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Influence of Temperature on the Egg Production and Hatching of *Microcotyle sebastis* (Monogenea : Microcotylidae), Parasitic on Rockfish, *Sebastes schlegeli*

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The influence of temperature on the rate of egg production and embryonic development of *Microcotyle sebastis* was investigated to determine the precise time of a second treatment. The survival time of the adults of *M. sebastis* was inversely proportional to temperature. The number of laid eggs per each replicate during the first 24 h was 39.3±4.0 at 10°C, 62.7±14.2 at 15°C, 101.0±5.6 at 20°C and 89.0±11.0 at 25 °C. The time required for egg hatching of *M. sebastis* was 31.30±4.88, 17.52±3.24, 11.59±3.02 and 10.76 ±3.10 days at 10, 15, 20 and 25°C, respectively. The regression models of the time required for the beginning and 50% point of hatching according to the different temperatures were as follows; Beginning of hatch: $D = 58.2000 - 4.2067 \times Temp + 0.0867 \times (Temp)^2$ ($P \leq 0.01$), 50% of hatch: $D = 91.3833 - 7.5767 \times Temp + 0.1767 \times (Temp)^2$ ($P \leq 0.01$).

Key words: *Microcotyle sebastis*, Rockfish, Egg production, Egg hatching, Temperature

Microcotyle sebastis Goto, 1894 is one of the most important ectoparasites in culturing rockfish, *Sebastes schlegeli*, in Korea. Recently, Kim and Choi (1998), Kim *et al.* (1998a) have reported that oral administrations of praziquantel, mebendazole and bithionol were very efficacious cures for *M. sebastis* infestation. However, treated rockfish can be reinfestated by *M. sebastis* oncomiracidia, which hatched from eggs layed before treatment. Therefore, a second treatment is essential to eradicate the new generation of *M. sebastis*.

In the present study, we investigated the influence of temperature on the rate of egg production and embryonic development of *M. sebastis* in order to determine the precise time of a second treatment.

Materials and Methods

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Survival and egg production of parasite

Living worms of *Microcotyle sebastis* were collected from the gills of infected rockfish cultured in an net-pen in Tongyoung, Korea. The 120 parasites were randomly divided into four different temperature groups (10, 15, 20 and 25°C) with 3 replicates, and were kept in 6-well plates (Corning) containing freshly filtered seawater. The life or death of worms was determined by observation of worm's response to the stimulus with a fine pincette. The number of layed eggs in each groups was counted under a stereomicroscope in 6 hour intervals.

Egg hatching

The worms removed from the infected rockfish were kept for 12 hours in a vessel containing freshly filtered seawater. The eggs layed by the worms in the vessel were collected using a pasteur pippet. The 120 eggs were randomly divided into four groups with

3 replicates, and incubated at different temperatures (10, 15, 20 and 25°C) with 16 h exposure to light and 8 h darkness. The eggs were kept in 6-well plates containing freshly filtered seawater and the seawater was changed daily. The hatching of eggs was examined under a stereomicroscope twice daily.

Statistics

Student's *t*-test was used for testing the differences in worm survival, egg production and hatching at different temperatures. The times of the beginning and 50% hatch of eggs at the experimental temperatures were regressed using SPSS statistical program.

Results

Worm survival

The survival time of the adults of *Microcotyle sebastis*, which were removed from the gills and kept in the seawater, was inversely proportional to temperature (Fig. 1). The mean survival time of worms at 10, 15 20 and 25°C was 9.93±1.68, 4.73 ±0.98, 3.31±1.25 and 1.17±0.23 days, respectively. Student's *t*-test showed that the mean survival time of worms was significantly different ($P \leq 0.01$) among each temperature groups.

Egg production

The parasites in each temperature group showed an active egg production during the first 6 h, and more than a half of the eggs were laid within 12 h in all experimental groups (Fig. 2). The number of laid eggs per each replicate containing 10 adult worms during the first 24 h was 39.3±4.0 at 10°C, 62.7± 14.2 at 15°C, 101.0±5.6 at 20°C and 89.0±11.0 at 25°C. At 20°C, the parasites laid significantly higher ($P \leq 0.05$) number of eggs than those at 10 and 15 °C. There were no statistically significant differences in egg production between 20 and 25°C group, but the parasites in 20°C group laid more eggs than those in 25°C group.

Egg hatching

The hatching time of eggs of *M. sebastis* was temperature- dependent, *i.e.* inversely proportional to temperature (Table 1). There were satistical differences ($P \leq 0.01$) in hatching times among different temperature groups except between 20 and 25°C group ($P \geq 0.05$). The success rates of egg hatching were higher at 15 and 20°C than at low (10°C) or high (25°C) temperature (Table 1).

The regression models of the time required for the beginning and 50% point of hatching according to the different temperatures (Fig. 3) were as follows;

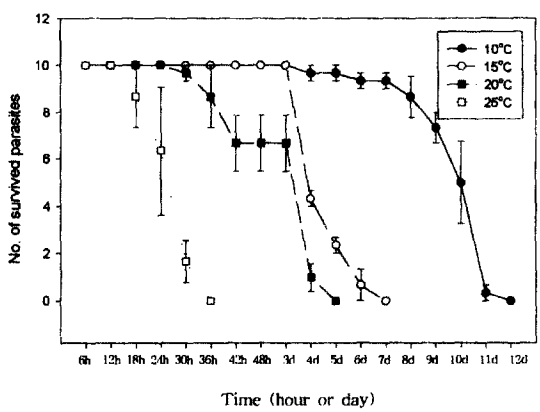

Fig. 1. The influence of temperature on the survival time of adult *M. sebastis*.

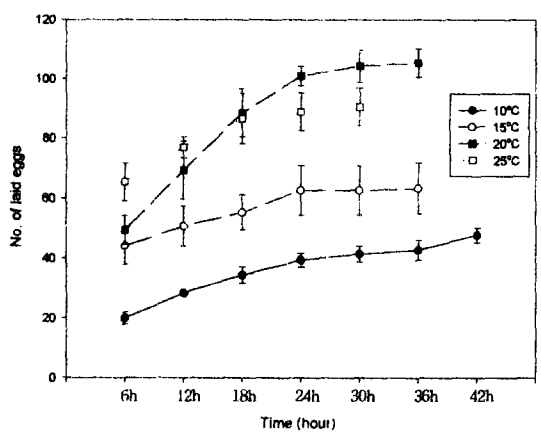

Fig. 2. The influence of temperature on egg production of *M. sebastis*.

Table 1. The influence of temperature on hatching of *M. sebastis* eggs

Temperature (°C)	Mean time (day) to hatch±S.D.	Range of hatching time (day)	Success rate of hatch± S.D.
10	31.30±4.88	17-39	76.67±11.55
15	17.52±3.24	15-31	90.00±10.00
20	11.59±3.02	8-19	90.00±10.00
25	10.76±3.10	6-16	70.00±10.00

Beginning of hatch

$D = 58.2000 - 4.2067 \times Temp + 0.0867 \times (Temp)^2$ $(P \leq 0.01)$

50% of hatch

$D = 91.3833 - 7.5767 \times Temp + 0.1767 \times (Temp)^2$ $(P \leq 0.01)$

where D is the number of days required for hatching and Temp is the water temperature (°C).

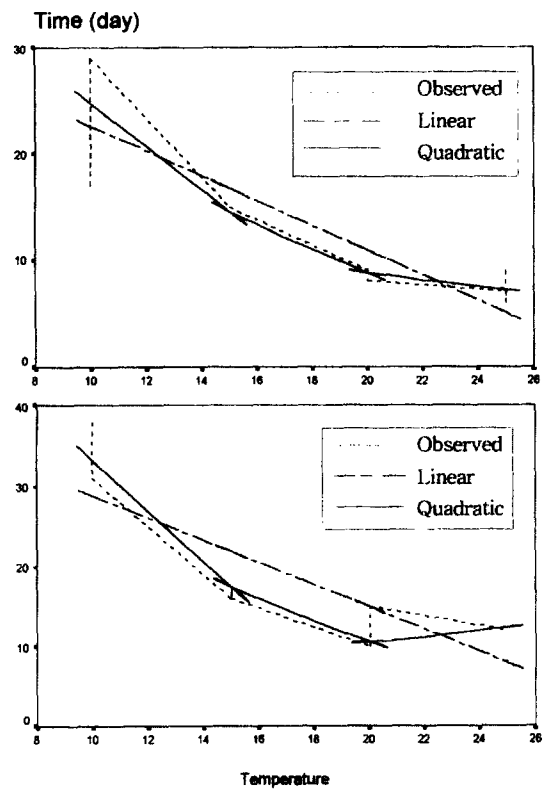

Fig. 3. The regression curves (linear and quadratic) of the time required for the beginning (A) and 50% point (B) of hatching according to different temperatures. Quadratic curve corresponds well to the observed curve.

Discussion

The results of the present study show that temperature affects significantly on the *in vitro* survival time of adults, egg production and hatching of *Microcotyle sebastis*.

In the present experiment, *in vitro* survival time of the adult worms was inversely proportional to temperature. It is well known that temperature affects the reproduction rate, growth rate and life span of monogeneans (Kamiso and Olson, 1986; Ogawa, 1988), and generally the growth rate of monogeneans has a positive correlation with the water temperature. Kim *et al.* (1998b), also, reported that the growth of *M. sebastis* was significantly affected by water temperature. From these facts, it can be conjectured that the metabolic rate of *M. sebastis* at high temperatures is faster than at low temperatures. Detached parasites, unable to renew their resources by feeding, therefore, might be expected to exhaust faster at higher temperatures than at lower temperatures. With regards to egg production, the experimental result show that adults of *M. sebastis* incubated at higher temperatures (20 and 25°C) laid more eggs significantly than those incubated at lower temperatures (10 and 15°C). The influence of temperature on egg output of dactylogyrid monogeneans had been investigated by many authors (Prost, 1963; Paperna, 1963; Molnar, 1971), and those results illustrate the tendency to produce more eggs as the temperature increases, however, at temperatures at the upper end of the range, egg output may fall. The present study, also, show that temperature has a profound influence on egg output of *M. sebastis*, and higher temperatures above 25°C would be inhibitory for egg production.

The time required for egg hatching of *M. sebastis* in the present experiment was 31.30±4.88, 17.52 ±3.24, 11.59±3.02 and 10.76±3.10 days at 10, 15, 20 and 25°C, respectively. Thoney (1986) report-

ed that the hatching time of *M. sebastis* at 12.2-17.0°C was 17.6±3.78 (12-31) days, which was similar to our result. The hatching time of *Bivagina tai* (26.2, 11.4 and 6.8 days at 10, 20 and 30°C, respectively, Ogawa, 1988) was similar to that of *M. sebastis*, also.

Generally, higher temperatures promote faster hatching of monogenean eggs, but higher temperatures also result in reduced hatching success (Kearn, 1986; Ogawa, 1988), and low temperatures are inhibitory (Llewellyn, 1957; Kearn, 1986). The result of the present study, also, show the lower success rates of egg hatching at the lowest (10°C) and the highest (25 °C) temperatures.

The regression model in the present study can be used to estimate the time interval between the first and the ensuing treatments for *M. sebastis* in different temperatures. This will make it possible to reduce unnecessary efforts and to elevate the efficacy of treatments.

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수온이 조피볼락에 기생하는 아가미흡충(*Microcotyle sebastis*)의 산란과 부화에 미치는 영향

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조피볼락에 기생하는 아가미흡충(*Microcotyle sebastis*)의 구제에 있어서 재투약시기를 정확히 산정하기 위해서 수온별 산란율 및 충란이 부화되는데 걸리는 시간, 부화율등을 조사하였다. *In vitro*상에서 성충의 생존시간은 온도가 증가함에 따라 감소하였다. 각 수온별 초기 24시간동안 성충 1마리당 산란한 충란의 수는 10℃에서 3.93±0.40개로 가장 적었으며, 15℃에서는 6.27±1.42개, 20℃에서는 10.10±0.56개로서 가장 많았다. 그러나 25℃에서는 8.90±1.10개를 산란하여 20℃에서 보다 적었으나, 유의차는 없었다. 각 수온별 충란이 부화되는데 걸린 시간은 10℃에서 31.30±4.88일, 15℃에서 17.52±3.24일, 20℃에서 11.59±3.02일, 25℃에서 10.76±3.10일로서 수온이 증가할수록 부화시간은 짧아지는 것으로 나타났으며, 충란의 부화성공률은 15℃와 20℃에서 모두 90% 이상을 나타낸 반면 10℃와 25℃에서는 70-77%의 낮은 부화율을 나타냈다. 수온에 따른 충의 초기 부화시점 및 50% 부화시점을 회귀분석하여 얻은 방정식은 다음과 같다. 초기 부화시점: $D = 58.2000 - 4.2067 \times Temp + 0.0867 \times (Temp)^2$, 50% 부화시점: $D = 91.3833 - 7.5767 \times Temp + 0.1767 \times (Temp)^2$.

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