KOREAN JOURNAL OF ICHTHYOLOGY, Vol. 33, No. 1, 8-14, March 2021
ISSN: 1225-8598 (Print), 2288-3371 (Online). DOI: https://doi.org/10.35399/ISK.33.1.2

Received: January 22, 2021
Revised: February 16, 2021
Accepted: February 24, 2021

Spawning Behavior of *Microphysogobio koreensis* (Cyprinidae) in Korea

By Jong Sung Park, Seung Woon Yoon[1], Jae Goo Kim[2], Hyun Tae Kim[1], Cheol Woo Park[2], Hyeong Su Kim[3] and Jong Young Park[1,]*

Research Center for Endangered Species, National Institute of Ecology, Yeongyang 36351, Republic of Korea
[1]Department of Biological Science, College of Natural Science, Chonbuk National University, Jeonju 54896, Republic of Korea
[2]Department of Ecology Research, Alpha Research Ecology Institue, Daehak-ro, Gunsan 54151, Republic of Korea
[3]Advanced Aquaculture Research Center, National Institute of Fisheries Science, Changwon 51688, Republic of Korea

ABSTRACT Spawning behavior of the endangered Korean fish, *Microphysogobio koreensis*, was investigated in the Seomjingang (river), Imsil-gun, South Korea, during the spawning season, April to May 2012. The mating system of *M. koreensis*, a broadcast spawner, was a primitive spawning mode, and involved one male and one female, unlike group spawning fishes. Spawning behavior of *M. koreensis* in the wild were observed in eight patterns as resting, male chase, body beating, parallel swim, female withdrawal, male competition, spawning and not guard while spawning behavior in the glass tank were verified in six patterns as resting, male chase, body beating, parallel swim, spawning and not guard. In particular, a behavioral attempt of the pre-spawning stage showed more frequently in the wild than in the glass tank. We assume that difference of spawning behavior might be implication on behavioral restrictions in small and narrow indoor glass tank.

Key words: Endangered species, pre-spawning behavior, mating system, spawning mode

INTRODUCTION

Microphysogobio koreensis is an endemic Korean freshwater fish belonging to the subfamily Gobioninae. This fish is restricted to only smaller areas of water systems, the Seomjingang (river) and Nakdonggang (river), in South Korea, and inhabiting gravel and sand river bottoms in areas with a rapid current (Kim and Park, 2002).

The genus *Microphysogobio* has currently 30 species worldwide (Huang *et al*., 2018) and most previous research has focused on its ecology in *M. longidorsalis* (Song and Son, 2003), egg development and early life history in *M. koreensis*, *M. yaluensis* and *M. rapidus* (Baek, 1978; Kim *et al*., 2012; Hong *et al*., 2015), spawning ecology in *M. koreensis* (Yoon *et al*., 2013; Park *et al*., 2017), and karyotype in *M. koreensis*, *M. longidorsalis* and *M. yaluensis* (Ueno and Ojima, 1984; Im *et al*., 2004; Park *et al*., 2018). Despite the fact that spawning behavior is closely related to reproductive success (Marimuthu *et al*., 2001; Yoon, 2012; Sousa-Santos *et al*., 2014), little is known about this genus.

A research on Korean freshwater fish's spawning behavior has been reported in as follows: building spawn tower in *Hemibarbus mylodon* (Choi and Back, 1970), brood parasitism in *Pseudopungtungia nigra* (Kim *et al*., 2004), spawning within the host mussel in genus *Sarcoheilichtys* (Kang *et al*., 2007). There are some additional researches of spawning behavior in inner glass tank (Park *et al*., 2005; Ko *et al*., 2012).

M. koreensis has been protected as an endangered fish by the Ministry of the Environment in South Korea (ME, 2005) as its population size and distribution are currently showing drastic decreases due to a variety of water pollutions and the destruction of rivers and streams in Korea. Amid the growing restoration plans for this species, there is no information on the spawning behaviors including a

저자 직위: 박종성 (전임연구원), 윤승운 (박사 후 연구원), 김재구 (대표이사), 김현태 (박사 후 연구원), 박철우 (연구원), 김형수 (해양수산연구사), 박종영 (교수)
*Corresponding author: Jong Young Park Tel: 82-63-270-3344, Fax: 82-63-270-3362, E-mail: park7877@jbnu.ac.kr

main spawning field and an exact time their eggs release. Our purpose in the present study, therefore, was to describe the spawning behavior of *M. koreensis* in the wild and in an indoor aquarium to obtain useful information for its conservation.

MATERIALS AND METHODS

M. koreensis is designated as an endangered animal by the Ministry of the Environment in South Korea, and we received permission from the ministry for collection from 2011～2012 (Permit number: 2011-5).

1. Study site in the wild

The spawning behavior of *M. koreensis* was observed from Deogam-ri, Sinpyeong-myeon, Imsil-gun, and Jeollabuk-do in Korea from April to May 2012. Observations were carried out from 8:00～10:00 pm by an underwater video camera (Sony, 420TVL CCD 6LED Outdoor Camera IP68). Sex determination was made as described by Park *et al*. (2017). Terminology and categories of substrates by particle size were based on Cummins' classification (Cummins, 1962).

2. Indoor observation

For observation of spawning behavior in an indoor glass tank, four males and two gravid females were collected from the same localities where the wild survey was performed. This experiment was designed for comparison with the wild survey. These fish were kept in an indoor glass tank (27 × 27 × 45 cm, 24 ± 0.5°C) illuminated with a 20-watt incandescent light bulb and equipped with air filters. A 14-h light/10-h dark (10:00 pm～8:00 am the next morning) cycle was maintained to provide optimum reproductive conditions. Fish were fed plant-feed (JBL GmbH & Co. KG) twice a day. We used an infrared video camera (Sony, Waterproof IR Camera) to observe spawning behavior at night and at dawn.

RESULTS

1. Spawning site

During the non-spawning season, *M. koreensis* inhabits deep and broad streams. As the peak spawning season approaches, they begin to move toward the edges of the main streams. Spawning sites consist of boulders, cobbles, and pebbles with a shallow depth of 0.4～0.6 m and a current velocity of 0.6～0.9 m/sec. During the spawning season, the fish show distinctive sexual dimorphism. Spawning behavior mostly takes place from 8:00～11:00 pm at a 20～21°C water temperature and a 24～25°C air temperature.

Table 1. Comparisons of the spawning behaviors of *Microphysogobio koreensis* in the wild and a glass tank

Behaviors	The wild	A glass tank
Pre-spawning behaviors	Resting	Resting
	Male chase	Male chase
	Body beating	Body beating
	Parallel swimming	Parallel swimming
	Female withdrawal	
	Male competition	
Spawning behaviors	Spawning	Spawning
Post-spawning behaviors	No guard	No guard

2. Spawning behaviors in the wild

1) Pre-spawning stage

This stage involves at least 6 behavioral patterns: resting, male chase, body beating, parallel swimming, female withdrawal, and male competition (Table 1).

Resting; Males and females stay in a small school without any movement in between the gravel in the spawning field.

Male courtship; Males aggregate in a small school near the bottom of the stream and then start to chase selected females. The male then stimulates the female's abdomen using its snout or body (Fig. 1A). During this process, the male swims parallel to the female and approaches close to the female's side or abdomen (Fig. 1B).

Female withdrawal; When the male approaches, other females not ready to spawn move away to avoid breeding attempts by the male.

Male competition; The male head-butt away other males in order to reach the lateral region of the selected female (Fig. 1C).

2) Spawning behavior

After repetitive pre-spawning behavior, *M. koreensis* starts to display the spawning stage. The male bends its body toward the belly of the female and simultaneously the female bends its body toward the male (Fig. 2). At this time, the gametes of both sexes are released. This cloud can usually be seen on the surface of pebbles or in the crevices between pebbles.

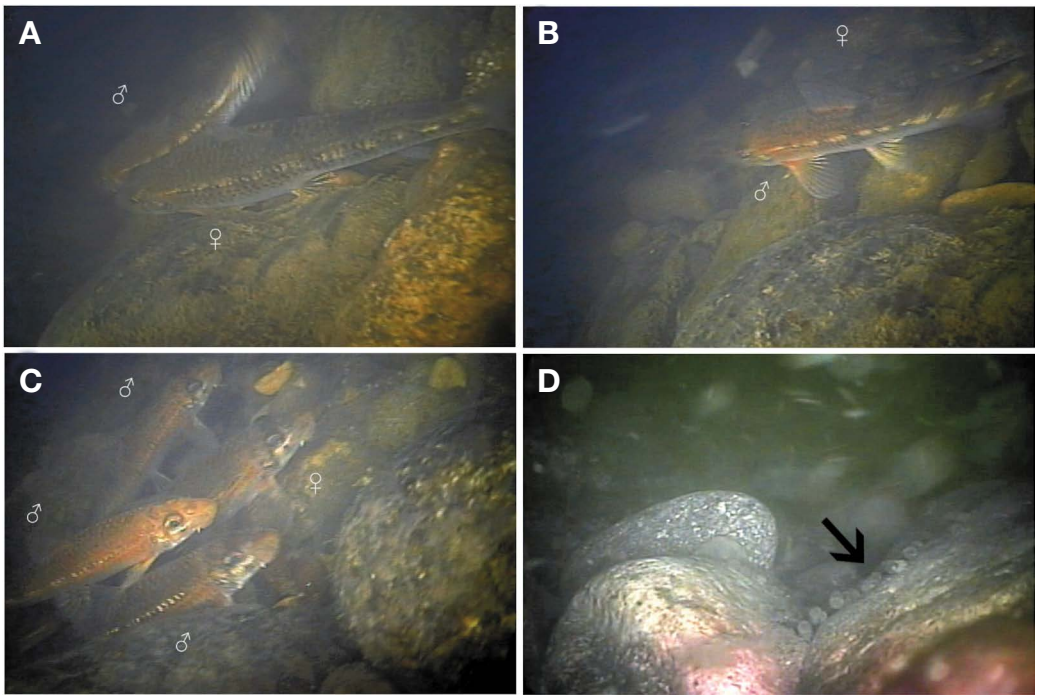

Fig. 1. Video clips of natural spawning behavior of *Microphysogobio koreensis* in the wild. Body beating (A), parallel swimming (B), male competition (C), adhesive-demersal eggs are not guarded by the parents (D). Arrow, fertilized eggs on stones.

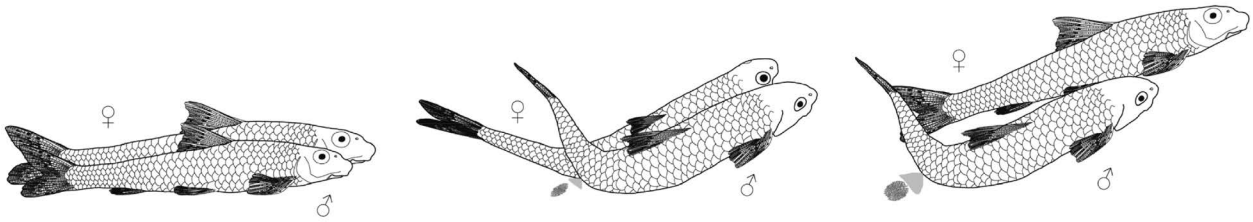

Fig. 2. The illustration showing typically spawning sequence of *Microphysogobio koreensis*.

3) Post-spawning stage

After spawning, the fertilized eggs are not guarded by the parents and sometimes are eaten by other fish. They are demersal eggs separated from one another and there are many adhesive-demersal eggs on the surface between substrates of the spawning field (Fig. 1D).

3. Spawning behaviors in a glass tank

Spawning behaviors in the glass tank also occurred at around midnight, and showed a similar pattern to those observed in the field. The pre-spawning stages consist of resting, male chase, body beating, and parallel swimming (Table 1 and Fig. 3). After chasing a female, the male stimulates the belly of the female using its head and then swim side-by-side with the female (Fig. 2). The male then bends its body laterally towards the female and quickly surrounds the latter's belly. In response, the female quickly approaches the male and releases its eggs while the male releases sperm. This spawning display continued for 1∼3 seconds and was repeated one minute later and then five minutes later. Afterward, mutual courtship ceased. The fertilized eggs were not guarded by the parents and conspecifics sometimes ate the eggs.

DISCUSSION

To date, it has been reported that *M. koreensis* does not show any sexual dimorphism during the spawning season. With the lack of information regarding nuptial coloration, there has been little research done on the spawning cha-

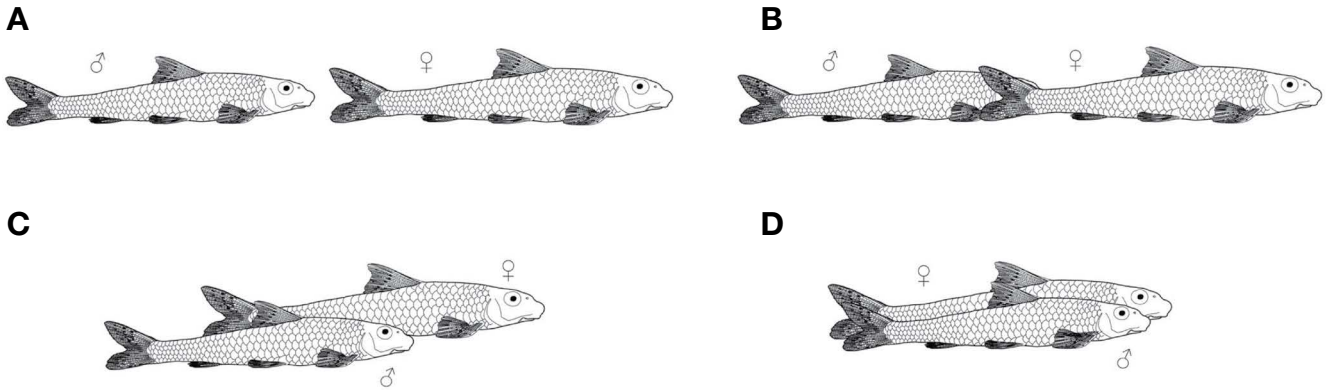

Fig. 3. An illustration of the pre-spawning behavior of *Microphysogobio koreensis* in a glass tank. Resting (A), male chase (B), body beating (C), parallel swimming (D).

racteristic of this species. Through this study, however, we confirmed that males exhibit a bright-orange body and a thick red horizontal line from the rear of the operculum to the caudal peduncle during spawning, along with a lateral line, while females have a dark-brown body (Park *et al*., 2017). Based on the male's lateral pink nuptial coloration, we were able to conduct a detailed investigation of its spawning behavior.

In the wild, *M. koreensis* displayed a pre-spawning stage consisting of male chase, body beating, parallel swimming, female withdrawal, and male competition. After spawning, the eggs settled on the surface between pebble and stone substrates. The parents did not guard the fertilized eggs, which were adhesive-demersal. Until now, the spawning behaviors of the subfamily Gobioninae were described in only some species: *Hemibarbus barbus* has demersal eggs, males form territories, and no parental care is provided in the wild (Katano and Hakoyama, 1997) and *Gobiobotia macrocephala* does not form territories and provides no parental care of demersal eggs in an aquarium (Ko *et al*., 2012). Park *et al*. (2005) opined that *Squalidus gracillis majimae* in an aquarium do not form territories, but provides partial parental care of demersal eggs, unlike *M. koreensis* who does not guard their eggs after spawning. In particular, Yoon (2012) stated that the male of *Ladislabia taczanowskii* builds spawning field using their tail and covers their fertilized eggs with sands for physical protection. However, once *M. koreensis* lays eggs on the bottom, they leave this place and does not guard. Some Gobioninae species utilize the ripple widely as a spawning field (Choi *et al*., 2001; Kim *et al*., 2014; Byeon, 2020). In this restricted area, it is possible to look like that several fishes remain grouped in this area for mating. However, in detailed, we observed, that they do a pair mate (male: female) with spawning competition and behaviors and spawn at the bottom of the stream area, where fluid-flow rate is starting to get faster, between the pool and the ripple (Yoon *et al*., 2013). In both wild and glass tank, we confirmed the behavioral evidence that *M. koreensis* play pair mating for spawning as well.

From observations in the wild, we could not determine the exact number of times that spawning occurred because it predominantly took place in the middle of the night. Thus, we carried out an indoor experiment, which showed spawning behavior that was similar to that observed in the wild, with the exception of the pre-spawning behaviors. In the wild, pre-spawning had 6 stages: resting, male chase, body beating, parallel swimming, female withdrawal, and male competition, while in captivity, pre-spawning had only 4 stages: resting, male chase, body beating, and parallel swimming (Table 1). Based on observations of *Dionda diaboli* spawning in the wild and in captivity, Phillips *et al*. (2011) reported that differences in spawning frequency may be caused by behavioral restrictions in small and narrow indoor glass aquariums. We assume that difference of spawning behavior might be implication on behavioral restrictions in small and narrow indoor glass tank.

The components of *M. koreensis* habitat bottom during a spawning season consists of boulders, cobbles, and pebbles with a shallow depth of 0.4~0.6 m and a current velocity of 0.6~0.9 m/sec. We suggest that such bottom structure and physical water condition for the fish's successful spawning. Unfortunately, from this study, we did not determine any relationship between the behavior frequency increase in pre-spawning stage and successful fertilization for their reproduction. So, it is furthermore needed to conduct any affection according to increase of behavioral frequency.

M. koreensis is a broadcast spawner, which is a primitive

mode of spawning in fish (Balon, 1975; Page, 1985; Page and Johnston, 1990a, 1990b; Johnston and Page, 1992). The spawning behavior of *M. koreensis* involves one male and one female and is not communal like in several species in the genera *Notropis*, *Hybognathus* (Platania and Altenbach, 1998), and *Epinephelus striatus* (Whaylen *et al*., 2004). Understanding the mode of spawning is important for conservation strategies (Marimuthu *et al*., 2001) and can predict how the species will respond to natural and artificial changes (Angermeier, 1995). Therefore, this data may be utilized for the conservation of endangered fish such as *M. koreensis*.

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모래주사 *Microphysogobio koreensis* (Cyprinidae)의 산란행동

박종성 · 윤승운[1] · 김재구[2] · 김현태[1] · 박철우[2] · 김형수[3] · 박종영[1]

국립생태원 멸종위기종복원센터, [1]전북대학교 자연과학대학 생명과학과, [2]알파생태연구원,
[3]국립수산과학원 첨단양식실증센터

요 약 : 멸종위기 담수어류 모래주사의 산란행동을 조사하기 위해 산란시기인 2012년 4월부터 5월까지 전북 임실군의 섬진강 일대에서 연구를 실시하였다. 모래주사의 산란은 무리산란(group spawning)하는 어류와 달리 암수 한 쌍에 의해 이루어졌으며, 수중으로 대량의 생식세포를 방출하여 산란하는 원시적인 생식모드(broadcast spawner)로 확인되었다. 자연에서 모래주사의 산란행동은 휴식(resting), 유혹(male chase), 자극(body beating), 평행유영(parallel swim), 암컷의 산란회피(female withdrawal), 경쟁(male competition), 산란(spawning), 산란 후 난을 보호하는 행동은 보이지 않아(not guard) 총 8가지 행동을 보인 반면 수조 내에서는 휴식, 유혹, 자극, 평행유영, 산란, 산란 후 난을 보호하지 않은 6가지 행동이 관찰되었다. 특히 산란전 행동은 수조 내에서 보다 자연상태에서 더 빈번하게 일어났다. 이와 같은 행동적 차이는 작고 좁은 수조에서의 행동적 제약에 의한 결과로 추정된다.

찾아보기 낱말 : 멸종위기종, 산란 전 행동, 교배방식, 생식모드