

Comparison of Reproductive Behaviors between Two Species of Eightspine Sticklebacks (Genus: *Pungitius*)

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The object of this study is to differentiate the reproductive behaviors of *Pungitius sinensis* and *P. kaibarae* inhabiting the southern part of the Korean Peninsula. We collected *P. sinensis* from Jusu stream, Okkye-myeon and *P. kaibarae* from Sacheon stream, Sacheon-myeon both in Gangwon province and subsequently raised and observed them in an aquarium. At the beginning of the reproductive season, male *P. sinensis* got tinged with dark green, made a territory, and built nests on the bottom. On the other hand, male *P. kaibarae* became black all over, its white ventral spines became conspicuous and built nests on the stems of waterweed off the bottom. In the courtship dance, male *P. sinensis* made frequent attempts to entice females into their nests after many bitings, while male *P. kaibarae* mostly did this with conspicuous jumpings. In courtship behaviors, the body's angle of male *P. kaibarae* with his head down was larger than that of male *P. sinensis* by 50-60 degrees. During courtship, the biting frequency as an index of aggressive behavior was greater in *P. sinensis* and the jumping frequency as an index of sexual behavior was greater in *P. kaibarae*. During the courtship dance, bitings tended to suppress jumpings, for *P. sinensis*, but not for *P. kaibarae*.

In considering an evolutionary trend in behaviors of a species, it should be kept in mind that many of behavioral characteristics form an adaptive complex suited for particular way of life. Furthermore, any change in one behavioral aspect can lead to other changes ramifying through the entire system of its characteristics (Hinde, 1970). The analysis of the reproductive behavior of *Pungitius* seems to provide a good illustration of this principle.

In the southern region of the Korean peninsula, *Pungitius sinensis* and *P. kaibarae* are distributed as fishes of the genus *Pungitius* belonging to the family Gasterosteidae. However, the two species have not been distinctively identified taxonomically. While Kim et al. (1989) consider the two species as distinct species based on the morphological differences in the numbers of dorsal fin rays, dorsal spines, and vertebrae and the body depth. On the other hand, Chae and Yang (1990) consider them as subspecies because it can be thought that no reproductive isolation has taken place; there is much chance that they are considerably differentiated morphologically or of any ecotype.

This study is to compare and analyze reproductive behaviors of the two species while their taxonomical positions are not distinctively identified. Differences in

reproductive behaviors between the two species could become an important cue to their taxonomical identification.

Materials and Methods

P. sinensis and *P. kaibarae* were collected before their propagation period over two years from March 1999 until June 2000, and their behaviors were observed while being raised in aquariums installed in a laboratory. Two habitats in Gangwon province were selected as places where *P. sinensis* and *P. kaibarae* were to be collected. Fishes of *P. sinensis* were collected from the Jusu stream at Okkye-myeon, Donhae city (37° 40'N, 129° 02'E) and fishes of *P. kaibarae* were collected from the Sacheon stream at Sacheon-myeon, Kangneung city (37° 49'N, 128° 51'E).

The fishes were taken to the laboratory where they were bred in respective aquariums (50 × 30 × 40 cm). The individuals were bred under the condition of 16 h light at about 16 °C.

Small pebbles and fine sand were laid on the bottom of the aquarium, and an air pump was installed and operated in order to supply oxygen. Also, a filtration system was installed therein. Considering the habits of *P. sinensis* and *P. kaibarae* to build a nest, waterweeds taken from their natural habitats were planted abundantly. They were fed with tubificids and frozen mosquito larvae.

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When a male built a nest completely in the aquarium, a female with spawn was put into it. Ten couples of *P. sinensis* each and *P. kaibarae* were observed under this condition. Behaviors of the male and female were photographed with a 8 mm video camera, and were later analyzed by replaying the video tape.

The male showed a behavior of enticing the female to come into the nest by jumping toward and biting at her repeatedly. The jumping and biting frequencies observed during his courtship for the first 5 min after the female was put into the aquarium was analyzed.

Differences in reproductive behaviors between *P. sinensis* and *P. kaibarae* were processed statistically. In the following two behavioral aspects. First, differences in the jumping and biting frequencies between two situations that; 1) the female was approaching the nest and 2) the female approached the nest but hesitated to enter the nest was analyzed by Mann-Whitney U test. Second, differences in the respective jumping and biting frequencies during by a unit time (5 min) between the two subspecies was analyzed Mann-Whitney U test.

Results

Coloration

Both female and male *P. sinensis* were tinged with yellowish brown in the whole body before the reproductive period, and their back wore green sheen between the yellowish brown. The male, at the peak of their reproductive period became tinged with dark green and the yellowish green sheen became deeper. The abdomen of the female during the reproductive period was bright silver yellow, and dark black spots appeared in their back. The fins and dorsal spines of *P. sinensis* were transparent, and the ventral spines thereof were transparent or with a blue tint.

Both female and male *P. kaibarae* were tinged with yellowish brown before the reproductive period, and their back had a dapple pattern with which a gray tint was mixed, which was darker than the abdomen. The male, at the peak of their reproductive period became black in the whole body and fins. While their dorsal spines were black, their ventral spines were white.

Their white ventral spines were conspicuous because they contrasted with their black body. The back of females with spawn showed dark and black spots (Table 1).

Nesting

All 10 male *P. sinensis* built a nest on the bottom, near the roots of a waterweed (Table 1; Fig. 1, upper). They brought some thin stem fragments or fallen root fragments in the mouth to stuff them into the nest. Then, they showed a fanning behavior, waving the pectoral fin fast, and repeatedly showed a boring behavior to creat an inlet to the nest. Also, they applied a mucus substance secreted from the kidney to the inner and outer surfaces of the nest to glue the nest materials (Morris, 1958). When they posed themselves horizontally and turned round several times with their body rolled up above the nest, a substance looking like a white thread was secreted from the kidney. They held it in the mouth and applied it to the inner surface of the nest through the inlet. Meanwhile, they applied the mucus substance to the outer surface by going around the nest with their abdomen abutting the outer surface. As they were picking the inlet part continuously, they bored a deep hole therein, but did not bore a separate hole for the nest's outlet. The shape of the nest was globular or oval. Once the nest was completely built, they hardly showed the nest-related behaviors such as boring, fanning and gluing, but showed a behavior as if they were waiting quietly among other waterweeds a little away from the nest. The male attempting to propagate a second time used the existing nest after mending it.

All 10 male *P. kaibarae* were observed to build a nest among stems or branches of a waterweed, and its inlet was off the bottom. The distance from the nest's inlet to the bottom was 3-11 cm (Table 1; Fig. 1, lower). The nest appeared to be globular or a little oval. Like *P. sinensis*, all 10 male *P. kaibarae* also showed the behaviors of boring, fanning and applying the mucus substance to the inner and outer surfaces of the nest for building the nest. While building the nest, they increasingly showed such behaviors. Upon completion of the nest, the frequency of such behaviors

Table 1. Differences between reproductive behaviours of *Pungitius sinensis* and *P. kaibarae*

Reproductive behaviour	<i>P. sinensis</i>	<i>P. kaibarae</i>
Color of the male	Bark-green on the head, back and sides shaded off to silvery on the belly. Dorsal spines were transparent and ventral spines were bluish white.	The whole body was jet-black. Dorsal spines were black and ventral spines were very prominent white.
Color of the female	Black blotches were formed on the back and sides. The swollen belly was bright silver.	The female making her territory was black on the back and sides, and ventral part was light. The female responding to male's courtship was black patch on the back and sides.
Nesting behaviour	Nest was built on the bottom.	Nest was built on the stems of waterweed off the bottom
Courtship behaviour	The male made frequent attempts to entice female into his nest after many a bite. Jumps were weak. The angle of the body with head-down posture was 10-20 degrees.	The dancing of the male consisted of a number of conspicuous jumps with the body held downwards, the angle of the body 50-60 degrees from the horizontal.

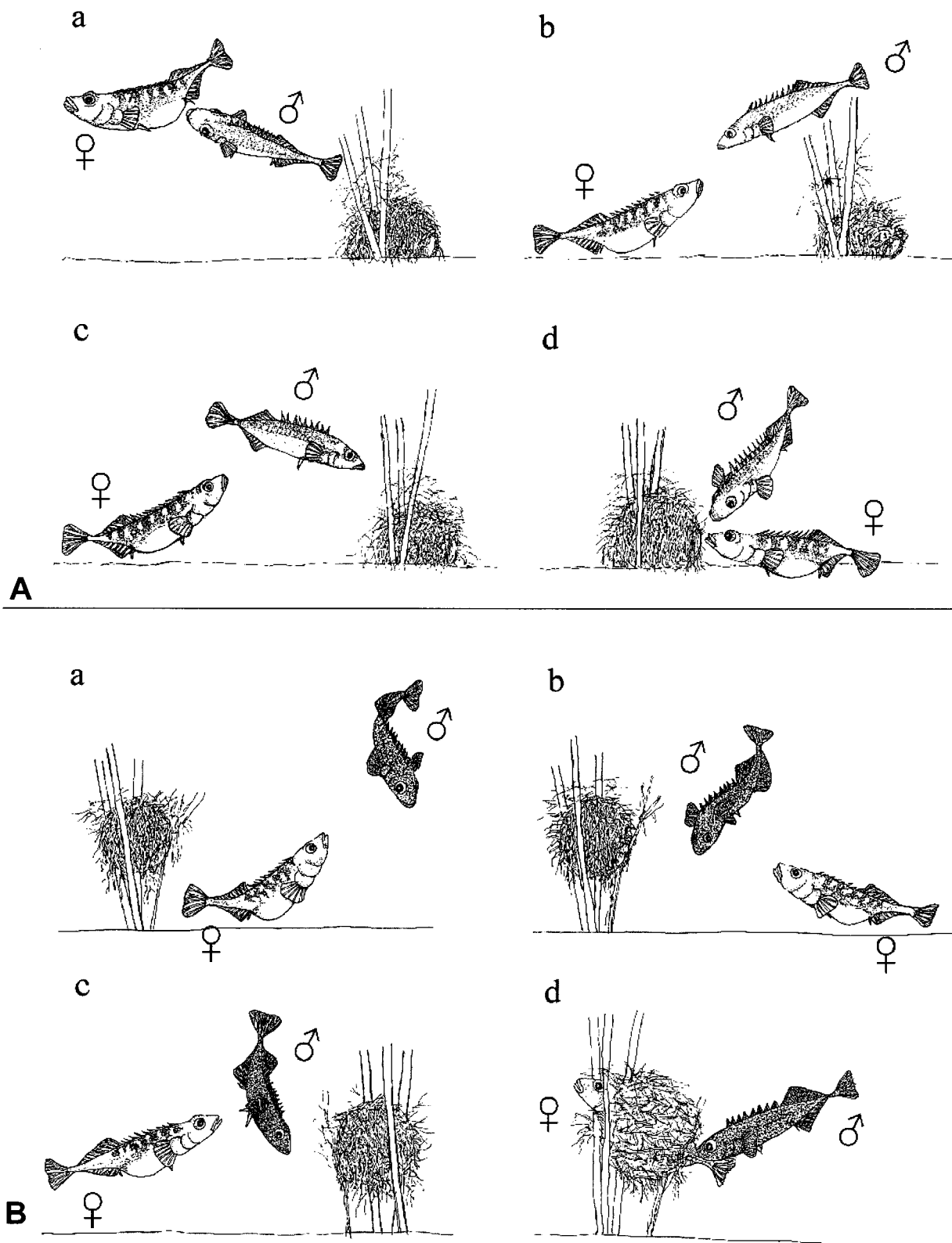


Fig. 1. The sequence (a-d) of courtship behaviors of the *Pungitius sinensis* (A) and *P. kaibarae* (B). The nests are located on the bottom (A) and on the stems of waterweed off the bottom (B). The body's angles of males are 10-20° (A) and 50-60° (B) respectively.

was decreased, but they continuously mended the completed nest. After they succeeded in propagation, once the nest was demolished, and males attempting a second propagation built a nest at a new place. At this time, they bit off the existing nest, collected stems or roots of other waterweeds, and brought the fragments to build the new nest. Two out of 10 individuals were observed to build a nursery by weaving waterweeds loosely a little above the nest when offsprings began to disperse and their nursing behavior was coming to an end. When building such nursery, they bit off their original nest and brought the bitten material more frequently rather than bringing the nursery material from other places. During this process they did not show the behavior of boring or applying the mucus substance to it, but simply laid the nest materials into a heap.

Courtship behavior of P. sinensis

When a female with spawn showed up, a male *P. sinensis* moved on toward her fast and bit at her abdomen strongly. When the female was still at the bottom, the male attempted to jump as a courtship dance. Such jumping distance or motion was smaller in *P. sinensis* than in *P. kaibarae*. When jumping, the male's body toward the female was kept with his head down and tail up, but the body's angle from the horizontal plane, which was 10-20° (Table 1; Fig. 1, upper), was much smaller in *P. sinensis* than in *P. kaibarae*. The male showed much of the jumping and biting behaviors at the same time. At this time, the female fled against such biting. The male attempted to entice her by tapping her on the abdomen with his mouth and returning to the nest rather than by jumping. The female, had a back with a dapple pattern and a bulgy abdomen at the peak of spawning season, was poised with her head directed a little upward and her tail downward. This female readily responded to the male's courtship dance. The jumping frequency and power of the male were quite different depending upon individuals. The larger the angle of the male's body with his head down was, the more the male attempted to jump rather than to bite, and the jumping frequency was higher than the biting frequency. When the female turned her head toward the male, he led her to the nest's inlet with his ventral spines raised up. She followed him, looking at his ventral spines. He guided her just directly to the nest, or after going around the nest. When the female entered the nest with only her tail out of the inlet, the male put his mouth on her flank and further shook her body. This behavior accelerated the female's spawning. When the female completed her spawning and came out of the nest, the male entered the nest and inseminated the spawn by strewing his semen. The female stayed in the nest for spawning for 20-30 sec, and the male entered the nest and came out fast as if he went

through the nest. The male subsequently became aggressive to ward the female, and the female showed a fleeing behavior.

Courtship behavior of P. kaibarae

The male *P. kaibarae* at the peak of the reproductive period was tinged with black in the whole body. Some female *P. kaibarae* showed a behavior of taking advantage of being on her own ground to act highhandedly, and at that time, her body color was black overall, but her abdomen was tinged with grey. The female with spawn responding to the male's courtship dance had a dark black dapple pattern appearing in the back. The male began to jump as a courtship dance while he was poised above the female. The male's body was poised with his head downward, and its angle from the horizontal plane was 50-60° (Table 1; Fig. 1, lower). Also, his black dorsal spines and white ventral spines were raised up. The male moved toward the female, bending his body in an S-shaped form several times, and then turned around and went back to the nest several times. When the female turned her head or faced the male in response to such jumping behavior, the male led the female to the nest, waving his tale fiercely. The female followed the male, looking at the male's white ventral spines which were raised up with her head upward. While the male was leading the female to the nest, the male's body was kept to be poised with his head being downward.

When they reached the nest, the male put his mouth on the upper part of the nest's inlet and showed the fanning behavior. Also, the female put her mouth into the nest's inlet and showed the fanning behavior for a while, and then entered the nest. When the male shook the flank of the female with his mouth, the female got to spawn. When the female came out of the outlet upon completion, the male immediately entered the nest and inseminated the spawn, and then came out of the nest.

Comparison of the courtship behaviors between Pungitius sinensis and P. kaibarae

In order to find any difference in sexual and aggressive tendencies between the males of *P. sinensis* and *P. kaibarae*, which they showed during courtship upon completion of nest building, the interactions between the male and female of after the female was put within the males' territory were analyzed. The male often showed a biting behavior in response to the female's fleeing behavior. Also, when the female showed positive response to the male's courtship dance, or was guided to the nest and attempted to enter the nest, the male showed continuous jumping or fanning behaviors at the nest's inlet. However despite the female's positive response, the male bit at the female aggressively. This behavioral difference between the two species was comparatively analyzed. As a result of analyzing the

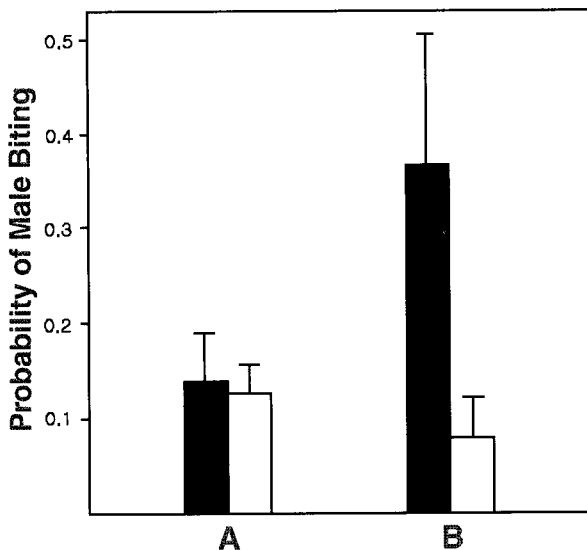


Fig. 2. The comparison of male's biting probability during the courtship in *Pungitius sinensis* (■) and *P. kaibarae* (□). A. In response to the positive approaching females (N = 10, U = 37.5, $p = n.s.$). B. In response to the hesitating females at the nest entrance (N = 10, U = 4.0, $p < 0.001$).

interactions between the male and female with spawn when the female was put into the aquarium by using 10 males of respective *P. sinensis* and *P. kaibarae*, the male's biting frequency in response to the female's positive approach was found to be 0.14 (SE = 0.07) in *P. sinensis* and 0.12 (SE = 0.04) in *P. kaibarae*. Therefore, no difference was found between the two species (U = 37.5, $p > 0.05$, Fig. 2A). On the other hand, when the male guided the female to the nest but the female hesitated to enter the nest, the male's biting frequency in response thereto was found to be 0.36 (SE = 0.20) in *P. sinensis* and 0.08 (SE = 0.05) in *P. kaibarae*. Therefore, the biting frequency of the male *P. sinensis* was found to be higher than *P. kaibarae* (U = 4.0, $p < 0.001$, Fig. 2B). Thus, the male's aggression caused by the female's hesitation to enter the nest was greater in *P. sinensis* than in *P. kaibarae*.

In order to find the jumping and biting frequencies of the male of respective *P. sinensis* and *P. kaibarae* after the female was put into the nest territory of the male, the frequencies during 5 min after the female with spawn was put into it were recorded by using 10 males of respective *P. sinensis* and *P. kaibarae* in this experiment. The average jumping frequency was found to be 115.4 ± 17.18 times in *P. sinensis* and 203.86 ± 11.01 times in *P. kaibarae*. The average biting frequency was found to be 19.49 ± 5.10 times in *P. sinensis* and 7.74 ± 2.77 times in *P. kaibarae*. During the courtship behavior, the male *P. kaibarae* was found to jump more than *P. sinensis* (U = 4.0, $p < 0.001$, Fig. 3). On the other hand, the male *P. sinensis* was found to bite more than *P. kaibarae* (U = 0.0, $p < 0.001$, Fig. 3) during the courtship behavior.

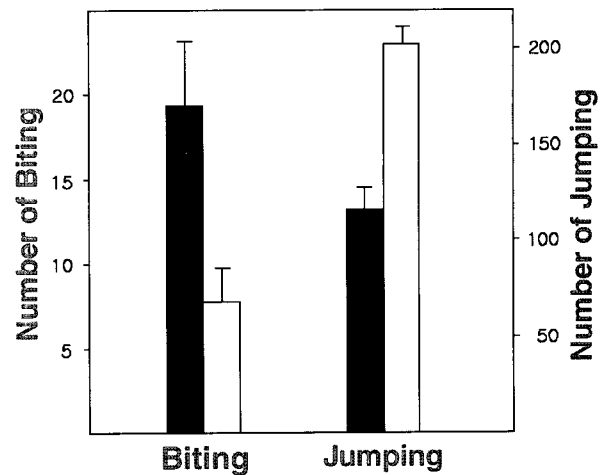


Fig. 3. The comparison of the number of biting and jumping in the *Pungitius sinensis* (■) and *P. kaibarae* (□) in 5 min. Biting: N = 10, U = 0.0, $p < 0.001$, Jumping: N = 10, U = 4.0, $p < 0.001$.

Discussion

Generally, morphological differences is evaluated as a basis of classifying any species. There is an obvious morphological difference between the species of the genus *Pungitius*. Nonetheless, there has been an opinion (Kim et al., 1989) that they should be treated as distinct species. Also, a different opinion (Chae, 1988) was ever suggested. In this study, obvious differences were found in the total length and number of dorsal fin rays between the two species. The difference in nuptial coloration could be also identified conspicuously by the naked eyes. As a result of researching on nuptial coloration in *Gasterosteus aculeatus* (Foster, 1994), it was found that nuptial coloration in *G. aculeatus* varied depending upon its habitat. From the evolutionistic viewpoint, it could be thought that *P. sinensis* and *P. kaibarae* originated from a single ancestor, but their different habitats caused the difference in nuptial coloration (McLennan, 1995). However, the relation between habitat and nuptial coloration should be identified through further studies under the natural condition. Anyhow, if the two species are under reproductive isolation, the respective species should respond to their own nuptial coloration. However, it remains to be researched whether the male's nuptial coloration of respective species will act on the female as a sign stimulus.

There was also an obvious difference in the location where the nest was built between the two species. While *P. sinensis* built their nest on the bottom by the roots of waterweed, *P. kaibarae* built their nest at a place off the bottom. As European *Pungitius pungitius* also built their nest off the bottom (Morris, 1970; Wootton, 1976), the European *P. pungitius* seems to be similar to *P. kaibarae* in the behavior of nest building. It was observed that *P. kaibarae* applied the

mucus substance secreted from its kidney to the outer surface of the nest, and this behavior is thought to have a close relation to the location where the nest is built. On the other hand, the nest of *P. sinensis*, was mostly existed between the roots or stems of waterweed (*Phragmites communis*) just over the bottom in the aquarium as well as under the natural condition. Therefore, it is interesting to compare the location of the nest between *P. sinensis* and North American *P. pungitius* (Nelson, 1968). In North American *P. pungitius*, nests were usually wedged underneath rocks, where the male could not swim over the outer surface and deposit glue (McKenzie and Keenleyside, 1970). These two species are the same in the behavior of building the nest just over the bottom. Thus, it could be thought that they lack the behavior of applying the mucus substance to the outer surface of the nest. McKenzie and Keenleyside (1970) reported that the North American *P. pungitius* in the aquarium never showed the behavior of applying the mucus substance to the outer surface of the nest (superficial gluing). The nest built on the bottom would be less damaged by turbulence of the water current or any intruder compared to the nest built on a waterweed (Potts and Wootton, 1984). In this study both species showed the superficial gluing during the nest-building. However, there was a difference in the frequency of the gluing in that. *P. kaibarae* showed higher frequency (Park and Lee, 1999). After the deposition of eggs in the nest, *P. kaibarae* continued to apply the mucus substance to the outer surface of the nest, while *P. sinensis* did not. The activity of superficial gluing seems to be associated with the site of nest-building. Therefore, it is thought that the frequency of gluing was much lower in *P. sinensis* than in *P. kaibarae*.

In the courtship dance, the male *P. kaibarae* showed jumping in a big motion and in noticeable moderation. On the other hand, *P. sinensis* showed rhythmic jumping in a petty motion. When the male was poised with his head down while he was dancing for courtship, *P. kaibarae* kept the body's angle from the horizontal plane at 50-60°, but *P. sinensis* kept it at 10-20°, almost level. The body's angle with the head down during the courtship reveals the aggressive and sexual tendencies. It is known that the smaller the body's angle is, the higher the aggressive tendency is (Morris, 1958). Accordingly, *P. kaibarae* was also similar to European *P. pungitius* in the courtship behavior, as in the nest building behavior (Wootton, 1976).

While the courtship dance of three-spined stickleback is composed of zigs and zags, the courtship dance of *Pungitius* is composed of continuous motions of jumping and biting (Wootton, 1976). The jumping frequency of *P. kaibarae* was found to be greater than

that of *P. sinensis*. On the other hand, the biting frequency of *P. sinensis* was found to be greater than that of *P. kaibarae*, suggesting a difference in the aggressive tendency between the two species. This is in conformity with the difference in the male body's angle with the head facing down (Morris, 1958). That is to say, if the biting frequency is high and the body's angle is small in a species with a high aggressive tendency, *P. sinensis* was found to have a higher aggressive tendency than *P. kaibarae*. The possibility that this difference is related to population density for the reproductive period can not be ruled out (Fitzgerald, 1993), but more studies on such possibility will have to be carried out under the natural condition.

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