Bulletin of the National Salmon
Resources Center, No. 4 : 7-11
December 2001

さけ・ます資源管理センター
研究報告　第4号　7-11頁
2001年12月

RESEARCH NOTE

Occurrence of *Kabatana takedai* (Microspora) in Juvenile Masu Salmon (*Oncorhynchus masou*) reared at Varying Water Temperatures in a Hatchery

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Abstract. ― Infections with the microsporidian parasite *Kabatana takedai* (Awakura, 1974) were monitored for juvenile masu salmon (*Oncorhynchus masou*) held at various water temperatures controlled using river and spring waters in the Chitose Hatchery, Hokkaido, Japan. In the summer of 1999, the hatchery water became over 15°C due to unusual increase of water temperature in the Chitose River. In consequence, heavy microsporidian infections occurred among juvenile masu salmon temporally transplanted from the Shiribetsu and Shizunai rivers, discontinuing back releases of these fish into their native rivers to prevent the parasite diffusion. These juveniles (approximately 100,000 fish) were killed by the late November. The present survey also indicated that the cyst formation of *K. takedai* could occur at low temperatures less than 13°C. A rearing experiment confirmed that the parasite cysts were residual within the muscle and heart of hosts over 7 months.

Key words: microsporidian parasite, *Kabatana takedai*, masu salmon, control of infection, water temperature

Introduction

Kabatana takedai (Awakura, 1974) is a highly pathogenic microsporidian parasite infecting the muscle and heart of various salmonid fishes. The parasite was first recorded as *Plistophora* sp. from diseased rainbow trout (*Oncorhynchus mykiss*) reared in the Chitose Hatchery, which used water from the Chitose River (Takeda 1933). Later, the parasite was also found in Lake Akan and Tokitonuma of Hokkaido (Awakura et al. 1966; Awakura 1978), and in the Taranay and Bryanka rivers on south Sakhalin Island (Vyalova and Voronin 1987; Vyalova 1999). The distribution of *K. takedai* is limited in these areas of Hokkaido and Sakhalin. The taxonomic position of this species has been altered several times such as *Glugea takedai, Nosema takedai, or Microsporidium takedai*, and finally it is assigned to the genus *Kabatana* Lom, Dyková and Tonguthai, 2000 by ultrastructural and molecular study (Lom et al. 2001).

The parasite infects only salmonids such as masu (*O. masou*), pink (*O. gorbuscha*), chum (*O. keta*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon, white-spotted char (*Salvelinus leucomaenis*), Dolly Varden (*S. malma*), and rainbow and brown trout (*Salmo trutta*)(Urawa and Awakura 1994). The parasite could be transmitted directly by the ingestion of spores added to food or by spore immersion (Awakura 1974), but following similar transmission experiments were unsuccessful (Fujiyama, unpublished data). Awakura (1974) suspected that small aquatic organisms in river or lake water might act as a vector for transmission of the parasite.

Awakura (1974) and Urawa (1989) found that the cyst formation occurred when the water temperature was above 15°C between July and September. Because the parasite development is retarded below

Contribution A No. 23 from the National Salmon Resources Center.

this temperature (Awakura and Kurahashi 1967), temperature manipulation has been believed as an effective method to avoid the disease outbreaks. However, critical temperature requirements in the development of *K. takedai* have not been determined yet. The purpose of this study is to examine *K. takedai* infections in juvenile masu salmon reared at varying water temperatures in the Chitose Hatchery, Hokkaido, Japan.

Materials and Methods

Fish

In 1999, juvenile masu salmon originating from the Shiribetsu and Shizunai rivers were reared in the Chitose Hatchery. Approximately 140,000 masu salmon eggs were transplanted from the Mena Hatchery along the Shiribetsu River System in southern Hokkaido. In addition, twenty thousand masu salmon eggs were transplanted from the Shizunai Hatchery. These Shiribetsu and Shizunai stocks were reared in ponds supplied with spring water after hatching from January 21 and March 9, respectively. From early April, the Chitose River water was supplied to the rearing ponds in addition to spring water with an ambient temperature of approximately 8℃. Water temperatures in the Chitose River and rearing ponds were recorded daily (Fig. 1). The number of dead fish was also recorded. The fish were sampled on September 29, and October 7 and 19, 1999 for parasite survey. To confirm the residual period of parasite cysts within hosts, two hundred masu salmon juveniles of Shizunai origin stock were held in two closed tanks supplied with circulating parasite free water for 7 months from October 7. The rearing water temperature was controlled at 8°C or 13°C by chiller or heater. The fish samples were collected from both groups 1, 4 and 7 months after the start of trial.

In 2000, juvenile masu salmon originating from

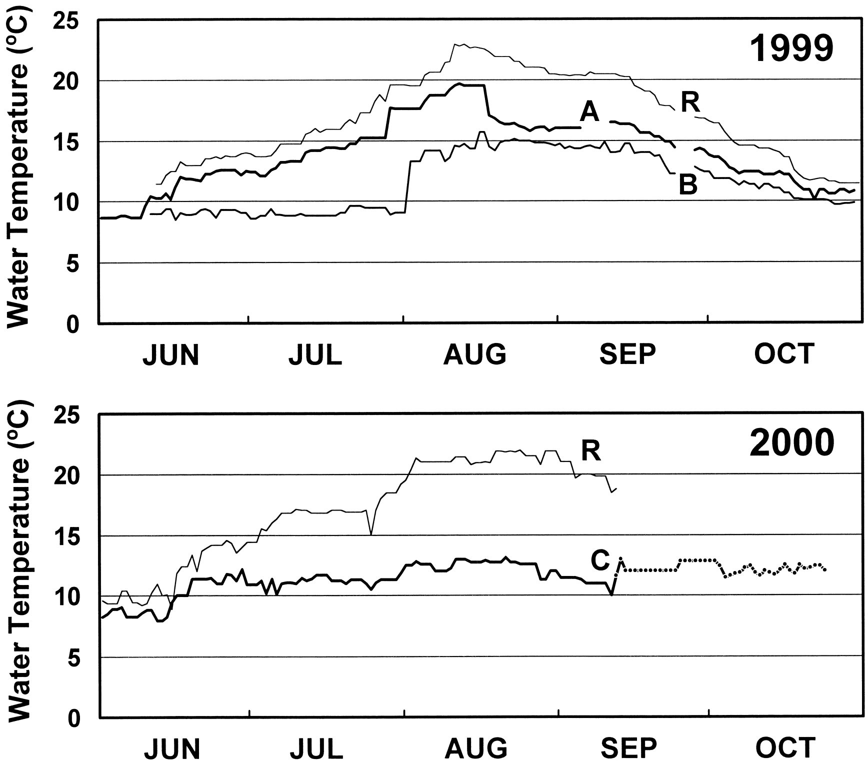

Fig. 1. Daily changes of water temperature at rearing ponds (A-C) in the Chitose Hatchery for masu salmon, and in the Chitose River (R). A, B, and C indicate water temperatures in ponds where Shizunai, Shiribetsu, and Chitose stocks were reared, respectively. A dotted line indicates water temperature in a closed tank to which masu salmon were moved from the pond C on September 11, 2000.

Table 1. Prevalence and number of cysts of *Kabatana takedai* in juvenile masu salmon reared in the Chitose Hatchery.

Origin of stocks	Sampling Date	No. samples	Fish fork length (cm)*[1]	Prevalence of parasite (%)	No. parasite cysts*[2]	
					Heart	Muscle
Shizunai R.	September 29, 1999	27	11.0 ± 1.0	88.9	3.7 ± 3.1 (11)	9.2 ± 12.1 (45)
	October 7, 1999	21	10.1 ± 0.6	85.7	4.9 ± 3.9 (12)	6.2 ± 9.7 (31)
Shiribetsu R.	October 7, 1999	10	10.0 ± 0.6	80.0	1.6 ± 2.5 (7)	9.9 ± 25.7 (83)
	October 19, 1999	22	10.3 ± 1.0	77.3	1.3 ± 1.7 (5)	8.3 ± 15.4 (53)
Chitose R.	September 11, 2000	30	8.1 ± 1.3	16.7	0.1 ± 0.2 (1)	0.6 ± 1.5 (5)
	October 3, 2000	20	8.2 ± 1.4	45.0	0.4 ± 1.1 (4)	1.6 ± 3.4 (13)
	October 25, 2000	30	9.4 ± 1.1	40.0	1.8 ± 1.3 (4)	1.0 ± 1.8 (7)

*[1] Mean ± SD

*[2] Mean ± SD (max)

the Chitose River were reared in the Chitose Hatchery. Approximately one hundred thousand juveniles were reared in a pond supplied with spring and river waters from the Chitose River. Water temperature and fish mortalities were recorded daily. Thirty fish were sampled on September 11, 2000 for parasite survey. In the same time, two hundred fish were stocked in a closed tank, where parasite free water was circulated at about 13℃. Fish were sampled on October 3 and 25 for parasite survey.

Parasite survey

After measuring fish fork length, the heart and muscle were examined to detect the white cysts of *Kabatana takedai* under a dissected microscope. The number of cysts were counted for each fish infected with the parasite.

Results

Parasite in hatchery fish

The prevalence of *K. takedai* cysts was 86-89% in masu salmon juveniles originating from the Shizunai River (Table 1). The number of cysts was averaged at 3.7-4.9 in the heart and 6.2-9.2 in the trunk muscle. The maximum number of cysts was 12 and 45 in the heart and muscle, respectively. The water temperature in the rearing pond increased gradually from mid June, being over 15℃ in mid July (Fig. 1A). It reached near 20℃ during mid August in proportion to unusual temperature rise in river water. The total mortality rate of fish was 43% between June and October.

In the Shiribetsu stock, the prevalence of parasite cysts was 77-80% and the mean number of cysts was 1.3-1.6 in the heart and 8.3-9.9 in the muscle (Table 1). The maximum number of cysts was 7 and

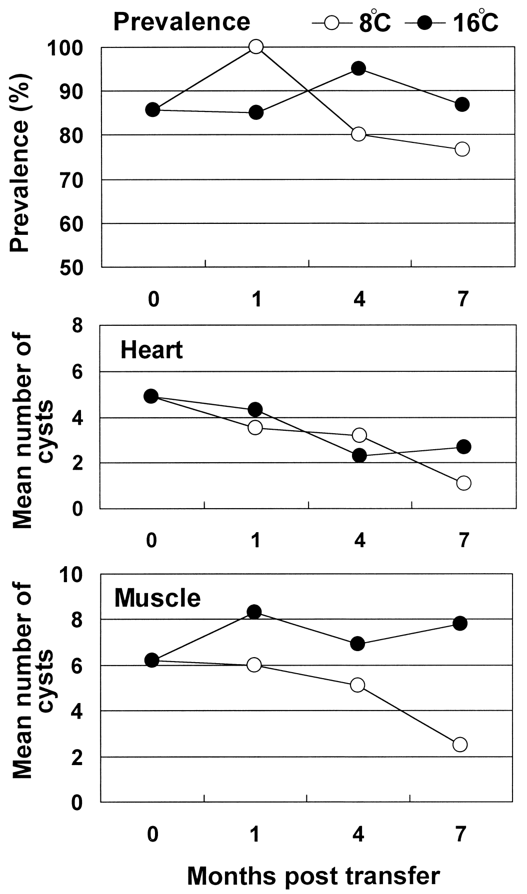

Fig. 2. Changes in the prevalence of *Kabatana takedai* and mean number of parasite cysts in the muscle and heart of juvenile masu salmon reared at low (8℃) and high (16℃) water temperatures.

83 in the heart and muscle, respectively. The rearing water temperature was below 10ºC until late July, but increased to approximately 15ºC in August and September (Fig. 1B). The fish mortality rate was accumulated to 9% from June to October.

In 2000, Chitose masu salmon were reared below 13ºC (Fig. 1C). Nevertheless, 17% of juveniles examined in mid September were infected with *K. takedai* (Table 1). The prevalence of parasite cysts increased to 40-45% 3-5 weeks after reared in a circulating tank at 13ºC.

Residual of parasite cysts

Juvenile masu salmon affected with *K. takedai* were reared in two closed tanks at low (8ºC) or high (16ºC) temperatures for 7 months. The prevalence of parasite cysts remained around 90% at 16ºC, but slightly decreased at 8ºC (Fig. 2). Mean number of cysts in the heart decreased in both groups, but in the muscle it decreased only in the low temperature group.

Discussion

It is well reported that temperature affects the development of microsporidian parasites. Temperatures above 20ºC or below 9ºC interrupt the life cycle of the gill intracellular microsporidian parasite *Loma salmonae* prior to sporogony, inhibiting the production of xenomas (Sanchez et al. 2000).

Kabatana takedai cysts appeared in salmonid hosts during summer when water temperature was above 15ºC both in the Chiotose River (Urawa 1989) and hatchery supplied with the river water (Awakura 1974). Thus temperature manipulation has been used for the control of parasite infection in the Chitose Hatchery, where the disease outbreak has been prevented by cooling the water to less than 15ºC in the rearing ponds during the summer season (Urawa and Awakura 1994).

In the summer of 1999, however, rearing water became over 15ºC due to unusual increase of water temperature in the Chitose River. In consequence, heavy microsporidian infections occurred in juvenile masu salmon originating from the Shiribetsu and Shizunai rivers. The fish mortality was 43% in Shizunai stock and 9% in Shiribetsu stock during summer and fall, but these mortalities might be caused by other factors in addition to heavy microsporidian infection. Back releases of these fish into their native rivers were discontinued in order to prevent the microsporidian diffusion, and the survived juveniles (approximately 100,000 fish) were killed by the late November.

In the next year, the parasite cysts were observed again in some masu salmon reared in the Chitose Hatchery, although the fish pond was kept less than 13ºC even in the summer. In 2001, the parasite cysts were observed in the muscle of juvenile sockeye salmon reared under 11ºC in the Chitose Hatchery for 4 months (Fujiyama, unpublished data). These observations suggest that *K. takedai* can adapt to cooler water conditions than we have expected.

Awakura and Kurahashi (1967) reported that *K. takedai* could not develop after the infected rainbow trout were transferred into cool water (8ºC), but they did not indicate how long the parasite remained in the host. The present study confirmed that the parasite cysts prolong within hosts for more than 7 months at low (8ºC) or high (16ºC) temperatures, although the number of cysts was slightly reduced by host's defense responses maybe such as macrophage activity. It suggests a difficulty in treating infected fish by temperature manipulations. Infection experiments should be designed to determine critical water temperatures which the parasite fails to develop within hosts.

Acknowledgements

I thank the staffs of Chitose Branch of the National Salmon Resources Center for their kind helps to provide fish samples and information.

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様々な水温で飼育されたサクラマス幼魚における武田微胞子虫 *Kabatana takedai* の寄生

浦和　茂彦

北海道の千歳事業所において河川水と湧水を混合した様々な水温下で飼育されたサクラマス幼魚における武田微胞子虫(*Kabatana takedai*)の寄生状況を1999年と2000年にモニタリングした．1999年夏, 千歳川の水温が異常に上昇したため，飼育水温が15°Cを超えた．その結果，一時的に飼育されていた尻別産と静内産サクラマス幼魚に武田微胞子虫が大量寄生した．未発生地域への微胞子虫の拡散を防ぐため原産地への放流は中止され, 約10万尾の生残魚は11月末までに処分された．また2000年の調査により武田微胞子虫のシスト形成は水温13°C以下でも起きることが始めて確認された．形成されたシストは宿主の筋肉や心臓に7ヵ月以上残ることが飼育実験で示された．