Comparison of Susceptibility to *Kabatana takedai* (Microspora) among Salmonid Fishes

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ABSTRACT—*Kabatana takedai* (Microspora) is known as an enzootic pathogen of salmonids in limited water systems such as the Chitose River in Hokkaido, northern Japan. In this study, susceptibility to *K. takedai* was compared among masu salmon *Oncorhynchus masou* originating from the Chitose River, Shiribetsu River and Shari River, and rainbow trout *O. mykiss* from Shizuoka Prefecture, Japan. After exposure of fish to the Chitose River water, cysts of *K. takedai* in the heart and trunk muscle were examined by microscopy. Prevalence and intensity of infection were not considerably different among the three populations of masu salmon, whereas rainbow trout was more susceptible to *K. takedai* than masu salmon, particularly in the heart. A newly developed PCR test showed the higher percentage of fish with pre-cyst stage of *K. takedai* in the heart of rainbow trout than in masu salmon, suggesting that *K. takedai* established the cardiac infection at early stages more in rainbow trout than in masu salmon. A histopathological observation indicated that *K. takedai* infection in the heart caused granulomatous inflammation characterized by fibrinoid degeneration, but there was no difference in the progress of the inflammation between the two fish species.

Key words: *Kabatana takedai*, microsporidian, susceptibility, *Oncorhynchus masou*, *Oncorhynchus mykiss*, salmonid, masu salmon, rainbow trout

*Kabatana takedai* (Microspora) is known as an enzootic pathogen infecting various salmonid fishes. It has been reported from the limited different water systems including the Chitose River, Lake Akan and Lake Tokito-numa in Hokkaido, northern Japan (Takeda, 1933; Awakura et al., 1966; Awakura, 1978) and the Taranay River and Bryanka River in Sakhalin, Far Eastern Russia (Vyalova, 1999). The diseased fish exhibits multiple cyst-like foci (hereinafter referred to as cysts) in the heart and the trunk muscle, and the heavy infection leads to host death (Awakura, 1965; Urawa, 1989). The Chitose Hatchery, which is located at the upstream of the Chitose River, has raised various salmon stocks from different geographical origins as well as the Chitose River stock, followed by releasing them into their native rivers. In 1999, juvenile masu salmon *Oncorhynchus masou* originating from the Shiribetsu River and Shizunai River were reared at the Chitose Hatchery, resulting in heavy infection of *K. takedai*. Eventually, approximately 100,000 fish were slaughtered to prevent the parasite spreading (Urawa, 2001). This incident has created a renewed interest in the potential effects of the parasite on hatchery-reared salmonids.

Until now, three populations of masu salmon derived from the Chitose, Shiribetsu and Shizunai rivers were found to be infected with *K. takedai* (Urawa, 1989, 2001), but it remains to be investigated whether the susceptibility variation exists between them. If *K. takedai* spreads into non-endemic regions, severe negative impacts on naïve salmonids are of practical concern. It seems that exotic or newly introduced fish species (e.g., rainbow trout *O. mykiss*) is highly susceptible to enzootic pathogens than native fish (Egusa, 1979; Hedrick et al., 2003). Nevertheless, Awakura (1974) reported that captive masu salmon was more susceptible to *K. takedai* than rainbow trout. In contrast, Urawa (1989) found that the prevalence of infection with *K. takedai* was...
higher in feral rainbow trout than in masu salmon. This inconsistency may be attributed to the observation of uncontrolled natural infection under wild environment. However, experimental infection using *K. takedai* spores to naïve fish was not successful (Fujiyama et al., 2002). The present study was designed to carry out the risk assessment of possible spread of the parasite to other areas by comparing the susceptibility of several populations of salmons to *K. takedai* in controlled exposure to the Chitose River water.

**Materials and Methods**

**Experimental fish**

Juvenile masu salmon originating from the Shiribetsu River (Shiribetsu population) and eyed eggs of masu salmon derived from the Chitose River (Chitose population) were received from the Chitose Hatchery. Eyed eggs of masu salmon originating from the Shari River (Shari population) were provided from the Shari Hatchery. Juvenile rainbow trout were purchased from Fuji Trout Hatchery and Fuji Trout Federation of Fisheries Cooperative Associations, Shizuoka Prefecture, in 2005 and 2006, respectively. All eggs and fish were reared in tanks supplied with specific-pathogen-free (SPF) well water at the National Salmon Resources Center, Sapporo, until the beginning of the experiment. Prior to the trial in 2005, thirty fish from each group were screened for the presence of cysts in the heart and trunk muscle using a stereo-microscope. To verify the SPF status of experimental fish in 2006, ten fish from each group were examined by both stereo-microscopy and PCR test described below. Mean fork length (± S.D.) of Chitose, Shiribetsu and Shari populations of masu salmon and rainbow trout used in 2005 were 5.73 (± 0.7) cm, 6.04 (± 1.0) cm, 5.81 (± 0.8) cm and 4.18 (± 0.4) cm, respectively. Mean fork length (± S.D.) of Shiribetsu masu salmon and rainbow trout used in 2006 were 5.93 (± 1.2) cm and 5.02 (± 0.3) cm, respectively. All fish were transferred to the Chitose Hatchery, where they were held for the following experiments in 2005 and 2006.

**Experimental design in 2005 (comparison among three populations of masu salmon and rainbow trout)**

From July 26 to September 6, 2005, Chitose, Shiribetsu and Shari populations of masu salmon and rainbow trout (n = 400 in each tank) were placed in four flow-through rectangular tank (180 cm long, 33 cm wide and 21 cm high). Incoming water was provided equally into each fish tank at 20 L/min from an 80-L water tank supplied with the Chitose River water. During the summer period, the water tank was cooled by addition of SPF well water, because too high temperature affects fish survival and parasite infection. Throughout the experiment, water temperature was maintained at 14.2 to 19.6°C (mean 17.2°C). Approximately 30 fish were randomly sampled at 2 and 4 weeks post-exposure (PE) and examined for presence of *K. takedai* cysts. At the termination of the trial (6 weeks PE), all surviving fish were sacrificed and examined for *K. takedai*. Sampled fish were killed with an overdose of tricainemethan sulphonate (MS222, Sigma, U.S.A.) and frozen at −20°C. After thawing of the samples, the number of cysts in the heart (ventricle) and the trunk muscle was counted according to the method described by Zenke et al. (2005). Briefly, heart was placed in a wet mount and compressed by applying pressure using a cover slip. Skin over the both sides of trunk muscle was peeled off using a forceps. These samples were examined for cysts of *K. takedai* using a stereo-microscope. If required, a compound microscope at a magnification of 400 was used to ensure the presence of *K. takedai* spores. Prevalence of infection was defined as the percentage of fish with cysts in the heart and/or trunk muscle. Intensity of infection was expressed as the number of cysts observed in the heart or trunk muscle.

**Experimental design in 2006 (comparison between masu salmon and rainbow trout in duplicate)**

From August 1 to September 26, 2006, duplicate groups of Shiribetsu masu salmon and rainbow trout were kept in four separate flow-through tanks (n = 400 in each tank) supplied with the Chitose River water. The rearing tanks were set up as the same to the previous experiment. Water temperature was controlled as described in the previous experiment, ranging from 14.0 to 18.1°C (mean 16.2°C). Fish from each tank were randomly sampled at 2, 3, 4, 6 and 8 weeks PE. At the two earlier sampling periods, 30 fish were collected, while approximately 60 fish were removed in the subsequent samplings. The fish were examined for presence of *K. takedai* cysts in the same way as the previous experiment. At the four latter sampling periods (3, 4, 6, and 8 weeks PE), microscopy-negative hearts in 30 fish out of 60 sampled fish were further investigated by PCR described below. At the end of the experiment (8 weeks PE), 50 fish of each group were fixed in 10 % phosphate buffered formalin for histology.

**PCR assay**

The hearts were homogenized in TNES-urea buffer (Asahida et al., 1996), followed by vortexing for 2 min with 0.4 g of 400–600 μm glass beads (Sigma-Aldrich Corporation, U.S.A.) and digested with proteinase K overnight at 37°C. DNA was extracted by phenol/chloroform/isoamyl alcohol twice and by diethyl ether. DNA was resuspended in sterilized H2O following to the ethanol precipitation, and DNA concentration was adjusted to 100 ng/μL. The primers Kt-fwd1, Kt-fwd2, Kt-rev2 and Kt-rev1-2 (Table 1) were designed from the SSU rDNA sequence of *K. takedai* (GenBank accession no. 150.
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AF356222). In the first PCR, each PCR mixture contained 3.7 μL Taq solution (Takara) [mix containing TaKaRa Ex TaqTM HS (5 U/μL), 10 × Ex Taq Buffer (including 20 mM Mg²⁺) and dNTP Mixture (2.5 mM each) in the ratio 1:20:16], 1.0 μL of primers Kt-fwd1 and Kt-rev2 (20 pmol/μL), 1.0 μL of DNA sample and sterilized H₂O to a final volume of 20 μL. The cycling conditions were 35 cycles of denaturation (95°C for 30 s), annealing (52°C for 30 s) and extension (72°C for 60 s), which were preceded by an initial 4 min denaturation step at 95°C and followed by a final 10 min extension at 72°C. In the second PCR, each PCR mixture contained 3.7 μL of Taq solution, 0.6 μL of the primers Kt-fwd2 and Kt-rev1-2 (20 pmol/μL), 1.0 μL of the product from the first amplification, and sterilized H₂O to a final volume of 20 μL. The cycling conditions were 30 cycles of denaturation (95°C for 10 s), annealing (62°C for 30 s) and extension (72°C for 15 s), which were preceded by an initial 4 min denaturation step at 95°C and followed by a final 10 min extension at 72°C. Taq polymerase was kept on ice until the preheating steps. PCR products were electrophoresed on an agarose gel with ethidium bromide and visualized under UV transillumination. This PCR method was able to detect a DNA fragment of 231 bp, which has 100 % homology to the sequence of the SSU rDNA of *K. takedai* (data not shown).

Detection thresholds of the PCR test was estimated as 10 spores of DNA / 20 μL-PCR tube (data not shown). Non-specific amplifications were not observed for other four microsporidians (*Heterosporis anguillarum, Glugea plecoglossi, Microsporidium seriolae, Microsporidium sp.* from red sea bream *Pagrus major*) and the heart of non-infected masu salmon.

**Histopathological observation**

The heart (ventricle) was removed from formalin-fixed fish, dehydrated in ethanol series, cleared and embedded in paraffin wax. Four-μm sections were stained with Azan stain or Mayer's hematoxyline and eosin (H & E) following to Uvitex 2B (Yokoyama et al., 1996). Sections were observed by light and fluorescent microscopy. The grade of severity of lesions associated with *K. takedai* infection was determined separately in each focus of fish using the following criteria:

- **Stage A** (minimal): A mass of parasite contains mostly immature stages. No host response associated with *K. takedai* is observed.
- **Stage B** (mild): A parasite mass contains immature and mature spore stages. Inflammatory cells are found around the lesion, but do not invade into the focus of infection.
- **Stage C** (moderate): Focus is infiltrated by inflammatory cells, followed by disintegration of the parasite mass.
- **Stage D** (severe): Encapsulation of focus is evident.

The relative abundance of each category was expressed as proportion of numbers of lesions among all lesions examined. Data of the duplicate groups of masu salmon (MS-1 and MS-2) were combined, because of the small sample size.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Forward</td>
<td></td>
</tr>
<tr>
<td>Kt-fwd1</td>
<td>5’-CGA ATG ATG AGA CCA ATG AAT-3’</td>
</tr>
<tr>
<td>Kt-fwd2</td>
<td>5’-TTA CCA CAG CCC GCA TTG G-3’</td>
</tr>
<tr>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>Kt-rev2</td>
<td>5’-CTC GCT CCT GTC GAT ATC TA-3’</td>
</tr>
<tr>
<td>Kt-rev1-2</td>
<td>5’-GCT CCT AAC TCG GAA CCA TAT CA-3’</td>
</tr>
</tbody>
</table>

**Table 1.** Primer sequences used for the detection of *Kabatana takedai*.

**Fig. 1.** Prevalence of infection with *Kabatana takedai* in the three populations of masu salmon and rainbow trout which were exposed to the Chitose River water in 2005. A, B and C indicates the prevalence of infection in the heart or the trunk muscle, the heart, and the trunk muscle, respectively. Different letters (a, b, c) above the bars indicate a significant difference between the fish groups at each week post-exposure.
Statistical analysis

Prevalence of infection and intensity of infection were analyzed with Fisher’s exact test and Kruskall-Wallis tests followed by Scheffe’s multiple comparisons, respectively. Percentages of results by PCR, microscopy and histology were analyzed with Fisher’s exact test. Probabilities of 0.05 or less were considered statistically significant.

Results

Comparison among three populations of masu salmon and rainbow trout (Experiment in 2005)

Cyst formations of K. takedai occurred among the fish groups only at 4 and 6 weeks PE except one individual of Shari masu salmon at 2 weeks PE in 2005 (Fig. 1A). Prevalence of infection at 4 weeks PE varied from 13.3 to 30.0%, but there were no significant differences among them. At 6 weeks PE, the prevalence was significantly higher in rainbow trout (45.0%) than 3 populations of masu salmon (22.3–36.3%). Among the three populations of masu salmon examined, Shari population was more susceptible than the others, but not statistically different from Chitose population. When the heart infections were compared (Fig. 1B), the prevalence at 6 weeks PE reached 35.3% in rainbow trout, which was significantly higher than masu salmon (9.2–18.0%). Regarding the trunk muscle infection (Fig. 1C), the prevalence at 6 weeks PE in rainbow trout (35.7%) was significantly higher than Chitose (20.9%) and Shiribetsu (18.8%) population, but not statistically different from Shari population (28.5%). Mean intensity of infection in the heart at 6 weeks PE was also significantly higher in rainbow trout, but the statistical differences were partly found.

Comparison between masu salmon and rainbow trout in duplicate (Experiment in 2006)

K. takedai appeared first in the duplicate groups of rainbow trout at 4 weeks PE in 2006 (Fig. 2A). Higher prevalence of infection in rainbow trout than in masu salmon was found at 6 weeks PE, and significantly noted at 8 weeks PE; the prevalence reached 35.0 and 37.7% in rainbow trout compared with 16.4 and 15.9% in masu salmon. The prevalence at 8 weeks PE in the heart of rainbow trout (30.0 and 34.4%) were significantly higher than masu salmon (4.9 and 4.8%) (Fig. 2B), while no significant differences in the trunk muscle infection were found at any week PE (Fig. 2C). As for the intensity of infection, there were no significant differences among the parallel groups examined (Table 3).

Comparison of K. takedai pre-cyst stage

The percentage of fish which were positive by microscopy, and the percentage of fish which were negative by microscopy but positive by PCR were illustrated in Fig. 3. The former indicates the prevalence of cyst formation, while the latter suggests the prevalence of pre-cyst stages. Thus, the total bars suggest ‘true’ prevalence of infection (positive by microscopy plus by PCR). PCR-positive results were obtained from several fish even at 4 weeks PE, in which only a few cysts were found by microscopy. At 6 weeks PE, the percentage of fish with pre-cyst stages was significantly higher in rainbow trout-2 (30.0%) than in masu salmon-1 and 2 (6.7 and 3.3 %). ‘True’ prevalence at 6 weeks PE was significantly higher in rainbow trout (36.7 and 30.0%) than in masu salmon (6.7 and 6.7%). At 8 weeks PE, the percentage of fish with pre-cyst stages and ‘true’ prevalence were significantly higher in rainbow trout-1 than masu salmon-1.

Table 2. Mean (± S.D.) intensity of infection with Kabatana takedai in the heart or trunk muscle of the three populations of masu salmon and rainbow trout following exposure to the Chitose River water in 2005.

<table>
<thead>
<tr>
<th>Fish group</th>
<th>Site of infection</th>
<th>Mean intensity of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
</tr>
<tr>
<td>Masu salmon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitose</td>
<td>Heart</td>
<td>0</td>
</tr>
<tr>
<td>Shiribetsu</td>
<td>Heart</td>
<td>0</td>
</tr>
<tr>
<td>Shari</td>
<td>Heart</td>
<td>1.0</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Heart</td>
<td>0</td>
</tr>
<tr>
<td>Masu salmon</td>
<td>Muscle</td>
<td>0</td>
</tr>
<tr>
<td>Shiribetsu</td>
<td>Muscle</td>
<td>0</td>
</tr>
<tr>
<td>Shari</td>
<td>Muscle</td>
<td>0</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Muscle</td>
<td>0</td>
</tr>
</tbody>
</table>

*: Values within heart or muscle infections with different superscript letters are significantly different between fish groups at each week post-exposure.
Comparison of susceptibility to *Kabatana takedai*

Histopathological observation of *K. takedai* cysts in the heart

In the early phase of *K. takedai* infection in the heart, no host reactions were observed around a mass of parasite developing in the endocardium (Stage A; Fig. 4A). In the next phase of *K. takedai* derived lesions, inflammatory cells composed of lymphocytes, macrophages and fibroblasts appeared around the parasite mass including immature stages (meront and/or sporont) and spores (Stage B; Fig. 4B). Foci were infiltrated by inflammatory cells, followed by spore phagocytosis (Stage C; Fig. 4C). At this stage, degeneration and necrosis of the peripheral tissues were evident, resulting in disintegration and encapsulation of cysts. Finally, fibrinoid degeneration (seen as eosinophilic ring by HE, and blue ring by Azan) occurred in the marginal area of foci (Stage D; Fig. 4D). Encapsulated granuloma contained necrotic parasite, proliferating connective tissue, lymphocytes and macrophages. Edema was not detected. Epicarditis including macrophages that phagocytized spores was occasionally found (data not shown). Such foci were found to project into the pericardial cavity, likely resulting in visible cysts under stereo-microscope. Higher percentages of Stage D were found in rainbow trout (Fig. 5), but no significant differ-

Fig. 2. Prevalence of infection with *Kabatana takedai* in the duplicate groups of masu salmon and rainbow trout which were exposed to the Chitose River water in 2006. A, B and C indicates the prevalence of infection in the heart or the trunk muscle, the heart, and the trunk muscle, respectively. No infection was found at 2 and 3 weeks post-exposure. Different letters (a, b) above the bars indicate a significant difference between the fish groups at each week post-exposure.

Fig. 3. Percentage of fish in the duplicate groups of masu salmon (MS-1, MS-2) and rainbow trout (RT-1, RT-2), which were positive for *K. takedai* infection in the heart. Open area in the bars represents the percentage of fish which were positive by microscopy (cyst formation), while shaded area represents the percentage of fish negative by microscopy and positive by PCR (probably detecting the pre-cyst stages). Thus, total bars (positive by microscopy plus by PCR) suggest ‘true’ prevalence of infection. Different letters above the bars indicate significant differences between the fish groups at each week post-exposure. Upper and lower cases of letters are for the ‘true’ prevalence and pre-cyst stages, respectively. Data at 3 weeks post-exposure were not included, because only a few positive results were obtained.
ences were noted between the two species.

**Discussion**

This study revealed that rainbow trout was more susceptible to *Kabatana takedai* than masu salmon, particularly in the heart. Considering the previous report (Awakura, 1974) showing that prevalence of infection in both rainbow trout and masu salmon from the Chitose River reached 100%, it is possible that the experimental period in the present study was too short to reach the maximum prevalence. Nevertheless, it is obvious that *K. takedai* infection in rainbow trout is more acute than masu salmon. As far as the intensity of infection in the heart, resulting data appear to reach a plateau at 6 weeks PE, and the mean intensity was almost consistently higher in rainbow trout than in masu salmon (Table 3). Categorization of host susceptibility to infection as ‘susceptible’ and ‘resistant’ is relative, and thus a reproducible method of exposure is important in demonstrating the differences between fish groups (Shaw et al., 2000). The present study employed the controlled exposure of fish to the river water containing the infective stage of *K. takedai*, and both experiments in 2005 and 2006 indicated significant differences between masu salmon and rainbow trout.

Various biological and environmental factors have been demonstrated to be responsible for a range of susceptibility to fish microsporidians; water temperature (Beaman et al., 1999; Becker et al., 2003; Zenke et al.,...
2005), flow rate (Becker et al., 2003), exposure method (Ramsay et al., 2001), spore dose (Speare et al., 1998), and fish strains (Shaw et al., 2000). In the present study, temperature and water flow are consistent among the fish groups. The rainbow trout used in both years were smaller than the other groups, and thus the possibility that size of fish affected the infection of *K. takedai* may be considered. However, the past literature demonstrated no relationship between fish size and microsporidian infections (Urawa, 1989; Speare et al., 1998; Shaw et al., 2000). Thus, it seems that there was little, if any, effect of fish size on susceptibility to *K. takedai*.

Varied host responses among fish species or populations may be related to differences in the susceptibility to microsporidian infections (Ramsay et al., 2002; Lovy et al., 2007). It has been reported that chinook salmon *O. tshawytscha* and coho salmon *O. kisutch* are more susceptible to *Loma salmonae* than rainbow trout (Ramsay et al., 2002). Xenomas degenerated more rapidly and reduced with time in rainbow trout, as compared with the former two species. A greater inflammatory response was found in chinook and coho salmon than in rainbow trout. Shaw et al. (2001) documented that macrophages of Atlantic salmon *Salmo salar*, which were resistant to *L. salmonae*, phagocytosed more spores than susceptible chinook salmon. Urawa (2001) found that mean number of *K. takedai* cysts in the heart of masu salmon gradually decreased during the 7 months-rearing period. This raised the possibility that cysts were cleared by protective host responses. However, the histopathological observation in the present study showed no remarkable differences in host reactions between the two fish species. Humoral and cellular immunological parameters of the salmonids should be further explored in relation to *K. takedai* infection.

Interestingly, *K. takedai* infection in rainbow trout was more conspicuous in the heart than in the trunk muscle. The sequential development of *K. takedai* infection, such as the portals of entry and migration route to the primary site of infection, has not been elucidated yet. As for *L. salmonae*, it has been demonstrated that the parasite penetrates through the gut epithelium and migrates to the heart, where early merogonic stages occur, followed by xenoma formation in the gills (Kent and Speare, 2005). Sanchez et al. (2001) suggested that Atlantic salmon and brook trout *Salvelinus fontinalis*, the resistant species to *L. salmonae*, did not block the parasite invasion at the entrance in the gut, and that the parasite failed to develop into xenoma inside the fish body. *K. takedai* is not likely to follow the same migration pattern to *L. salmonae*, because cyst formation occurred simultaneously at the heart and trunk muscle. Nonetheless, it is highly probable that the parasite invasion has occurred to a similar extent in masu salmon and rainbow trout, because the prevalence in the trunk muscle was similar between the two species. The higher percentage of fish with pre-cyst stages of *K. takedai* in the heart of rainbow trout than in masu salmon suggested that *K. takedai* established the cardiac infection more in rainbow trout than in masu salmon. The mechanisms responsible for parasite establishment in the heart were not determined. The structural and functional differences or the host-parasite affinity between the heart and trunk muscle may be involved. The PCR method newly developed in the present study will be useful for tracing early developmental stages of *K. takedai* and for clarifying the underlying mechanisms.

We expected a lower susceptibility to *K. takedai* in Chitose masu salmon population than in the other masu populations, which were naïve to *K. takedai*. However, in the present study, the susceptibility difference among the three masu populations was unclear. This may be explained by difference in health status of the experimental fish used. The Chitose population had relatively lower survivals than the other populations (data not shown), likely resulting in high susceptibility of Chitose population to *K. takedai*. Alternatively, the susceptibility to *K. takedai* may not be closely associated with difference in fish population, although considerable differences in biological characteristics among masu salmon populations in Hokkaido were reported (Mayama, 1989). This disease was found after several dams had

### Table 3. Mean (± S.D.) intensity of infection with *Kabatana takedai* in the heart or trunk muscle of duplicate groups of masu salmon (MS) and rainbow trout (RT) following to the Chitose River water in 2006.

<table>
<thead>
<tr>
<th>Fish group</th>
<th>Site of infection</th>
<th>Mean intensity of infection</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
</tr>
<tr>
<td>MS -1</td>
<td>Heart</td>
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<tr>
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</tr>
<tr>
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been built in the Chitose River in 1919, and the lowest reservoir was suspected as the source of *K. takedai* infection. It implies that the host-parasite relationship for *K. takedai* established recently even in Chitose population. The severity of a given disease is dependent on the interaction of numerous variables of the host, the parasite, and the environment (Hedrick, 1998). The limited geographical distribution of *K. takedai* is likely related to the environmental factors, including the lacustrine conditions or the availability of the potential intermediate hosts.

Because of the similar susceptibility to *K. takedai* in the three populations of masu salmon examined, there seems a risk of parasite spreading by transports of infected fish through stocking programs. Inadequate fish stocking may result in severe losses of highly susceptible salmonids such as rainbow trout. Indeed, Urawa (1989) found that feral rainbow trout in the Chitose River were dead due to the heavy infection of *K. takedai*. It is unknown whether *K. takedai* can establish the infection in new locations, because the life-cycle of this parasite has not been elucidated yet. Awakura (1974) and Fujiyama et al. (2002) suggested the involvement of the intermediate host in the life-cycle of *K. takedai*. Further studies are required to clarify the life-cycle and the mode of infection for *K. takedai*.

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**References**


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