Sinogastromyzon szechuanensis*, *Shizothorax Grahami* (juveniles), *Hypophthalmichthys molitrix* (juveniles), *Carassius auratus var. Pengza*. The half lethal concentrations of *Sinogastromyzon szechuanensis* at 24 h, 48 h, 72 h and 96 h are 10.50 g/L, 10.15 g/L, 9.83 g/L and 9.46 g/L respectively, and the safe concentration is 2.32 g/L, which is only higher than that of *Shizothorax grahami* when compared with other fish (adult). The salt concentration of fresh water on the earth is 0.01~0.5 g/L, and the salt concentration of sea water is 16~47 g/L (generally 35 g/L). The safe concentration for the tolerance of *Sinogastromyzon szechuanensis* shows that it cannot adapt to Mariculture, but can adapt to all fresh water and bear low-salinity water environment. In the protection work for *Sinogastromyzon szechuanensis*, the appropriate brackish water can reduce the disease of freshwater fish. It is of great significance to domesticate the *Sinogastromyzon szechuanensis* with low salinity and use the brackish water for artificial culture and fry rearing.

4.2. Effects of salinity stress on respiratory rate of *Sinogastromyzon szechuanensis*

The change of fish respiratory system can reflect the change of body function. Through the index of respiratory frequency, we can preliminarily judge whether fish are sensitive to the change of some factors in the water environment. Wang Hongjun et al successfully monitored the heavy metals such as Hg$^{2+}$, Cu$^{2+}$, Zn$^{2+}$ in the water with the respiratory parameters of *Brachydanio rerio* [31]. MichallA. Cairns et al measured the respiratory rate to monitor the safe concentration of zinc for *Salmo gaiscl*, and found that the respiratory rate of unaffected *Salmo gaiscl* was normal, and the respiratory rate of affected *Salmo gaiscl* was significantly changed [32].

This research shows that with the increase of salinity, the respiratory rate of *Sinogastromyzon szechuanensis* increases. The respiratory rate of *Sinogastromyzon szechuanensis* in the 0h group reaches 177.4±10.42 times/min in the 4 g/L salinity water, significantly higher than 156.3±10.97 times/min in the 0 g/L salinity water (P<0.05). When the salinity is 12 g/L, the respiratory rate is highest, which is 215.3±7.51 times/min. *Sinogastromyzon szechuanensis* continuously consumes energy to adjust its body skills to maintain the balance of osmotic pressure. The respiratory frequencies of *Sinogastromyzon szechuanensis* in each 0~7 g/L salinity group after 4h of adaptation are all lower than that in the initial stage (0h). The respiratory frequency of the group with salinity above 2 g/L is significantly higher than that of the group with salinity above 0 g/L (P<0.05), which shows that in the range of low salinity, with the extension of time, the tolerance is lower. In each 8~12 g/L salinity group, the respiratory frequency of *Sinogastromyzon szechuanensis* increases significantly compared with the initial period (0 h), which indicates that the high osmotic pressure environment may cause damage to the body skills of *Sinogastromyzon szechuanensis*, which requires *Sinogastromyzon szechuanensis* to consume a lot of energy to regulate itself, so the respiratory frequency increases abnormally, resulting in life-threatening and even death. This is consistent with the results of tolerance experiment, and mortality was positively correlated with salinity concentration and action time. Therefore, after 4 hours, the respiratory frequency of *Sinogastromyzon szechuanensis* is seriously affected in the group with salinity greater than 7 g/L, and the respiratory frequency is more than 163.3±7.77 times/min.

4.3. Effect of salinity on asphyxia point of *Sinogastromyzon szechuanensis*

Fish asphyxia point can be used as an important parameter to judge the hypoxia tolerance of fish under different environmental conditions, and it is of great significance to culture and production. In the natural state, *Sinogastromyzon szechuanensis* lives in streams and adsorbs on rocks, so it has a high
demand for dissolved oxygen in water environment. From table 4, we can see that the asphyxia point of *Sinogastromyzon szechuanensis* is close to *Mugil cephalus*, *Mylopharyngodon piceus*, *Onychostoma sima*, *Megalobrama pellegrini*, *Pelteobagrus fulvidraco*, and is much higher than the asphyxia point of common freshwater fish, such as *Erythroculter ilishaeforis* and *Spinitubarbus sinensis*, *Cyprinus carpio*, *Hypophthalmichys molitrix*, *Ctenopharyngodon idellus*, *Aristichthys nobilis*, *Carassius auratus* and so on, but when compared with the asphyxia point of the sea fish such as *Pagrosomus major*, the asphyxia point is much lower.

The energy of aquatic animals consumed by the standard metabolism is used for the repair and renewal of tissues and the maintenance of the stability of the environment in body [33]. In this experiment, under 0–7 g/L salinity stress, the asphyxia point of *Sinogastromyzon szechuanensis* decreases first and then increases in each salinity group of 4h adaptation. At 2 g/L, the asphyxia point is the lowest, which is 0.81±0.02 mg/L, which is consistent with the results of *Oreochromis niloticus* [34], *Procypris merus* [33] and *Mugil cephalus* [35]. Under the salinity stress of 0–7 g/L, after 96h adaptation, the asphyxiation point of *Sinogastromyzon szechuanensis* in each salinity group decreases compared with that of 4h, and the asphyxia point of 0–1 g/L is the lowest, which is 0.60±0.06 mg/L, which is probably caused by the decrease of metabolism level caused by the continuous hunger. The suitable salinity value in a short period of time can decrease the asphyxia point of *Sinogastromyzon szechuanensis*. With the extension of time, the asphyxia point further decreases, but the asphyxia point of each group with salinity greater than 3 g/L is significantly higher than 0 g/L group and 1 g/L group (P<0.05).

5. Conclusions
The study on the tolerance of *Sinogastromyzon szechuanensis* to salinity is beneficial to the protection of resources, and can improve the survival rate in the process of living transportation and temporary keeping. According to the results of this experimental study, the half lethal concentrations of *Sinogastromyzon szechuanensis* at 24 h, 48 h, 72 h and 96 h are 10.50 g/L, 10.15 g/L, 9.83 g/L and 9.46 g/L respectively, and the safe concentration is 2.32 g/L. The respiratory frequency of *Sinogastromyzon szechuanensis* when the salinity is 4 g/L is 177±10.42 times/min, significantly higher than that of 0 g/L group (P<0.05). In the 4h group, when the salinity is in the range of 0–8.56 g/L, the respiratory frequency of *Sinogastromyzon szechuanensis* increases with the increase of salinity. The respiratory rate when the salinity is over 2 g/L is 150.0±5.57 times/min, which is significantly higher than that of 0 g/L group (P<0.05), and the salinity is close to the safe concentration, 2.32 g/L. Under 0–7 g/L salinity stress, in each salinity group of 4 h adaption, the asphyxia point of *Sinogastromyzon szechuanensis* first decreases and then increases, and the asphyxia point of 2 g/L is the lowest, 0.81±0.02 mg/L. After 96 h adaptation, the asphyxia point of *Sinogastromyzon szechuanensis* in each salinity group decreases, and the asphyxia point of 0–1 g/L is the lowest, which is 0.60±0.06 mg/L. We suggest controlling the salinity to be 0–2 g/L, which is helpful to enhance the hypoxia tolerance and survival rate of *Sinogastromyzon szechuanensis*. In view of its high asphyxia point compared with general fresh water fish, we should ensure sufficient dissolved oxygen during breeding and transportation.

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