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Prevalence of *Diphyllobothrium hottai* Plerocercoids in Three Osmerid Fishes in Japan and Morphologic Features of the Cestoda (Diphyllobothriidae, Cestoda)

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Abstract

Japanese surf smelt, *Hypomesus pretiosus japonicus*, from 8 localities in Hokkaido and 7 localities in the Tohoku district, olive rainbow smelt, *Osmerus eperlanus mordax*, from 2 localities in Hokkaido, and Japanese smelt, *H. transpacificus nipponensis* from 7 localities in Honshu and 2 localities in Hokkaido in Japan were examined for plerocercoids of the family Diphyllobothriidae. Plerocercoids were found in Japanese surf smelt in all of the 15 localities in which they were surveyed. The prevalence of plerocercoids ranged from 4.4 to 31.3% (avg. 25.0%) in fish from Hokkaido and from 10.0 to 83.6% (avg. 49.3%) in fish from the Tohoku district. We also noted that the prevalence of plerocercoids increases as the length of the fish increased, with marked increases in fish of body length of 8, 11, and 13 cm. Plerocercoids were also found in olive rainbow smelt in both of the localities in which they were surveyed. The prevalence of plerocercoids was 5.5% in the fish taken from the coast of Muroran and 51.3% in those taken from Lagoon Notoro in Hokkaido. No plerocercoids were found in Japanese smelt. All of the plerocercoids were found in the abdominal cavity of the infected fish; the intensity of plerocercoids per fish ranged from 1 to 15 (total avg. 2.13). We found very few morphometric or histologic differences between the results of our studies of plerocercoids, including adult worms reared in golden hamsters and cestode eggs, and those of Yazaki *et al.*, (1988). Therefore, we identified this tapeworm as *Diphyllobothrium hottai*.

Key word: *Diphyllobothrium hottai*, osmerid fish, plerocercoid, prevalence, morphology

Introduction

Hotta *et al.* (1978) first completed morphologic studies on plerocercoids found in osmerid fishes in Japan and on adult worms from experimentally infected golden hamsters, and reported that these tapeworms bear a close morphological resemblance to *Diphyllobothrium ditremum*. Further studies have been made on the morphology and mode of development and biochemistry by Hasegawa *et al.* (1979), Yazaki *et al.* (1986), and Fukumoto *et al.* (1987). Yazaki *et al.* (1988) described this tapeworm as a new species, *D. hottai*.

This species of plerocercoids has been found in Japanese surf smelt, *Hypomesus pretiosus japonicus* (Hotta *et al.*, 1978), and in olive rainbow smelt, *Osmerus eperlanus mordax* (Hotta *et al.*, 1978; Yazaki *et al.*, 1986). Moreover, Hotta *et al.* (1978) reported that plerocercoids obtained from Japanese smelt, *H. transpacificus nipponensis*, have the same morphologic features as those from the two smelts mentioned above.

Japanese surf smelt are widely distributed along the coasts of Hokkaido and the Tohoku district, while olive rainbow smelt inhabits only the coasts of Hokkaido in Japan. Previous surveys of *D. hottai* plerocercoids have been made of fish found only at Lagoon Saroma and along the coasts of Nemuro, Kushiro, Wakkanai, Odaito (Hotta *et al.*, 1978) and Hiro-o (Yazaki *et al.*, 1986) in Hokkaido. We initiated the present study to clarify the prevalence of *D. hottai* plerocercoids in 3 osmerid fishes in Japan and to verify the morphologic features of the plerocercoids, adult worms, and eggs.

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Materials and Methods

A total of 2179 Japanese surf smelt, 798 olive rainbow smelt and 3186 Japanese smelt were collected during 2 periods, from 1985 to 1987 and 1990 to 1992, with the help of the fishing cooperative unions or the fishing industries in each locality (Table 1). Japanese surf smelt were collected from 8 localities in Hokkaido and 7 localities in the Tohoku district, olive rainbow smelt were collected from 2 localities in Hokkaido, and Japanese smelt were collected from 9 localities in Honshu and Hokkaido. Japanese surf smelt and olive rainbow smelt were captured on the coasts, while Japanese smelt were captured from freshwater lakes only, since those from the coasts are not available in the markets.

The body length of all the fish obtained were measured and immediately examined macroscopically for plerocercoids throughout the body cavity, visceral organs and muscles being pressed between glass plates. Some of the plerocercoids obtained

Table 1 Prevalence and intensity of plerocercoids in three osmerid fishes at various localities

Species of fish examined	Localities surveyed	No. of fish examined	Body length of fish (Range in cm)	No. of fish infected	Prevalence (%)	No. of plerocercoid detected		Intensity per fish
						non-cyst	cyst	
Japanese surf smelt	**Hokkaido**							
	Wakkanai coast	45	7.0–12.7	2	4.4	1	1	1 (1.00)
	Abashiri coast	56	9.2–13.5	11	19.6	11	2	1–2 (1.18)
	Notoro-ko (Lagoon)	128	13.0–17.0	25	19.5	21	11	1–2 (1.28)
	Nemuro coast	486	6.8–13.8	149	30.7	238	44	1–3 (1.89)
	Notsuke coast	516	7.9–14.9	111	21.5	217	157	1–7 (3.37)
	Akkeshi coast	198	6.9–13.6	51	25.8	48	41	1–6 (1.75)
	Ishikari coast	179	7.7–13.3	56	31.3	87	29	1–5 (2.07)
	Hakodate coast	21	8.8–15.9	2	9.5	2	0	1 (1.00)
	Total	1629		407	25.0	625	285	(2.24)
	Tohoku							
	Bay Mutsu	30	9.2–12.3	3	10.0	3	1	1–2 (1.33)
	Kamaishi coast	53	8.3–13.2	26	49.1	16	16	1–3 (1.23)
	Rikuzentakada coast	73	8.8–14.2	61	83.6	104	95	1–15 (3.26)
	Ohfunato coast	72	7.8–13.5	29	40.3	26	16	1–5 (1.45)
	Kesennuma coast	110	7.5–15.1	55	50.0	62	51	1–11 (2.05)
	Shizukawa coast	64	8.8–17.2	45	70.3	12	88	1–6 (2.22)
	Onagawa coast	148	6.7–13.5	52	35.1	52	23	1–4 (1.06)
	Total	550		271	49.3	275	290	(2.08)
Olive rainbow smelt	**Hokkaido**							
	Muroran coast	761	14.7–30.9	42	5.5	42	10	1–3 (1.24)
	Notoro-ko (Lagoon)	37	17.5–23.5	19	51.4	48	3	1–9 (2.68)
	Total	798		61	7.6	90	13	(1.69)
Japanese smelt	**Hokkaido**							
	Barato-ko (Lake)	308	5.6–12.4	0	0	0	0	0
	Abashiri-ko (Lake)	727	5.7–15.3	0	0	0	0	0
		1035		0	0	0	0	0
	Honshu							
	Kogawara-ko (Lake)	483	5.6–11.4	0	0	0	0	0
	Jusan-ko (Lake)	258	5.1–9.2	0	0	0	0	0
	Hachiro-gata (Lake)	162	4.8–10.6	0	0	0	0	0
	Kasumiga-ura (Lake)	654	4.5–10.5	0	0	0	0	0
	Ashino-ko (Lake)	186	5.2–10.8	0	0	0	0	0
	Suwa-ko (Lake)	283	5.0–11.2	0	0	0	0	0
	Shinji-ko (Lake)	125	5.2–11.8	0	0	0	0	0
	Total	2151		0	0	0	0	0

were used to infect golden hamsters for further study, the remaining plerocercoids were first washed and immersed in 1% saline solution for 60 min at 4°C to subdue their movement and then fixed in a solution of 4% formalin diluted with 1% saline. Some fixed plerocercoids were embedded in paraffin, cut into 7 μm cross sections at mid-body and stained with hematoxylin and eosin. All fixed specimens and the cross sections were subjected to morphometric and historogic studies for identification. Adult worms that had been experimentally introduced at the larval stage into golden hamsters and eggs that had been collected from both feces of the host and the uterine gravid sections of adult worms were also subjected to morphologic and histologic studies.

We removed adult worms from golden hamsters 20 days after they had been infected and placed them in tap water for 4 hrs at 4°C to subdue their movement. We then measured whole body length, and the number and maximum width of the segments of adult worms. One group of adult worms were fixed in 80% ethanol and stained with Mayer's ulum-carmin and Heidenhain's iron-hematoxylin. The remaining adult worms were fixed in a 10% formalin solution, cut into 7 μm sections after being embedded in paraffin, and stained with hematoxylin and eosin for histologic study. We measured microscopically the lengths and widths of 50 eggs and observed plerocercoid and egg surfaces using scanning electron microscopy (SEM) after they were treated following the methods of Yazaki *et al.* (1988).

Results

We categorized the results of the present study into four general areas: prevalence of plerocercoids in each fish type per locality; prevalence of plerocercoids per body length of fish; the site of infection; and overall morphology of the plerocercoids, both adult woms and eggs. Each of these areas is outlined in Table 1 through 4.

Prevalence of plerocercoids in each fish type per locality

Japanese surf smelt

Plerocercoids were found in Japanese surf smelt at all of the 15 localities in Hokkaido and the Tohoku district. The prevalence of plerocercoids ranged from 4.4 to 31.3% (avg. 25.0%) in fish from Hokkaido and from 10.0 to 83.6% (avg. 49.3%) in fish from the Tohoku district. The intensity of plerocercoids per fish ranged from 1 to 7 (avg. 2.24) in Hokkaido and from 1 to 15 (avg. 2.08) in the Tohoku district (Table 1).

Olive rainbow smelt

Plerocercoids were found in olive rainbow smelt taken from both the coast of Muroran and Lagoon Notoro. The prevalence of plerocercoids in this fish varied greatly per locality: only 5.5% of the fish from the Muroran coast were infected as compared to 51.3% of the fish from Lagoon Notoro. The intensity of plerocercoids per fish ranged from 1 to 3 (avg. 1.24) on the coast of Muroran and from 1 to 9 (avg. 2.68) at Lagoon Notoro (Table 1).

Japanese smelt

The number of Japanese smelt captured from each of the 9 freshwater lakes in Hokkaido and Honshu ranged from 125 to 727. We found no plerocercoids in any of these fish (Table 1).

Prevalence of plerocercoids in fish per body length

Nine-hundred and seventy-two of the Japanese surf smelt obtained from Hokkaido (body from 6.8 to 14.9 cm in length) were divided into 9 different groups based on body length, categorized by one-centimeter increments. The prevalence of plerocercoids was calculated for each group (Table 2). We found that the prevalence of plerocercoids increased as the body length of the fish increased; this increase was marked at body lengths of approximately 8, 11, and 13 cm. We therefore grouped these fish into four classes according to the prevalence of plerocercoids per body length: fish with body lengths between, (1) 6–8 cm in the first class (4.8% and 8.3% prevalence); (2) 8.1–11 cm in the second class (21.9%, 19.7%, and 19.3%); (3) 11.1–13 cm in the third class (39.4% and 40.7%); and (4) 13.1–15 cm in the fourth class (61.1% and 66.7%). We found that differences in the prevalence of plerocercoids were not significant within each of the four body length classes, but that significant differences existed between certain classes, i.e., between the 1st and 2nd class (7.0% and 19.8%), the 2nd and 3rd

Table 2 The prevalence of plerocercoids with different body length of Japanese surf smelt

Class	Body length of fish ranged in mm	Number of fish examined	Number of fish infected	Prevalence (%)	Mean prevalence in class (%)
1st	6.8–7.0	21	1	4.8	7.0
	7.1–8.0	36	3	8.3	
	8.1–9.0	64	14	21.9	
2nd	9.1–10.0	71	14	19.7	19.8
	10.1–11.0	259	50	19.3	
3rd	11.1–12.0	355	140	39.4	39.8
	12.1–13.0	145	59	40.7	
4th	13.1–14.0	18	11	61.1	61.9
	14.1–15.0	3	2	66.7	
Total		972	298	30.2	

(19.8% and 39.8%), and the 3rd and 4th (39.8% and 61.9%), ($\chi^2 = 5.467, 41.232$ and 4.080, respectively; P=0.05).

Site of infection with plerocercoids

In the Japanese surf smelt and olive rainbow smelt, all plerocercoids found were either moving freely or encysted on the surface of visceral organs in the abdominal cavity, such as the liver and/or the digestive tract. Encysted plerocercoids outnumbered non-encysted ones in fish from Hokkaido, whereas more non-encysted ones were found in fish from the Tohoku district (Table 1).

Morphologic features of plerocercoids, adult worms and eggs

Morphometric data on the plerocercoids, adult worms, and eggs are shown in Tables 3 and 4. Figures 1 to 10 show typical morphologic and histologic features of those originated from Japanese surf smelt taken from the Tohoku district.

Plerocercoid

We saw very few differences in morphology between plerocercoids found in Japanese surf smelt and those found in olive rainbow smelt from both Hokkaido and the Tohoku district (Table 3). The

Table 3 Measurements of plerocercoids from two osmerid fishes and of its cross sections in microns

	Japanese surf smelt origin from		Olive rainbow smelt origin
	Hokkaido	Tohoku	
Body length (mm)	3.0–13.0 (8.7)	7.0–21.4 (10.3)	8.5–13.5 (9.5)
Height of section	478–746 (577)	618–872 (652)	606–651 (629)
Breadth of section	780–1153 (964)	855–1355 (1167)	1056–1167 (1108)
Microvilli length	15–27 (22.3)	10–20 (17.6)	10–21 (16.7)
Thickness of E. L. M	25–36 (30.1)	24–40 (32.9)	20–32 (28.9)
Thickness of P. L. M	68–132 (93.0)	53–108 (87.5)	60–113 (86.6)
Thickness of P. T. M	26–63 (43.7)	20–42 (33.2)	30–51 (36.5)

Range (mean) measured the sections of five plerocercoids from each origin.
E. L. M.; Epidermal longitudinal musculature.
P. L. M.; Parenchymal longitudinal musculature.
P. T. M.; Parenchymal transverse musculature.

Table 4 Measurements of adult worms originated from osmerid fishes

	Japanese surf smelt origin from		Olive rainbow smelt origin
	Hokkaido	Tohoku	
Total length of strobila (mm)	185–309 (245)	213–356 (278)	206–342 (226)
Maximum width of segments (mm)	6.2–8.4 (7.9)	9.6–11.6 (10.4)	8.8–10.2 (9.0)
Length of widest segments (mm)	1.6–2.2 (1.9)	2.5–2.9 (2.8)	2.1–2.6 (2.4)
Scolex length (mm)	1.2–1.6 (1.4)	1.3–1.7 (1.4)	1.3–1.6 (1.5)
Neck length (mm)	0.8–2.0 (1.2)	1.3–2.3 (1.5)	1.5–1.9 (1.7)
Total number of segments	168–265 (201)	183–286 (235)	179–238 (212)
No. of segments anterior to primordia	15–25 (22)	18–25 (23)	19–23 (22)
No. of loops of uterus	5–10 (6.8)	4–8 (6.3)	4–10 (7.1)

Range (mean) measured on 5–10 specimens of each origin.

body of plerocercoids was generally flat, milky white in color, and ranged from 3 to 21.4 mm (avg. 9.5 mm) in length. The maximum body width was noted at the anterior one third of body. Body width grew rapidly from the anterior end to a maximum, then gradually decreased toward the posterior end (Fig. 1). The body surface had no transverse wrinkles and was covered with microvilli (Fig. 2). Histologic structures observed in a mid-body cross section of plerocercoids included the parenchymal transverse musculature and longitudinal musculature, the dorso-ventral musculature, the epidermal cell layer, and the epidermal longitudinal musculature. All structures had well-developed circular layers of muscle fibers or cells and were thicker at the midline than at the lateral end in the cross section (Figs. 3, 4).

Adult worm

We noted very few differences in morphology between adult worms originated from olive rainbow smelt and from Japanese surf smelt (Table 4). Strobilae with spatula-like scolex and short neck ranged from 185 to 356 cm (avg. 250.0 cm) in length. Segments increased in width along the length of strobila to maximum width at its central part and then decreased gradually toward its end. Segments were more wide than long (Figs. 5 and 6). The relative positions and/or sharps of the genital pore, genital atrium, cirrus sac, seminal vesicle, vitellaria, ovaries, gravid uterus, and testes were observed with stained segments and its cross sections (Figs. 7 and 8). The results of the present study are very similar to those described by Yazaki *et al.* (1988).

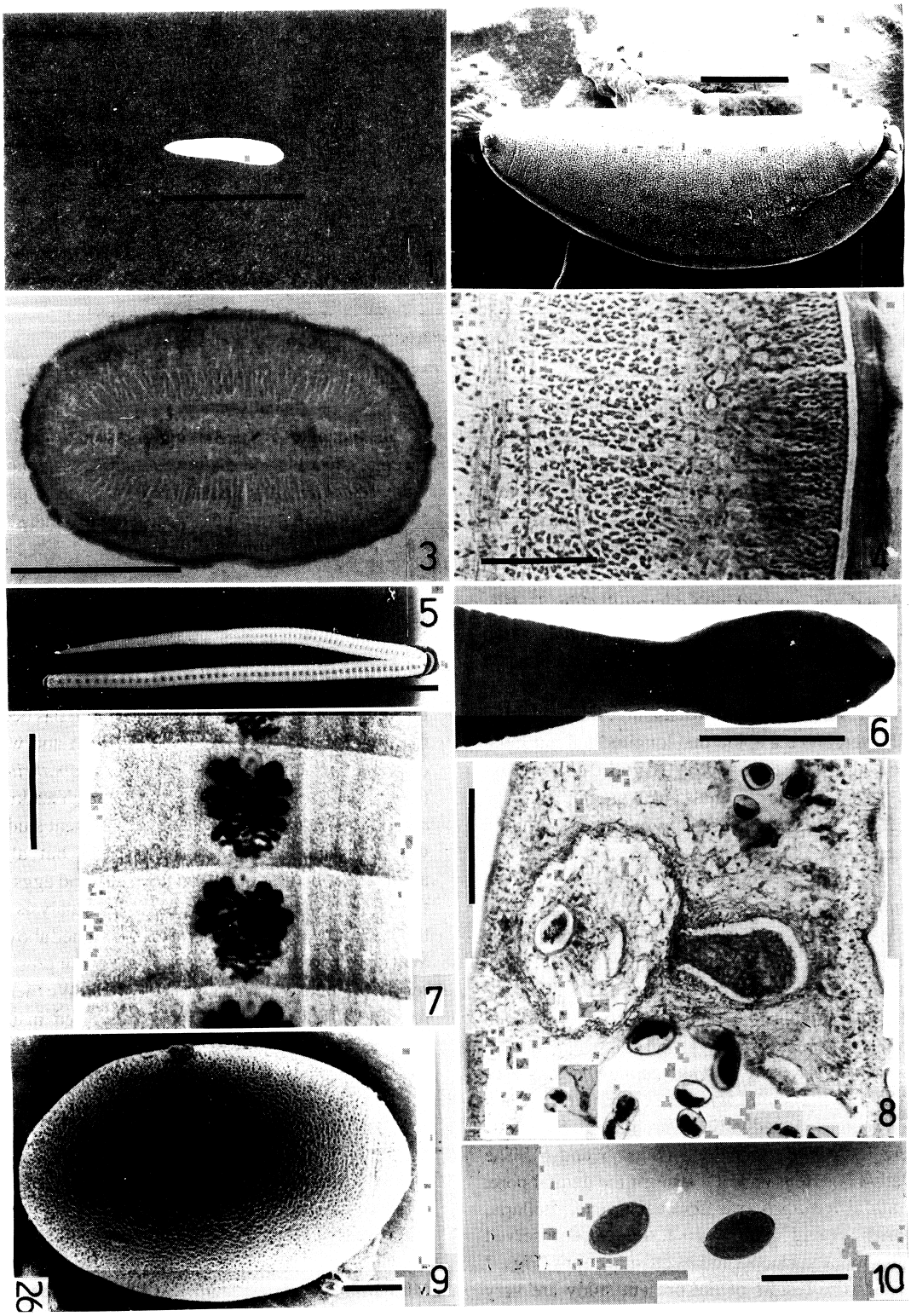
Eggs

The average sizes of the eggs obtained from both feces of the host and the gravid uteri in segments were 65.5×43.5 μm and 62.3×41.4 μm, respectively. We observed pinpoint pits covering the surfaces of the eggs (Figs. 9, 10).

Discussion

The taxonomic criterion of the family Diphillobothriidae was reviewed and arranged precisely by Kamo (1978). *Diphyllobothrium hottai* has been investigated on the basis of this criterion and was distinguished from other species of *Diphillobothrium* (Hotta *et al.*, 1978; Hasegawa *et al.*, 1979; Yazaki *et al.*, 1986; Yazaki *et al.*, 1988). In the present study, we subjected not only plerocercoids, but also adult worms reared in golden hamsters and eggs to the same morphometric and histologic analysis as those reported by the investigators mentioned above. We found very few differences between our present results and those of Yazaki *et al.* (1988). We therefore identified the plerocercoids observed in the present study as *D. hottai*.

Plerocercoids of *D. hottai* had previously been detected only in fish collected from Lagoon Saroma and along the coast of Nemuro (Hotta *et al.*, 1978) and along the coast of Hiro-o (Yazaki *et al.*, 1986) in Hokkaido. In the present study, however, we discovered that this tapeworm is widely distributed in other areas of Hokkaido, such as in Lagoon Notoro and along the coasts of Abashiri, Nemuro, Akkeshi, Muroran, Hakodate, and Ishikari as well as the coasts of the Tohoku district. From these results, we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hypothesized that this tapeworm is commonly distributed in the areas inhabited by Japanese surf smelt and/or olive rainbow smelt.

Diphyllobothrium hottai seems to complete its life cycle in a marine environment (Hotta *et al.*, 1978; Yazaki *et al.*, 1986), and so it is reasonable that fish obtained from Japanese freshwater lakes were negative for *D. hottai* infection. Japanese smelt belong to the same family Osmeridae as do Japanese surf smelt and olive rainbow smelt. Therefore, Japanese smelt in marine waters may also play a role as the second intermediate host of *D. hottai*. Moreover, Hotta *et al.* (1978) detected similar plerocercoids in Japanese smelt collected from marine waters, but not from those in freshwater although the plerocercoids were not identified since investigators did not obtain adult worms by experimental infection. In the present study, we attempted to examine Japanese smelt from marine waters as well as those from freshwaters, but saltwater Japanese smelt could not be obtained since all Japanese smelt on the market are captured in freshwater lakes to which they were introduced. Some Japanese smelt seem to be anadromous, however and to inhabit coastal waters. Also, in the present study, Japanese surf smelt infected with plerocercoids included some specimens that closely resembled Japanese smelt. We did not discriminated between these and Japanese surf smelt since the differences between the discriminative morphologic features are too subtle to differentiate the species (dorsal to ventral fin relationship, length of the upper jaw, number of vertebrae, and shape of the stomach). More studies should be done on Japanese smelt captured in marine waters and on shishamo smelt, *Spirinchus lanceolatus*, (Osmeridae), which is sympatric with Japanese surf smelt and olive rainbow smelt in the Hokkaido region.

There was a significantly higher prevalence of plerocercoids in Japanese surf smelt that measured 8, 11, and 13 cm in length within the group of fish with body lengths ranged from 6.8 to 14.9 cm. Therefore, we statistically divided the fish into four classes according to their body length and the prevalence of plerocercoids. Japanese surf smelt completes its life span in about 4 years. One year after hatching, it matures in the open sea to a body length of 6–7 cm (Yanagawa, 1989), and thereafter grows to a maximum body length of about 20 cm (Miyadi *et al.*, 1976). The yearlings migrate between coastal and off coastal waters together with the elder fish to spawn (Yanagawa, 1989). Judging from the life history of the fish, the subjects in the present study apparently included individuals of different ages; the above four classes of fish, grouped as per body length, seem to correspond to the age groups of the fish. The higher prevalence of plerocercoids in larger smelt is apparently due to the fact that elder, or larger fish may have a higher probability of ingesting infected copepods than yonger, or smaller ones.

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Fig. 1 Plerocercoids found in Japanese surf smelt. (scale bar: 1 cm)
Fig. 2 Surface of plerocercoid as seen using SEM. (scale bar: 2 mm)
Fig. 3 Cross section of plerocercoid. (scale bar: 500 μm)
Fig. 4 Higher magnification of cross section of plerocercoid. (scale bar: 100 μm).
Fig. 5 Whole strobila of *D. hottai*. (scale bar: 3 cm)
Fig. 6 Scolex and neck of *D. hottai*. (scale bar: 1 mm)
Fig. 7 Mature segments of *D. hottai*. (scale bar: 2 mm)
Fig. 8 Longitudinal section of mature segment. (scale bar: 200 μm)
Fig. 9 Eggshell surface as seen using SEM. (scale bar: 10 μm)
Fig. 10 Eggs of *D. hottai*. (scale bar: 100 μm)

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