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The viability of free-living glochidia of the freshwater pearl mussel (*Margaritifera laevis*) was studied in the laboratory at water temperatures of 10°C, 15°C and 20°C. To obtain glochidia, gravid female mussels were collected from the Chitose River, inhabited by adult and juvenile mussels, and from the Abira River, where only adult mussels were found. Daily survival rates of glochidia from each population at various water temperatures were significantly different, and survival time was longest at the lowest temperature in each population. Maintenance of some field mussel populations might become difficult at higher water temperatures due to the short survival time of glochidia and expected low density of host fish. Daily survival rates of glochidia were compared between the Abira population at 15°C and the Chitose population at 20°C, since these temperatures were close to the mean water temperature during the period of glochidial release in the respective rivers. Daily mean survival rates were significantly different between the Abira population at 15°C and the Chitose population at 20°C. Mean glochidial survival rate for the Chitose population changed from 85.3% to 66.2% from 9 to 13 h, whereas that for the Abira population dropped suddenly from 80.4% to 34.2% from 10 to 14 h after the initiation of experiment. Absence of juveniles in the Abira River might have been caused by the low glochidial viability. Survival times of free-living glochidia in *Margaritiferidae* tend to be shorter than in other families in Unionoida. A trade-off is suggested between high fertility and low glochidial survival rate in *Margaritiferidae*.

Key words: glochidium, *Margaritifera laevis*, survival, temperature effect, population

INTRODUCTION

Unionoida (Bivalvia) all live in freshwater, and their larvae, known as glochidia, are ectoparasitic on the gills and/or fins of fish (Wachtler et al., 2001) and the gills of amphibians (Watters and Scott, 1998), except for Mycetopodidae and Mutelidae, whose larvae are called lasidia and/or haustoria, with a different history from other Unionoida (Wachtler et al., 2001). *Margaritifera laevis* belongs to Unionoida and its maximum age reaches 79 years (Awakura, 1969). This species lives in cool water (<23°C) (Yoshida, 1971). The glochidial larvae drift in water after being released from a gravid female mussel (Kobayashi and Kondo, 2005), infect gills of suitable hosts such as masu salmon (*Oncorhynchus masou masou*), red-spot masu trout (*Oncorhynchus masou ishikawae*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) and rainbow trout (*Oncorhynchus mykiss*) for about a month (Awakura, 1964; Habe, 1982), and transform into free-living juveniles that drop and settle in sediment. Glochidia of *M. laevis* cannot parasitize the white-spotted char, *Salvelinus leucomaenis*, which serves as a host for a closely related mussel, *Margaritifera togakushiensis* (Kondo and Kobayashi, 2005).

*Margaritifera laevis* is listed in Endangered Species Category II by the Ministry of the Environment, Japan (Kondo, 2005). The major causes of its extinction are alterations of habitat (Kondo, 1995), such as damming that limits host-fish density (Awakura, 1969) and river improvement (Yoshida, 1973). The glochidial survival rate for *M. margaritifera* becomes lower with increasing rearing temperature (Jansen et al., 2001). A similar phenomenon probably occurs in *M. laevis*. Glochidia cannot survive without infecting host fishes, because they do not feed during the free-living glochidial stage (Jansen et al., 2001). Since the free-living glochidial stage is the most vulnerable in the life history (Bauer, 2001), the population dynamics of *Margaritiferidae* perhaps depend strongly on the survival rate at this stage. Therefore, a shift in water temperature over a long period may affect *Margaritiferidae* as greatly as human-induced disturbances.

Two margaritiferid species, *Margaritifera laevis* and
Margaritifera togakushiensis are distributed widely in rivers of Hokkaido, Japan (Kondo and Kobayashi, 2005). Margaritifera laevis was confirmed in the Chitose River (Kondo and Kobayashi, 2005). In our preliminary survey, we did not find S. leucomaenis in the Abira River. Furthermore, infection by glochidial larvae of mussels is only observed for O. masou masou (Akiyama, unpublished data). By observation of shell morphology, mussels in the Abira River were identified as M. laevis (Kondo, personal communication). These facts indicate that only M. laevis is distributed in the Chitose and Abira Rivers. Adult and juvenile mussels are found in the Chitose River, whereas in the Abira River, only adult mussels are found (Akiyama unpublished data). For purposes of conservation, a comparative study is needed to clarify the reason for the absence of juvenile mussels in the latter river. River water was slightly alkalinity in both rivers (pH=8.27±0.43 in the Abira River and 8.19±0.16 in the Chitose River, mean±SD) and slightly polluted in the Chitose River, according to the high conductivity (11.26±0.36) compared to that in the Abira River (4.26±0.22 mS m⁻¹).

In the present study, survival times of free-living glochidia of M. laevis were compared in the laboratory between the population in the Chitose River and that in the Abira River at three levels of water temperature.

MATERIALS AND METHODS

Gravid mussels were collected from the Abira and Chitose Rivers, central Hokkaido, Japan, on 5 August 2005 during their breeding period. In the field, the valves of each individual were opened slightly with a shell opener, and the soft parts of the muscles were checked by eye for the condition of the marsupium. Gravid mussels were collected from the Abira and Chitose Rivers in the containers were 4,015 and 838 individuals/ml, respectively. From each container containing 900 ml, 300 ml aliquots were poured into 525 ml containers. Three containers each for the Abira and Chitose Rivers were respectively kept in incubators at 10, 15 and 20 °C under constant aeration. The survival of glochidia was observed nearly daily from 1 ml of water sampled with a measuring pipette from each container. The water was dropped on a counting glass chamber, and live and dead individuals were counted under a binocular microscope one to three times for up to an 0.2 ml sample. Larval death and survival were judged by the presence or absence of damage in cells.

In the present experiment, the survival rate at each temperature was expressed as the ratio of the mean number of live glochidia at each observation time (L) to the mean number of live glochidia in triplicate containers at the start of the rearing experiment (L₀). The number of dead glochidia at each observation time (D) was obtained by D=L₀–L. The mean numbers of live and dead glochidia observed daily were compared by Pearson’s χ² test between the two populations at the same temperature, and within each population between rearing-water temperatures. In the former case, the Yates correction for continuity (Zar, 1999) was applied to the calculation of χ². Statistical analyses were performed using the software R-2.4.1 (R Development Core Team, 2006). A p-value less than 0.05 was considered to be statistically significant.

RESULTS

Glochidia of both the Abira and Chitose River populations survived for 1 day at 15°C and 20°C, but they survived much longer at 10°C. 11 days in the Abira population and 4 days in the Chitose population (Fig. 1). Survival time of glochidia was thus longest at the lowest temperature, 10°C. Daily survival rates differed with water temperature within both the Abira population (0–24h, χ²=1615.2, df=2, p<0.01; 24–48h, χ²=9071.4, df=2, p<0.01) and the Chitose population (0–24h, χ²=55.2, df=2, p<0.01; 24–48h, χ²=728.8, df=2, p<0.01), but were not always higher at lower temperatures. Mean water temperature during the breeding period was...
14.5±2.0°C (mean±SD) in the Abira River and 19.0±1.1°C in the Chitose River (Fig. 2). Based on these mean water temperatures, mean glochidial survival rates were compared between the Abira River at 15°C and the Chitose River at 20°C. Daily mean survival rates on the first day were significantly lower in the Abira population, but on the second day were significantly higher in the Abira population than in the Chitose population (0–24h, $\chi^2=140.1$, df=1, $p<0.01$; 24–48h, $\chi^2=31.9$, df=1, $p<0.01$). Mean glochidial survival rate for the Abira population at 15°C was 80.4% 10 h after the initiation of the experiment, whereas that for the Chitose population at 20°C was 85.3% 9 h after the initiation of the experiment (Fig. 1). During the 4 h following these times, the mean glochidial survival rate in the Chitose population changed to 66.2%, whereas that in the Abira population dropped sharply to 34.2% (Fig. 1).

**DISCUSSION**

In the present study, an increase in water temperature induced a decrease in the survival time of *M. laevis* glochidia. The density of *Oncorhynchus masou masou*, a host fish species for *M. laevis*, is also expected to decrease with increasing maximum summer water temperature (Inoue et al. 1997; Inoue and Nakano 2001). This suggests that an increase in water temperature may reduce the chance for glochidial infection of the host, which in turn causes a decrease in the density of the mussel population. Higher water temperatures are observed in narrow lowland rivers lacking vegetation cover above the water surface (Inoue et al. 1997). The life span of mussels in such warm habitats tends to be short (Bauer, 1992), and maintenance of their population may become difficult. Extinction of freshwater pearl mussel populations has been reported mainly for southern Japan (Matsuoka, 1979; Naito, 1989). Groundwater temperature is also higher in low-latitude and low-altitude areas (Nakano et al., 1996). Extinction of mussel populations and restriction of the mussel distribution to high altitudes in southern Japan might be related to water temperature.
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Wellmann G (1943) Fischinfektionen mit glochidien der *Margaritifera margaritifera*. Z Fischerei 41: 385–390


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