Studies on helminth parasites of salmonoid fishes in Hokkaido, especially the plerocercoid of *Diphyllobothrium latum*

by N. Seki

Original title: *Hokkaido-san sake, masu-rui no kisei zenshu, tokuni kosetsu retto jochu no plerocercoid ni tsuite*


Translated by the Translation Bureau (KFM)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries and Marine Service
Pacific Biological Station
Nanaimo, B.C.

1977

12 pages typescript
Studies on helminth parasites of salmonoid fishes in Hokkaido, especially the plerocercoid of *Diphyllobothrium latum*

STUDIES ON HELMINTH PARASITES OF SALMONOID FISHES IN HOKKAIDO,

ESPECIALLY THE PLEUCERCOID OF Diphyllobothrium latum

Naoki SEKI
Department of Animal Parasitology, Faculty of Veterinary Medicine, Hokkaido University

Introduction
Salmonoid fishes are infested with a variety of helminths that cause parasitic diseases in man and hence pose a hazard to public health. Plerocercoid larvae of the broad fish tapeworm Diphyllobothrium latum (LINNAEUS, 1758) and larvae of the genus Anisakis are examples of these parasites. As for the former species, fish are its second intermediate hosts where this tapeworm lives in the form of plerocercoid larvae. Freshwater fleas known as 'Ken-mijinko' serve as first inter-mediate host where the tapeworm exists in the procercoid larval form. Fish become infested with D. latum as they feed on these water fleas. In other words, infestation of fish with this tapeworm takes place in fresh water. The final hosts of D. latum are fish-eating mammals

UNEDITED TRANSLATION
For information only

TRAD U CTION NON REVISÉE
Information soulemont
including man, dogs and bears. On the other hand, man becomes infested with larvae of the *Anisakis* genus by eating raw fish. However, the final hosts of this ascarid nematode are marine animals such as whale and seals, and adult worms parasitize the digestive tract of these animals. In the human body, *Anisakis* larvae, without being able to grow into adult worms, invade the wall of the digestive tract to produce pathological changes including granulomatous foci. These changes can be considered to belong to "a nematode larval transmission syndrome" occurring in the body of improper hosts and in an acute case patients complain of a severe stomachache. However, salmonoids are not considered to be so important as the other marine fishes as the origin of anisakiasis in man.

Studies on the broad fish tapeworm in Japan have been carried out by IJIMA (1889), EGUCHI (1922-1932) and a few others. However, all of these studies are old and in recent years hardly any investigation has been conducted on this tapeworm. Patients infested with this tapeworm are frequently found in the districts of Hokuriku and Tohoku and along the coastal area of Hokkaido facing the Japan Sea. All these districts are salmonoid-fishing areas and also the fishes are often eaten uncooked there.

The author recently conducted a parasitologic survey on freshwater salmonoids caught at various sites of Hokkaido and obtained the results of some interest which are presented in this publication. Detailed accounts of the present report are given in a thesis for a master's degree submitted to the Postgraduate School (Course of Veterinary Medicine), Hokkaido University, in the academic year of 1974.
Materials and Methods

The present survey was conducted from June to October of 1974 and covered a total of eight freshwater sites, seven lakes and one river; i.e., Mashu Lake, Panketo, Shikaribetsu Lake, Kinzan Lake, Seinai Lake, Okushinkan Lake, Toyohira Gorge and Mokume River. Of these, four sites - lakes of Kinzan, Seinai and Okushinkan and Toyohira Gorge - are artificial lakes formed as a result of dam construction.

Five species of salmonoids were examined; i.e., rainbow trout *Salmo gairdneri irideus* (GIBBONS), 'ame' trout *Salvelinus leucomaenis* (PALLAS), a salmonoid known as 'Oshorokoma' *Salvelinus malma* (WALBAUM), kokanee salmon *Oncorhynchus nerka adonis* (JORDAN et McGREGOR) and cherry trout *O. masou* (BREVOORT). Of these, the cherry trout is a land-locked form of the sockeye salmon.

All sample fish were caught with a gill net and dissected to examine helminths at the sampling sites or at the author's laboratory. In order to detect plerocercoid larvae of *D. latum* that were the main target of this investigation, flesh of every fish was sliced to a proper thickness and each slice was sandwiched between two glass plates for examining parasites with the naked eye and stereoscopically. Plerocercoid larvae upon collection were placed in a physiological saline solution and after storage in a refrigerator at about 4° C for a few days they were fed to about 3-month-old puppies to obtain adult worms.

Results and Discussion

A total of 15 genera and 17 species of helminths were detected. Of these, six species were trematodes, eight were nematodes, two tapeworms
and one hookworm. The six trematodes were as follows: *Azygia lucii* (MUELLER, 1776), *Crepidostomum farionis* (MUELLER, 1874), *C. metoecus* (BRAUN, 1900), *C. sp.*, *Tubulovesicula lindbergi* (IAYMAN, 1930) and *Allocreadium sp.*. The eight nematodes were *Metabronema salvelini* (FUJITA, 1922; BAYLIS, 1935), *Ezonema bicornis* (BOYCE, 1971), larvae of *Rhabdochona sp.*, *Capillaria sp.* and *Cucullanus sp.*, *Anisakis sp.* larvae, *Philonema sp.*, and *Contracaecum sp.* larvae; the two tapeworms were plerocercoid larvae of *Diphyllobothrium latum* and larvae of *Pelichnibothrium sp.*; and the one hookworm was *Neoechinorhynchus sp.*.

Distribution patterns of these helminths in the natural and artificial lakes are shown in Tables 1 and 2, respectively. There was a marked difference in the pattern between the two and this difference appears to have originated in dissimilar sources, both lake and fish. The lakes from which the sample fishes were obtained can be divided into two - natural and artificial. These fishes are also classified into indigenous and naturalized species or landlocked and anadromous forms. The naturalized species included kokanee salmon and rainbow trout in Mashu Lake, kokanee salmon in Panketo, rainbow trout in Shikaribetsu Lake and kokanee salmon in Okushinkan Lake, whereas cherry trout in the Mokume River were anadromous and had migrated from the sea up the river to spawn. The other fishes were all indigenous.

As can be seen from the two tables, the helminths detected in samples from the artificial lakes were all nematode parasites and neither trematode, tapeworm nor hookworm was found. This also applies to the case of Mashu Lake which, though a natural lake, no fishes had inhabited until recently. The naturalized fishes generally showed low parasitization rates with the exception of the rainbow trout in the lakes of Mashu and Shikaribetsu. However, it is possible in the artificial lakes that in
Table 1 Profile of helminths in salmonoids sampled from natural lakes and river (top)

<table>
<thead>
<tr>
<th>I Sampling site</th>
<th>a Mashu Lake</th>
<th>b Panketo</th>
<th>c Shikaribetsu Lake</th>
<th>d Mokume River</th>
<th>e Kinzan Lake</th>
<th>f Seinai Lake</th>
<th>g Okushinkan Lake</th>
<th>h Toyohira Gorge</th>
</tr>
</thead>
<tbody>
<tr>
<td>II Host fish</td>
<td>1) kokanee salmon</td>
<td>2) rainbow trout</td>
<td>3) Salvelinus leucomaenis</td>
<td>4) Salvelinus malma</td>
<td>5) cherry trout</td>
<td>6) 'yamabe' trout</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III Helminth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Ratio of positive parasitization cases to total examined cases (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Number of helminths per each case</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Profile of helminths in salmonoids sampled from artificial lakes (bottom)

<table>
<thead>
<tr>
<th>I Sampling site</th>
<th>a Mashu Lake</th>
<th>b Panketo</th>
<th>c Shikaribetsu Lake</th>
<th>d Mokume River</th>
<th>e Kinzan Lake</th>
<th>f Seinai Lake</th>
<th>g Okushinkan Lake</th>
<th>h Toyohira Gorge</th>
</tr>
</thead>
<tbody>
<tr>
<td>II Host fish</td>
<td>1) kokanee salmon</td>
<td>2) rainbow trout</td>
<td>3) Salvelinus leucomaenis</td>
<td>4) Salvelinus malma</td>
<td>5) cherry trout</td>
<td>6) 'yamabe' trout</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III Helminth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Ratio of positive parasitization cases to total examined cases (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Number of helminths per each case</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

future the life cycle of helminths other than the nematodes is established and also that the pattern of parasitization in the naturalized fish species is altered. Although the sex and the age of a host often play an important role in establishing parasitization in the host - parasite relationship, no significant differences were observed in this respect in the present survey.
Photo plates 1 to 5  Broad fish tapeworm
1 Plerocercoid larva (arrow marked) in the fish muscle.  2 Plerocercoid larva in the muscle (section specimen, H-E staining).
3 Plerocercoid larvae immediately after collection from a muscle (in a physiological saline solution).  4 Plerocercoid larvae expanding and contracting in a physiological saline solution.  5 Adult worms obtained from an experimentally infected dog (by oral administration of plerocercoid larvae).
Two Helminth Species Important to Public Health

Of the aforementioned helminths, plerocercoid larvae of *D. latum* and larvae of *Anisakis* sp. appear to be important from a public health viewpoint and hence they will be discussed in detail.

Parasites of these two species were detected in the cherry trout (anadromous form, they were at the spawning stage) caught from the Mokume River.

*Anisakis* larvae were found in 54.2% of the cherry trout, the number ranging from 1 to 5 per fish. Ninety % of the larvae were located in the abdominal cavity, especially the mesentery of the fish and every larva was encapsulated. The remaining 10% parasitized the skeletal muscles, especially the abdominal muscle, and none of these larvae was encapsulated. It is considered that only those larvae which are in the flesh of fish are ingested by man. However, the observed low infestation rate suggests that the possibility of infestation to man is fairly low. It should be noted here that the sample cherry trout were caught during the month of October, since a number of investigators have found that *Anisakis* larvae alter their parasitizing locus as the season changes. According to these investigators, during a certain season *Anisakis* larvae move from the abdominal cavity into the muscle, resulting in an increase in the number of larvae in the flesh of fish. Further surveys are needed on the seasonal fluctuation of *Anisakis* larvae parasitizing cherry trout. Judging from the known development cycle of *Anisakis* larvae, it appears that they invade cherry trout while the fish live in the ocean.

As for *D. latum*, patients infested with this tape-worm are usually found in cherry trout-fishing areas, as has already been mentioned
herein. In the other areas nearly all fishes are stored frozen or pickled for a long time before they are used for food, and raw salmon and trout are seldom eaten. Hence when fish are eaten, any plerocercoid larvae in them are considered to have already been dead. The Mokume River in which plerocercoid larvae of *D. latum* were detected in the present survey is a tributary of the Shiribetsu River draining into the Japan Sea.

In Japan second intermediate hosts of the broad fish tapeworm are limited to a few salmonoids including cherry trout, pink salmon (*Oncorhynchus gorbuscha*), salmon (*O. keta*) and sockeye salmon (*O. nerka*). According to EGUCHI, *O. rhodurus* and rainbow trout can be experimentally infested with early plerocercoid larvae. In the present survey however, no plerocercoid larva was detected in the kokanee salmon, the landlocked form of the rainbow trout and the red salmon. The cherry trout, unlike the other salmonoids, lives in freshwater for two years; i.e., eggs that are spawned in fall remain undeveloped over winter and hatch in the following spring. The fry live in upper streams or lakes for more than a year including the second winter. As has aforementioned, 'Ken-mijinko' freshwater waterfleas are first intermediate host of *D. latum* and it is believed that cherry trout fry are infested with plerocercoid larvae of this tapeworm during their freshwater life. After this, the fry run downward into the sea. When these ecological characteristics are considered, the cherry trout has higher chances of being infested with *D. latum* than the other salmonoids, and hence this fish species is considered to be the most important intermediate host in Japan. As the final host, the brown bear *Ursus arctos* is regarded as the most important in
Hokkaido. Although the number of this animal has decreased in recent years, numerous cases of parasitization with _D. latum_ in brown bears are known. There is a possibility that northern foxes distributed widely in Hokkaido serve as final host of the parasite, but no case of parasitization has been reported yet.

The rate of parasitization with plerocercoid larvae of _D. latum_ and the number of parasites per fish were reported by EGUCHI (1929) to be 40% and 1 to 5, the maximum being 15, respectively, for cherry trout in waters near Otaru. The corresponding values found in the present survey were 37.5% and 1 to 5, and these are nearly the same as the results of EGUCHI.

In the fish body, plerocercoid larvae of _D. latum_ are found mostly in the muscles but rarely in the abdominal cavity. According to EGUCHI (1924), the largest number has been found in the abdominal muscle. However, the results of the present survey showed the following figures: 74.1% for the dorsal muscle, 18.5% for the abdominal muscle and 3.7% each for the tail muscle and the abdominal cavity. In Europe nearly all cases of plerocercoid larval parasitization occurred in the abdominal cavity, especially the liver, and also nearly every larva was encapsulated. Also, the number of parasites per fish was very high and as large as more than 50 worms in the liver have been reported. On the other hand, all the reported Japanese cases including the present paper have shown that plerocercoid larvae of _D. latum_ are found almost exclusively in the flesh of fish and that the number of parasites per fish is small. EGUCHI (1924) has observed that 10.1% of plerocercoid larvae sampled from cherry trout were encapsulated. However, in none of the cases in the present survey
were plerocercoid larvae encapsulated. Such a discrepancy presumably
arised from differences in the host fish species, parasitization site and
parasitizing period. In Europe freshwater pikes are the main second
intermediate host of D. latum but in Japan salmonoids, especially cherry
tROUT are the one.

In order to obtain adult worms of D. latum, plerocercoid larvae
collected from the fish samples in this survey were orally given to 3-
month-old puppies and the puppies were subjected every day after admin-
istration to stool examination. As soon as parasite eggs were detected
in feces of an animal, it was killed to obtain adult worms. Three pup-
pies were used in this experiment and the numbers of plerocercoid larvae
administered to these three were 9, 4 and 4. All of these three puppies
were found to be parasitized with the broad fish tapeworm; i.e., the
parasitization rate was 100% as opposed to that of 66.6% obtained by
EGUCHI (1926) in dogs. In his experiment, eggs of this tapeworm were
excreted in feces after 14 and 17 days of plerocercoid administration.
However, in the present survey, egg excretion occurred after 4 and 13
days in two of the three puppies. The time of 4 days is indeed very
short. In the remaining one animal, however, egg excretion was not
observed even after 17 days and hence the animal was autopsied. The
four given plerocercoid larvae were recovered alive but they did not grow
at all and remained at the plerocercoid stage. Although it is extremely
difficult to explain the cause(s) of the nondevelopment of these larvae,
of extreme interest is the finding that a great difference was noted in
the development of plerocercoid larvae among individual final hosts.
While EGUCHI stated that adult worms obtained in his experimental infection
in dogs varied in body length from 250 to 1960 mm, those secured in the present survey, after fixation with formalin, were 190 to 1300 mm long and the eggs were 0.052 to 0.068 by 0.036 to 0.044 mm large. In the one dog that excreted eggs after 4 days, it is estimated that each larva grew more than 300 mm a day.

Postscript

In this publication the profile of helminths in freshwater salmonoids sampled in various parts of Hokkaido has been described. However, it appears that further investigations from a variety of angles are needed on D. latum. These include a survey on the state of infestation of salmonoids during their freshwater life with its plerocercoid larvae, examination of adult worms in a variety of possible final hosts of this tapeworm and investigation on the profile of helminths in salmonoids during their marine life with due consideration on the unique ecology of these fish species.

The author is grateful to Associate Professor M. OHBAYASHI for guidance and valuable suggestion in the conduct of this survey. Thanks are also due to Mr. M. NAGAUCHI and Dr. T. AWAKURA of Hokkaido Fisheries Hatchery; to Mr. H. KAMIYA and Mr. Y. TADA of the Department of Animal Parasitology, Faculty of Veterinary Medicine, Hokkaido University; to Mr. M. YASUTAKE of Faculty of Engineering, Hokkaido University; and to the officials of Akan Lake Fishermen's Co-operative Association and the Townships of Shikaoi, Minami-Furano, Seinai and Shinkan, for the supplies of materials used in the present survey.

References
