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Spawning and Growth of eightspine stickleback, *Pungitius sinensis kaibarae* (Tanaka) (Gasterosteidae, Pisces), in the Chayang Stream, a tributary of Kumho River, Korea

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The spawning and growth of eightspine stickleback, *Pungitius sinensis kaibarae* (Tanaka, 1915), were investigated in the Chayang Stream from March 1990 to February 1991 as a part of study on its life history. Nest building, spawning and parental behaviors were similar to those known on the sticklebacks of Europe, North America and Japan. But *P. sinensis kaibarae* in the Chayang Stream, peculiarly, showed only superficial gluing behavior in nest building phase and made two nurseries just behind the nest in parental phase. The individuals with fully mature eggs were found from late February to late June. The fecundity of an adult female was about 21 to 110 (mean 47.07) eggs and the diameter of fully matured ovarian eggs ranged 1.3 to 1.5 mm. The smallest female with fully mature eggs was 25.8 mm in body length and had 21 eggs. The relationship between the number of mature eggs in ovary and body length was No. of eggs = 2.857BL - 55.134 (r = 0.890). The number of deposited eggs in a nest was 27 to 637 and they were composed of several clusters of various developmental stages. The distribution pattern of gonad-somatic index (GSI) against the body length shown that in females 1+ year-old fishes mainly participate in spawning but in males 1+ and 2+ year-old fishes equally participate. From the fluctuation pattern of GSI and the number of mature eggs in ovary, it was assumed that the spawning season was from February to June and the peak of spawning was early March to late April. The newly hatched young was found at late April and their body length was about 10 mm. The young (0+) grew rapidly until late August. Then their growth was slowed down and finally ceased in mid-autumn. The young reached about 35 mm BL in their first year of life. The halting of growth was lasted to next June when the spawning season would be nearly closed. They (1+) began to grow again from late June, grew rapidly until late September and reached about 45 mm BL. Then there was no more growth until to die as 2+ year-old fish. Therefore it was assumed that the life span of *P. sinensis kaibarae* in the Chayang Stream would be about two years. The relationship between the body length and the body weight was logBW = 2.954logBL - 4.802 (r = 0.998).

KEY WORDS: Spawning, Growth, Reproductive behavior, *Pungitius sinensis kaibarae*, Korea

The distribution of eightspine stickleback, *P. sinensis kaibarae* (Tanaka), is very restricted. They inhabit only in some rivers of Korean Peninsula drained into Sea of Japan and at near Kyoto and Hyogo in Japan (Kobayashi, 1933; Jeon, 1982, 1987; Chae, 1988). This species is now extincted in Japan (Miyadi *et al.*, 1976). It is also apprehended that the habitats in Korean Peninsula may be destroyed due to the water pollution.

The complex reproductive behavior of sticklebacks has attracted the attention of ethologists and there were numerous studies on the spawning ecology of the genus *Pungitius* (Leiner, 1931; Kobayashi, 1933; Barraud, 1955; Hall, 1956; Morris, 1958; Griswold and Smith, 1973; Wilz, 1971; Wootton, 1976; McKenzie and Keenlyside, 1970; Goto *et al.*, 1979).

Since the first record as *Pygosteus kaibarae* by Tanaka (1915), there were a few descriptions on the morphology and ecology (Kobayashi, 1932, 1933) and on the development of scutes (Igarashi, 1969). Some studies on the Korean populations were conducted in the field of taxonomy, morphology, karyotype and genetic variations (Jeon, 1987; Chae, 1988; Chae and Yang, 1988; Kim *et al.*, 1989; Yang and Min, 1990) but their life history was not investigated yet. Thus it is necessary to reveal the ecology of this species including its life history. The present paper deals with the spawning and growth of this fish species in the Chayang Stream, a tributary of the Naktong River Basin.

Materials and Methods

Study area and the environment

Observation and sampling of *P. sinensis kaibarae* were conducted in the Chayang Stream, which is a tributary of the Kumho River, Naktong River Basin (Fig. 1). This stream is approximately 100 m to 150 m wide but the water occupies usually 30m to 50m width. In this stream *P. sinensis kaibarae* inhabited sporadically from the confluence to 7 km upstream but the size of population was relatively small. Of those habitats the most abundantly inhabiting site was a pond

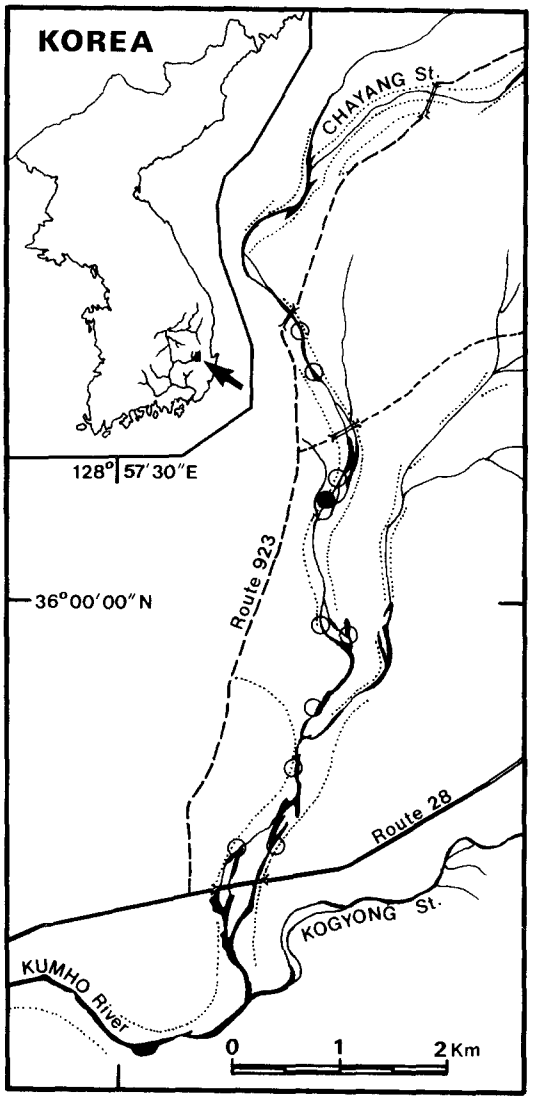

Fig. 1. Map showing the location of the sampling site in the Chayang Stream. Open circles indicate the habitat of *Pungitius sinensis kaibarae* and solid circle represents the sampling and observation site.

under the banks of the stream not a flowing region. This pond was approximately 50 m-long, 10 m-wide and 1 m-deep, and the substratum was composed of gravel and mud. Because the water flows through the stacked gravels, water velocity was very slow and the water temperature was relatively stable throughout the year, with fluctuation between 10° C and 20° C (Fig. 2). This

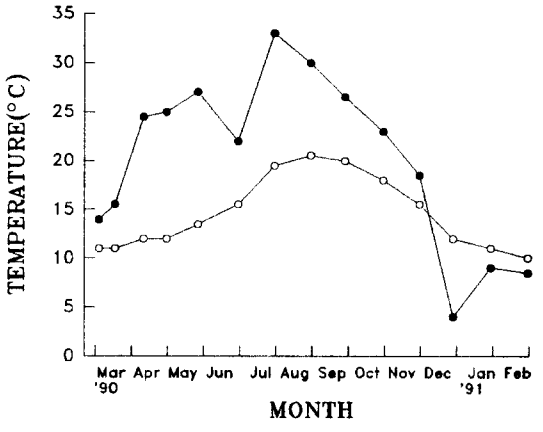

Fig. 2. Monthly changes of the atmospheric temperature (open circles) and water temperature (solid circles) of the sampling site.

pond was flooded only in rainy season or when there was heavy rain. The water was very clear except for the rainy season from late June to mid July.

In this pond, the aquatic rooted plants such as *Ceratophyllum demersum*, *Hydrilla verticillata* and *Typha orientalis*, and the green algae such as *Spirogyra* sp. and *Hydrodictyon reticulatum* flourished through the spring to late autumn. Some other fish species cohabit in this pond such as *Carassius auratus*, *Rhodeus uyekii*, *Pungtungia herzi*, *Pseudorasbora parva*, *Moroco oxycephalus*, *Squalidus gracilis majimae*, *Zacco platypus*, *Zacco temmincki*, *Misgurnus anguillicaudatus*, *Cobitis taenia taenia*, *Lefua costata*, *Liobagrus mediadiposalis*, *Pseudobagrus brevicorpus*, *Odontobutis platycephala*, *Oryzias latipes* and *Macropodus chinensis*.

Materials and methods

The collection of samples was made once a month from early March 1990 to late February 1991 but was made twice a month in spawning season. Approximately one hundred fishes per one survey were caught with a hand net of 5 mm meshes. The collection of spawning nest was made by pulling out the aquatic plants. The specimens collected were fixed immediately with 10% formalin.

After standard length and body weight were measured, the gonads were removed and weighed. Then the sex and the maturation stage of ovary were noted. The standard length was measured to nearest 0.1 mm with vernier caliper (accuracy 1/20 mm; Mitutoyo, Japan) and the weight of body and gonads were measured to 1 mg with digital chemical valance (accuracy 1/10 mg; Chyo Balance Corp., Japan). The age of the fishes was determined by the length frequency of catches at successive months. The gonad-somatic index (GSI) was calculated by the equation, (weight of gonad/body weight) $\times 100$.

It was difficult to observe the behavior of this fish in the field. Thus we transferred several males and females to the laboratory in April 10, 1990. These fishes were kept in a glass aquarium ($45 \times 35 \times 30$ cm); the bottom of the aquarium was covered with sand, planted some water plants (*Ceratophyllum demersum* and *Hydrilla verticillata*) and put down *Spirogyra* and old leaves. The water temperature was kept at about 15° C. We observed the nest building, spawning and parental behaviors in this aquarium.

Results

Spawning

1) Nesting site and size of nest

The nests were made with threads of *Spirogyra* and old leaves or stems, and positioned among the branches and leaves of aquatic plants such as *Hydrilla verticillata*, *Ceratophyllum demersum* and *Typha orientalis*. The nest were ellipsoid and approximately 25×35 mm in size (Table 1). There was an entrance at the anterior part of the nest but no exit at the upper posterior part. The diameter of the opening was 5-6 mm.

2) Nest building, spawning and parental behavior

In aquarium, a male established a territory around a water plant and became very aggressive. The territory covered about a half or all of the bottom. The male started building a nest from early morning in April 12, 1990. The male carried nest materials (threads of *Spirogyra* or

debris of old leaves) with his mouth and attached to the stem of a plant. The site of nest was about 5 cm above the bottom. Nest materials were glued together with the mucus secreted from kidney. At this time, the male shown the behavior called superficial gluing (Wootton, 1976). With the accumulation of a mass of material, the male started pushing and boring and then fanning with his pectoral fin. These behaviors were repeated sometimes until the nest completion. The nest was completed during the daytime (within about 12 hours). During the nest building phase, the male tried often to court gravid female but failed everytime. After completion the nest and before spawning, the male guarded the nest and sometimes tried pushing, fanning and superficial gluing.

The next day, a gravid female spawned in the nest. The spawning behavior was the same as the previous reports (Morris, 1958; Wootton, 1976). Then the male entered in parental phase. The male became more aggressive and shown pushing, fanning and superficial gluing behavior more frequently than in nest building phase. The eggs hatched six days after spawning. The young remained motionlessly in the nest for one or two days. And then the young started to jump out of the nest. But the male sucked up the young with his mouth and retrieved to the nest. Till at this time, the young had yolk sac in its belly. The male made a loose nursery behind the nest two days after hatching and returned the straying young to the nursery. At three days after hatching, the male made a second nursery just behind the first one. This second nursery was small and very loose network. The young absorbed their yolk almost completely four days after hatching and start to visit the surface to fill their swim bladder with air. They then hid in the vegetation near the surface. The next day the male ceased to show any parental behavior.

3) Fecundity

The fecundity was decided by the number of mature eggs in the ovary. The number of fully mature eggs in an ovary were 21 to 110 (mean 47 eggs). But the number of eggs deposited in the nest were 27 to 637. The deposited eggs in a nest were grouped into several clusters. The cluaters in a nest were different each other in developmental stage (Tables 1 and 2). The diameter of fully mature eggs in ovary was 1.3-1.6 mm (mean 1.4 mm) and the diameter of deposited ones in the nest was 1.5-1.8 mm (mean 1.6 mm). The eggs

Table 1. Size of nest, number of eggs and number of different developmental stage in a nest of *Pungitius sinensis kaibarae* observed in the Chayang Stream

No. of sample	Size of nest (mm)	Number of eggs in a nest	Number of different developmental stage
1	23.1 × 34.2	143	2
2	28.5 × 37.1	290	4
3	25.7 × 31.6	27	1
4	23.0 × 32.0	637	6
5	26.0 × 36.0	617	6

Table 2. Fecundity and egg diameter of fully mature female of *Pungitius sinensis kaibarae* taken from the Chayang Stream

	Range	Mean ± SD	Number of samples
Number of eggs in an ovary	26 ~ 110	47.1 ± 20.4	71 specimens
Diameter of ripe eggs (mm)	1.3 ~ 1.6	1.4 ± 0.1	201 eggs
Diameter of deposited eggs (mm)	1.5 ~ 1.8	1.6 ± 0.1	90 eggs

became larger after spawning. This was because the egg membrane swelled with absorbed water. The relationship between the body length and the number of mature eggs in ovary was No. of eggs = 2.857BL − 55.134 (r = 0.890) (Fig. 3). This shows a tendency that the number of eggs increases proportionately according to increment of body size.

4) Spawning season

Monthly change of gonad-somatic index (GSI) was monitored for assumming the spawning season of *P. sinensis kaibarae* in this stream (Fig. 4). In females the value of GSI from early March to late May ranged from 2.5 to 20.5. Almost all females with GSI value more than 9 had mature eggs. Thereafter the value of GSI decreased to 1 and in late July most females have GSI value less than one. From August the values of GSI began to increase slowly and in late January of next year GSI value reached 2-5. Until late February the value increased abruptly and the distribution

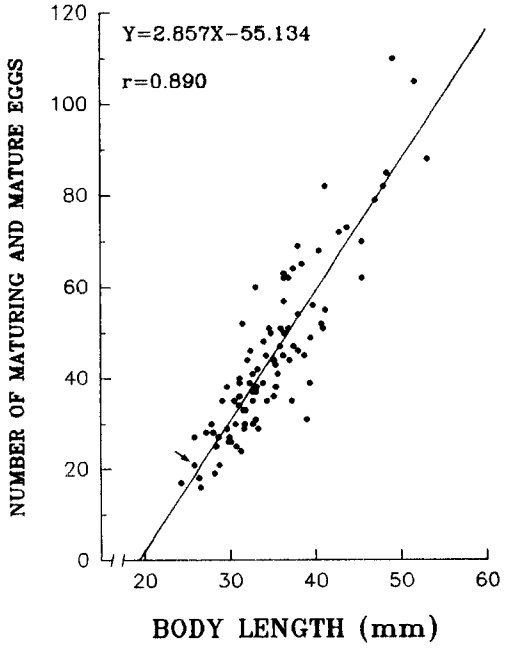

Fig. 3. The relationship between body length and the number of mature eggs in an ovary. Arrow indicates the smallest individual with fully mature eggs.

pattern of GSI resembled that of early march of previous year. Furthermore a few females had fully mature eggs.

The male's GSI value were lower than female's and also the fluctuation pattern of GSI was different from female's one. The mean value of GSI in early March was 0.35. Then the GSI value decreased slowly and reached to minimum mean value of 0.02 in late July. From August the GSI value increased rapidly. The males that collected in late October and late November had the largest testes and their GSI value was 0.5-0.7. Thereafter their GSI value decreased again until next February. It was noted that the female had maximum GSI value in spring but the male had a peak of GSI value in autumn.

Fig. 5 shows a distribution pattern of the value of GSI against body length. In both sexes some individuals were fully matured at less than 30mm BL. Females with eggs of fully mature or maturing stage (GSI values greater than 5) distributed from 25 mm to 50 mm BL and majority of them were concentrated at 27-40 mm BL. Female individuals greater than 40mm were few in number and also had somewhat low GSI value. In spawning season, males with GSI value greater than 0.2 distributed from 25 mm to 50 mm and majority of them were concentrated at 35-47 mm BL. Therefore the range of body length of individuals participating in spawning was similar in both sexes but the body length of the main group were larger in males than in females. According to the age determination by the distribution of body length, this result means that in females 1+ year-old fishes participate in spawning mainly but in males 1+ and 2+ year-old fishes equally participate.

Fig. 6 shows monthly change of the number of fully mature eggs in ovary of the fish and shows change of the ratio of females with fully mature eggs to all females captured each month. The females with mature eggs were captured from early March to late June. Thereafter there were no females with mature eggs in their ovary. From late February of next year, females with mature eggs appeared again. The smallest female with fully mature eggs was 25.8 mm in standard body length and she had 21 eggs. Relatively many females reached sexually mature adult stage before

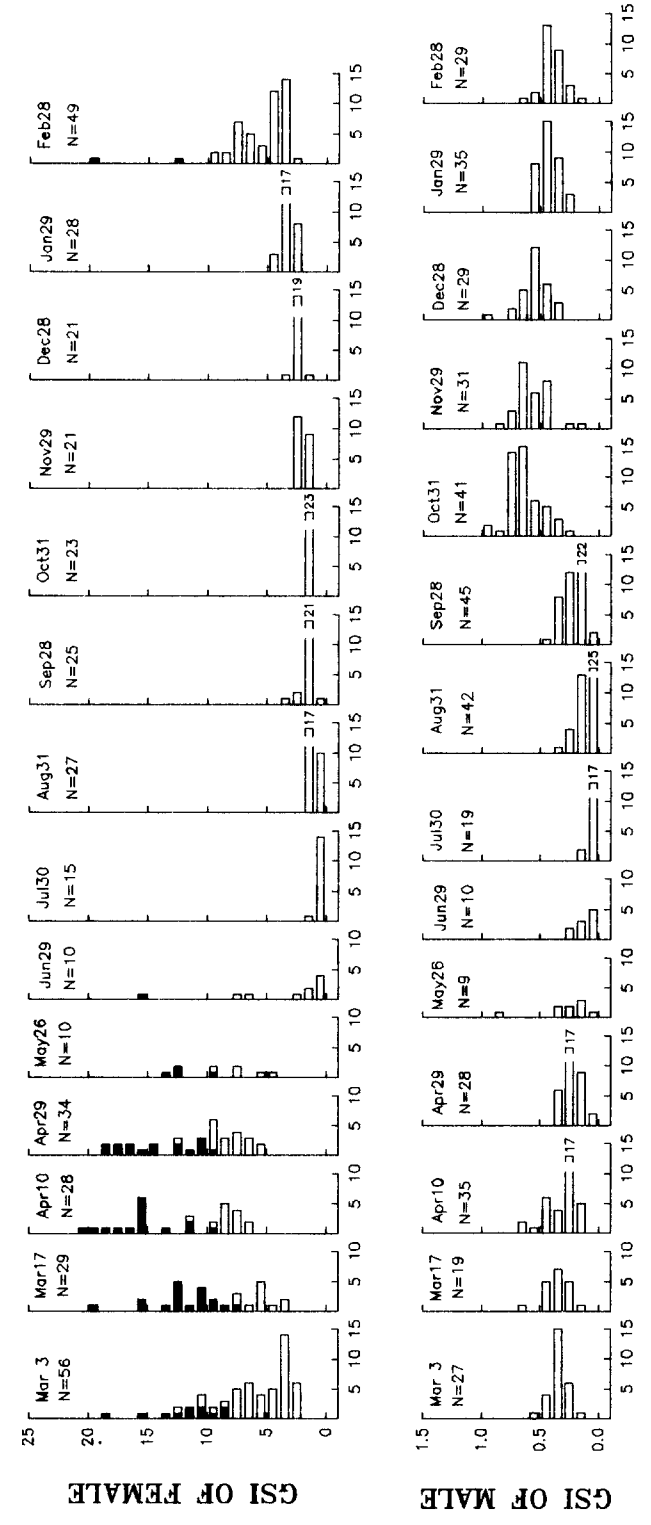

Fig. 4. Monthly changes of gonad-somatic index (GSI) of *Pungitius sinensis kaibarae* in the Chayang Stream. Black bars indicate the females with fully mature eggs.

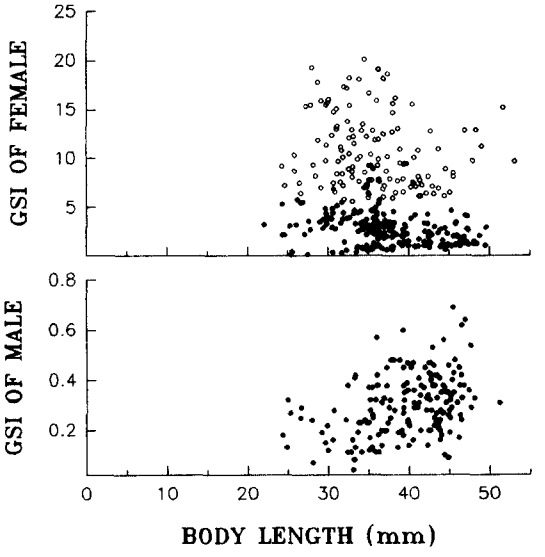

Fig. 5. Distribution patterns of GSI against body length. In upper figure open circles indicate females with fully mature or maturing eggs and solid circles indicate females with immature eggs. Lower figure shows GSI of males in the breeding season (February to June).

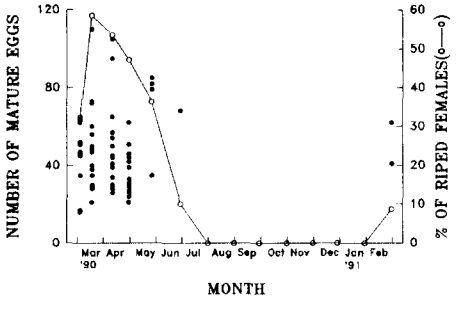

Fig. 6. Monthly changes of fecundity and of the number of females with mature eggs. Solid circles indicate the number of mature eggs in ripe females and open circles indicate the ratio of the females with mature eggs to all females in each month.

to grow to 30 mm in standard body length.

Seasonal growth

The distribution patterns of standard body length of *P. sinensis kaibarae* collected monthly are presented in Fig. 7. The standard length of the fishes in early March distributed from 25 to 50 mm. Such pattern was not changed until late May. In this period there were no significant difference in the frequency of the individual number of each length group. But it seemed that two peaks appeared at April 10: one was at 30-35 mm and the other at 40-45 mm. Thereafter, up to July a peak was found at 40-45 mm length group and no fishes longer than that were captured. In late August there was a peak at near 45 mm and this pattern was lasted to next April.

The 0-year old fishes were captured at late April and a peak of standard length was found at 10-15 mm. After that there was rapid growth in length and in the number of individuals. They were grown to 35 mm in standard length until September and then there was no change in the distribution pattern of the body length until next April. The standard length of mode of estimated normal distribution of each month was plotted in Fig. 8. This curve shows the average seasonal growth of *P. sinensis kaibarae* in the Chayang stream.

Based on the specimens collected during spring to summer months, the logarithms of body weight were plotted against the logarithms of standard length (Fig. 9). As is seen in the figure, one regression is applicable for the data. The regression equation is as follows: log BW = 2.954 log BL – 4.802 (r = 0.998), where BW is body weight in gram and BL is standard body length in millimeter.

Discussion

In the nest building behavior, it was known that two forms of gluing behavior occur: superficial and insertion gluing. *Pungitius pungitius* in Europe and *Culaea inconstans* in North America show both forms of the behavior. In these species, superficial gluing occurs only during the nest building phase and not subsequently, but insertion gluing appears towards the end of nest building phase and persists until a day or two after spawning (Hall, 1956; Morris, 1958; Wootton, 1976). However, it was already described that only superficial gluing behavior occured in *P. sinensis*

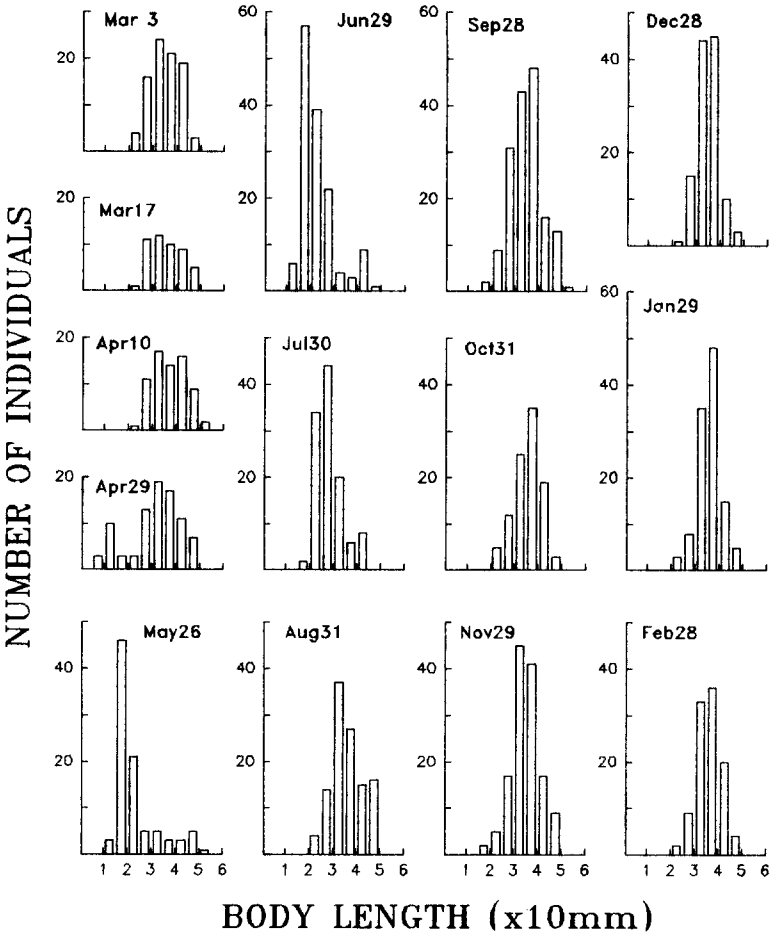

Fig. 7. Length frequency distribution of catches of *Pungitius sinensis kaibarae* in the Chayang Stream from March 1990 to February 1991.

kaibarae from Japan and *Gasterosteus aculeatus* in Europe (Kobayashi, 1933; van Iersel, 1953). In the present observation, *P. sinensis kaibarae* showed only superficial gluing behavior from nest building phase to the end of parental phase. The significance of such difference in gluing behavior among species or among populations is not yet known.

In late parental phase, *P. pungitius* in Europe shows two behaviors: the male retrieve any young that jump out of the nest and make a nursery behind the nest (Morris, 1958). We also observed the two behaviors in the present study but our fish made two nurseries. Males of *P. sinensis kaibarae* in Japan and of *P. pungitius* in Lake Huron do not build a nursery but straying young are returned to the nest (Kobayashi, 1933; McKenzie and Keenlyside, 1970). *P. pungitius* in Hokkaido of Japan neither make a nursery nor retrieve the young (Goto *et al.*, 1979). These differences in parental behavior among local populations of sticklebacks are very interesting in relation to the survival of the young but the reason is not yet known. In our observation, unlike other reports, the males made two nurseries. But it is a question whether or not this behavior may be occurred normally in the field because the observation was made on fish kept in an aquarium with artificial environment.

The fecundity was decided by the number of

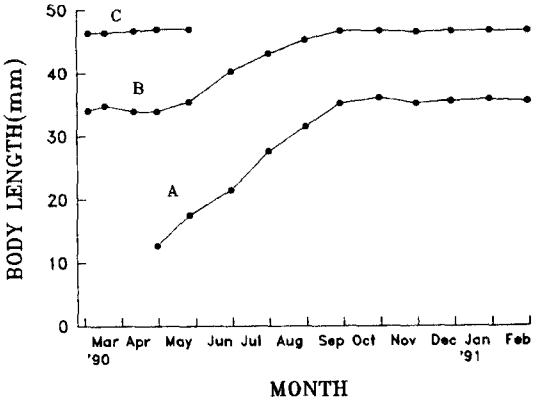

Fig. 8. Growth curve of *Pungitius sinensis kaibarae* in the Chayang Stream estimated from the successive change of length frequencies. Each curve represents the growth curve of 0-year old (A), 1-year old (B) and 2-year old fishes (C).

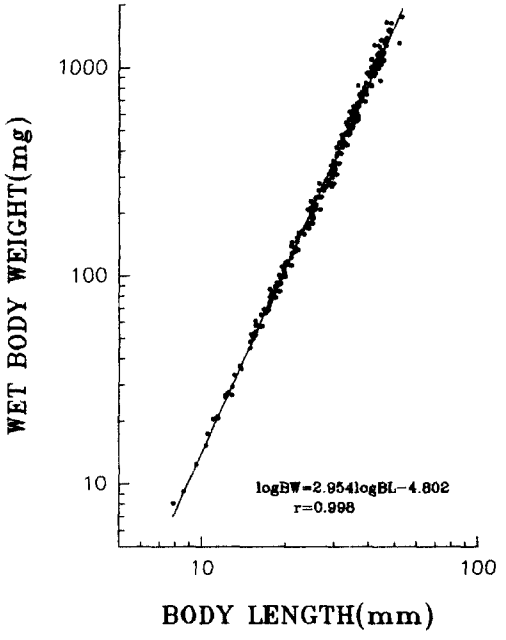

Fig. 9. Length-weight relationship of *Pungitius sinensis kaibarae* in the Chayang Stream.

matured eggs in the ovary. In the present observation, females of *P. sinensis kaibarae* had 21 to 110 eggs in their ovary and their standard body length was 26 to 53 mm. *P. pungitius* in Lake Superior had 61 to 112 eggs in their ovary and the total body length was 58 to 81 mm (Griswold and Smith, 1973). And *P. pungitius* in Hokkaido had 44 to 82 eggs in their ovary and their body length was 37 to 61 mm (Goto *et al.*, 1979). In the aspect of egg number, it seems that there are no significant difference among the above three cases. When compared the body length, however, the size of *P. sinensis kaibarae* is the smallest. It is known that the number of eggs in ovary increases according to increment of body size (Coad and Power, 1973; Griswold and Smith, 1973) and present result is not an exception. Based on this, therefore, *P. sinensis kaibarae* have more eggs for size than *P. Pungitius*. On the other hand, *P. sinensis kaibarae* in Japan had 58 to 186 eggs (mostly 70-150 eggs) (Kobayashi, 1933). In this case the body length of the females with mature eggs was not mentioned but there was some comments on the maturity and life span (1 year): larval fish – 30 mm, adult fish – 45 mm or more. This means that *P. sinensis kaibarae* in Japan starts to spawn after growing more larger than the fish of Korea. It is believed, therefore, that the fecundity of Japanese fish is not higher than Korean one. These facts must be considered in the aspect of the reproductive tactics related to the size of eggs.

We observed that the *P. sinensis kaibarae* in the Chayang Stream had life span for about two years at least. This population were consisted of three age-classes in the months of spring: the young of the year (0+), the fishes born in the previous year (1+) and a few fishes born before two years (2+). In relation to the life span of sticklebacks, some authors reported instances of three and more years (Jones and Hynes, 1950; Griswold and Smith, 1973) and the other reported instances of one or two years (Kobayashi, 1933; Goto *et al.*, 1979; Tanaka and Hoshino, 1979; Kawanabe and Mizuno, 1989). The cause of this discrepancy in the life span among populations has remained unknown but this is caused probably by the difference of the conditions of life like the environmental factors.

In our observation, the young (0+) hatched in late April and grew rapidly until late August. Then their growth was slowed down and finally ceased in mid-autumn. They began to grow again from

next June when the spawning season would be nearly closed. They (1+) grew rapidly until late September and then there was no more growth until death as 2+ year-old fish. The halt of growth in autumn and winter seasons was also appeared for *P. pungitius* in Hokkaido (Goto *et al.*, 1979). But in this case, unlike our result, the sticklebacks grew rapidly before spawning season. On the other hand, *P. sinensis* in Honshu of Japan grew slowly but continuously in autumn and winter (Tanaka and Hoshino, 1979). In the case of *P. sinensis* of Honshu the foods were supplied abundantly throughout the year but in our survey area the water vegetation almost disappeared and the food material was scarce in late autumn and winter season. Therefore such difference of growth pattern is seemed to relate to the abundance of food in the cold season.

P. sinensis kaibarae in the Chayang Stream did not grow in prespawning season as stated above and GSI value of male was abruptly increased in late October and November. The increase of GSI value of male in autumn was also recorded in other reports (Goto *et al.*, 1979; Tanaka and Hoshino, 1979) but the cause was not commented. It was stated that a fish spent most of the energy taken from the food to grow until maturing, but after maturing to produce eggs or sperms and to spawn (Nikol'skii, 1963). According to this statement, our result is interpreted as that the fish grow fully and mature physically until early autumn and then use all the energy for maturing sexually until next spring. This seems true in males at least. However, in relation to this respect some problems remain to be solved. Why the females do not grow in the cold season in spite of the absence of ovarian increase in size? What is the reason that the fluctuation pattern of GSI is different between both sexes, unlike the other fish species (Hayashi, 1971, 1972)?

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잔가시고기 ***Pungitius sinensis kaibarae*** (Tanaka) (큰가시고기과, 어강)의 산란과 성장

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잔가시고기, *Pungitius sinensis kaibarae* (Tanaka), 의 생활사를 밝히기 위한 연구의 일환으로 낙동강수계, 금호강의 한 지류인 자양천에서 1990년 3월부터 1991년 2월까지 산란과 성장을 조사하였다. 營巢, 산란 및 보호행동은 기존의 보고와 거의 유사하였다. 그러나 본 수역의 *P. sinensis kaibarae*는 특이하게 영소행동에서 superficial gluing behaviour만을 보여주었으며 보호행동에서는 두개의 육아장(nursery)을 만들었다. 완숙란을 지닌 개체는 2월말부터 6월말까지 발견되었다. 성숙한 암컷의 잉란수는 21-110개 (평균 47.07개)였으며 난소내 성숙란의 직경은 1.3-1.5 mm였다. 완숙란을 지닌 최소체장의 암컷은 25.8 mm였으며 21개의 알을 지니고 있었다. 난소내 완숙란의 수와 체장과의 관계는 No. of eggs = 2.857BL - 55.134 (r = 0.890)였다. 한 巢내에 산란된 알의 수는 27-637개였으며 발생단계가 다른 여러 개의 卵塊로 이루어져 있었다. 산란에 참여하는 연령을 보면 암컷에서는 주로 1년생 (1+ year-old)이, 그리고 수컷에서는 1년생과 2년생 (2+ year-old)이 다같이 참여하는 것으로 나타났다. 난소내 완숙란의 수와 GSI의 변동양상을 볼 때 산란시기는 2월에서 6월까지이며 산란성기는 3월초에서 4월말까지인 것으로 추정된다. 부화된 자어는 4월말부터 발견되었으며 이들의 체장은 10 mm정도였다. 부화자어는 8월말까지 빠르게 성장하였으며 그 후 성장이 둔화하여 10월말에는 완전히 중지되었다. 이때의 체장은 약 35 mm에 달하였다. 이러한 성장의 중지는 다음 해의 산란시기가 거의 끝날 무렵인 6월말경까지 지속되었다. 1년생 어류는 6월말부터 다시 성장하기 시작하여 9월말까지 빠르게 성장하였으며 약 45 mm의 체장에 이르렀다. 그 이후로는 2년생 (2+ year-old)으로서 죽을 때까지 더 이상 성장하지 않았다. 그러므로 본 수로에 서식하는 *P. sinensis kaibarae*의 수명은 약 2년인 것으로 여겨진다. 체장과 체중과의 관계는 logBW = 2.954logBL - 4.802 (r = 0.998)였다.