

The Effect of Temperature and Salinity on Maturation and Hatching of *Fibricola seoulensis* eggs

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INTRODUCTION

The eggs of *Fibricola* spp. are discharged in immature state of unembryonated germ cell with yolks from definitive host. After intraovular embryonation to mature miracidia in the water, the miracidia hatch out. This process of maturation or embryogenesis is dependent upon various physical or chemical factors, which may affect differently by the species of flukes. The effects of temperature on hatching of the eggs of *F. texensis* (Chandler, 1942), *F. cratera* (Hoffman, 1955) and *F. intermedium* (Pearson, 1961) were already known. In *Fasciola hepatica*, hatching of the eggs are known to be influenced by light and salinity (Wilson, 1968). As for the mechanism of egg hatching, there are 3 hypotheses; 1) presence of a hatching factor or a proteolytic enzyme, 2) osmotic pressure due to the entry of water, 3) mechanical activity of the miracidium (Xu and Dresden, 1986).

In case of *Fibricola seoulensis*, a favourable condition for maturation and hatching of the eggs was described as incubation of eggs at 26°C in distilled water (Seo *et al.*, 1986). The miracidia began to emerge on the 9th day, however, more detailed data were not known.

This study was undertaken to observe the effects of temperature, salinity and aeration on maturation and hatching of *F. seoulensis* eggs, which were essential factors to keep the life cycle in laboratory and to understand the life cycle in natural environment. In this context,

the eggs were laid under various temperatures and salinities, and with aeration. Development and/or hatching of the eggs under given conditions were observed by day after incubation.

MATERIALS AND METHODS

The metacercariae of *F. seoulensis* were obtained by peptic digestion of snakes, *Natrix tigrina lateralis*, and adult worms were collected from experimentally infected mice. Adult worms were incubated in Tyrode solution for 12 hours at 37°C. The eggs discharged by them were collected under a stereoscope and preserved at 4°C until used.

1. The effects of temperature and aeration

The eggs were divided into 5 groups, each consisted of more than 100 eggs; 1) the first group was incubated at 4°C, 2) the second at 5~15°C room temperature (RT I), 3) the third at 11~26°C room temperature (RT II), 4) the fifth at 28°C with aeration. The appearance of eyespots in the miracidia was used as an indication for maturation of the embryos in this study. Their appearance was examined daily using 40 eggs from the 2nd day of incubation to the 18th day except for group 3.

From the 5th day of incubation to the 18th day and on 30th day, 50 eggs in each group were exposed to sunlight for an hour at room temperature under a stereomicroscope to observe their hatching.

2. The effect of salinity

Each group of more than 100 eggs was dispensed in 6 petri dishes containing distilled water, 0.15%, 0.3%, 0.6%, 0.9% and 1.2% saline respectively. They were incubated at 28°C without aeration. From the 3rd day to the 14th day and on the 20th day after incubation, 40 eggs were examined every day for the appearance of eyespots. After exposure to sunlight for an hour at room temperature, hatching status was observed under a stereomicroscope.

3. The effect of light

More than 100 eggs were incubated in a test tube containing distilled water in dark at 28°C. On the 10th day, the rate of their hatching was observed every 10 minutes after exposure to sunlight at room temperature up to 2 hours.

RESULTS

1. The effect of temperature

The eggs incubated at 4°C and at 5~15°C (RT I) remained in immature state and showed no appearance of eyespots(Fig. 1). Also they did not hatch out throughout the observation period. However, more than 90% of the eggs

kept at RT II(11~26°C) showed the eyespots on the 8th day. Contrary to this, hatching rate was 2.5% on the 11th day and increased up to 35% on the 30th day. In the eggs which were incubated at 28°C, the eyespots began to appear on the 4th day(Fig. 2). Mature miracidia began to hatch on the 9th day(Figs. 3 & 4). The rates of eyespots appearance and of hatching were not influenced by aeration(Tables 1 & 2).

2. The effect of salinity

In 0~0.6% salines, the eyespots began to appear in the eggs on the 4th incubation day, and the rates increased up to 87.5~92.5% on the 7th day. In 0.9% saline, however, the rate of the eggs with eyespots was 2.5% on the 7th day and 55% on the 20th day. The eggs in 1.2% saline showed no appearance of eyespots throughout the period of observation (Table 3). The hatching rates in 0, 0.15% and 0.3% salines on the 14th day were in range of 90~92.5%. However, the rate in 0.6% saline was 47.5% on the 14th day and none of eggs in 0.9% and 1.2% salines hatched throughout the period of observation(Table 4).

3. The effect of light

Table 1. The rates of eyespots appearance in *F. seoulensis* eggs* by incubation day

Temperature	The rates(%) on day after incubation									
	3	4	5	6	7	8	9	10	11	18
4°C	0	0	0	0	0	0	0	0	0	0
Room temp. I (5~15°C)	0	0	0	0	0	0	0	0	0	0
28°C	0	76	96	96	94	**				
28°C with aeration	0	0	50	90	96	96	**			

* No. of eggs : 50

** Begin to hatch out

Table 2. The hatching rates of *F. seoulensis* eggs* by incubation day in each group of temperature

Temperature	Hatching rate (%) on day after incubation														
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	30
4°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Room temp. I (5~15°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—
Room temp. II (11~26°C)	0	0	0	0	0	0	2	4	2	6	4	2	0	4	34
28°C	0	0	0	0	74	92	94	86	92	96	88	92	86	88	94
28°C with aeration	0	0	0	0	62	76	92	92	88	90	92	90	86	97**	—

* No. of eggs : 50

** No. of eggs : 34

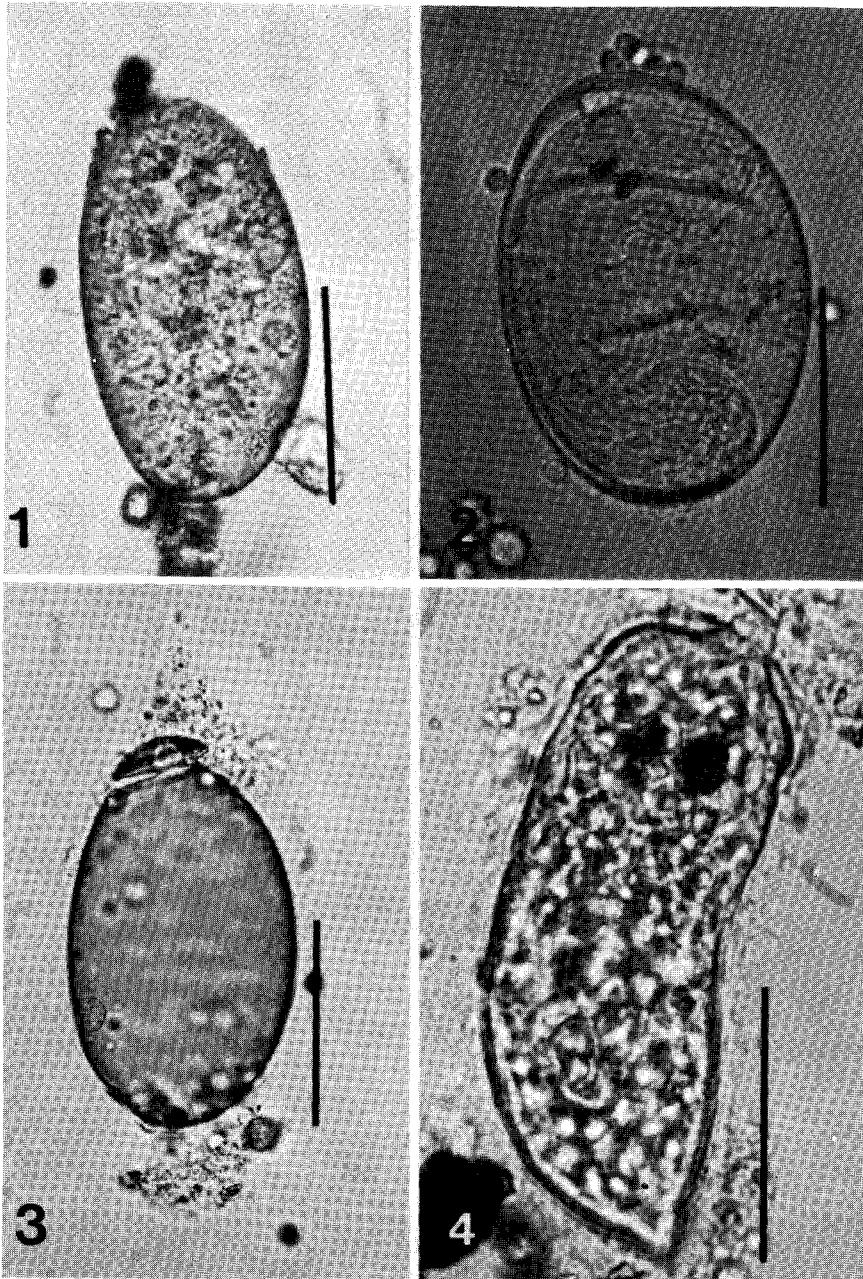


Fig. 1. An immature egg of *F. seoulensis*, bar=50 μm .
Fig. 2. A mature egg of *F. seoulensis* with a miracidium, bar=50 μm .
Fig. 3. An empty egg shell of *F. seoulensis*, bar=50 μm .
Fig. 4. A hatched miracidium of *F. seoulensis*, bar=50 μm .

On 10 days after incubation at 28°C in distilled water without light, 12.5% of the eggs hatched already. The hatching rate increased up to 75% by 120 minutes after exposure to light

(Table 5). A half of unhatched eggs were immature but another half contained the miracidia. The hatching rate of mature eggs was 85.7% at 120 minutes after exposure to light.

Table 3. The rates of eyespots appearance in *F. seoulensis* eggs* by incubation day in various salines

Salinity (%)	The rates(%) on days after incubation												
	3	4	5	6	7	8	9	10	11	12	13	14	20
0	0	40	45	87.5	95	—	—	—	—	—	—	—	—
0.15	0	7.5	70	95	92.5	—	—	—	—	—	—	—	—
0.3	0	12.5	60	92.5	87.5	—	—	—	—	—	—	—	—
0.6	0	32.5	35	82.5	90	—	—	—	—	—	—	—	—
0.9	0	0	0	0	2.5	2.5	0	2.5	0	65	42.5	70	55
1.2	0	0	0	0	0	0	0	0	0	0	0	0	0

* No. of eggs : 40

Table 4. The hatching rates of *F. seoulensis* eggs* by incubation day in various salines

Salinity (%)	Hatching rate (%) on days after incubation												
	3	4	5	6	7	8	9	10	11	12	13	14	20
0	0	0	0	0	0	80	95	87.5	95	95	92.5	90	95
0.15	0	0	0	0	0	90	92.5	95	95	95	97.5	92.5	97.5
0.3	0	0	0	0	0	60	70	77.5	77.5	85	95	92.5	95
0.6	0	0	0	0	0	15	10	15	12.5	12.5	32.5	42.5	47.5
0.9	0	0	0	0	0	0	0	0	0	0	0	0	0
1.2	0	0	0	0	0	0	0	0	0	0	0	2.5	0

* No. of eggs : 40

Table 5. Hatching rate of *F. seoulensis* eggs* after illumination

Minutes	Hatching rate (%)	Minutes	Hatching rate (%)
0	12.5	70	57.5
10	12.5	80	67.5
20	12.5	90	70.0
30	27.5	100	72.5
40	42.5	110	75
50	47.5	120	75
60	55.0		

* No. of eggs : 20

DISCUSSION

Results of the present study indicated that maturation and hatching of the eggs of *F. seoulensis* was influenced greatly by temperature, the optimal being about 28°C. In temperature under 15°C, none of the eggs matured. However, aeration showed no influence on maturation nor hatching.

The incubation period for hatching of *F. seoulensis* eggs was similar with that of other

Fibricola spp. The eggs of *F. texensis* or *F. cratera* hatched at room temperature after 9 days of incubation (Chandler, 1940; Hoffman, 1955). The eggs of *F. intermedium* were known to hatch after 10~12 days at 18.3~26.6°C (Pearson, 1961).

The temperature in range of 5~15°C is the air temperature in March to April and 11~26°C is the temperature from May to June in Korea. Also in May, aquatic snails and tadpoles, the first and the second intermediate hosts of *F. seoulensis*, appear in rice paddies. From same seasonal period, the miracidia of *F. seoulensis* begin to hatch out into water. Therefore the connection of life cycle might begin from May in a year. In summer, when average temperature is about 28°C, *F. seoulensis* eggs are expected to hatch on the 8-9th day after discharge in water. Considering the air temperature and harvest of rice, the connection of its life cycle can hardly occur after September in Korea.

Maturation and hatching of *F. seoulensis* eggs is also expected to be sensitive to salinity. In this study, maturation was not affected by

salinity under 0.6%. However, maturation was delayed or suppressed in 0.9% saline and did not occur in 1.2% saline. Upon hatching the salines under 0.3% showed little effect, the hatching rates over 90% after 2 weeks. However, hatching rate decreased to nearly half in 0.6% saline and to 0% in 0.9% saline. This result indicated that hatching was more sensitive to salinity than maturation was. The effect of salinity on egg hatching was also observed in *Schistosoma mansoni* by Xu and Dresden (1986). They revealed that salinity inhibited the activity of leucine aminopeptidase which was proved as a hatching factor. Whether the effect of salinity is due to solute itself or to dehydration by hyperosmolarity is not proved yet.

It was known that the light initiated hatching process of mature eggs although hatching could occur without light. This was also proved in the present experiment. Most of mature eggs hatched within 2 hours after exposure to light, but 12.5% of the eggs hatched already in the dark. The light is known as a strong external stimulus triggering the hatching mechanism of *F. hepatica* eggs (Wilson, 1968).

As for the hatching mechanism, there have been several experiments on a few digenetic trematodes (Xu and Dresden, 1986). The studies are summarized into 3 categories of hatching mechanism; enzyme as hatching factor, osmotic pressure and mechanical activity. However, they might play synergistically, not separately. Only one of them might be demonstrated of its major role by the species. The hatching mechanism of *F. seoulensis* eggs should be pursued in the future.

SUMMARY

This study was carried out to reveal the effect of temperature, salinity and aeration on maturation and hatching of *Fibricola seoulensis* eggs. The eggs were incubated and were observed daily for the appearance of eyespots and hatching.

The results were summarized as follows.

1. From 4~5 days after incubation in distilled water at 28°C or at 11~26°C, the eyespots began to appear and the rates of eggs with eyespots were over 90% in 28°C on the 7th or 8th day. However, eyespots did not appear in 5~15°C or 4°C by the 18th day.

2. The mature eggs began to hatch at the 8th day, and hatching rate 2 weeks after incubation was over 90% at 28°C, but it was below 5% at 11~26°C, and 0% at 5~15°C and at 4°C.

3. Aeration did not influence the appearance of eyespots nor hatching.

4. In salines under 0.6%, the rates of eyespots appearance were over 90% on the 7th day. The rate was 55.0% in 0.9% at 20 days, and 0% in 1.2%.

5. The hatching rates in salines below 0.3% concentration were over 90% by 14 days of incubation. However, the rate decreased to 44% in 0.6% saline and to 0% over 0.9% salinity.

6. The eggs incubated in the dark hatched in 12.5% on the 10th day, but hatching rate of mature eggs increased to 85.7% within 2 hours after exposure to light.

Above results demonstrated that the best temperature for maturation and hatching of *F. seoulensis* eggs was 28°C, and the miracidia began to hatch at 8~9 days after incubation. In the field, hatching and invasion into snails of the miracidia may occur from May to September in Korea. In salines under 0.3% concentration maturation and hatching were not influenced, but as salinity increased hatching was inhibited more than maturation was.

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==국문요약==

온도 및 염도가 *Fibricola seoulensis* 충란의 성숙과 탈각에 미치는 영향

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Fibricola seoulensis 충란의 성숙과 탈각에 대하여 미치는 온도, 염도, 공기주입 및 빛의 영향을 관찰하고자, 몇 가지 조건 하에서 충란을 배양하고 eyespots의 출현과 탈각 상태를 관찰하였다.

그 결과는 아래와 같다.

1. 증류수에 넣고 실온 II (11°~26°C)와 28°C에서 배양한 충란 중에서는 4~5일부터 eyespots이 관찰되어 7일 이후에는 90% 이상에서 관찰할 수 있었다. 그러나 4°C와 실온 I (5°~15°C)에서는 배양 18일까지도 eyespots이 관찰되지 않았다.
2. 충란의 탈각은 배양 8~9일에 일어나기 시작하여 배양 2주에 탈각율이 28°C에서는 90% 이상이었으나, 실온 II (11°~26°C)에서 5%, 실온 I (5°~15°C)과 4°C에서 0%이었다.
3. 28°C에서 공기를 주입하면서 배양한 충란의 eyespots출현율과 탈각율이 공기주입 없이 배양한 충란의 것과 유의한 차이를 보이지 않았다.
4. 염도 0.6% 이하에서는 7일 째에 90% 이상의 충란이 성숙하여 eyespots가 관찰되었으나, 0.9% 식염수에서는 20일에 55.0%에서 나타났고 1.2%에서는 전혀 관찰되지 않았다.
5. 증류수와 0.15% 식염수에서는 9일에 90% 이상의 충란이 탈각하였으나 0.3% 용액에서는 70%, 0.6% 용액에서는 10%에 지나지 않았고, 0.9% 이상 농도의 식염수에서는 전혀 탈각하지 않았다.
6. 빛을 차단한 채 10일간 28°C에서 배양한 충란의 경우 12.5%가 탈각하였고, 이들을 빛에 노출시키고 2시간 후에는 성숙한 충란의 85.7%가 탈각하였다.

이상의 결과와 우리나라의 외계 온도를 고려하면, 논에서 *F. seoulensis*의 생활사가 실제로 이어지는 시기는 5월부터 9월의 사이일 것으로 추정된다. 또한 염도가 0.3% 이하일 경우 별 지장을 받지 않으나 염도가 증가함에 따라 탈각과 성숙이 모두 억제되는데 특히 탈각이 더 예민하게 영향을 받았다.