

Original Research

Zoosporic Fungi Growing on Eggs of *Coregonus lavaretus holsatus* Thienemann, 1916 from Lake Wdzydze in Kaszuby

B. Czczuga*, B. Kiziewicz, A. Godlewska

Department of General Biology, Medical University, Kilińskiego 1, 15-089 Białystok, Poland

Received: 12 September 2003

Accepted: 14 November 2003

Abstract

The authors investigated fungal communities associated with eggs of *Coregonus lavaretus holsatus* species Lake Wdzydze in Kaszuby. A total of 15 fungus species were found to grow on the studied eggs: *Achlya americana*, *A. oblongata*, *A. treleaseana*, *Aphanomyces frigidophilus*, *A. irregularis*, *A. parasiticus*, *Aplanes androgynus*, *Saprolegnia ferax*, *S. parasitica*, *S. salmonis*, *S. shikotsuensis*, *Pythium aquatile*, *P. pulchrum*, *P. thalassium*, and *P. torulosum*. Worth a special note was the finding of *Aphanomyces frigidophilus* and *Saprolegnia salmonis*, new to Polish waters. The results obtained may be important to ichthyopathologists, because 8 of the above-mentioned fungi are known as necrotrophs or parasites of fishes.

Keywords: zoosporic fungi, whitefish, *Coregonus lavaretus holsatus*, hydrochemically

Introduction

Among several dozen fish species which inhabit inland waters of Poland there are three coregonids, namely *Coregonus albula*, *Coregonus peled*, and *Coregonus lavaretus*. In Polish waters, the last species is represented by four subspecies, *generosus*, *lavaretus*, *maraena* including *Coregonus lavaretus holsatus* living in Lake Wdzydze [1,2]. It was first described by Thienemann [3], a well known German limnologist. These four forms differ not only in the structure of skeletal bones [4,5,6,7], but also in the number of branchial processes on the first branchial arc, but also in their biology, e.g. in the type of food [8,9,10]. The analysis of species composition of aquatic zoosporic fungi growing on eggs of three forms of *Coregonus lavaretus*, except for *holsatus*, revealed substantial differences [11,12,13]. In this context, to provide a complete picture of the fungi growing on eggs of all the four forms of *Coregonus lavaretus*, we decided to

investigate the eggs of *Coregonus lavaretus holsatus* from Lake Wdzydze.

Material and Methods

A female of *Coregonus lavaretus holsatus* Thienemann, 1916 was caught in Lake Wdzydze in December 2002. This female had 22 gill rakers on the first branchial arc.

Water samples for the experiments were collected from six different bodies of water: Cypisek spring, Fosa and Akcent ponds, the Biała and Supraśl rivers, and the Komosa Lake. Nineteen parameters of these water samples were determined (Table I) according to the generally accepted methods [14].

The following procedure was followed while determining the presence of aquatic fungus species on the eggs. The eggs (50-75) were transferred to thirty 1.0- dm³ vessels (altogether 18 vessels) and placed in the laboratory at a temperature approaching that of a respective body of water. The subsamples from each vessel were observed under a microscope and the presence of mycelium (forming zoospores, antheridia and oogonia) of aquatic fungi

*Corresponding author; e-mail: czcz@amb.edu.pl

Table 1. Chemical composition (in mg l⁻¹) of water from the different sites (mean from 3 samples).

Parameter	Water bodies					
	Cypisek Spring	Biała River	Supraśl River	Akcent Pond	Fosa Pond	Komosa Lake
Temperature (°C)	6.0	2.0	0.5	3.0	1.0	1.8
pH	7.69	7.31	7.36	7.48	7.02	7.88
O ₂	10.46	11.04	11.88	0.05	1.82	12.24
BOD ₅	3.8	4.82	3.64	6.12	9.22	5.02
COD (Oxidability)	2.5	9.02	8.80	29.78	15.09	8.25
CO ₂	13.2	15.4	6.6	37.4	22.0	8.8
Alkalinity in CaCO ₃ (mval l ⁻¹)	5.2	4.7	4.0	6.8	5.5	4.3
N-NH ₃	0.045	0.642	0.324	1.470	0.854	0.245
N-NO ₂	0.027	0.011	0.012	0.292	0.112	0.008
N-NO ₃	0.021	0.05	0.06	1.04	0.05	0.036
P-PO ₄	0.804	1.504	1.108	7.182	3.590	0.735
Sulphates	50.60	68.0	30.85	90.50	23.04	32.91
Chlorides	22.5	40.0	18.0	43.0	44.0	14.0
Total hardness in Ca	117.36	92.16	68.42	128.16	79.20	76.32
Total hardness in Mg	16.77	22.34	14.19	28.80	26.23	19.84
Fe (total)	0.60	0.90	0.90	1.95	1.05	0.85
Dry residue	456.0	532.0	236.0	604.0	429.0	230.0
Dissolved solids	420.0	496.0	199.0	593.0	370.0	218.0
Suspended solids	36.0	36.0	37.0	11.0	59.0	12.0

growing on eggs was recorded. The methods were described in detail by Seymour and Fuller [15]. The samples of eggs were examined for one (or one and a half) week. The experiments were carried out for three weeks.

The fungi were identified using the following keys: Johnson [16], Sparrow [17], Seymour [18], Batko [19], Dick [20], and Watanabe [21].

Results

The water samples used for analysis differed in nutrient content being the index of trophicity in these water reservoirs, as well as in other parameters (Table 1). The highest nitrogen content (the three forms altogether) was found in the ponds Akcent and Fosa. The lowest content of nitrogen was noted in Cypisek Spring, and that of phosphates in the Komosa Lake. Sulphates and chlorides serving as chemical pollution indices were also found in greatest amounts in the both ponds. The highest oxidability was observed in the ponds, and the lowest in the spring.

15 zoosporic fungus species were found to grow on the eggs of *Coregonus lavaretus holsatus*, including 11 representatives of Saprolegniales and 4 of Peronosporales (Table 2). Worth a special note was the finding of *Aphanomyces frigidophilus* and *Saprolegnia salmonis*, new to

Polish waters (Fig. 1). Only 3 fungus species were found to grow on the eggs in the Fosa Pond, 5 in the Supraśl River and 5 in Komosa Lake.

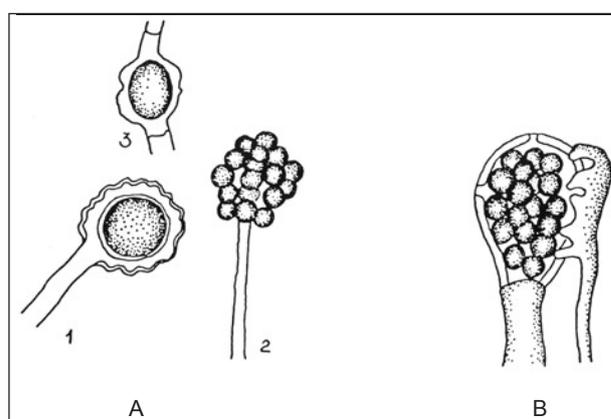


Fig. 1. New to Polish hydromycology fungi species. A - *Aphanomyces frigidophilus*: 1 - oogonium (16-25 µm); 2 - sporangium with zoospores (8-12 µm); 3 - elongated oogonium (25-45 µm) between hyphae; B - *Saprolegnia salmonis*: elongated oogonium (90-150 x 50-72 µm) with declinuous antheridial attachment.

Table 2. Aquatic fungi found on eggs of *Coregonus lavaretus holsatus*.

Fungi taxa	Cypisek Spring	Biała River	Supraśl River	Akcent Pond	Fosa Pond	Komosa Lake
Saprolegniales						
* <i>Achlya americana</i> Humphrey	x		x		x	
<i>Achlya oblongata</i> de Bary						x
<i>Achlya treleaseana</i> (Humphrey) Kauffman					x	
* <i>Aphanomyces frigidophilus</i> Kitancharoen et Hatai	x			x		
<i>Aphanomyces irregularis</i> Scott					x	x
<i>Aphanomyces parasiticus</i> Coker		x				
<i>Aplanes androgynus</i> (Archer) Humphrey						x
* <i>Saprolegnia ferax</i> (Gruith.) Thuret			x			
* <i>Saprolegnia parasitica</i> Coker			x			x
* <i>Saprolegnia salmonis</i> Hussein et Hatai		x				
* <i>Saprolegnia shikotsuensis</i> Hatai et al.	x		x			
Peronosporales						
<i>Pythium aquatile</i> Höhnk	x	x	x	x		
* <i>Pythium pulchrum</i> Minden						x
<i>Pythium thalassium</i> Atkins				x		
* <i>Pythium torulosum</i> Coker et Patterson		x		x		
Number of species	4	4	5	4	3	5

*known in literature as parasites or necrotrophs of fish

Discussion

A comparison of the number of zoosporic fungus species found on eggs of *Coregonus lavaretus holsatus* comine from Lake Wdzydze with the remaining three forms of *Coregonus lavaretus* found in Polish waters [11,12,13] revealed similarity to *Coregonus lavaretus generosus*. On the eggs of *Coregonus lavaretus lavaretus* we observed 28 and on those of *Coregonus lavaretus maraena* 32 fungus species. The number of fungi growing on eggs of a respective population is the result of the environmental conditions in a particular water reservoir. The less is the eggs are vulnerable to zoosporic fungi, the fewer stressogenic factors, known to reduce fish and eggs resistance to bacterial and mycotic infections [22]. This would suggest good environmental conditions in Lake Wdzydze for the population of *Coregonus lavaretus holsatus*. It is worth remembering that the annual increase in its population in Lake Wdzydze is high [2].

Among the Saprolegniales found, *Aphanomyces frigidophilus* and *Saprolegnia salmonis* appear new to Poland, *Aphanomyces irregularis* and *Aphanomyces parasiticus* are new to *Coregonus lavaretus*. These latter two fungi were observed on eggs of certain cyprinoid fish species [23] and other families [24,25,26,27]. *Aphanomyces frigidophilus* was found on eggs of whitefish in Cypisek and

Fosa. This species was previously described from eggs of Japanese charr (*Salvelinus leucomaenis*) from Tochigi Prefectural Fisheries Experimental Station, Utsonomiya, Japan [28]. It has also been observed on the eggs of masu salmon (*Oncorhynchus masou*) in Oshino Tront Hatchery, Yamanashi [29,30]. The eggs of *Coregonus lavaretus holsatus* is a new substrate for this fungus, which is also new to Polish hydromycoflora.

In our study *Saprolegnia salmonis* was found to grow on eggs of *Coregonus lavaretus holsatus* in the water collected for the experiment from the Biała River. It was first described from cultured sockeye salmon (*Oncorhynchus nerka*) raised in Hokkaido, Japan [31]. In later years, *Saprolegnia salmonis* was found in hatcheries in Hokkaido, Japan [32] and at immature stages (fingerlings) of rainbow trout (*Oncorhynchus mykiss*), masu salmon (*Oncorhynchus masou*), sockeye salmon (*Oncorhynchus nerka*), brown trout (*Salmo trutta*) and Japanese charr (*Salvelinus leucomaenis*) [33]. The eggs of *Coregonus lavaretus holsatus* is a new substrate for the growth of *Saprolegnia salmonis*.

Of the representatives of Peronosporales growing on eggs of *Coregonus lavaretus holsatus*, worth note is the finding of *Pythium aquatile* and *Pythium thalassium*, new to salmonids and other fish species in Poland. *Pythium aquatile* was first described from an animal

substrate in the water of salinified peatbogs near Kiel (Germany) [34]. It was also observed in several springs, rivers, ponds, and lakes of northeastern Poland [35]. Moreover, its growth was noted on animal substrates, including 19 species of aquatic insects [36] and on the carapace of 17 dead aquatic plankton crustacean species [37]. *Pythium thalassium* was first described by Atkins [38] in salty estuary water in North Cornwall in Great Britain as a parasite and saprophyte of crab *Pinnotheres pisum* eggs. We observed its growth in Akcent Pond and Komosa Lake on hairs of several species of mammals [39]. Generally, the species included in the genus *Pythium* are better known as parasites of root systems of wild and cultivated plants [40], rather than animals. As for fish, the occurrence of fungi of the genus *Pythium* has been mentioned since the mid '70s of the previous century [41], without giving their species affiliation [29,30,42,43]. Our study on eggs of fishes of northeastern Poland [44] revealed 16 species of the genus *Pythium*, including *Pythium torulosum*.

Of the 7 zoospore fungus species known in literature as fish parasites and growing on eggs of *Coregonus lavaretus holsatus* worth note are four, namely *Achlya americana*, *Saprolegnia ferax*, *Saprolegnia parasitica* and *Saprolegnia shikotsuensis*, known to cause great losses in fish farms, both in ponds and river-lakes. The parasitic nature of *Achlya americana* was reported by Scott and Warren [41] for tropical fish. Its growth was observed by El-Sharouny and Badran [45] on specimens of two species of *Tilapia* in Egypt. In Polish waters, *Achlya americana* was found to grow on muscles of *Neogobius gymnotrachelus*, *Perccottus glenii*, and *Pseudorasbora parva* introduced to Polish waters [46]. *Saprolegnia ferax* and *Saprolegnia parasitica* are species which most frequently cause saprolegniosis of eggs in hatcheries [47,48], of fry [49], and grown-up fish [50,51]. These two fungus species are most frequently encountered not only on eggs of salmonid fish [52,53], but also on eggs of other fish species [54,55], and lampreys [56]. *Saprolegnia shikotsuensis* was first described in Japan from the salmon *Oncorhynchus nerka* var. *adonis* caught in Lake Shikotsu on Hokkaido [57]. We found it on eggs of various fish species, including two forms of *Coregonus lavaretus*, *generosus* and *maraena*.

In conclusion, the number of zoospore fungus species growing on the eggs of *Coregonus lavaretus holsatus* from Lake Wdzydze in Kaszuby, particularly fish parasites is same as on the eggs of *Coregonus lavaretus generosus*, but smaller than on eggs of the two other forms - *Coregonus lavaretus lavaretus* and *Coregonus lavaretus maraena*.

Acknowledgements

The authors wishes to thank Professor L. Żmudziński from the Pomeranian Pedagogical University of Słupsk for help in obtaining the investigated materials.

References

1. KULMATYCKI W. Studien an Coregonen Polens. Arch. Hydrobiol. Ryb. **1**, 275, **1926**.
2. SZCZERBOWSKI J.A. (ed.). Rybactwo śródlądowe [Inland fisheries]. IRS, Olsztyn, **1993**. (In Polish).
3. THIENEMANN A. Unterschiede zwischen der grossen Maräne des Madüses und Selentersees. Zool. Anz. **48**, 97, **1916**.
4. GAŚOWSKA M. Analytic survey of Coregonids of three Polish lakes: Miedwie, Pełcz and Wielkie Okonińskie. Annal. Zool. **24**, 343, **1967**.
5. GAŚOWSKA M. Genus *Coregonus* L. discussed in connection with a new systematic feature that of shape and proportion of os maxillare and os supramaxillare. Annal. Zool. **18**, 471, **1960**.
6. HEESE T. Whitefish, *Coregonus lavaretus* L. (1758) of Polish water bodies. I. Systematics. Prz. Zool. **34**, 291, **1990**.
7. HEESE T. Systematic of Polish populations of European whitefish, *Coregonus lavaretus* (L.), based on skull osteology. Pol. Arch. Hydrobiol. **39**, 491, **1992**.
8. ZAWISZA J., KAPIŃSKA-WALUŚ B. Wzrost ryb w jeziorze Wdzydze [Growth of fishes in Lake Wdzydze]. Roczn. Nauk Roln. **93D**, 163, **1961**. (In Polish).
9. SZCZERBOWSKI J.A. Odżywianie się siei (*Coregonus lavaretus* L.) w jeziorach o różnym charakterze limnologicznym [Nutrition of whitefish (*Coregonus lavaretus* L.) in different limnological character lakes]. Roczn. Nauk Roln. **90H**, 689, **1969**. (In Polish).
10. SZCZERBOWSKI J.A. Biologiczne podstawy produkcji siei w jeziorach [Biological bases production of whitefish in lakes]. Wyd. IRS Olsztyn, **46**, 1, **1971**. (In Polish).
11. CZECZUGA B., MUSZYŃSKA E. Aquatic fungi growing on coregonid fish eggs. Acta Hydrobiol. **40**, 239, **1998**.
12. CZECZUGA B., PIETRUCHA M., MUSZYŃSKA E. Zoospore fungi growing on the eggs of *Coregonus lavaretus maraena* (Bloch, 1779) from Lake Miedwie in Pomerania. Acta Ichthyol. Piscat. **31**, 141, **2001**.
13. CZECZUGA B., KIZIEWICZ B., MUSZYŃSKA E. Grzyby zoosporowe rozwijające się na ikrze siei z jeziora Gołdopiwo na Mazurach. [Zoospore fungus species growing on eggs of whitefish from Lake Gołdopiwo in Mazury.] Med. Wet. **60**, 379, **2004** (In Polish).
14. GREENBERG A.E., CLESCERI L.S., EATON A.D. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington **1995**.
15. SEYMOUR R.L., FULLER M.S. Colletion and isolation of water molds (Saprolegniaceae) from water and soil. In : Fuller M.S., Jaworski A. (eds.) Zoospore fungi in teaching and research. Southeastern Publishing, Athens 125, **1987**.
16. JOHNSON T.W. jr, The genus *Achlya* : Morphology and taxonomy. The University of Michigan Press, Ann Arbor, Michigan **1956**.
17. SPARROW F.K. Aquatic Phycomycetes. The University of Michigan Press, Ann Arbor, Michigan **1960**.
18. SEYMOUR R.L. The genus *Saprolegnia*. Nowa Hedwigia, **19**, 1, **1970**.
19. BATKO A. Zarys hydromikologii. [Aquatic mycology - an overview.] PWN, Warszawa **1975**. (In Polish).
20. DICK M.W. Keys of *Pythium*. College Estate Management Whiteknights, Reading, U.K. **1990**.
21. WATANABE T. Pictorial atlas of soil and seed fungi : morphologies of cultured fungi and key to species. Second edition. CRC Press, Boca Raton, Florida **2002**.
22. IWAMA G.K., PICKERING A.D., SUMPTER J.P.,

- SCHREK C.B. Fish stress and health in aquaculture. Cambridge Univ. Press, Cambridge 1997.
23. CZECZUGA B., MUSZYŃSKA E. Aquatic fungi growing on the eggs of fishes representing 33 cyprinid (Cyprinidae) in laboratory conditions. *Acta Ichthyol. Piscat.* **29**, 53, 1999.
 24. CZECZUGA B., MUSZYŃSKA E. Growth of zoosporic fungi of the eggs of North Pacific salmon of the genus *Oncorhynchus* in laboratory conditions. *Acta Ichthyol. Piscat.* **26** (1), 25, 1996.
 25. CZECZUGA B., MUSZYŃSKA E. Aquatic fungi growing on the eggs of some anadromous fish species of the family Clupeidae. *Acta Ichthyol. Piscat.* **27**, 83, 1997.
 26. CZECZUGA B., MUSZYŃSKA E. Aquatic fungi growing on the eggs of various fish families. *Acta Hydrobiol.*, **41**, 235, 1999.
 27. CZECZUGA B., MUSZYŃSKA E. Aquatic fungi growing on percid fish eggs (Percidae) in Poland. *Pol. J. Envir. Stud.* **8**, 31, 1999.
 28. KITANCHAROEN N., HATAI K. *Aphanomyces frigidophilus* sp. nov. from eggs of Japanese charr, *Salvelinus leucomaenis*. *Mycoscience* **38**, 135, 1997.
 29. KITANCHAROEN N., HATAI K., YAMAMOTO A. Aquatic fungi developing on eggs of salmonids. *J. Aquat. Anim. Health* **9**, 314, 1997.
 30. KITANCHAROEN N., HATAI K. Some biochemical characteristics of fungi isolated from salmonid eggs. *Mycoscience* **39**, 249, 1998.
 31. HUSSEIN MORTADA M.A., HATAI K. *Saprolegnia salmonis* sp. nov. isolated from sockeye salmon, *Oncorhynchus nerka*. *Mycoscience* **40**, 387, 1999.
 32. HUSSEIN MORTADA M.A., HATAI K., NOMURA T. Saprolegniosis in salmonids and their eggs in Japan. *J. Wild. Dis.* **37**, 204, 2001.
 33. HUSSEIN MORTADA M.A., HATAI K. Pathogenicity of *Saprolegnia* species associated with out breaks of salmonid saprolegniosis in Japan. *Fish. Sci.* **68**, 1067, 2002.
 34. HÖHNK W. Studien zur Brack- und Seewassermykologie. 3. Oomycetes Zweiter Teil. Veröffentl. Inst. Meeresf. in Bremerhaven **2**, 52, 1953.
 35. CZECZUGA B., SNARSKA A. *Pythium* species in 13 various types of water bodies of north-eastern Poland. *Acta Soc. Bot. Pol.*, **70** (1), 61, 2001.
 36. CZECZUGA B., GODLEWSKA A. Aquatic insects as vectors of aquatic zoosporic fungi parasitic on fishes. *Acta Ichthyol. Piscat.* **31**, 87, 2001.
 37. CZECZUGA B., KOZŁOWSKA M., GODLEWSKA A. Zoosporic aquatic fungi growing on dead specimens of 29 fresh water crustacean species. *Limnologica* **30**, 180, 2002.
 38. ATKINS D. *Pythium thalassium* sp. nov. infecting the egg – mass of the pea – crab, *Pinnotheres pisum*. *Trans. Brit. Mycol. Soc.* **38** (4), 31, 1935.
 39. CZECZUGA B., MUSZYŃSKA E. Aquatic fungi growing on the hair of wild and domestic animal species in diverse water bodies. *Pol. J. Envir. Stud.* **10**(5), 313, 2001.
 40. YU Y., MA G. Z. The genus *Pythium* China. *Mycosystema* **2**, 1, 1989.
 41. SCOTT W.W., WARREN C.O. Studies on the host range and chemical control of fungi associated with diseased tropical fish. *Agric. exp. Stn., Blackburg. Tech. Bull.* **171**, 1, 1964.
 42. SRIVASTAVA R.C. Studies in fish – mycopathology – a review. *Mykosen* **23**, 325, 1980.
 43. DILER O. *Pythium* spp. on infected rainbow trout eggs and fry. *Ir. J. Biol.* **19**, 317, 1995.
 44. CZECZUGA B. Species of *Pythium* isolated from eggs of freshwater fish. *Acta Mycol.* **31**(2), 151, 1996.
 45. EL-SHAROUNY H.M., BADRAN R.A.M. Experimental transmission and pathogenicity of some zoosporic fungi to *Tilapia* fish. *Mycopathologia* **132**(2), 95, 1995.
 46. CZECZUGA B., KIZIEWICZ B., DANILKIEWICZ Z. Zoosporic fungi growing on the specimens of certain fish species recently introduced to Polish waters. *Acta Ichthyol. Piscat.* **32**(2), 117, 2002.
 47. HATAI K., WILLOUGHBY L.G., BEAKES G.W. Some characteristics of *Saprolegnia* obtained from fish hatcheries in Japan. *Mycol. Res.* **94**, 182, 1990.
 48. CZECZUGA B., WORONOWICZ L. Aquatic fungi developing on the eggs of certain fresh-water fish species and their environment. *Acta Ichthyol. Piscat.* **23**, 39, 1993.
 49. SATI S.C., MER G.S., KHULBE R.D. Studies on parasitic water molds: some new host records for *Saprolegnia parasitica* Coker. *Mycosen* **25**(11), 638, 1982.
 50. MENG H.J. Über die Urachen von Saprolegniosen in sekwizerischen Gewässern. *Eidgnoss. Techn. Hochschule, Zurich* 1980.
 51. HATAI K., HOSHIAI G. Mass mortality in cultured coho salmon (*Oncorhynchus kisutch*) due to *Saprolegnia parasitica* Coker. *J. Wild. Dis.* **28**, 532, 1992.
 52. MUELLER G.J.(ed.). Salmon Saprolegniasis. Bonneville Power Administration, Portland. 1994.
 53. CZECZUGA B., MUSZYŃSKA E., TRYGGVADOTTIR S.V. Aquatic fungi growing on the eggs of nine salmonid species of the genus *Hucho*, *Salmo* and *Salvelinus*. *Acta Ichthyol. Piscat.* **26**(2), 113, 1996.
 54. SCHÄPERCLAUS W. Fish Diseases. Oxonlan, New Delhi 1991.
 55. NOGA E.J. Fish Disease: Diagnosis and Treatment. Mosby Yearbook, St. Louis, Missouri 1996.
 56. CZECZUGA B. Aquatic fungi growing on lamprey eggs (Petromyzontidae). *Bull. Lampetra* **3**, 7, 1997.
 57. HATAI K., EGUSA S., AWAKURA T. *Saprolegnia shikotsuensis* sp. nov. isolated from kokane salmon associated with fish saprolegniasis. *Fish Pathol.* **12**(2), 105, 1977.