Effects of Water Temperature on Infection of the Microsporidian *Kabatana takedai* in Salmonids

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ABSTRACT—Effects of water temperature on infection of the microsporidian *Kabatana takedai* were investigated in salmonids under field and experimental conditions. “Cysts” of *K. takedai* appeared in the heart and the skeletal muscle of wild juvenile masu salmon *Oncorhynchus masou* from the Chitose River, Hokkaido, during summer in 2003 and 2004, when the river water temperature exceeded 15°C. Following the exposure of naïve juvenile sockeye salmon *Oncorhynchus nerka* to river water (18–19°C) for 3 days, the fish were transferred to separate tanks where the temperatures were set to 11, 13, 15 and 17°C. Prevalence of infection reached more than 70% in the 13, 15 and 17°C groups, but in the 11°C group, it remained less than 30%. However, shifting a part of the 11°C group to 18°C increased the prevalence to 71% by 23 days after the elevation of the temperature. When juveniles were exposed to 11°C river water and subsequently kept at 9°C for 42 days, no development of *K. takedai* occurred even after the temperature was elevated to 15°C. These results indicate that temperature manipulation is partially effective as a preventive method of *K. takedai* infection.

Key words: *Kabatana takedai*, microsporidian, salmonid, water temperature

Kabatana takedai, an intracellular microsporidian parasite, causes endemic disease of salmonids in the Chitose River, Lake Akan and Lake Tokito-numa, Hokkaido, in Japan (Takeda, 1933; Awakura *et al.*, 1966; Awakura, 1978) and the Taranay River and Bryanka River in Russia (Vyalova and Voronin, 1987; Vyalova, 1999). This disease is characterized by multiple cyst-like foci in the heart and the skeletal muscle of salmonid fishes. The parasite was first reported by Takeda (1933) from rainbow trout *Oncorhynchus mykiss* in the Chitose Hatchery, where growers used the Chitose River water for rearing. Since then, this disease has occurred in various species of salmonid fishes, e.g., masu salmon *Oncorhynchus masou* and sockeye salmon *Oncorhynchus nerka*, bred in the hatchery during summer when the temperature of river water exceeded 15°C (Awakura, 1974; Urawa, 1989). Currently, disease management is based solely on keeping a rearing

water in the hatchery under 13°C by the addition of spring water (8°C). However, Urawa (2001) found that “cysts” of *K. takedai* were formed at low temperatures even those less than 13°C. Thus, determination of the critical temperature for development in *K. takedai* is important to prevent this disease.

In the present study, we monitored seasonal changes of prevalence of *K. takedai* infection in wild masu salmon from the Chitose River to estimate the relationship between water temperature and parasite infection. Additionally, in order to examine the effects of water temperature on the infection of *K. takedai*, we designed several experiments, where exposed sockeye salmon were held at various temperatures.

Materials and Methods

Field investigation of K. takedai in juvenile wild masu salmon

Between June and November of 2003 and 2004, approximately 60 juvenile masu salmon (0+) were

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caught monthly from the Chitose River with a casting net. Mean fork lengths of fish collected in 2003 and 2004 were 6.7–10.1 cm and 7.4–8.4 cm, respectively. The river water temperature was recorded at 1 h interval throughout the period of the survey using a temperature data logger (T&D Corporation, Japan). Half of the fish was immediately killed upon collection and examined for *K. takedai* infection. To determine the “true” prevalence of infection, the other fish were reared for three weeks in a tank receiving specific-pathogen-free (SPF) well water at 15–18°C to promote the parasite development.

Parasitological examination

Sampled fish were killed with an overdose of tricainemethansulphonate (MS222, Sigma, U.S.A.). Examination of fish for *K. takedai* was done according to the method described by Fujiyama *et al.* (2002). To visualize the presence of “cysts” under a stereo-microscope, the heart was pressed between a slide glass and a cover slip. The skin over the trunk muscle was peeled off, and the muscle was then observed by stereo-microscopy and the number of “cysts” counted. Prevalence of infection was defined as the percentage of fish with “cysts” of *K. takedai* in the heart and/or trunk muscle among all fishes examined. Mean intensity of infection was expressed as the average number of “cysts” observed in the trunk muscle of infected fish.

Experimental design of effects of temperature control on infection of *K. takedai*

Fish: Naïve juvenile sockeye salmon (five-six months old) were provided from the Chitose Hatchery and Shizunai Hatchery, where no previous history of occurrence in *K. takedai* has been reported. Mean body weight of fish used for Trial 1 was 5.9–7.5 g at the end of the experiment.

Trial 1 (control of rearing water at four different temperatures): 507 fish were exposed to Chitose River water (18–19°C) for three days in August, 2001, and then separated into four tanks where the temperatures of SPF well water were set to 11, 13, 15 and 17°C. The number of fish kept in each tank was 100, except in the 11°C tank, which held 207 fish. Exposed fish were reared for 62 days, whereas the 11°C group was kept for additional 55 days. On 62 days post-exposure (PE), 31 fish of the 11°C group were shifted to 18°C, while the remaining fish were left at 11°C. Ten fish from each group were randomly sampled periodically and examined for *K. takedai* infection as described above.

Trial 2 (control of rearing water at a lower temperature, 9°C): One hundred fish were exposed to Chitose River water (19–20°C) for 8 days in August, 2004, and split into two tanks (50 fish in each tank) supplied with SPF well water of 9 or 15°C. The tank of fish at 15°C was used as a positive control group because fish are optimally susceptible to infection at this temperature. On

21 days PE, 20 fish from the 9°C tank were transferred to another tank at 15°C for 21 days to determine if the parasite was killed at low temperature or if development was simply retarded.

Trial 3 (control of river water and rearing water at low temperatures): Fish were exposed to temperature-regulated water from the Chitose River for 14 days in August, 2004. The river water was adjusted at $10.8 \pm 0.6^\circ\text{C}$ by adding well water (8°C) combined with using a water chiller. Fish were then held for 84 days in three tanks (300 fish in each tank) with well water at 9, 11 and 13°C, respectively. Forty-two days PE, 40 fish from the 9°C group were shifted to 15°C and held for additional 42 days.

Results

Seasonal changes of *K. takedai* infection in juvenile wild masu salmon

“Cysts” of *K. takedai* were first observed in late July, 2003, when water temperature was about 14°C (Fig. 1). Prevalence of infection increased during the summer of 2003 and peaked at 92.9% in late September. In late October, the prevalence declined, when water temperatures fell below 13°C. Prevalence of infection

Fig. 1. Seasonal changes of the prevalence of *Kabatana takedai* in juvenile wild masu salmon *Oncorhynchus masou* collected from the Chitose River in 2003 (the upper graph) and 2004 (the lower graph). Open and closed circles show the prevalence of infection immediately upon the collection and after the subsequent rearing at 15–18°C for three weeks, respectively. Subsequent rearing was not done for fish sampled in June and October 2003 and July 2004, due to insufficient number of collected fish.

in fish after subsequent rearing at 18°C for three weeks was higher than those of the parallel groups examined just after the collection, with the one exception of the late September sample. Mean intensity of infection reached the maximum (18.0 ± 14.0) in late July and then decreased gradually (data not shown).

Field investigations in 2004 revealed that overall prevalence was relatively lower than that in the same period of 2003. The seasonality in prevalence appeared to be similar to that in 2003, but the prevalence declined earlier than that in 2003. The seasonal pattern in water temperature was considerably different from that of 2003. Water temperature exceeded 15°C

in mid-July and reached more than 22°C in early August. Mean intensity of infection peaked at 16.7 ± 17.6 in mid-August (data not shown).

Effects of experimentally regulated temperature on *K. takedai* infection

Trial 1: Development of *K. takedai* occurred more rapidly at warmer temperatures (15 and 17°C) than at the lowest temperature tested (11°C) (Fig. 2). Prevalence reached more than 70% in the 13, 15 and 17°C groups, but in the 11°C group, it remained less than 30% even on 62 days PE. Mean intensity of infection in the trunk muscle of the 13, 15 and 17°C groups varied at around 10 per fish, while it was nearly two or three in the 11°C group until 62 days PE (Fig. 2). However, fish of the parallel group, which were shifted from 11 to 18°C, showed a prevalence of 71% by 23 days after transfer to the higher temperature (Fig. 3).

Trial 2: Prevalence of infection at 15°C reached 83% at 35 days PE, while no "cyst" formation occurred at 9°C by 35 days PE (Table 1). However, shifting a portion of the 9°C group to 15°C increased the prevalence to 40%

Fig. 2. Prevalence (closed circles) and mean intensity (open squares) of infection with *Kabatana takedai* in juvenile sockeye salmon *Oncorhynchus nerka* held at four different temperatures. Bars show the standard deviations.

Fig. 3. Prevalence (closed circles) and mean intensity (open squares) of infection with *Kabatana takedai* in juvenile sockeye salmon *Oncorhynchus nerka* kept at 11°C for 117 days (continued from the top graph of Fig. 2). Bars represent the standard deviations. Arrow shows the time point when 31 fish were transferred to 18°C, resulting in an increase of the prevalence (indicated by the broken line).

Table 1. Prevalence of infection with *Kabatana takedai* in juvenile sockeye salmon *Oncorhynchus nerka* reared at 9 or 15°C after exposure to ambient river water of 18–19°C. Twenty fish from the 9°C group was shifted to 15°C at 21 days post-exposure. Numbers in parentheses show the mean intensity of infection.

	Day 0	Day 21	Day 35	Day 42
15°C	0	55% (3.6)	83% (3.3)	nd*
9°C	0	0	0	nd
From 9 to 15°C at Day 21			nd	40%

* nd: not determined.

Table 2. Prevalence of infection with *Kabata takedai* in juvenile sockeye salmon *Oncorhynchus nerka* reared at low temperatures after exposure to river water controlled at 11°C. Part of the 9°C group was moved to 15°C at 42 days post-exposure. Numbers in parentheses show the mean intensity of infection.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
13°C	0	0	0	0	0	20% (10.0)	20% (12.8)
11°C	0	0	0	0	0	0	nd*
9°C	0	0	0	0	0	0	nd
From 9 to 15°C at Day 42					0	0	nd

* nd: not determined.

by 21 days after the elevation of the temperature.

Trial 3: The parasite slowly developed over time at 13°C, resulting in as low as 20% prevalence at 70 and 84 days PE (Table 2). No “cysts” were observed in fish constantly kept at 9 and 11°C by 70 days PE. Even after the temperature was elevated from 9 to 15°C, no infection appeared by 70 days PE.

Discussion

It is widely documented that water temperature is a primary factor influencing the development of fish microsporidians (Olson, 1981; Antonio and Hedrick, 1995; Kim *et al.*, 1997; Beaman *et al.*, 1999; Speare *et al.*, 1999). The previous studies also described that *K. takedai* is dependent on water temperature above 15°C for development (Awakura, 1974; Urawa, 1989). A seasonal trend with a peak in summer was observed in the field investigation of the present study, supporting the hypothesis of temperature-dependency of *K. takedai* development, however, a time lag between the parasite's invasion and “cyst” formation should be considered. For example, in the 2003 survey, *K. takedai* “cysts” were first noticed on late July. Nevertheless, it is likely that these fish might have been infected by *K. takedai* at least three weeks prior to sampling, because “cyst” formation of *K. takedai* requires about three weeks at 13–18°C. As water temperatures in early July were about 13°C, it is probable that the “infective stage” of *K. takedai* emerged and penetrated into the fish host at 13°C.

The decreases in prevalence of infection during the autumn might be caused by degeneration of “cysts”. Similar declines also occurred in fish kept at a high temperature after collection from the Chitose River. Urawa (2001) found that the mean intensity of infection with *K. takedai* reduced during the 7 month-rearing of infected fish, probably due to host's immune response, such as macrophage phagocytosis. Although “cyst” dissolution might not affect the prevalence of infection while a new invasion occurs, the prevalence would probably decrease during the later period because of the termination of *K. takedai* invasion. This suggests that the water temperature at the end of invasion period of *K. takedai* is

nearly 13°C. Although an upper limit of water temperature for the invasion of *K. takedai* is unknown, the early decline of prevalence in 2004 might have been a consequence of the unusually high water temperature. In the case of *Loma salmonae*, which causes xenoma formation in the gills of salmonids, xenoma degeneration after development was accelerated at higher temperatures (Becker and Speare, 2004).

The present study demonstrates that the optimal temperature range for the development of *K. takedai* is between 13 and 17°C, although the rate of “cyst” formation was somewhat influenced by water temperature. Awakura (1974) reported that “cyst” formation in salmonids exposed to river water controlled at an average of 11.4°C was delayed by one month, by comparison to those fish exposed to ambient river water (15.3–19.8°C). Beaman *et al.* (1999) suggested that water temperature had no significant effect on the prevalence and intensity of infection with *L. salmonae* within the permissive temperature range, but there was a high correlation between temperature and the time required for xenoma formation in the gills.

Results of temperature shifting in the present study show that *K. takedai* suspended development at temperatures below 11°C. Arrested development of fish microsporidians in low water temperature has been also documented in other species. Olson (1981) reported that *Glugea stephani* can remain dormant within infected English sole *Parophrys vetulus* for 42 days at 10°C and recommence the development once temperatures rise above 19–20°C. Antonio and Hedrick (1995) showed that chinook salmon *Oncorhynchus tshawytscha* infected with *Nucleospora salmonis* (= *Enterocytozoon salmonis*) maintained a low degree of infection at 9°C, while a temperature shift to 15°C at 12 weeks PE resulted in severe infections with higher mortalities. In contrast, temperatures below 9°C interrupted the development of *L. salmonae*, and parasite DNA was no longer detected by 4 weeks PE (Beaman *et al.*, 1999; Sanchez *et al.*, 2000). In the present study, it should be noted that the overall prevalence of infection in Trial 3, where the fish were exposed to river water controlled at 11°C, was relatively low, and that no development of *K. takedai* occurred in the fish reared at 9°C even after they were

moved to 15°C. It is possible that addition of well water caused the dilution of the infective stage in river water, resulting in the decrease of the prevalence of infection. These results also suggest that low temperature adversely affects the initial stages of *K. takedai* following penetration of parasite. It is presumed that the spore germination and/or the delivery of sporoplasm into host cells might be blocked or interrupted by low temperatures.

The resumption of *K. takedai* development after temperature increment is of practical concern, because releases of salmonids carrying latent infection would result in the spread of the parasite to non-endemic regions. However, considering that hatchery-based strategies of temperature manipulation have to be designed in relation to the fish growth and susceptibility to other infectious diseases too, a temperature of 9°C might be too low to conduct in hatcheries. Until now, the life cycle and the mode of transmission of *K. takedai* have remained unknown, partially because infection experiments using *K. takedai* spores to naïve fish failed (see Fujiyama *et al.*, 2002). Thus, involvement of an intermediate host and the existence of unknown infectious stage has been suggested (Awakura, 1974; Fujiyama *et al.*, 2002). To develop more effective prevention methods as an alternative to temperature regulation, clarification of the life cycle of *K. takedai* is required.

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