The effect of pH value on fertilized eggs, larva fish and juvenile fish of *Takifugu obscurus*

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Abstract: pH value is one of the most significant ecological factors affecting the survival and growth of aquatic organisms. But the effect and tolerance of pH value on many aquatic species are still unknown. This study mainly investigated the effects of pH value on the hatching rate of eggs, the adaptability of newly hatched larva fish and the survival of 3-months-old juvenile fish of *Takifugu obscurus*. Water pH values can cause a great impact on *Takifugu obscurus* lives, when the fertilized eggs of *Takifugu obscurus* were incubated at pH 5.0, 6.0, 7.0, 8.0, 8.3 (control group), 9.0, 10.0 and 11.0 respectively. Under the pH of 5.0, 6.0, 10.0 and 11.0, the hatching rate and speed is greatly reduced and the deformity rate of hatching larvae is greatly increased; When the hatch-pH value was 6.0, the acid adaption of hatched larvae was significantly enhanced, but alkali adaptation was weakened; Under the condition of pH gradient of 6.0, 6.4, 6.8, 7.0, 8.0, 8.3, 9.0, 10.0, 10.2, 10.5, 11.0 and 12.0, survival time and survival rate of juvenile fish show a significant difference. Especially when the pH value was 6.0 and 12.0, juvenile fish die immediately after swimming quickly. Within 24 h, half of the fish died at the pH of 6.4 and 10.3, and within 48 h, half died at the pH of 7.1 and 9.9. In addition, the gradual pH value group in which the pH value gradually decreased or increased had stronger acid-base adaptability and a higher survival rate than the acute experimental group. The above results show the relationship between pH value and survival of *Takifugu obscurus*, which can provide more reference for the introduction of *Takifugu obscurus* into saline-alkali land in the future. Additionally, the incubated pH value can influence the pH tolerance of *Takifugu obscurus*, which can be applied to the breed improvement into an appropriate water environment.

Keywords: *Takifugu obscurus*; pH; Fertilized egg; Larva fish; Juvenile fish; Saline–alkaline land

Introduction

The pH value of water is crucial for fish growth, survival, and metabolic characteristics [1]. Hatch rate, abnormal rate and viability of newly hatched larvae will be significantly affected by the pH valve [2]. Both the temperature and pH have an impact on the amount of unionized ammonia in the water column, and influence the embryo development of *Takifugu obscurus* [3]. Due to the burning of fossil fuels and factory emissions, the rapid increase in the concentration of acid gases in the air, and ocean acidification are unavoidable major problems currently facing. Ocean acidification can affect aquatic communities and cause ecological connection change, fish communities will make a change at the same time. For example, the growth of coral reefs is disturbed by climate warming and acidification, and coral reefs cannot maintain normal carbonate production[4, 5]. Most river and sea water pH levels fall between 6.5 and 8.5, however, this range is gradually changing [5]. The chemistry of water carbonate is significantly altered by the rise in CO_2 concentration on the water surface, which may result in a sea pH decrease of 0.2 to 0.4 units this century [6]. Additionally, excessive sulfur and nitrogen pollution in the atmosphere can generate acid rain, which can result in an acidic water environment and affect aquatic metabolisms. The ideal pH range for fish is 6.5–9.0, and the great climate change has devastated some commercial aquatic products, which results in huge economic loss [3, 7]. Acidemia may occur due to reduced excretion of metabolically produced H^+ and CO_2 when fish are stressed with debilitating acids, thereby increasing permeability to H^+ and Na^+. When the capacity of the buffering mechanism is exceeded, the pH of the blood drops and the ability of hemoglobin to transport oxygen decreases. Oxidative stress, DNA damage and antioxidant enzyme gene express when exposed to acute pH stress [8]. A low or high pH value has a great impact on fish reproduction, and unsuitable pH conditions can reduce sexual maturity, spawning frequency and quality [3]. When cultured in outdoor ponds, due to the forceful photosynthesis at noon, the pH value of the water will increases. When the fish exceeds the tolerance range of the pH value, it will lead to rapid death of fish, which is also the main cause of collective death.

A stable water environment is significant for *Takifugu obscurus* to survive, grow and reproduce. Numerous environmental factors like salinity, temperature, heavy metals, pH and other factors can influence the growth and survival of *Takifugu obscurus* [9-11]. As a typical euryhaline species, *Takifugu obscurus* can survive and thrive at salinities between 0 ppt and 32 ppt, that is why it could be cultivated in both freshwater and seawater, according to research by Gao, Quan Xin, et al. But excessive salinity environment can still contribute to the death of *Takifugu obscurus* [12, 13]. Temperature is another crucial ecological factor because it can increase oxygen radicals, the concentration of free calcium ions in the cytoplasm, and the rate of apoptosis, all of which can cause fish to lose their ability to function normally and eventually die [9, 14]. The optimal temperature range for *Takifugu obscurus* is between 22 and 23℃, and the maximum temperature that can be tolerated is 32℃. As the temperature rises between 20 and 29℃, the growth rate of *Takifugu obscurus* larvae will be accelerated accordingly [15]. Additionally, extensive ammonia exposure can induce relevant oxygen genes, interrupt intracellular Ca^{2+} homeostasis and subsequently lead to DNA damage and cell apoptosis in pufferfish [16]. Except for salinity, temperature and ammonia, organic pollutants, low oxygen, pathogens and heavy metals can infect physiological reactions as well. for example, cadmium as an industrial pollutant that can be enriched by the food chain, cause damage to growth and respiratory by inducing numerous reactive oxygen species (ROS) [2]. Interestingly the light will affect the growth of *Takifugu obscurus* as well. In dark conditions, the larvae have a high mortality rate and cannot grow and eventually perish. The larvae can survive in the majority of brackish waters due to their high salinity tolerance [17]. However, the effect of pH on *Takifugu obscurus* is still unknown.

The area of saline-alkali land in the world accounts for about one-third of the land area. China is a big country with saline-alkali land, ranking third among the top 10 countries in terms of saline-alkali land area. Saline-alkali land of China is distributed in 17 provinces and autonomous regions including northwest, northeast, north China and coastal areas. In recent years, due to the limitation of land use and water ecological environment protection, the freshwater aquaculture industry and its future development are facing huge difficulties. More researchers focus on the development and utilization of saline-alkali water resources to alleviate the shortage of freshwater resources. and ecological protection. The total area of saline-alkali wasteland and saline-alkali land affecting arable land exceeds 351,000 square kilometers in China [18]. At present, many methods have been adopted for the management and utilization of saline-alkali land, including planting salt-tolerant crops, microbial management, and aquaculture. Rice-fishing comprehensive breeding systems in low-lying saline-alkali land were studied, which provided data support and a scientific basis for the sustainable development of rice-fishing comprehensive breeding in saline-alkali land [19]. Using idle saline-alkali water resources for fishing can not only reduce the surrounding soil salinity, improve the ecological environment, and increase the area of arable land, but also provide economic benefits for the fishery [20]. At present, the main economic aquatic species cultured in saline-alkali land are *Piaractus brachypomus, Cyprinus carpio, Oreochromis niloticus, Carassius auratus gibelio and Macrobrachium rosenbergii*, etc [21]. Since pH is related to the effect of alkalinity on organisms, it is necessary to pay attention not only to alkalinity but also to the pH value in saline-alkali water cultivation [22]. Through the implementation of the integration of fish and agriculture in saline-alkali land, the cultivation of excellent varieties of saline-alkali-tolerant, the vigorous development of aquaculture, and the promotion of local economic development, a series of measures have been taken to scientifically improve the saline-alkali land. The pH value has been reduced from 8.8 to 8.2. And the alkalinity has been reduced from 6‰ dropped to about 2‰ [23]. The results show that for different salinity levels, taking measures such as digging ponds for precipitation, raising fields for land reclamation, and fishery in saline-alkali land can significantly improve soil salinization [19].

Takifugu obscurus is widely distributed in the Yellow Sea, the East China Sea, and the inland waterways of China and the Korean Peninsula. As a typical anadromous fish, *Takifugu obscurus* is 20–40 cm long, inhabits the bottom of nearshore and inland waterways, and migrates to freshwater rivers to reproduce from February to May every year. The freshly hatched fish larvae spend several months in freshwater and migrate to the sea and take over several years at sea until sexual maturity [24]. According to Figure 1, *Takifugu obscurus* has a nearly oval body with black dots, a reasonably long head, hard rostra between the upper and lower jaws and small eyes. The artificially farmed *Takifugu obscurus* typically has a rounder body shape, more fat content, and a faster growth rate; before sexual maturity, the male identities growth rate is faster than the female identity, and when the female is getting close to sexual maturity, the female's growth rate surpasses the male's [25]. *Takifugu obscurus* is referred to as the "crown of dishes" because of its rapid growth and nutritional quality. Whereas internal organs and blood of *Takifugu obscurus* are often very poisonous, especially during maturity. The pufferfish toxin has significant economic and therapeutic value, which is an important medicine that can be used for analgesia, and it has an important role in the treatment of cancer and drug rehabilitation [26] [27]. While the ovaries of *Takifugu obscurus* are toxic and inedible, the gonads are edible and nutrient-rich containing micronutrients [28].

Figure 1. The appearance of *Takifugu obscurus* (scale bars represent 50 mm).

Furthermore, the demand for *Takifugu obscurus* is gradually increasing and far exceeds the yield. Due to overexploitation and environmental contamination, the population of this species has gradually declined in recent decades. Consequently, groundwater or river water is used for the majority of *Takifugu obscurus* culture, which is also practiced in a few traditional outdoor earthen ponds and factories. *Takifugu obscurus* larvae were reported to have a greater survival rate in low salinity environments than in freshwater [29]. Due to environmental damage and exploitation, the number of wild individuals of this fish has drastically decreased in recent years despite its great economic worth. In this study, we aim at the effect of pH value on fertilized eggs, larva fish and juvenile fish of *Takifugu obscurus*. The early life stage of mangy fish will be close to their survival threshold and only a slight change in water quality might result in death [30]. Fertilized eggs are the most vulnerable stage in the life cycle of *Takifugu obscurus*. And larva fish is a very significant part of choosing excellent breeds according to the corresponding pH environment. When the *Takifugu obscurus* is three-months-old, they are moved from indoor pools to outdoor pools. During this transfer process, the physical and chemical properties of the water, especially the pH will greatly change. Therefore, investigation of *Takifugu obscurus* reactions to changes in ecological conditions in their early stages is important for the conservation of this species. This experiment also established the *Takifugu obscurus* pH tolerance range, which can be used as a guide when picking breeding waters in the future. The findings of this study demonstrated a correlation between the hatching of fertilized eggs and the pH level of *Takifugu obscurus*, as well as the ability of hatchlings to tolerate different pH levels. Finally, the range of pH values tolerated by hatchlings was determined, providing a basis for saline-alkali farming, and it was discovered that the acute experiment has more significant mortality than progressive groups, which illustrates the importance of respite before releasing fish into pounds.

Material and methods

2.1. Materials

2.1.1. Acquisition of fertilized eggs

The broodstock was selected to be vigorous, disease-free and morphologically intact for more than three years (weight over 2kg). And the broodstock was injected with LRH-a to induce maturation, with a total injection amount of 10 μg/kg body weight, the first injection of 2 μg/kg body weight and the second injection of the remaining amount. During the insemination, live *Unionidae* will be released as nutrients of broodstock. After injection, the fish will be observed and checked every eight hours. And when the abdomen of the broodstock is soft and a few eggs or semen are discharged by light pressure on the abdomen of the female or male, artificial insemination can be performed. During fertilization, a few amount of water will be placed in the bucket primarily, and the sperm and eggs will be squeezed into the container with a feather to ensure that the eggs and sperm can be fully stirred [31, 32]. Then the fertilized eggs are immediately transferred to a continuously oxygenated and mobile incubator, and when the embryos reach the blastocyst stage in 24 hours, the fertilized and well-developed eggs are taken as experimental materials. Compared with fertilized eggs, unfertilized eggs are much heavier than fertilized eggs. Thus, unfertilized eggs and dead eggs will land on the bottom of the incubator. Additionally, fertilized eggs are transparent. Thus, we select the floating and transparent fertilized eggs as experiment materials [33, 34].

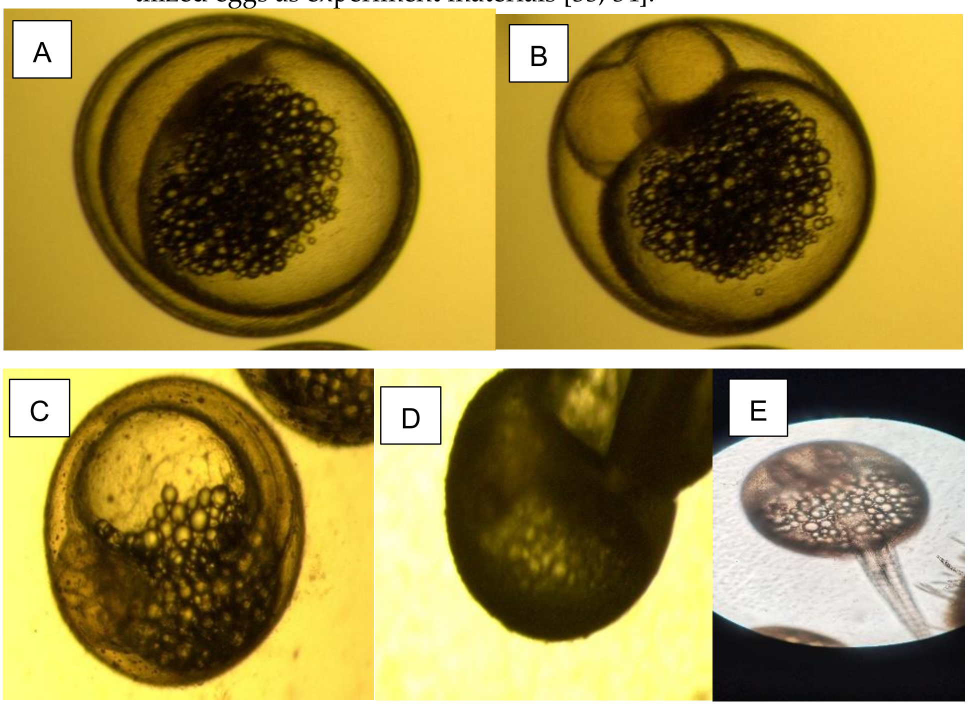

Figure 2. Embryonic development period of *Takifugu obscurus*, (A) Blastaea period (B) Gastrulae period(C) Segmentation period (D) Dead eggs (E) Hatched fish.

2.1.2. Acquisition of larva fish

Artificially inseminated fertilized eggs were incubated at the pH of 5, 6, 7, 8, 8.3 (control), 9, 10, and 11 under the condition of continuous oxygen. To get larva fish with similar

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hatching times, incubated eggs were selected for 2000 in each group. The larva fish used in the experiment is vigorous and own a normal shape. We use a pH meter (METTLER TOLEDO FE20/FE20K) to record the pH value in the aquaculture ponds at 8 a.m due to photosynthesis will reflect the Carbon dioxide content in water, and regard the tested value (pH 8.3) as the control group.

2.1.3. Acquisition of juvenile fish

The experimental fish were chosen from Jiangsu Zhongyang Groups *Takifugu obscurus* juvenile fish with a breeding time of three months, almost the same size, and a healthy body with an average weight of 4.665g/tail. During 8:00 and 9:00 p.m, the experimental juvenile fish was caught. The juvenile fish would frequently congregate at the side of the pool to wait for feeding because the juvenile fish was typically fed brine shrimp at that time of night. Before capture, the juvenile fish were fed frozen brine shrimp into the pond along the edge. When the brine shrimp thawed after a short period, the fish collected around them to feed and were then pulled up with a fishing net. Juvenile fish between three and four centimeters in length were chosen and those that were too big or little were returned to the pond.

Weighing the dry weight of the juvenile fish, which meant that they had been wrapped in gauze for five to six seconds before being weighed to remove any remaining water from their surface. Weighing procedures should be quick to minimize fish stimulation in the air, and the fish were then kept in a plastic bucket filled with swimming pool water for three days while receiving constant oxygen. The total weight of the fish in each experimental group was virtually equal.

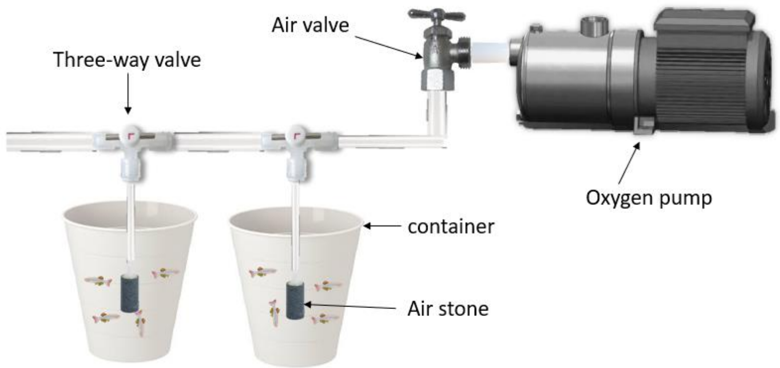

Figure 3. The system of pH tolerance experiment for juvenile fish.

2.1.4. Experiment materials

The 4L plastic buckets, 500ml beakers and 1L plastic buckets used for the experiment were cleaned with sodium hypochlorite solution before use. All the containers were submerged in the povidone-iodine solution for two hours to kill microorganisms, and then rinsed three times with water, and then air-dried for use. The oxygenator (YuEr F-7900) supply continuous oxygen to the fish during this experiment. Analytical balance (Sartorius GL224i-1SCN) can be capable of reaching an accuracy of 1/1000 gram to the weight of juvenile fish. Acid-base adjustment uses HCl (0.1 mol/L) and NaOH (0.1mol/L). The experimental water comes from the pond water and is placed in the laboratory one day in advance to ensure the stability of physical and chemical properties such as water temperature.

2.2. Methods

2.2.1. Egg hatching rate of Takifugu obscurus at different pH values

There were 8 distinct pH gradients set up in total: 5, 6, 7, 8, 8.3 (the control group), 9, 10, and 11. Then 200 fertilized eggs with normal development were placed in each beaker

(500ml) and each gradient is set up with 5 parallel groups. 400 ml of aeration water with various pH values was added to make sure there was enough dissolved oxygen and supplied with continuous oxygenation. To maintain water quality and enough dissolved oxygen, the water was changed once every eight hours at a volume of 1/3. The temperature was kept at 22℃, the light intensity was 2000lx; the light/dark ratio was 12/12; the dissolved oxygen was kept above 8mg/L.

Before hatching, we check every three hours to identify dead eggs and remove them promptly. When membranes began to emerge, the observation was modified to once an hour, and the number of hatching fish was promptly recorded. Through the embryo development experiment, it was learned that the fertilized eggs started to hatch out as hatchlings at a temperature of 22°C for 139h10min and all embryos hatched at 210h [35]. So the total incubation time of the experiment was set at 240h to ensure sufficient hatching time for fertilized eggs. And the standard of dead embryos refers to the acute fish experiment, the dead eggs will become white or black and stop growing [36].

To determine the hatching rate of each group within 240 hours, the total number of hatchlings that hatched during the experimental period was counted. The hatching rate is expressed as the proportion of the normal development of newly hatched larvae within 240 hours to the total egg number, and the deformity rate is expressed as the proportion of the total number of deformed larvae 240 hours after the membrane emerges.

2.2.2. Influence of pH on the survival of larvae fish hatched at different pH values

Larvae fish were hatched at different pH values 6, 7, 8, 8.3, 9. Then the larvae fish was placed in experiment water with pH values of 6, 7, 8, 9, and 10. In each experiment water with 3 parrel groups, and each parallel group was placed with 20 larvae fish. The larvae fish are placed in 1L plastic buckets with 800ml of water. After 24 hours, the survival rate was counted. Throughout the experiment, the deceased fish were cleaned every three hours. Within the experiment, oxygen was continuously passed to ensure that the dissolved oxygen in the water was maintained at a level of 8 ppm. To maintain water quality, water was changed every 8 h. The water was changed by siphoning, and the volume of each water change was 1/3.

2.2.3. Influence of pH on the Takifugu obscurus juvenile fish

A total of 13 pH gradients were set, respectively 6.0, 6.4, 6.8, 7.0, 8.0, 8.3, 9.0, 10.0, 10.2, 10.5, 11.0, 11.5 and 12.0. Each gradient was set up with five parallel groups. In addition, each pH gradient was then set to reach the corresponding pH gradient group by increasing or decreasing the pH value by 0.1 each hour.

Throughout the experiment, the temperature was regulated with a constant ambient temperature of 26°C and a water temperature of 22°C. The dissolved oxygen concentration in the water was kept at 8 ppm by constantly oxygenating containers. Each bucket containing water was chosen from the ZhongYang ocean Group for cultivation. The water had the following characteristics: pH 8.3, salinity 0 mg/L, ammonia nitrogen content 0.210 mg/L, nitrite nitrogen content 0.062 mg/L, alkalinity 4 mmol/L and hardness 170 mg/L. 5 parallel groups in each pH level and 8 identically sized and vigorous juvenile fish were in each parallel group. Before the experiment, the juvenile fish was kept in the lab for three days to let them adjust to the water temperature and other environmental factors. During temporarily reared, dead fish or fish with poor living conditions were picked out and replaced with healthy juvenile fish of the same size (the fish replaced were those fished at the same time as the experimental fish and temporarily reared under the same conditions).

As a control group, the original pond water (pH 8.3) was chosen. During the experiment, the fish will not be fed to maintain the water quality. After temporarily rear, the juvenile fish was then placed in the corresponding pH bucket. The juvenile fish stop swimming, sink to the bottom, show no signs of life-like breathing and have no response when picked up, they can be regarded as dead [37]. The death time of each fish was noted and dead fish should be taken out quickly. The death time of each fry was recorded and the dead fish were taken out in time. During the experiment, in order to maintain the water quality and pH value, the water was changed every 3 hours. The indicators of the new water were consistent with the corresponding experimental groups, and a siphon was used when changing the water. 1/3 of the water volume is changed each time to reduce the impact on the juvenile fish. The lighting conditions are indoors, and the light intensity is 2000lx.

2.3. Statistical methods

Hatching rate (HR): Number of fertilized eggs/ total numbers of eggs.

Deformity rate (DR): Number of malformed young fish / total number of hatchlings.

Survival rate (SR): Number of survival juveniles / total number of experimental fish.

Death rate (DR): Number of dead fish after experiment / total number of experiment fish.

Alkalinity determination: The semi-lethal pH value obtained by fitting. To configure the corresponding semi-lethal pH solution, and the experimental conditions are consistent with the previous experimental treatment. Alkalinity in water is determined using acid-base titration experiments, using phenolphthalein as the indicator.

3. . Results

3.1. Phylogenetic analysis of Takifugu

In order to acquaint with the phylogenetic relationship between *Takifugu species*, we selected *Takifugu* 16S rRNA from the NCBI database and use muscle version 5.1 with default settings to align the 16S reads [38]. The aligned reads were imported into IQ-TREE version 1.6.12 with 1000 bootstrap to build a phylogenetic tree [39]. The tree results were visualized and optimized by FigTree version 1.4.4 [40].

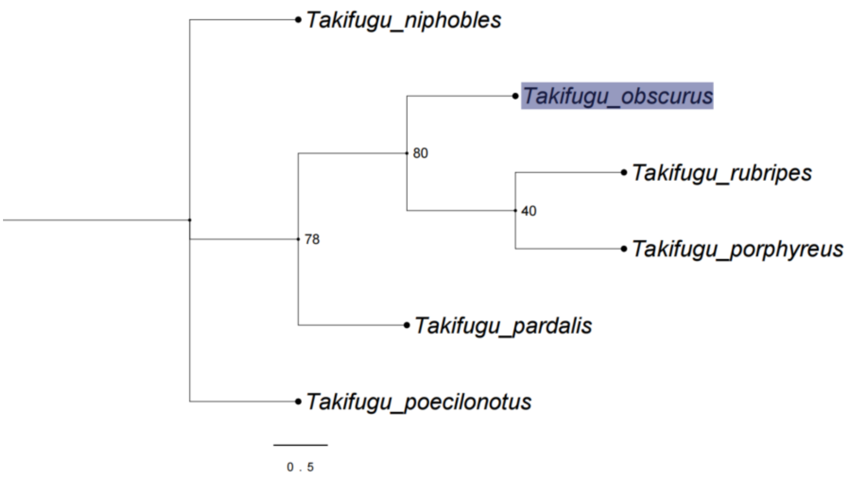

Figure 4. Maximum-likelihood-based phylogenetic analysis of *Takifugu* species based on 16S rRNA gene sequences of *Takifugu* species.

By analysis, *Takifugu obscurus* show great similarity with other *Takifugu* species, and In the 16S tree, *Takifugu obscurus* has the closest relationship with *Takifugu rubripes* and *Takifugu porphyreus*,16S reads own 99.4% and 99.35% similarity. Thus, *Takifugu obscurus* can be regarded as model creates to research *Takifugu* and protect or cultivate them.

3.2. Influence of pH on the hatching rate of Takifugu obscurus eggs

The pH level and the development of *Takifugu obscurus* embryos are correlated. Figure5 illustrates that at pH levels of 5 and 11, fertilized eggs immediately perished and embryos were unable to develop. Few fertilized eggs could hatch at pH levels of 6 and 10, and the majority of them were malformed. As illustrated in Figure 5, the majority of the malformed fish had underdeveloped, curled spines, very low survival, poor swimming skills, and high death within 24 hours of hatching.

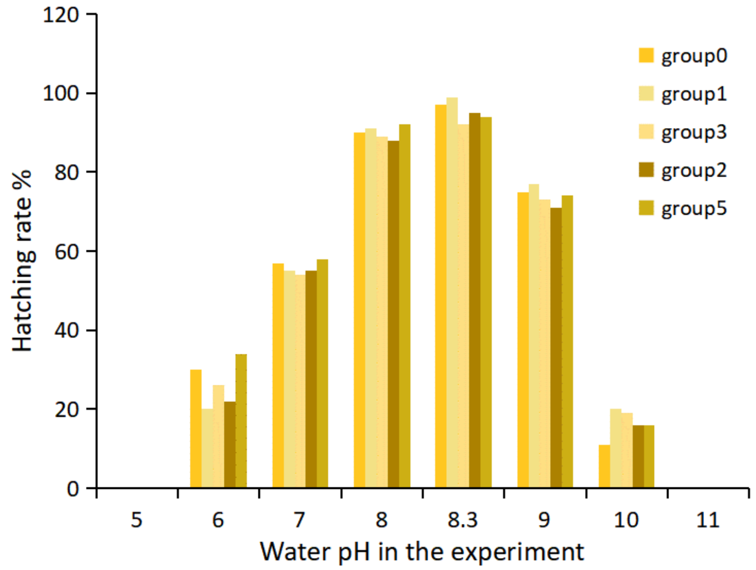

Figure 5. Hatching rate of eggs of *Takifugu obscurus* at different pH values in 240h,5 parallel groups in each gradient.

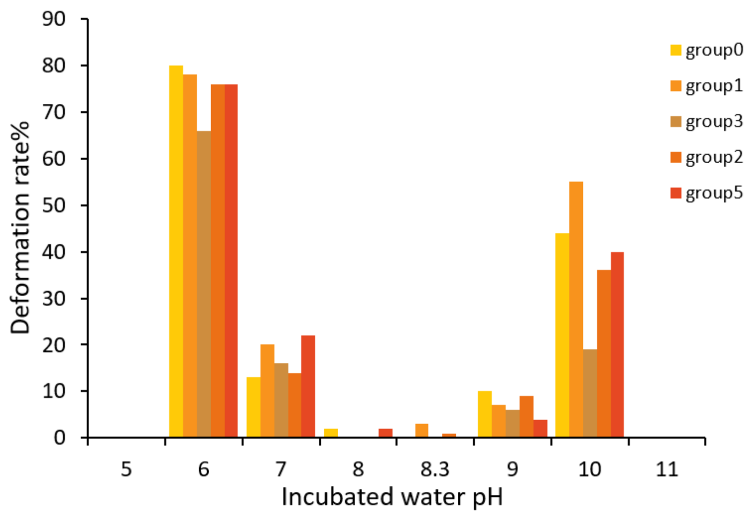

Figure 6. Deformation rate of hatchlings at different pH values, 5 parallel groups in each gradient.

3.2. *Influence of pH on the survival of larvae fish hatched at different pH values*

Figure 7 illustrates hatchlings at pH 8, 8.3, and 9 for various pH values have nearly the same tolerance to different pH conditions; When incubated water at pH 6, the hatchlings from pH 6 were significantly more tolerant to acidic conditions than the rest of the group; Experiment water at pH 9 and 10, for weakly alkaline conditions, the survival rate of the hatchlings from pH 6 and 7 was significantly lower than that of the other groups. Regardless of the incubated pH, there was no appreciable improvement in their ability to withstand excessively acidic or too alkaline pH circumstances (pH 5 or pH 11). Both groups of fish will immediately die when exposed to pH 5 and 11.

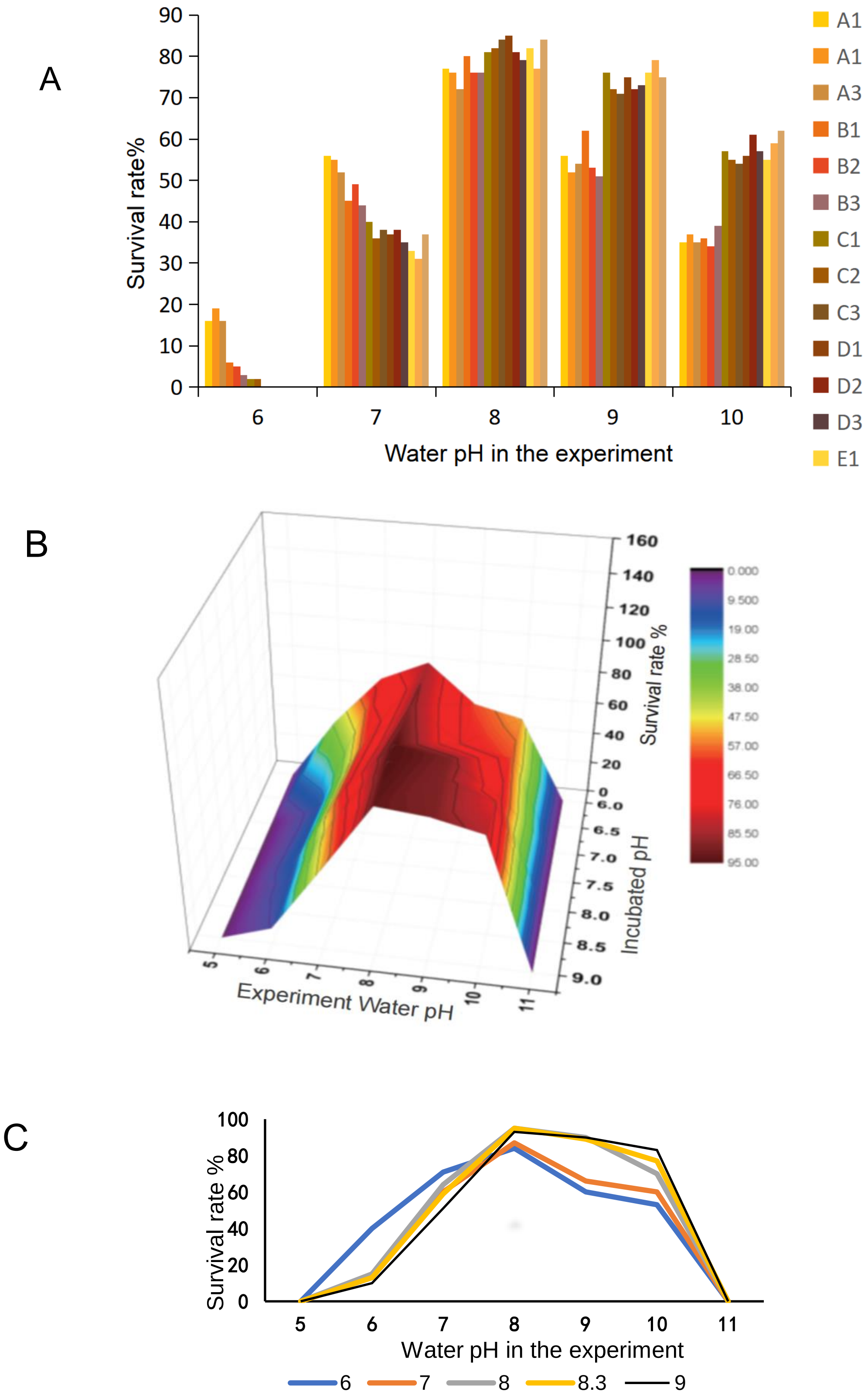

Figure 7. A) At different pH, the survival rate of hatchlings at different pH values for 24h.Group A, B, C, D, and E respectively correspond to incubated conditions pH 6, 7, 8, 8.3, 9. (B) The survival rate of hatchlings in different pH under different pH conditions (3D plot). (C) The average survival rate in each pH value.

3.3. Influence of pH on the survival of Takifugu obscurus juvenile fish

Death rate (DR) can be regarded as a standard to evaluate tolerance capacity. As illustrated in Figure 8, young fish died after a brief period of wild swimming at pH 6 and

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11. Under pH 8.3 conditions, *Takifugu obscurus* had the highest survival rate, none of the individuals died during the experiment. During the experiment, under the pH 11 and 12 conditions of alkaline, the juvenile fish swam vigorously after being put into the water and stopped swimming after less than 30 seconds. Under the condition of pH 10, after some fish enter the water, they first swim wildly, then slow down and finally sink to the bottom. After being paralyzed for about 20 minutes, they gradually return to normal swimming and have a certain ability to adapt to the environment. The survival rates of the progressive pH value groups and the acute experimental groups are greatly different, as shown in Figure 8. The death rate of progressive groups was much lower at pH 7 to 10 than that of the acute experimental group, but at pH 6 or 11, there was little difference between the two groups. Finally, we use SPSS to conclude the relationship between pH and death rate can be expressed as $y = -3.1705x^4 + 109.74x^3 - 1384.2x^2 + 7542.6x - 14941$, y is the death rate and x is the pH of the incubation conditions, $R^2 = 0.9958$ [41]。

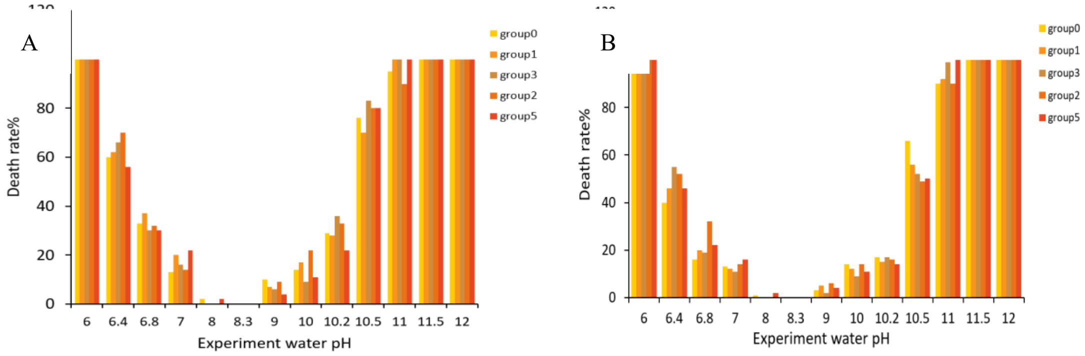

Figure 8. A) Mortality rate of *Takifugu obscurus* juvenile within 24h. (B) Mortality in the gradual change group within 24h.

4. Discussion

Along with the right levels of salinity and dissolved oxygen, certain pH criteria must also be met for *Takifugu obscurus* eggs to hatch. Exceeding the appropriate range will cause embryonic development difficulties, which will cause abnormalities and other difficulties and harm the survival of subsequent larva. Excessively extreme pH like pH 5 and pH 11 circumstances can directly cause the mortality of eggs. Studying the effects of pH on the *Takifugu obscurus* is crucial because, as a result of environmental degradation in recent years, CO_2 is absorbed by the ocean and causes ocean acidification, which has a significant impact on marine animals and may even cause the extinction of some species [42]. In our study, *Takifugu obscurus* will begin to die under pH 8. At present, the pH value of the global ocean is between 8 and 8.4, which is close to the optimum range for the growth of *Takifugu obscurus*. However, due to the emission of CO_2 and industrial waste gas, global ocean acidification will be intensified, which will further affect the growth of *Takifugu obscurus*. Additionally, saline-alkali land is a problem faced by the whole world, and various methods have been applied to the treatment of it. Within these methods, aquaculture on the saline-alkali land is an effective and economical method [43]. In this study, we primarily study the pH tolerance of *Takifugu obscurus*, which can provide a standard in the future. Compared with the traditional freshwater aquaculture environment, the saline-alkali land has the characteristics of high salt, high alkali and high pH, and few organisms can live normally in this extreme environment. China is one of the countries with the largest area of saline-alkali land, ranking third among the top ten countries in the world. Due to the low rainfall and long sunshine time in many areas, conditions are provided for the formation of saline-alkali land. For example, the salinity of the northeast saline-alkali land salinity can reach 10g/L, the pH can reach 8.5. From our conclusion, it can be seen that the *Takifugu obscurus* meets the basic conditions for breeding in this area.

The ability of juvenile fish to tolerate pH levels depends on the incubation environment, and there is a direct correlation between the pH of the incubation environment and the development of fertilized eggs [44]. Low or high pH conditions can slow down or kill eggs from hatching, and they can also cause more deformed young to be born, for example, groups pH 6 and 10 incubate more deformed larva fish than other groups. Especially, under pH 6, deformed larva fish account for 70%. Larva fish that hatched in alkaline conditions (pH 9, 10) were more tolerant of alkalinity but less tolerant of acid, whereas hatchlings that hatched in acidic conditions (pH 6) were more tolerant of acid but less tolerant of alkalinity. The optimal survival environment is still under weakly alkaline conditions, regardless of the incubated pH levels. In actual production, the pH value of the hatching water can be adjusted appropriately according to the pH value of the breeding water in order to adapt to the corresponding breeding environment, improve the adaptability of fish to the water environment, and increase the survival rate and growth speed. But it is worth noting that this method does not greatly improve the tolerance to extreme conditions such as pH 6 and 11. According to a study on whelk, a high pH value can cause congestion and the death of the fish, while too low of a pH can impair the blood oxygen transport ability of the fish [45, 46]. The survival of *Takifugu obscurus* is extremely correlated with the pH of the water quality. The preliminary findings of the pre-experiment on the initial ambiguous concentration gradient in this experiment considerably lowered the gradient. Calculating the initial pH level necessary for *Takifugu obscurus* viability offers a certain benchmark for choosing a future aquaculture water environment.

Table 1. Semi-lethal concentrations (LC50) of salinity for economic species in China (g/L) [47-52].

Species	24 h	48h	pH value	Temperature/℃
Colossoma brachypomus Cuvier	11.99	11.41	8.21	23.0
Oreochromis niloticus	13.69	13.64	8.60	25.0
Hypophthalmichthys molitrix	11.24	8.96	8.60	23.0
Oreochromis niloticus	18.37	14.66	8.91	23.0
Carassius auratus gibelio	11.53	10.77	8.80	24.5
Eriocheir Sinensis Milne-Edwards	8.12	6.47	6.47	20.3
Macrobrachium rosenbergii	8.58	5.32	5.32	23.6
Chalcalburnus chalcoides aralensis	11.32	9.16	8.30	24.0
Carassius Auratus	9.99	6.87	8.24	23.0
Carassius auratus Linnaeus	11.80	11.1	8.32	24.0
Schizothorax biddulphi	4.01	3.62	8.36	24.0

Table 2. Semi-lethal concentration (LC50) of alkalinity for economic species in China (mmol/L) [53-60].

Species	24h	48h	pH value	Temperature/°C
Colossoma brachypomus Cuvier	83.25	56.99	8.85	23.0
Oreochromis niloticus	142.1	128.28	8.32	20.0
Hypophthalmichthys molitrix	55.91	25.94	8.59	25.0
Silver carp	51.41	27.06	8.74	23.0
Carassius auratus gibelio	98.74	79.49	8.84	25.0
Eriocheir sinensis Milne-Edwards	52.97	42.44	8.33	20.3
Macrobrachium rosenbergii	51.02	37.02	8.59	23.6
Penaeuschinensis	22.00	11.66	8.60	23.0
Chalcalburnus chalcoides aralensis	54.30	23.63	8.30	24.0
Carassius Auratus	71.71	69.85	8.24	23.0
Carassius auratus Linnaeus	69.83	68.77	8.32	24.0
Schizothorax biddulphi	97.14	86.75	8.36	24.0

At an appropriate temperature of 22°C and pH 8.3, the semi-lethal salinity of *Takifugu obscurus* approached 35 g/L after 24 hours [13, 17]. In this study, *Takifugu obscurus* had a semi-lethal pH of 10.3 and water alkalinity of 99 mmol/L at a water temperature of 22°C, and a semi-lethal pH of 9.95 and water alkalinity of 89 mmol/L at 48 hours. Low-lying salt flats often have a pH variance between 7.08 and 9.64, with an average of 8.29 (usually below 9), which is exactly close to the optimum pH for *Takifugu obscurus* growth. For instance, the pH of salt flats in Gaocheng County, Shandong Province, varies between 7.78

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and 9.2, thus *Takifugu obscurus* can adapt to the majority of the saline-alkaline condition from pH point [21].

Takifugu obscurus can adapt to the majority of saline environments because it has a higher salinity tolerance of over 35 g/L, while in northeast saline-alkali land in China, salinity is about 10g/L. In terms of salinity tolerance, for instance, *Hypophthalmichthys molitrix* had a semi-lethal salinity of 9.996 g/L at 24 hours [61], whereas *Takifugu obscurus* can tolerate salt up to 35 g/L. In addition to salinity tolerance, Although *Cyprinus carpio* and *Carassius auratus gibelio* have excellent alkalinity tolerance, *Takifugu obscurus* have a semi-lethal concentration of 99 mmol/L after 24 hours, as shown in Table 2. This makes it more tolerant than most species like *Hypophthalmichthys molitrix and Penaeuschinensis*. In addition, *Takifugu obscurus* have a higher commercial worth than the majority of current species.

IK Jang discovered that shrimp mixed with carnivorous fish, like *Takifugu obscurus*, can effectively prevent and control the spread of white spot disease because *Takifugu obscurus* will choose to consume diseased and dead shrimp, cutting off the transmission pathway [62], and the previous pond farming when the WSSV outbreak caused incalculable losses to the shrimp farming industry. *Takifugu obscurus* generally offers benefits over traditional farmed species, such as high tolerance, and good meat quality, in addition to high economic value and diverse feeding habits. The breeding habitat can be improved and better economic benefits can be attained by introducing *Takifugu obscurus* into saline areas [63]. *Takifugu obscurus* generally offers benefits over traditional farmed species, such as high tolerance, and good meat quality, in addition to high economic value and diverse feeding habits. The breeding habitat can be improved and better economic benefits can be attained by introducing *Takifugu obscurus* into saline areas.

Conclusion

In this study, we examined the pH impacts on the growth and development of fertilized eggs, larva fish, and juvenile fish of *Takifugu obscurus*. Weak alkaline pH conditions (pH 8.3), which have the highest survival rates, are most comfortable for *Takifugu obscurus* growth. pH value has a great influence on the survival and growth of *Takifugu obscurus.* The incubation of fertilized eggs is correlated with pH value, and hatching speed and the hatching rate of fertilized eggs of *Takifugu obscurus* will be significantly reduced. Under pH values 6 and 10, extreme conditions lead to deformity and death of fertilized eggs. Larva fish from different pH incubation conditions demonstrate that fish hatched in acidic conditions are more adaptable to acidity and fish hatched under alkaline conditions are more adaptable to alkaline conditions. For example, larvae fish incubated from a pH of 6 have a more survival rate compared with other groups. *Takifugu obscurus* juvenile fish show that has a very high mortality rate under extremely acid or alkaline conditions. Especially when the pH value was 6.0 and 12.0, juvenile fish die immediately after swimming quickly. Last, by this study, we can acquaint with *Takifugu obscurus* more comprehensively, and provide a reference for future conservation and breeding.

Declaration of interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval: The study was approved by OCEAN UNIVERSITY OF CHINA ACADEMIC COMMITTEE (approval number: OUC-AE-45BE, approval time: 2021.05.23). Ensure that relevant research works follow humanitarian principles, ensure the welfare and ethics of experimental animals, and abide by relevant laws and regulations.

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