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Influence of Water Temperature on Spawning of Chinese Bleak, *Aphyocypris chinensis*

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Studies were conducted to determine the effects of water temperature on the number of spawning, egg production, spawning intervals and hatching success of Chinese bleak, *Aphyocypris chinensis*. Adult fish were exposed to water temperatures of 19, 22, 25 and 28°C for two months in the laboratory. The spawning number, egg productivity, and hatching rate increased as water temperature increased between 19°C and 25°C. Maximum egg production and hatching rate occurred at 25°C, whereas the spawning intervals decreased as water temperature increased except for the treatment of 28°C. The shortest spawning interval observed at 25°C.

Key words : Chinese bleak, *Aphyocypris chinensis*, Optimum water temperature, Spawning, Egg productivity

INTRODUCTION

The Chinese bleak, *Aphyocypris chinensis* (Cyprinidae), is common in small streams, agricultural waterways and ponds in Korea. The Chinese bleak has been selected as a possible test species among the 145 freshwater fishes of Korea because of some advantages of distribution, abundance, adult size and easiness of culturing (Yeom *et al*., 1999). Only two studies have dealt with the biological characteristics of *A. chinensis*: Park *et al*. (1998) describe the development of egg and larva and Yeom *et al*. (2000) describe the annual reproductive cycle.

In order to develop a new test organism, information on the reproductive conditions, such as water temperature and photoperiod, for a fish is needed to develop a culturing method in the laboratory. Most of the studies published on reproductive biology of a fish were concentrated on determining reproductive cycle, breeding season, seasonal change of fecundity, ovarian cycling, and length of intervals between spawning events (Taylor and Burr, 1977; Fernández-Delgado and Herrera, 1995; Gale and Buynak, 1982).

In this study, to determine the optimum water temperature of spawning for Chinese bleak, *A. chinensis*, we examined the effects of various water temperatures on the spawning, egg productivity, spawning interval, and hatching rate of Chinese bleak by inducing natural spawning in the laboratory for two months.

MATERIALS AND METHODS

Chinese bleak used in the experiment were obtained from the laboratory (Korea Research Institute of Chemical Technology, KRICT)-reared F_1 and F_2 males and females and were the same age groups (about 6 month old). Before the fish begun breeding, Identification of the sex of fish was nearly impossible using external morpholo-

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gical characteristics. For this study, the sex of the Chinese bleak was determined by visually abdomen. At the end of the experiment, sex of the fish was confirmed by observation of gonads. Chinese bleak discharged the eggs in the water regardless of spawning substrates. Since eggs sank to the bottom of the tank and weakly adhesive, all the spawning tanks had to be covered with a black and transparent acrylic plate substrates (25×16 cm) for easier collection of eggs.

Four groups of fish, each containing five pairs, were bred daily at various temperature ranges. One group was bred at 19°C. Other groups were bred at 22, 25, and 28°C, respectively. The temperature of experimental room was maintained at 19°C and water temperature was controlled by heating the filtered ground water. Each pair of fish was put in a separate spawning tank (25×32×35 H cm) and was observed through the two months study. Each spawning tank was aerated and filtered using tetra brillant filter (Tetra®, Germany). The fish were maintained on 15 : 9 h light : dark cycle. Fish were fed brine shrimp and tetramine flakes *ad libitum* 2 times daily.

During the experimental periods, the spawning tanks were checked daily between 9 : 00 and 10 : 30 a.m. Any eggs were removed from the acrylic plates using a soft brush, and then we counted the number of eggs. Then the substrates were placed back to the spawning tanks. The spawned eggs were placed in crystallizing dish (150×75 mm) and incubated at 23∼26°C. All dishes were examined for dead eggs and hatched larvae, and the water was aerated weakly using air stone during the incubation period. The spawning intervals were recorded as the number of days from the day of one spawning to the day of the next.

The tank with dead fish and same sex were removed from group of test water temperature and were not included in our data. Statistical analyses were performed using SYSTAT statistical software (SYSTAT, 1997).

RESULTS

During the experimental period, fish bred at 25°C showed maximum numbers in spawning. However, there were no significant differences (ANOVA, $p<0.05$) in the average numbers of spawning among test water temperature groups of 19, 22, 25 and 28°C. The number of spawning increased as the water temperature increased between 19°C and 25°C, although Chinese bleak showed big individual difference in spawning numbers at 19°C and 22°C. Further increase of the water temperature from 25°C to 28°C resulted in a decline in number of pair spawning (Table 1).

The number of eggs spawned each time varied considerably among fish of same replicates for all breeding water temperatures. While it is uncommon to have 1,182 fertilized eggs deposited at one spawning from a single female, some fish laid only less than 10 eggs at a time. In our study, batch fecundity, number of eggs per spawning, was estimated from naturally spawned eggs. The usual range of batch fecundity was between 110 and 160 eggs regardless of numbers of spawning and water temperatures (Table 1). To test for an effect of number of spawning, and water temperature on batch fecundity, Pearson correlation coefficients were calculated. Batch fecundity was not significantly associated with number of spawning act ($r=-0.442$, $df=14$) and water temperature of breeding ($r=-0.391$, $df=14$). When the egg productivity of Chinese bleak was estimated from naturally spawned eggs, the total yield of eggs laid per pair varied considerably according to water temperature. Based on the total number of eggs released per pair for 60 days, the egg productivity of fish bred at four different water temperatures followed the order: 25°C > 22°C > 19°C > 28°C (Table 1).

Although the outlier was present, frequency

Table 1. The influence of different water temperatures on spawning characteristics of the *Aphyocypris chinensis* kept under 15 hr light condition for 60 days.

Water temperature (±1°C)	No. of pairs tested	No. of spawning/pair		Batch fecundity		Total No. of eggs/pair	
		Mean	Range	Mean	Range	Mean	Range
19	4	5.4	2∼11	160	4∼665	932	508∼1,022
22	4	9.3	2∼20	135	2∼702	1,248	228∼2,990
25	3	14.3	12∼16	129	12∼1,182	1,803	1,262∼2,102
28	3	4.3	5∼7	110	2∼357	587	371∼810

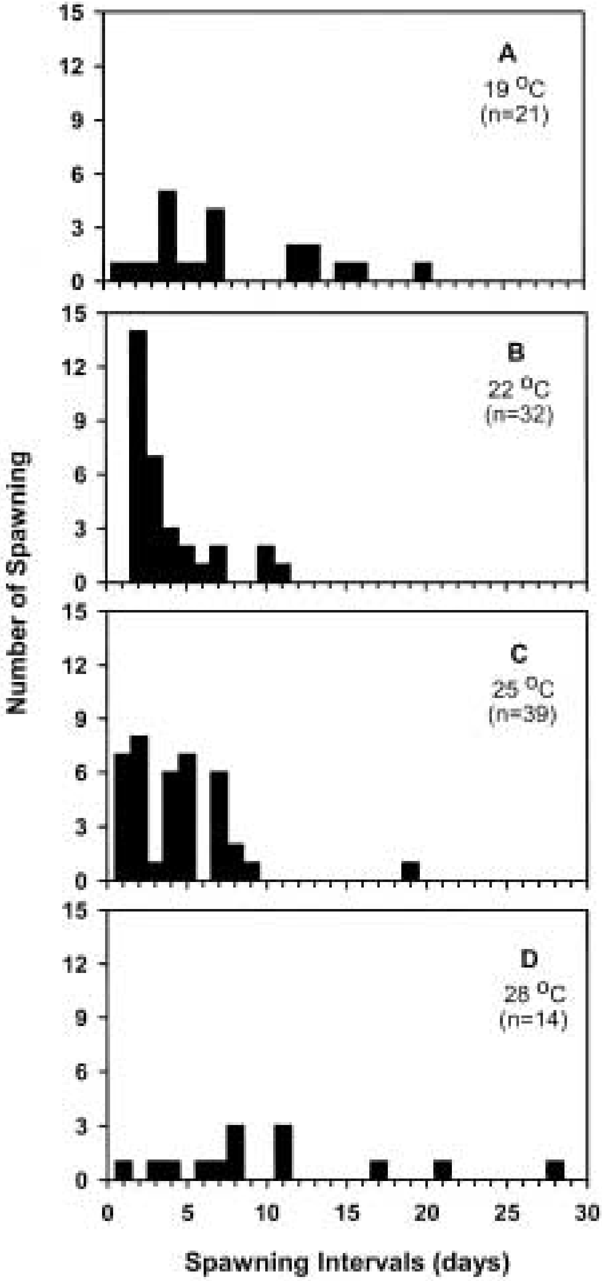

Fig. 1. Effects of various water temperatures on the spawning interval of Chinese bleak, *A. chinensis*.

distribution of spawning intervals showed a distinct difference in response of fish to various water temperatures. At 19°C, the frequency distribution of spawning intervals ranged predominantly between 4 and 7-day (mean: 8.3 day, Fig. 1A). The distribution of spawning intervals has a strong peak at 2~3 days at 22°C. The spawning intervals were mainly found between 2 and 7 day

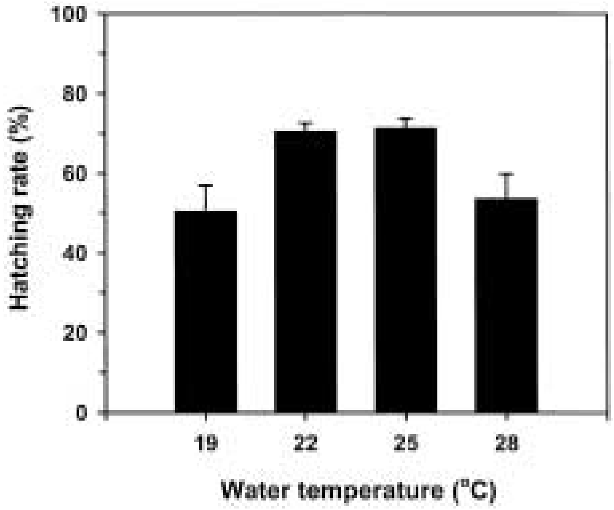

Fig. 2. Effects of various water temperatures on the hatching rate of eggs spawned under a 15 hr light: 9 hr dark photoperiod. Columns and bars indicate the means and standard errors.

and the spawning interval was far shorter than that obtained at 19°C (mean: 4.6 day, Fig. 1B). Spawning on consecutive days was observed 7 times of 39 spawning at 25°C. The frequency distribution of intervals was consisted predominantly 1, 2, 4, 5, and 7-day intervals, respectively (mean: 4.0 day, Fig. 1C). At 28°C, the frequency did not show any particular peak, and was evenly distributed ranging from 1 day to 42 day (mean: 9.7 day, Fig. 1D). The spawning intervals of the fish bred at 22°C and 25°C were far shorter than those fish bred at 19°C and 28°C.

Hatching usually occurred about two days after incubation of eggs at 23~26°C. 100% of the egg mortality occurred 5 times, but average 50% of the eggs succeeded in hatching when the fish were bred at 19°C. The hatching rate of fish bred at 22°C was 70.4% and fish bred at 25°C showed maximum hatching rate (71.2%). The average 47% of the eggs failed to hatch when the fish were bred at 28°C (Fig. 2).

DISCUSSION

Generally, the water temperature for spawning of fish has been determined by investigating monthly changes of goandosomatic index (GSI) and water temperature of sampling site (Taylor and Burr, 1997; Weddle and Burr, 1991; Heins and Baker, 1989). This method has a limitation in the determination of optimum water temperature for spawning of fish because ranges of optimal water temperatures vary highly. Our results also showed that the Chinese bleak, when kept between 19°C and 28°C, is capable of laying eggs. However, the results of this study demonstrate that the number of spawning, spawning interval, and hatching rate were very different depending on the water temperature of breeding.

The spawning number, egg productivity, and hatching rate increased as water temperature increased between 19°C and 25°C. Maximum egg production and hatching rate occurred at 25°C (Table 1, Fig. 2), whereas the spawning intervals decreased as water temperature increased except for the treatment of 28°C. The shortest spawning interval observed at 25°C (mean: 4.0 day, Fig. 1). Based on the results, we believe that the optimum water temperature for spawning is around 25°C for Chinese bleak.

Sexual development and spawning in cyprinids is modulated both by temperature and photoperiod although temperature is the predominant influence in most species (Potts and Wootton, 1984). In Chinese bleak, we found that the maturation of the gonad was initiated by the combination of water temperature of >19°C and photoperiod of >13 hr light condition. The highest spawning number, egg productivity, and egg hatching rates are observed under 15 hr light condition (unpublished data). In the zebrafish, *Brachydanio rerio*, it is also demonstrated that the length of the ovarian cycle varies with temperature, and the optimum water temperature for spawning is 25.3~25.7°C (Hisaoka and Firlit, 1962; Eaton and Farley, 1974).

If the Chinese bleak are bred in accordance with its precisely controlled reproductive conditions, viable eggs can be obtained in large numbers throughout the year. Thus, further studies are needed to assure the other reproductive conditions and ovarian cycle of the Chinese bleak.

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<국문적요>

왜몰개(*Aphyocypris chinensis*)의 산란에 미치는 수온의 영향

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왜몰개, *Aphyocypris chinensis*의 산란에 미치는 수온의 영향을 알아보기 위하여, 19, 22, 25, 28°C 수온에서 2개월 동안 사육을 하면서 산란횟수, 생산된 총 수정란 수, 산란간격 및 부화율 등을 조사하였다. 19°C와 25°C 사이에서는 수온이 증가하면 할수록 산란횟수, 생산된 총 수정란 수 및 수정란의 부화율이 증가하여 25°C에 노출된 암컷과 수컷에서 가장 많은 산란횟수, 난 생산 및 부화 성공률과 가장 짧은 산란간격을 나타냈다. 그러나 28°C에서는 25°C 보다 수온이 증가했음에도 오히려 4개의 실험수온 중에서 가장 적은 산란횟수, 난 생산 및 부화율과 가장 긴 산란간격을 나타냈다.