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Geographical Distribution and Seasonal Occurrence of *Myxobolus kisutchi* (Myxozoa: Myxosporea) in the Central Nerve Tissues of Chinook and Coho Salmon in the Columbia River and Its Vicinities

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Abstract.— The myxosporean parasite *Myxobolous kisutchi* Yasutake and Wood, 1957 infected in the medulla oblongata and spinal cord of Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon. The parasite occurred in restricted areas of the Columbia River basin and its vicinities (Minter Creek and Chehalis River) in Washington, but not in other major Chinook salmon populations in North America and Asia. The prevalence of parasite spores in Chinook salmon smolts migrating down through the McNary Dam in the Columbia River was 20-37% in May (spring type), dropped to 1.3-1.7% in June (mainly fall type), and increased again to 29% in July (fall type). The prevalence of spores in adult Chinook salmon captured in the lower mainstream of the Columbia River was 43-65% for spring runs, but lower (8-11%) for fall runs. Among juvenile coho salmon reared at the Minter Creek Hatchery, the spores of *M. kisutchi* first appeared in June, and the prevalence increased to 97% in July, being sustained at almost 100% until the smolt stage in the next spring. The parasite was redescribed based on fresh specimens.

Key words: Myxozoa, *Myxobolus kisutchi,* nerve tissue, description, geographical distribution, seasonal change, Columbia River, Minter Creek, Washington

Introduction

The myxosporean parasite *Myxobolus kisutchi* Yasutake and Wood, 1957 was first found in the spinal cord of wild juvenile coho salmon (*Oncorhynchus kisutch*) in Minter Creek, Washington. However, there has been no record since the first finding by Yasutake and Wood (1957), except for Wyatt (1978) who found this parasite in the brain of Chinook salmon (*O. tshawytscha*) from the McKenzie River of the Columbia River system. Several other *Myxobolus* species are recorded from the nerve tissues of salmonids (Schuberg and Schröder 1905; Pugachev and Khokhlov 1979; Gonzalez-Lanza and Alvarez-Pellitero 1984; Hedrick et al. 1991). However, the original description of *M. kisutchi* was based on sectioned materials, causing difficulties in taxonomic comparisons with other species. Some brain myxosporean species are known to be useful as biological tags for the stock identification of host fishes (Margolis 1982; Quinn et al. 1987; Bailey et al. 1988; Urawa 1989; Moles et al. 1991; Awakura et al. 1995; Urawa and Nagasawa 1995; Urawa et al. 1998). The aims of present study are to clarify the geographical distribution and seasonal occurrence of *M. kisutchi* in the U.S. Pacific Northwest, and to redescribe its morphological characteristics.

Materials and Methods

Fish

Hatchery-reared Chinook (n=447) and coho (n=437) salmon were collected from nine salmon rearing facilities in the Columbia River basin, the Minter Creek (Puget Sound tributary), and the Che-

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Table 1. Prevalence of *Myxobolus kisutchi* in Chinook and coho salmon from the Columbia River Basin and its vicinities.

Fish	Type	Stage	River	Locality	Date of capture	No. of fish examined	No. of fish infected	Prevalence (%)
Chinook	spring	adults	Columbia R.	Woody Island test fishery	Apr. 1987	62	27	43.5
Chinook	spring	adults	Columbia R.	Corbett test fishery	Apr. 1987	17	11	64.7
Chinook	spring	adults	Columbia R.	Mouth test fishery	Apr. 1988	83	36	43.4
Chinook	spring	adults	Columbia R.	Mouth test fishery	Apr. 30, 1989	65	33	50.8
Chinook	fall	adults	Columbia R.	Mouth test fishery	Sep. 14, 1987	44	5	11.4
Chinook	fall	adults	Columbia R.	Mouth test fishery	Sep. 1988	24	2	8.3
Chinook	fall	smolts	Columbia R.	Jones Beach	Jun. 16-18, 1987	60	11	18.3
Chinook	fall	smolts	Columbia R.	McNary Dam	Jul. 14-18, 1988	62	18	29.0
Chinook	fall	smolts	Columbia R.	McNary Dam	Jun. 21-24, 1988	59	1	1.7
Chinook	fall	smolts	Columbia R.	McNary Dam	Jun. 21-29, Jul. 6, 1989	80	1	1.3
Chinook	spring	smolts	Columbia R.	McNary Dam	May 6 & 7, 1988	62	23	37.1
Chinook	spring	smolts	Columbia R.	McNary Dam	May 8-10, 1989	59	12	20.3
Chinook	spring	smolts	Columbia R. (Deschutes R.)	Round Butte Hatchery	Feb. 5, 1991	50	0	0
Chinook	spring	smolts	Columbia R.	Warm Springs Hatchery	Mar. 6, 1991	60	0	0
Chinook	spring	smolts	Columbia R. (Wenatchee R.)	Leavenworth National Fish Hatchery	Feb. 27, 1991	90	0	0
Chinook	spring	smolts	Columbia R. (Clearwater R.)	Dworshak National Fish Hatchery	Mar. 27, 1991	54	0	0
Chinook	spring	smolts	Columbia R. (Clearwater R.)	Dworshak National Fish Hatchery	Mar. 13, 1991	91	0	0
Chinook	spring	smolts	Columbia R. (Willamette R.)	Dexter Ponds	Apr. 12, 1991	30	0	0
Chinook	spring?	smolts	Columbia R. (Cowlitz River)	Cowlitz Salmon Hatchery	Mar. 12, 1990	50	50	100
Chinook	spring	smolts	Minter Creek	Hupp Springs Hatchery	Aug. 5, 1990	22	1	4.5
Chinook	spring?	adults	Minter Creek		Sep. 23 & 30, 1996	37	15	40.5
coho	spring	smolts	Chehalis R. (Satsop R.)	Simpson Hatchery	Apr. 12, 1996	28	18	64.3
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Aug. 5, 1990	5	3	60.0
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Feb. 1, 1996	31	26	83.9
coho	spring	smolts	Minter Creek	Minter Creek Hatchery	Apr. 11, 1996	42	42	100
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Apr. 1996	30	0	0
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	May 4, 1996	30	0	0
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Jun. 7, 1996	6	3	50.0
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Jul. 3, 1996	31	30	96.8
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Aug. 1996	30	30	100
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Sep. 1996	30	30	100
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Oct. 15, 1996	31	31	100
coho	spring	juveniles	Minter Creek	Minter Creek Hatchery	Nov. 17, 1996	27	26	96.3
coho	spring	smolts	Minter Creek	Minter Creek Hatchery	Jan. 1997	53	53	100
coho	spring	smolts	Minter Creek	Minter Creek Hatchery	Mar. 1997	35	35	100
coho	spring	smolts	Minter Creek	Minter Creek Hatchery	Apr. 1997	28	28	100

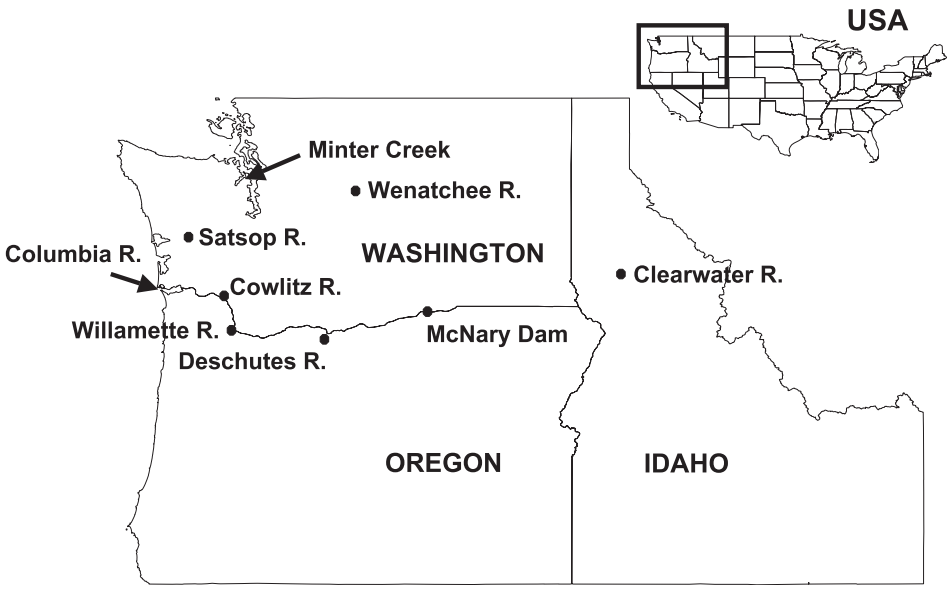

Fig. 1. Maps indicating sampling locations.

Table 2. Spore characters of *Myxobolus kisutchi* from the central nerve tissues of Chinook and coho salmon**.**

Host (Infection site)	Location	No. spores measured	SL[*1]	SW[*1]	ST[*1]	PCL[*1]	PCW[*1]	NC[*2]	SW/SL
Spring Chinook salmon	Columbia River	60	10.0±0.5	9.0±0.4	6.8±0.3	5.3±0.4	3.0±0.2		0.90±0.04
(medulla oblongata)	1987		(9.0-11.0)*[3]	(8.0-9.8)	(6.0-7.2)	(4.0-6.0)	(2.5-3.8)		(0.80-1.00)
Fall Chinook salmon	Columbia River	40	10.1±0.5	9.4±0.4	6.4±0.2	5.1±0.3	2.9±0.2	7.6±0.7	0.93±0.04
(medulla oblongata)	1988		(9.4-10.9)	(8.6-10.1)	(6.2-7.0)	(4.3-5.5)	(2.3-3.3)	(7-9)	(0.82-1.00)
Chinook salmon	Minter Creek, 1990	30	10.4±0.5	8.9±0.5	6.5±0.4	5.2±0.4	2.9±0.2		0.85±0.05
(medulla oblongata)			(9.5-11.0)	(8.0-10.0)	(6.0-7.2)	(4.5-6.0)	(2.5-3.5)		(0.76-1.00)
Coho salmon	Minter Creek, 1990	30	8.9±0.6	7.2±0.5	5.2±0.3	4.8±0.4	2.7±0.4		0.82±0.07
(medulla oblongata)			(8.0-10.0)	(6.2-8.5)	(4.9-6.0)	(4.0-5.2)	(2.0-3.1)		(0.69-0.98)
Coho salmon	Minter Creek, 1996	30	9.4±0.4	8.0±0.5	5.6±0.2	5.2±0.3	2.8±0.3	7.3±0.5	0.86±0.05
(medulla oblongata)			(8.6-10.1)	(7.2-9.0)	(5.2-6.2)	(4.5-6.0)	(2.3-3.1)	(6-8)	(0.76-0.97)
Coho salmon	Minter Creek, 1996	30	9.7±0.3	7.8±0.3	5.6±0.3	5.1±0.3	2.7±0.2	7.2±0.5	0.81±0.04
(spinal cord)			(9.4-10.1)	(7.0-8.6)	(4.9-6.2)	(4.7-5.5)	(2.3-3.1)	(6-8)	(0.72-0.88)
Coho salmon[*4]	Minter Creek	-	(7.5-8.5)	(6.5-7.0)	(3.5-3.8)	(3.8-5.5)			
(spinal cord)									

[*1]SL, spore length; SW, spore width; ST, spore thickness; PCL, polar capsule length; PCW, polar capsule width; in μm.
[*2]NC, number of coils per polar filament. [*3]Mean±SD (range). [*4]Measurements from sectioned material by Yasutake and Wood (1957).

halis River (coast of Washington tributary) during 1990-96 (Table 1, Fig. 1). For the survey of seasonal occurrence of parasites, juvenile coho salmon reared in the Minter Creek Hatchery were collected monthly from April 1996 to April 1997. These juveniles were reared in pond #17 supplied with water from Minter Creek. The water temperature was recorded daily during the survey period. Seaward migrating Chinook smolts (n=432) were captured at McNary Dam and Jones Beach in the lower Columbia River in the spring and early summer of 1987-89. Adult spring and fall Chinook salmon were captured in the lower Columbia River (n=295) and in Minter Creek (n=37). The fish samples were frozen or fixed with 10% formalin and stored until the tissues were examined for parasites.

Parasite examination

The medulla oblongata and spinal cord were removed from the fish samples and smeared on slides. The smeared slides were examined for the presence of *M. kisutchi* spores with a microscope (200 × magnification). In addition, the monthly samples from the Minter Creek Hatchery were observed for

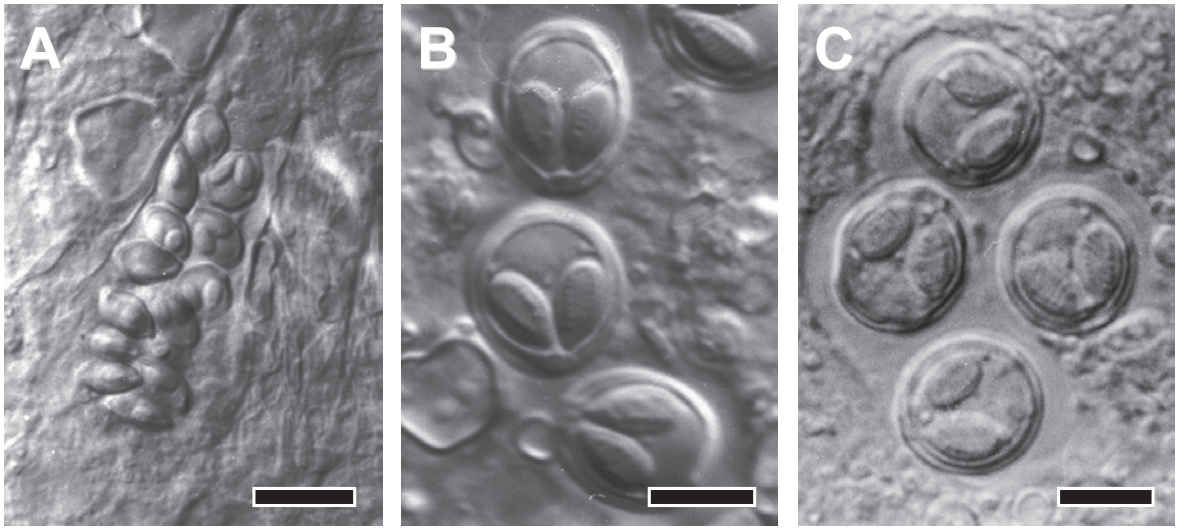

Fig. 2. Photomicrographs of *Myxobolus kisutchi.* (A) Spores in the spinal cord of coho salmon from the Minter Creek Hatchery. Scale bar = 25 μm. (B) Same. Scale bar = 5 μm. (C) Spores in the medulla oblongata of Chinook salmon from the Columbia River. Scale bar = 5 μm.

parasites in the lateral peripheral nerve. Detected myxosporean spores, viewed by a Normansky microscope, were described according to guidelines proposed by Lom and Arthur (1989). Spore measurements were made from fresh specimens.

Results

Parasite

Myxosporean spores of *M. kisutchi* were detected among the neurofibrils of the central nerve tissues (medulla oblongata and spinal cord) of coho and Chinook salmon, but not in the lateral peripheral nerve. No apparent host response was observed in the infection sites. Trohozoites (n=6) were irregular in shape, usually elongated, 20-64 × 16-29 μm in size, and contained 5-23 spores (Fig. 2A). The spore shape was oviform or almost round in the front view (Fig. 2B, C). The spore size was slightly different depending on the host fishes (Table 2): the spores from Chinook salmon were significantly larger than those from coho salmon ($P<0.05$). The width of shell membranes was 0.6-0.8 (mean 0.74) μm (n=100). Two pyriform polar capsules located at the anterior end occupied almost the half of the spore length. Polar filaments coiled inside forming 6-9 loops. The filaments extended with 5% KOH measured 52.5-87.5 (mean 66.6) μm (n=60). A small triangular intercapsular process was present.

Geographical distribution

Myxobolus kisutchi spores were detected in Chinook salmon smolts and adults captured in the lower Columbia River (Table 1). The prevalence of infection in smolts migrating seaward past the McNary Dam, which is located 467 km upstream from the river mouth, was 20-37% in May, dropped to 1.3-1.7% in June, and increased again to 29% in July. The prevalence of infection in adult Chinook salmon captured in the lower main stream of the Columbia River was 43-65% for spring runs, but lower (8-11%) for fall runs. Among six hatcheries in the Columbia River Basin, the parasite was found only in salmon from the Cowlitz Salmon Hatchery, where all Chinook salmon juveniles examined (n=50) were heavily infected. Chinook and coho salmon juveniles reared in the Minter Creek Hatchery and the Simpson Hatchery were also infected with *M. kisutchi.*

Seasonal occurrence

In coho salmon juveniles reared at the Minter Creek Hatchery, spores of *M. kisutchi* were not present in April and May, but appeared in the central nerve tissues in June (Fig. 3). The prevalence of spore infections increased to 97% in July, and remained at almost 100% until the next spring, when fish reached the smolt stage.

Discussion

Myxobolus kisutchi was first found by Yasukate

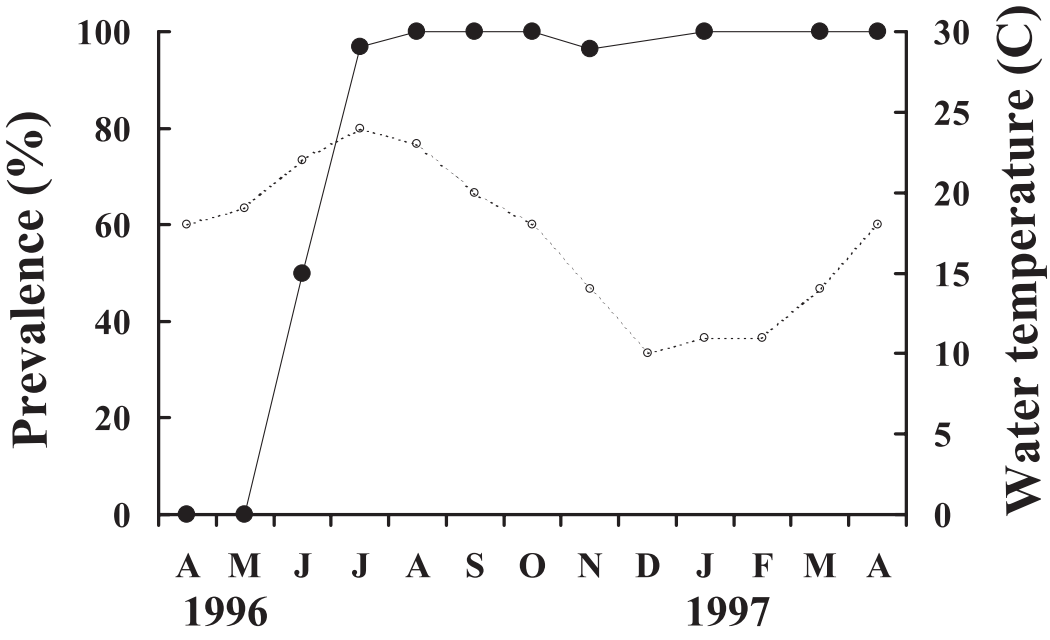

Fig. 3. Seasonal changes in prevalence of *Myxobolus kisutchi* spores (closed circles) in juvenile coho salmon reared at the Minter Creek Hatchery. Open circles indicate the average rearing water temperature in each month

and Wood (1957), but their description was based mainly on histological materials. The present description of *M. kisutchi* indicates that the fresh spore size is larger than that in the original description, even though the host species (coho salmon) and location (Minter Creek) were the same (Table 2). This is apparently attributable to spore shrinkage during the processing of sectioned materials. It is noteworthy that spores from Chinook salmon were larger than spores from coho salmon. Hine (1979) also observed variations in the size and shape of *Myxidium zealandicum* spores among host species.

Several other myxosporeans are found in the nerve tissues of salmonids (Schuberg and Schröder 1905; Pugachev and Khokhlov 1979; Gonzalez-Lanza and Alvarez-Pellitero 1984; Hedrick et al. 1991). Among these species, *Myxobolus kisutchi* is morphologically very similar to *M. neurobius*. The first record of *M. neurobius* was of specimens found in the spinal cord of brown trout *(Salmo trutta*) in Europe (Schuberg and Schröder 1905), and later *M. neurobius* was also found in several salmonid species from the Pacific coast of Asia (Pugachev and Khokhlov 1979; Awakura et al. 1982, 1995; Urawa and Awakura 1994) and the Atlantic coast of North America (Maloney et al. 1991). The spores of *M. neurobius* were found in the lateral peripheral nerve of Norwegian brown trout and Japanese masu salmon (*O. masou*), as well as in the brain and spinal cord (Urawa and Egil, unpublished data). However, spores of *M. kisutchi* were not present in the peripheral nerve tissues of coho and Chinook salmon. Thus, we propose that *M. kisutchi* remains as an independent species at the present time. Future molecular and life cycle studies are necessary for the taxonomy of *Myxobolus* spp. in the nerves of salmonids.

Many papers show that the life cycle of myxosporeans involves an actinosporean stage in aquatic oliogochaetes or polychaetes. For example, the oligochaetes *Stylodrilus heringianus* and *Lumbricullus variegatus* serve as the alternate host of *M. arcticus*, which infects the brain and spinal cord of salmonids (Kent et al. 1993; Urawa and Awakura 1994). We surveyed several species of oligochaetes in the Minter Creek during the early February of 1996, but could not find any actinosporeans. On the other hand, the seasonal survey indicated that spores of *M. kisutchi* appeared in the central nerve tissues of juveniles in June, several months after hatching. The prevalence immediately increased to almost 100% in the next month, and the spores remained in the nerve tissues throughout the host life. The spore formation of *M. arcticus* occurs within 3 months after infection at 10 ˚C (Urawa and Awakura 1994). Thus salmon juveniles may become infected with actino-

sporeans of *M. kisutchi* in late February at the Minter Creek Hatchery. It is interesting that the prevalence of *M. kisutchi* spores decreased in June (1-2%) but increased in July (29%) among Chinook smolts migrating past the McNary Dam in the Columbia River. Peak timing varies from year to year depending primarily on hatchery releases, but in general, spring Chinook smolts migrate in May, while fall smolts migrate in June and July (Dawley et al. 1980). The relatively high prevalence (20-37%) of *M. kisutchi* in May may be related to the longer freshwater residence (one year) of spring smolts compared to fall smolts. On the other hand, the prevalence in underyearling fall smolts was low in June but high in July, perphaps because the spore formation is almost completed in July, as indicated by our seasonal survey.

Our study confirms that *M. kisutchi* occurs in restricted areas of the Columbia River and Washington State waters (Minter Creek and Simpson River). Among six hatcheries located in the Columbia River basin the parasite was found only in Chinook salmon sampled at the Cowlitz Salmon Hatchery. However, Chinook smolts were already infected when they migrated past the McNary Dam on the mainstem of the Columbia River above the junction with the Cowlitz River. This suggests that the parasite might also occur in other areas within the river. *Myxobolus kisutchi* has not been found in other major stocks (33 populations) of Chinook salmon in North America and Asia (Urawa et al. 1998). Thus *M. kisutchi* is a good biological tag for identification of Chinook salmon originating from the Columbia River and Washington State waters. The past high-seas survey indicated that Chinook salmon infected with *M. kisutchi* was distributed in the Gulf of Alaska (55˚00´N, 143˚48´W) (Urawa and Nagasawa 1995, in appendix as *M. neurobius*).

No host response against *M. kisutchi* spores was observed, as mentioned in Yasukate and Wood (1957). Other *Myxobolus* species also do not cause any host response in the nerve tissues, but Moles and Heifetz (1998) found that *M. arcticus* significantly reduced the swimming performance of wild sockeye salmon (*O. nerka*). Hatchery-reared coho and Chinook salmon juveniles frequently suffer from heavy *M. kisutchi* infections. Thus the impact of *M. kisutchi* on host fishes should be evaluated.

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コロンビア川流域とその周辺河川のマスノスケとギンザケの中枢神経系に寄生する粘液胞子虫類*Myxobolus kisutchi*の地理的分布と季節変化

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マスノスケとギンザケの延髄や脊髄神経に寄生する粘液胞子虫類*Myxobolus kisutchi*の分布と寄生率の季節変化を北アメリカ北西域で調べた．この粘液胞子虫はコロンビア川と周辺域（ワシントン州のMinter CreekとChehalis川）の限られた水域で発生し，他の北米やアジアの主要なマスノスケ河川個体群ではみられなかった．コロンビア川のMcNaryダムを通過するマスノスケスモルトにおける*M. kisutchi*の寄生率は，5月（春タイプ）に20-37％で，6月（主に秋タイプ）は1.3-1.7％に減少し，7月（秋タイプ）に29％と再び増加した．コロンビア川河口で採集された回帰マスノスケ親魚における寄生率は，春タイプで43-65％であるのに対し，秋タイプでは8-11％と低かった．Minter Creekふ化場で飼育されたギンザケ幼魚では，*M. kisutchi*の胞子が6月に出現し，7月には寄生率が97％で，その後翌春のスモルト期までほぼすべての魚に寄生がみられた．生鮮標本に基づき*M. kisutchi*の再記載を行った．