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Studies on the artificial propagation of hybrid bream in brackish water

Dingbin Gong [a,b,c], Min Tao [a,c], Xueyan Wang [a], Jiayi Liang [a], Jingyun Yang [a], Siyu Fan [a], Tian Zhou [d], Zijian Guo [d], Shi Wang [a,c], Fangzhou Hu [a,c], Chenchen Tang [a,c], Chun Zhang [a,c], Ming Ma [b,**], Yuequn Wang [a,***], Shaojun Liu [a,c,e,*]

[a] State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University, Changsha, 410081, Hunan, PR China
[b] Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research, Ministry of Education, Hunan Normal University, Changsha, 410081, Hunan, PR China
[c] Hunan Yuelu Mountain Science and Technology Co. Ltd. for Aquatic Breeding, Changsha, 410081, Hunan, PR China
[d] Guangzhou Chengyi Aquaculture Co., Ltd., Guangzhou, 511462, Guangdong, PR China
[e] College of Marine Sciences, South China Agricultural University, Guangzhou, 510642, Guangdong, PR China

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ABSTRACT

Hybrid bream (BBTB) is a high-quality bream derived from a hybrid lineage of blunt snout bream (*Megalobrama amblycephala*, $2n = 48$, BSB, ♀) × topmouth culter (*Culter alburnus*, $2n = 48$, TC, ♂). In this study, to explore the laws of artificial propagation of BBTB in a brackish water environment, 150 pairs of BBTB with an average weight of 1000 g were selected as brood fish, and a successful artificial propagation experiment was carried out at the Freshwater Fish Nansha (Nanfan) Breeding Center (2‰–5‰ brackish water). The fertilization and hatching rate was 86% and 78%, respectively, and a total of 1 800 000 larvae were obtained. After 26 days of cultivation, 400 000 larvae survived, showing a survival rate of 22%. This study is of great significance regarding the basic theories and applications of fish genetics.

1. Introduction

In China, aquaculture can be classified into freshwater aquaculture, mariculture and brackish water aquaculture. Freshwater contains almost no salt, while seawater has a high salinity of approximately 35 g per liter. The salinity of brackish water is between freshwater and seawater. Specifically, water containing 0.5‰–30‰ salt per liter is usually called brackish water [1–3]. Freshwater aquaculture and mariculture have yielded remarkable results, but brackish water aquaculture is still being promoted. The Freshwater Fish Nansha (Nanfan) Breeding Center is located in Nansha District, Guangzhou, China. It has abundant water resources, mainly brackish water, with salinity between 2‰ and 5‰.

Blunt snout bream (*Megalobrama amblycephala*, $2n = 48$, BSB) belongs to *Megalobrama*, Cultrinae, and is an important freshwater cultured economic fish in China [4–8]. According to the 2021 China Fishery Statistical Yearbook, in 2020, the production of *Parabramis* and *Megalobrama* was 780 000 tons [9]. BSB has the characteristics of herbivory, fast growth rate, and delicious meat and thus it's widely favored by farmers and consumers. However, with the deterioration of the environment and continuous inbreeding, the germplasm resources of BSB have been degraded, as shown by a decline in growth rate, a reduction in resistance to stress, precocious maturity, and degeneration of meat quality, among other changes [10]. To genetically improve the germplasm resources of BSB, based on the establishment of an allodiploid fish lineage (BT, $2n = 48$, F_1–F_6) derived from distant hybridization between female BSB and male *Culter alburnus* (TC, $2n = 48$) [8,11], backcross progeny (BTB, $2n = 48$) were derived by backcrossing female F_1 of BT and male BSB [12]. A new type of hybrid bream (BBTB) was obtained by a second round of backcrossing of female BSB and male BTB [6]. BBTB has the characteristics of greater stress resistance, higher muscle protein content and lower muscle carbohydrate content [6]. In addition, disease occurs in BSB constantly due to the reduction in freshwater aquaculture

Abbreviations: BSB, blunt snout bream; TC, topmouth culter; BTB, A backcross progeny of female (blunt snout bream (♀) × topmouth culter (♂)) and male blunt snout bream; BBTB, hybrid bream.

* Corresponding author. State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University, Changsha, 410081, PR China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: mingma@hunnu.edu.cn (M. Ma), yuequnwang@hunnu.edu.cn (Y. Wang), lsj@hunnu.edu.cn (S. Liu).

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Fig. 1. Broodstock of BBTB artificially propagated in brackish water. Bar = 2 cm.

area, the decline in water quality of the aquaculture area, and the multiplication of a large number of pathogens. The cultivation of freshwater fish in brackish water has become an important trend in aquaculture. Therefore, artificial propagation of freshwater fish in brackish water is essential. The main purpose of this study is to explore the artificial propagation of BBTB in brackish water. In 2021, 150 pairs of BBTB brood fish were introduced from the State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, China, to the Freshwater Fish Nansha (Nanfan) Breeding Center for artificial propagation, and a total of 1 800 000 larvae were obtained.

2. Material and methods

2.1. Ethics statement

Animal experimenters were certified through a professional training course for laboratory animal practitioners by the Institute of Experimental Animals, Hunan Province, China. The fish were treated humanely following the regulations of the Administration of Affairs Concerning Experimental Animals for the Science and Technology Bureau of China.

2.2. Sample collection and rearing

In May 2021, healthy and robust two-year-old BBTB were introduced from the State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, China, to the Freshwater Fish Nansha (Nanfan) Breeding Center. These fish owned an average weight of 1000 g. These fish were cultured in a special pond for adaptation and were fed with commercial pellet feed three times a day for intensive culture.

2.3. Broodstock selection

Morphological deformities were carefully checked before selecting brood fishes including a range of measures, namely nasal erosion, caudal fin damage, eyesight loss, skin injury, abnormal skeleton and deformed operculum because it has a significant problem in the aquaculture industry [13–20]. Moreover, it was easy to distinguish between the BBTB spawner sexes. The females' scales during the spawning period are smooth or slightly rough. The abdominal section is swollen with a distinctly protruding papilla. Males will ooze milt if the abdomen is gently pressed. Then, the fish with obvious sexually mature characteristics were selected as broodstock for carefully reared in a special pond. The appearance of broodstock of BBTB was shown in Fig. 1. In this study, 150 pairs of BBTB with an average weight of 1000 g were selected as broodstock, and artificial propagation experiment was carried out at the Freshwater Fish Nansha (Nanfan) Breeding Center (2‰–5‰ brackish water).

2.4. Artificially induced spawning

Human chorionic gonadotropin (HCG), luteinizing hormone releasing hormone A_2 (LHRH), and domperidone (DOM) were obtained from the Ningbo No. 2 hormone factory. For induction of spawning, HCG and LHRH were dissolved in 0.7% physiological saline, following manufacturer instructions. In this study, the hormone doses of HCG, LHRH and DOM were 500 IU/kg, 6 μg/kg and 1 μg/kg body weight in females, respectively. A combination hormone of HCG, LHRH and DOM was administered intramuscularly in a single injection. At the same time, males were injected with half the dose used for females.

2.5. Natural fertilization and eggs incubation

After injection, the ratio of male to female fish was 1:1, and the fish were transferred to a breeding pond fitted with a recirculatory aquaculture system. A fish nest made of disinfected and sterilized screens (80 mesh) and palm was placed in the breeding pond. Running water was introduced into the pond 3–4 h before spawning. Afterwards, these fishes were kept in still water for easier spawning. After spawning, the broodstock were transferred to a special pond for further breeding, and the fish nest containing the fertilized eggs was transferred to hatching tank for hatching. The ovulation rate (%) of BBTB was calculated as ovulation rate (%) = (number of ovulating females/number of injected females) × 100. The ovulation period lasted from injection until the onset of ovulation was estimated, and the incubation period was also estimated at the time from the observation of fertilized eggs in water until the larvae hatched. Running water was used for incubation, and the temperature was controlled between 27 °C and 28 °C. The fertilization rate (%) = (number of embryos at the gastrula stage/number of eggs) × 100 and the hatching rate (%) = (number of hatched larvae/number of eggs) × 100 was calculated by using 2000 embryos. The hatched larvae were cultivated in hatching tank for 3 days. Three-day-old larvae were transferred to a nursery pond for further cultivation.

2.6. Larval pond preparation and management

The area of the nursery pond was 666 m^2–2000 m^2. Before stocking larvae in nursery pond, the pond was cleaned and disinfected, then filled with water to 0.6 m–0.8 m. Furthermore, 0.4 kg/m^2-0.7 kg/m^2 green manure was applied to the pond 6–7 days before stocking. After 6–7 days, the rotifers in the nursery pond reproduced vigorously. At this time, the larvae were stocked in nursery pond. Soybean milk was spread evenly into nursery pond 2–3 times every day after stocking the larvae. Moreover, the pond should be patrolled every morning and afternoon for careful observation of the water colour and larval activities including surfacing to determine the amount of feeds and manures to be administered and whether it is necessary to fill the pond with brackish water for the next day. Five days later, during the rearing period, commercial feed should be supplemented. Afterwards, the feeding quantity may be increased in accordance with the growth of larvae. When the larvae had been cultivated for 26–30 days, their body length was approximately 3 cm, and the larvae were divided into different ponds. Then, the survival rate (final number of larvae/initial number of larvae) was calculated.

3. Results

3.1. Courtship behavior and spawning

During May 2021, the reproductive behavior was observed at a regular interval, and it was found that the male initiated courtship activity beginning 4 h post-injection (hpi). The males started exhibiting active

movements around the females, with occasional synchronized swimming with the female at 4.5 hpi. At 5 hpi, frequently coordinated movements were exhibited by the fish. Subsequently, the intensity of movement increased, and the male repeatedly contacted the female's abdominal area close to the urogenital aperture with the snout. At 6 hpi, the pair of fish frequently moved to the surface of the water. The frequency of nipping or butting activity by males increased during spawning. In this study, spawning was achieved within a latency period of 6–7 h at an ambient temperature of 27–28 °C (Table 1).

3.2. Breeding performance calculation

The ovulation rate (%), fertilization rate (%), hatching rate (%) and survival rate (%) are shown in Table 1. All females ovulated successfully, and a 100% ovulation rate was found in this study. The broodstock responded well to hormones and started to release eggs and sperm at 6–7 h after hormonal injection. In this study, a high fertilization rate (86%) and hatching rate (78%) was observed, and a total of 1 800 000 larvae were obtained. Moreover, the incubation period was 24–28 h at an ambient temperature of 27–28 °C (Table 1).

3.3. Larval rearing

After 26 days of cultivation, the average body length of the larvae was 3.5 cm. The appearance of BBTB larval at 26 days was shown in Fig. 2A. A total of 400 000 larvae survived, showing a survival rate of 22% (Table 1). Finally, these larvae were divided into different ponds for cultivation, the appearance of 60-day-old fry of BBTB was shown in Fig. 2B.

4. Discussion

Freshwater fish live in fresh water, which has a very low salinity or even lacks salt. However, previous study has found that some freshwater fish can grow normally in brackish water [2]. Considering the capture fisheries' stagnations and the growing demand for fish as animal protein source, aquaculture plays an important role in augmenting production [21]. Therefore, the development of aquaculture has become imperative and the cultivation of freshwater fish in brackish water has become an important trend in aquaculture. Aquaculture in brackish water ecosystems in coastal ponds is assuming greater significance in recent years [21]. BBTB is a high-quality bream derived from a hybrid lineage of BSB (♀) × TC (♂), which has the characteristics of high muscle protein content, low muscle carbohydrate content, and strong stress resistance [6]. BBTB reaches sexual maturity at an age of 2 years in China [6]. In this study, BBTB was used for artificial propagation and cultivation in 2‰–5‰ brackish water. BBTB was found to spawn, fertilize, hatch and grow normally in brackish water, which has not been reported before. This study provides a theoretical basis for the large-scale breeding of BBTB in brackish water.

In terms of breeding performance, in this study, high fertilization and hatching rate was observed in BBTB. Moreover, the latency period of BBTB was 6–7 h at an ambient temperature of 27–28 °C, and the incubation period of BBTB was 24–28 h at an ambient temperature of 27–28 °C. During the breeding season in 2021, a large number of BBTB populations were obtained through self-mating in brackish water. Based on this, it could be inferred that the embryos of BBTB have an ability to adapt to certain salinity [22].

Saprolegniasis is an important and widespread fungal disease encountered in aquaculture, especially in fish eggs and larvae [23]. Although it commonly occurs on dead eggs during incubation, the main economic loss occurs when it spreads rapidly to live eggs [23,24]. However, in this study, there was no outbreak of saprolegniasis during the incubation of fertilized eggs. The reasons why saprolegniasis did not occur remain unknown. However, the following factors may help us to understand this phenomenon. One possibility is that brackish water contains a high salt content, which can kill most bacteria and fungi that cause saprolegniasis [23–28]. Another possibility is that water temperature during the incubation period was between 27 °C and 28 °C, whereas the suitable temperature for the growth of *Saprolegnia* fungi is between 13 °C and 18 °C. An excessively high water temperature might inhibit the growth of *Saprolegnia* [23,24,29]. Furthermore, this study revealed that BBTB larvae grow normally in brackish water, which indicates that BBTB larvae have a certain tolerance to salt. During the experiment, the brood fish did not receive any antibiotic injections, and the mortality rate was not excessively high. The reason may be that natural fertilization does not cause too much harm to the brood fish, and secondly, the higher salt content in the brackish water may function in disinfection and sterilization [23,25].

In summary, in this study, the artificial propagation of freshwater fish (BBTB) in brackish water was successful. A large number of BBTB larvae were obtained, and the larvae were cultivated. The BBTB larvae grew normally. This is the first report of the artificial propagation and larvae cultivation of BBTB in brackish water. This study is of great significance regarding the basic theories and applications of fish genetics.

Table 1
Observation of breeding performance through the ovulation rate (%), latency period (h), fertilization rate (%), incubation period (h), hatching rate (%), and survival rate (%) of BBTB.

Time of injection	Ovulation rate (%)	Latency period (h)	Fertilization rate (%)	Incubation period (h)	Hatching rate (%)	Survival rate (%)
May 2021	100	6–7	86	24–28	78	22

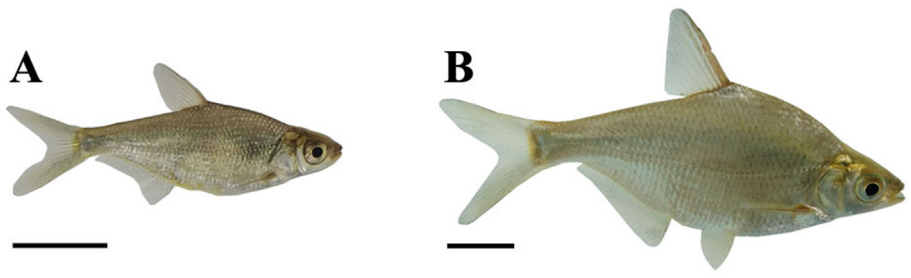

Fig. 2. Larval development of BBTB in different days. (A) Larval development of BBTB at 26 days; (B) Larval development of BBTB at 60 days. BBTB larvae were cultivated in a brackish water environment. Bar = 1 cm.

Declaration of competing interest

The author(s) declare that they have no conflict of interest.

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