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Spawning Behavior of the Kissing Loach (*Leptobotia curta*) in Temporary Waters

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The natural spawning behavior of the kissing loach, an endangered species of Botiidae, was investigated in the wild in early June for two years in relation to several environmental factors. Kissing loaches spawned in temporary waters after elevation in water level. All spawnings observed (n=163) occurred within 3–5.5 hours from late afternoon to night after formation of the temporary water. These spawnings were performed by one female and one (71%) or two (29%) males in densely vegetated lentic waters. The female and following male(s) swam into dense grasses, where they vibrated to spawn intermittently. After the vibration continuing for 3–20 seconds, they moved to other parts of the dense grassy area and began vibration again. This sequence of spawning behavior was usually repeated several times, and the eggs were thus scattered widely. The spawning behavior and the rapid larval development of this species appear to be adaptations for the use of temporary waters as a spawning ground. The rise in water level and the consequent formation of temporary waters appear to be crucial triggers for reproduction of the kissing loach.

Key words: teleost, fish, *Leptobotia curta*, endangered species, spawning, behavior, temporary waters, conservation

INTRODUCTION

The kissing loach (*Leptobotia curta*; one of the 47 species of Botiidae) is a fresh-water fish surviving only in a few rivers around Kyoto (the Lake Biwa-River Yodo system) and Okayama in Japan (Yuma *et al*., 1998). Due to its reduced habitat in artificially reconstructed rivers or irrigation channels with temporary waters, this species is now threatened with extinction (Maehata, 2003). Since 2004 and 2003 it has been recognized as a national endangered species of wild fauna and flora by the Ministry of the Environment Government of Japan and a critically endangered species in the Red Data Book of Japan, respectively; since 1977 it has been designated a national natural monument by the Agency for Culture Affairs, Japan.

For Botiidae, including the kissing loach, there are a few reports on development and growth (David, 1961; Nakamura and Motonobu, 1971), though there have been many reports on classification or genetic studies (*e.g*., Fang, 1936; Nalbant, 1963; Kottelat, 2004; Tang *et al*., 2006; Šlechtová *et al*., 2006). Ecological information, especially in relation to reproduction, is scarce, but it is essential for the appropriate conservation of the endangered species (Washitani and Yahara, 1996). Although it is known that mature kissing loaches suddenly appear in early summer around temporary waters (*e.g*., rice fields) for spawning (Nakamura and Motonobu, 1971; Saitoh *et al*., 1988), which is the key process for the kissing loach to persist, the reproductive ecology of the kissing loach has been unclear. For the spawning and growth, temporary waters are important for many fishes, such as the silver crucian carp (*Carassius* sp.), Asian pond loach (*Misgurnus anguillicaudatus*), striped spined loach (*Cobitis* sp. S), and Far-Eastern catfish (*Silurus asotus*), which have also been reduced recently (Saitoh *et al*., 1988).

In the present study, we report the natural spawning behavior of the kissing loach for the first time. Furthermore, we discuss possible triggers for spawning based on the relationship between frequency of spawning and environmental parameters at the spawning sites.

MATERIALS AND METHODS

Study site

Channel Y1 is an irrigation channel of the Yoshii-River system, Okayama, Japan (Fig. 1a). The exact location of study site will remain undisclosed for conservation purposes. Water is supplied to Channel Y1 from Channel Y2 directly and through ditches and reservoirs, and flows into the Yoshii River. Some of the ditches and reservoirs are covered with a luxuriant vegetation of terrestrial grasses (*e.g*., *Phalaris arundinacae* and *Persicaria maackiana*), which become submerged after elevations in water level caused by the closure of water gates during irrigation periods (from early June to the end of September) (Fig. 1a, Fig. 2).

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Observations on spawning behavior

The observation areas and periods were selected based on our preliminary observations; these areas were temporary waters covered with a dense growth of terrestrial grasses and submerged after elevation in the water level (>55 cm) of Channel Y1. In 2004, two people observed the spawning behavior of kissing loaches in the area (550 m^2) shown in Fig. 1b, from 17:00 on June 9 to 22:00 on June 10. In 2005, an area (6 m×5 m) was selected and observed by one person from 22:00 on June 8 to 0:00 on June 10 from a fixed point overlooking the observation area. Two adjacent areas of the same size were similarly examined from 16:00 on June 9 to 0:00 on June 10 in 2005. Spawning behavior could be assessed from the shore, even in the luxuriant grasses, by the vibration of grasses and the presence of spawned eggs, because the current was very slow during both years. Observation areas were illuminated indirectly at night. The time and location of spawning behavior (including repeated spawnings by individual females) were recorded, and spawned eggs were collected at the site of spawning behavior. The completion of spawning was confirmed by the collection of the eggs. These eggs were identified as those of the kissing loach by the hatched larvae, according to the description by Nakamura and Motonobu (1971).

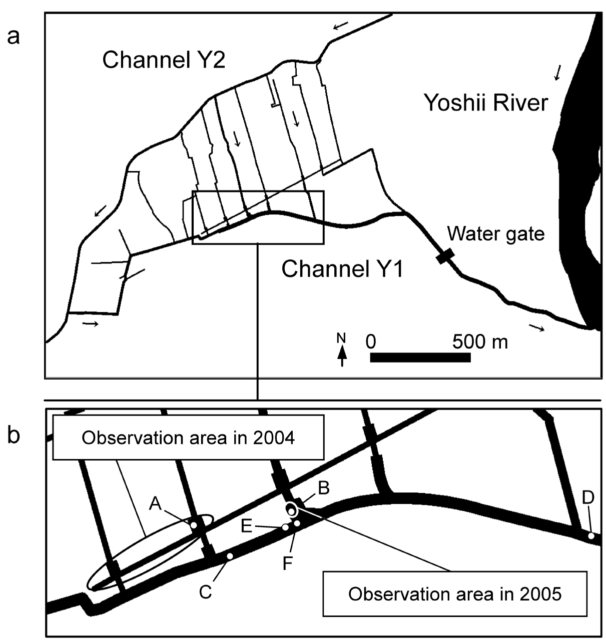

Fig. 1. Map of the study area **(a)** and enlargement showing the study site **(b)**. The arrows indicate directions of flow. Some of the ditches and reservoirs between Channel Y1 and Channel Y2 are temporary waters. Water temperature was measured in temporary waters (A, 2004; B, 2005) and the main stream (C, 2004; D, 2005). Water level was measured both years (E). Other parameters were measured in temporary waters (B) and the main stream (F) in 2005.

Measurement of water parameters

Water temperature was measured hourly using a water thermometer with a data logger (Onset Computer Corporation, MA) at the bottom of observation area (Fig. 1b, A-2004, B-2005) and in Channel Y1 (Fig. 1b, C-2004, D-2005). The water level in Channel Y1 was measured with a pressure transmitter connected to a data logger (Sensor Technik Sirnach AG, Switzerland) every ten minutes (Fig. 1b, E), and converted into the level of temporary waters based on measurements taken manually in the temporary waters every 30–120 minutes. In 2005, dissolved oxygen, pH, conductivity, and turbidity were measured with a multi-parameter water-quality meter (DKK-TOA Corporation, Japan) in the observation area (Fig. 1b, B, two points) and at Channel Y1 (Fig. 1b, F, two points).

RESULTS

Spawning behavior

In 2004 and 2005, 163 spawning events of kissing loaches (including repeated spawnings by individual females) were observed in temporary waters. During these seasons, more than 90% of the adult fish appeared to be fully mature and were presumed to be involved in spawning (unpublished data). In a spawning event, a female, distinguished from males by her swollen abdomen, was observed swimming in the temporary waters with one to four male(s) behind; some male(s) drove away other males. The female then swam into the dense grasses, followed by one or more males close behind. The female and male(s) continued into the grasses and vibrated intermittently for 3–20 seconds to spawn, not only on the bottom but also on the grasses; in 24 observations in which the number of male(s) could be counted, females were accompanied by one (71%) or two (29%) males. After intermittent spawning vibrations continuing for 3–20 seconds, all observed females and males moved (>50 cm) to other dense grasses and repeated this sequence of spawning behavior (≤5 times). In four cases, the female turned immediately after vibration and paused at the bottom for 1–7 minutes; the male(s) swam around. After spawning, no parental egg care was observed. Behavior in which a male embraced the female, as reported for the Far-Eastern catfish spawning in similar temporary waters and for a spined loach (*Cobitis taenia*) (Katano, 1988; Bohlen, 1998; Maehata, 2002), was not observed.

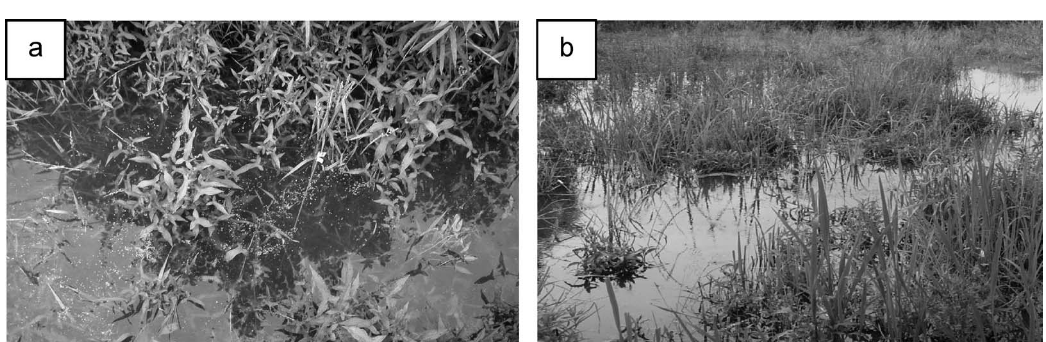

Fig. 2. Temporary waters after an elevation in water level. **(a)** Close-up view of the observation area; **(b)** perspective of the observation area.

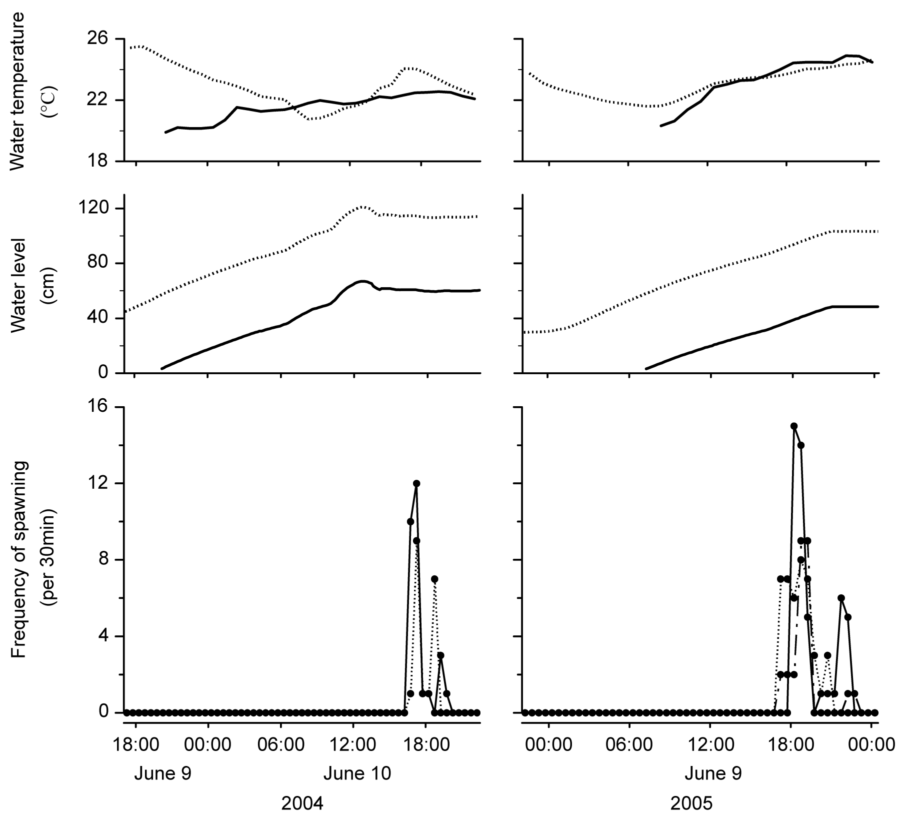

Fig. 3. Changes in water temperature, water level, and frequency of spawning. Solid and dotted lines indicate measurements for temporary waters and the main stream, respectively. A spawning vibration for 3–20 seconds was counted as one spawning event. The number of spawnings in 30-minute intervals recorded by all observers are shown. The water gate was closed at 6:00 on June 9 and 19:00 on June 8 in 2004 and 2005, respectively, and temporary waters were formed after 20:00 on June 9 and 7:00 on June 9 in 2004 and 2005, respectively.

Frequency of spawning behavior and spawning environment

Changes in the frequency of spawning as well as in water temperature and water level are shown in Fig. 3. Of the 163 spawnings observed, all were performed several hours after submersion of the spawning ground and were limited to 3–5.5 hours. All spawning by kissing loaches was observed from late afternoon to night, despite different timing of the rise in water level. There were no essential differences between 2004 and 2005, even though different methods of observation were employed in the two years. No significant difference in water temperature was detected between temporary waters and the main stream in 2005 (Wilcoxon's signed rank test, P=0.83), whereas a significant difference was detected in 2004 (P<0.01); the water temperature during spawning was 22.5–24.9°C. As shown in Fig. 4, dissolved oxygen, pH, conductivity and turbidity fluctuated after submersion of the spawning ground. The current was very low at the sites where spawning behavior was observed, and the bottom was more than 80% covered by vegetation.

Eggs and larvae

Around the points where vibration behavior was observed, fertilized eggs of kissing loaches were not concentrated, but were scattered. Egg were demersal and weakly adhesive via the smooth, transparent chorion; they were almost spherical in shape and 1.8 mm in diameter after formation of the perivitelline cavity. Eggs hatched within 25 hours after spawning at 25°C. Hatched larvae (3.5 mm long) often hung from the grass by a thread projecting from the frontal edge of the yolk.

DISCUSSION

The present study has reported for the first time the natural spawning behavior of the kissing loach, a botiid species. We found that after the formation of temporary waters, a female and associated male(s) spawned several times in several locations.

Because temporary waters are unstable and ephemeral, the short-term spawning of kissing loaches immediately after submersion of the spawning area appears to be reasonable. Furthermore, the widely scattered spawned eggs and the rapid larval development of this species increase larval survival (Katano *et al*., 1988; Saitoh, 1990). These traits appear to be adaptations of this species for the use of temporary waters as a spawning ground by decreasing larval mortality in an unstable ephemeral environment. There are few aquatic predators in temporary waters, and the dense vegetation shelters the fishes. In addition, possibly abundant zooplankton, as suggested by Molls (1999) for a bream (*Abramis brama*) on the floodplain of the River Rhine, is suitable for larval growth.

The rise in water level and the consequent formation of temporary waters appeared to induce spawning in kissing loaches in 2004 and 2005. Similar environmental stimulation of spawning was also observed in 2003 (unpublished observation). For a Biwa catfish (*Silurus biwaensis*), Maehata

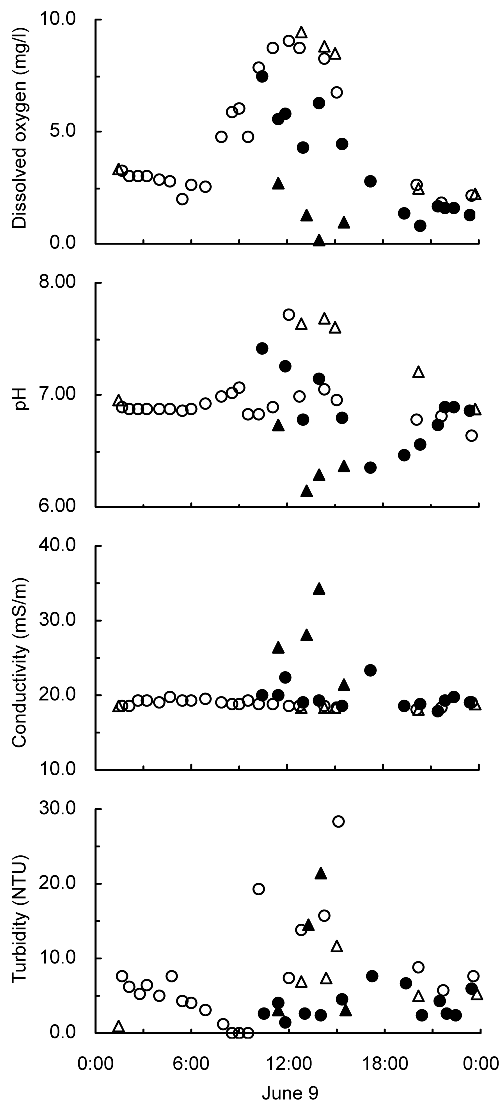

Fig. 4. Dissolved oxygen, pH, conductivity, and turbidity measured in temporary waters covered with luxuriant vegetation (solid circles), temporary waters not covered with vegetation (solid triangles), the shore side of the main stream (open circles), and the center of the main stream (open triangles).

(2001) suggested that a rapid rise in water level might be a main factor in inducing spawning in temporary waters. However, all spawning by the loaches was observed from late afternoon to night, despite different timing of the rise in water level, and so a circadian factor may also play a role. Since there was no consistent difference in water temperature between the temporary waters and main stream, this factor may not play an important role in the spawning of the kissing loach. Although changes in some water-quality parameters were detected at the spawning ground just before spawning (Fig. 4), the proximate trigger is still unclear, and experiments with artificial temporary waters might show changes in water pressure or chemical component(s) to be involved.

In conclusion, the conservation of appropriate spawning areas and triggers in temporary waters connected with rivers are called for to protect the existence of the endangered kissing loach, since modern rivers are often artificially controlled and temporary waters are seldom formed.

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