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Original Communication

Aquatic fungi developing on eggs of Chinook salmon *Oncorhynchus tshawytscha* and some of their biochemical characteristics

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ABSTRACT

The authors investigated the growth of hydromycoflora on the eggs of Chinook salmon (*Oncorhynchus tshawytscha*) from Małki hatchery on Bystraja River, Kamchatka, Russia. Twenty two straminipilous organisms (fungus-like organisms) were found on eggs of females of Chinook salmon. The most commonly encountered species on the Chinook salmon eggs were *Saprolegnia parasitica*, *S. salmonis*, *S. ferax*, *S. diclina*, *S. hypogyna*, *Aphanomyces frigidophilus*, and *Dictyunchus monosporus*. However, such species as *Scoliolegnia asterophora*, *Leptomitus lacteus*, *Pythium diclinum* and *P. monospermum* are rare to salmonid fish. Amino acid, carbohydrate and urease tests were used. All analysed species from genus *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* assimilated alanine, glucose and starch. They did not assimilate glycine, leucine, lysine, ornithine, arabinose and salicin. Urease was assimilated only by specimens from *Leptolegnia*, *Pythium* and *Saprolegnia* genus.

KEYWORDS: Chinook salmon, *Oncorhynchus tshawytscha*, eggs, hydromycoflora, straminipilous organisms

INTRODUCTION

Water pollution and intensive fishing are the main causes of the drop in population of many fish species, including those of anadromous salmonids. Thus, the natural reproduction places of those species are shrinking. As a result, from year to year, fish farms in which artificial reproduction takes place become more and more important. Economically important fish species such as Chinook salmon [1] that provide valuable proteins are mainly faced with this situation. Salmon species belonging to the *Oncorhynchus* genus, which has not had a big population in recent years [2], enter the rivers of the Far East, Alaska, Canada, and the west coast of the USA for spawning. More than 20% of salmon from the *Oncorhynchus* genus population enter the rivers of Kamchatka for spawning [3], and nowadays, the smallest population entering the rivers of Kamchatka for spawning is the Chinook salmon [4, 5]. As a result, about one million Chinook salmon fry are released from the Małki hatchery every year. Of the six species of Pacific salmon, Chinook salmon grows to the largest size, and its meat is very tasty; hence, it is often called king salmon [6]. Studies on the aquatic fungi growing on eggs of the *Oncorhynchus* species were performed by Taylor and Bailey [7], Czeczuga and Muszyńska [8], and Kitancharoen *et al.* [9]. Only Czeczuga and Muszyńska [8] mentioned an infection in the eggs of *Oncorhynchus tshawytscha.* In many countries, saprolegniasis is the most problematic fungal infection of cultured freshwater salmonid fish [10]. Some species of straminipilous organisms occur most often in wild and farmed mature salmonids and their eggs, and are responsible for broad economic impacts.

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As such, the search for new remedies to prevent economic losses caused by fungi is still ongoing [11, 12]. We have determined the fish parasite fungus species that grow on the eggs of Chinook salmon species, whose population has been dropping very rapidly in recent years.

MATERIAL AND METHODS

We investigated infected eggs of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), which were obtained from the Małki hatchery on the Bystraya River in south Kamchatka, Russia. For examination, 325 eggs covered with fungal mycelia were collected from the hatching trays and placed in a thermos flask with physiological solution, and then transported by airmail.

The fungi were isolated according to the procedures of Kitancharoen *et al.* [9]. The egg samples were washed several times in sterilized well water, and egg membranes covered by fungal mycelia were separated. The egg membranes were placed in 30-40 mL sterilized well water in petri dishes and incubated at a temperature close to that of the water at the hatchery of origin (10-15ºC) for 2-3 days, to induce zoospore formation. Simultaneously, autoclave- sterilized hemp seeds (*Cannabis sativa*) were added to the petri dishes. The number of zoospores was estimated on an inverted microscope. The germination type of zoospores was selected and classified according to Yuasa *et al.* [13]. Samples of 0.5 mL of the selected dilutions were spread on individual glucose-yeast extract (GY: 1% glucose from Hoffman-La Roche Co., Basel, Switzerland; 0.25% yeast extract from Difco Laboratories, Detroit, MI; and 1.5% agar from Hoffman-La Roche Co.) agar plates, air-dried, and incubated at 10-15ºC for 24 h. To prevent the growth of bacteria, ampicillin and streptomycin (Sigma Chemical Co., St. Louis, MO), in concentrations of 100 µg/mL each, were applied to the selected dilation. Bacteria-free germinating thalli from single spores were then transferred to fresh GY agar, and 20-30 thalli from single spores were collected to determine the fungal species.

Forming zoospores, antheridia, and oogonia of aquatic straminipilous organisms growing on the eggs were recorded. The methods used are those described in detail by Seymour and Fuller [14] and Willoughby [15].

Carbohydrate, urease, and amino acid tests were performed on the genera *Achlya*, *Aphanomyces, Leptolegnia, Pythium,* and *Saprolegnia,* according to Yuasa and Hatai [16]. The details of those methods are described in our previous paper [17].

The straminipilous organisms were identified using the following keys: Johnson [18], Seymour [19], Johnson *et al.* [20], and Pystina [21], and according to the works of the authors who were first to describe the respective species. *Aphanomyces frigidophilus* was identified according to Kitancharoen and Hatai [22], *Saprolegnia parasitica* was identified from bundles of long hairs on secondary cysts and their indirect germination [15], *S. salmonis* was identified according to Hussein and Hatai [23], and *S. shikotsuensis* was identified according to Hatai *et al.* [24]. The systematics of straminipilous organisms was used following the work of Dick [25].

RESULTS

Twenty-two straminipilous organisms, including 19 belonging to Saprolegniales, two to Pythiales, and one to Leptomitales, were found growing on the eggs of *Oncorhynchus tshawytscha* (Table 1). The *Achlya* and *Saprolegnia* genera were the most common. The most commonly encountered species on the Chinook salmon eggs were *Saprolegnia parasitica* (124 eggs, 38.2%), *Saprolegnia salmonis* (121 eggs, 37.2%), *Saprolegnia ferax* (109 eggs, 33.5%), *Saprolegnia diclina* (91 eggs, 28.0%), *Saprolegnia hypogyna* (82 eggs, 25.2%), *Aphanomyces frigidophilus* (74 eggs, 22.8%), and *Dictyuchus monosporus* (72 eggs, 22.2%). Other species, such as *Scoliolegnia asterophora*, *Isoachlya anisospora*, *Dictyuchus sterilis*, and *Pythium diclinum* were also observed on some eggs. However, *Scoliolegnia asterphora* (syn. *Saprolegnia asterophora* de Bary), *Leptomitus lacteus, Pythium diclinum* (syn. *Pythium gracile* Schenk), and *Pythium monospermum* are rare to salmonid fish. The results of amino acid, carbohydrate, and urease utilization are shown in Table 2. All stated specimens from the genera *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium*, and *Saprolegnia* assimilated alanine, glucose, and starch, but they did not assimilate glycine, leucine, lysine, ornithine, arabinose, or salicin. Urease was assimilated only by specimens from the *Leptolegnia*, *Pythium*, and *Saprolegnia* genera.

Table 1. Straminipilous organisms obtained from Chinook salmon eggs (n = 325 eggs).

Taxa	On eggs	
	Number	**%**
Straminipila		
Peronosporomycetes		
Leptomitales		
1. *Leptomitus lacteus* (Roth) C. Agardh	24	7.4
Pythiales		
2. *Pythium diclinum* Tokun.	19	5.8
3. *P. monospermum* Pringsh.	62	10.1
Saprolegniales		
4. *Achlya caroliniana* Coker	24	7.4
5. *A. dubia* Coker	32	9.8
6. *A. klebsiana* Pieters.	41	12.6
7. *A. radiosa* Maurizio	27	8.3
8. *A. treleaseana* (Humphrey) Kauffman	37	11.4
9. *Aphanomyces frigidophilus* Kitanch. et Hatai	74	22.8
10. *A. laevis* de Bary	37	11.4
11. *Dictyuchus monosporus* Leitgeb	72	22.2
12. *D. sterilis* Coker	14	4.3
13. *Isoachlya anisospora* (de Bary) Coker	12	3.7
14. *Leptolegnia caudata* de Bary	24	7.4
15. *Saprolegnia australis* Elliott	32	9.8
16. *S. diclina* Humphrey	91	28.0
17. *S. ferax* (Gruith.) Thur.	109	33.5
18. *S. hypogyna* (Pringsh.) de Bary	82	25.2
19. *S. parasitica* Coker	124	38.2
20. *S. salmonis* Hussein et Hatai	121	37.2
21. *S, shikotsuensis* Hatai *et al.*	28	8.6
22. *Scoliolegnia asterophora* (de Bary) M. W. Dick	7	2.2

DISCUSSION

The straminipilous organisms found on fish eggs are mostly representatives of Saprolegniales, especially species from the *Achlya*, *Saprolegnia*, and *Aphanomyces* genera [10]. The most frequently found mycota on the eggs of Chinook salmon include species of the genus *Saprolegnia*, such as *S. parasitica*, *S. salmonis*, *S. ferax*, *S. diclina*, and *S. hypogyna*. These species are commonly encountered in salmonidae [26], with the exception of *S. salmonis*, and were first described in Japan, on the eggs of sockeye salmon, *Oncorhynchus nerka* [23]. The most frequently encountered organisms also include

Table 2. Amino acids, carbohydrate and urease assimilation by aquatic fungi isolated from Chinook salmon eggs.

Species of genus	Amino acids	Carbohydrate	Urease
Achlya	Asp, Glu, Arg, Ala	Fru, Glu, Man, Raf, Suc, Mal, Lac, Mel, Cel, Tre, Sta, Dex, Rha, Gly	-
Aphanomyces	Glu, Ala, Cys	Glu, Sta	-
Leptolegnia	Asp, Glu, Ala	Fru, Glu, Man, Mal, Mel, Cel, Tre, Sta, Dex, Gly	+
Pythium	Ala, His	Fru, Glu, Man, Gal, Raf, Suc, Mal, Lac, Mel, Cel, Tre, Sta, Dex, Rha, Gly	+
Saprolegnia	Asp, Glu, Arg, Ala, His	Fru, Glu, Man, Mal, Cel, Tre, Sta, Dex, Gly	+

Abbreviations: Amino acids = Ala - Alanine, Arg - Arginine, Asp - Aspargine, Cys - Cysteine, Glu - glutamine, His - Histidine; Carbohydrate = Fru - Fructose, Gal - Galactose, Glu - Glucose, Man - Mannose, Mal - Maltose, Mel - Melibiose, Cel - Cellobiose, Dex - Dextrin, Gly - Glycerol, Lac - Lactose, Rha - Rhamnose, Raf - Raffinose, Sta - Starch, Suc - Sucrose, Tre - Trehalose. + positive; - negative.

Aphanomyces frigidophilus and *Dictyuchus monosporus*. *Aphanomyces frigidophilus* was first detected on the eggs of Japanese char, *Salvelinus leucomaenis* [22]. In addition, *Dictyuchus monosporus* has been observed on eggs of lamprey [27], coregonid [28], and cyprinid taxa [29], as well as other fish families [30].

Also worth noting is the occurrence of such straminipilous organisms as *Scoliolegnia asterophora*, *Leptomitus lacteus*, *Pythium diclinum*, and *Pythium monospermum* on the eggs of Chinook salmon. According to Seymour [19], *Scoliolegnia asterophora* is found in freshwater and acid soil habitats. This species is a facultative parasite of algae [31]. Its growth also has been noticed on dead fish [32]. In our previous studies, we observed it on the eggs of rainbow trout (*Oncorhynchus mykiss*) [8]. *Leptomitus lacteus*, commonly known as a sewage mycotal organism, was found to grow on only 24 of Chinook salmon eggs. This straminipilous organism has been observed on the eggs of cyprinid taxa [29], other fish families [30], and lamprey eggs [27]. *Leptomitus lacteus* has also been found on eggs of Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) [33, 34]. *Pythium diclinum* is known as a parasite of aquatic algae [21], especially green algae [31]. We have also observed its growth on the eggs of crucian carp (*Carassius carassius*) [28] and on the skin of piranha *Pygocentrus nattereri* [35]. It should be noted that Sati and Khulbe [36] observed the growth of *Pythium diclinum* as a parasite on the eggs of a few fish species in India. *Pythium monospermum* is known as a phyto- and zoosaprotroph [37]. This species has also been isolated in nematodes [38]. *Pythium monospermum* has been observed on the eggs of rainbow trout in Japan [9] and Atlantic salmon in Poland [34].

Studies on aquatic fungi growing on the species of the genus *Oncorhynchus* concern mainly young and adult individuals. Kolgajev and Ivanova [39] mentioned an infection in embryos of chum salmon (*Oncorhynchus keta* var. *autumnalis*), while Hatai and Egusa [40] described saprolegniasis in the young amago salmon (*Oncorhynchus rhodorus*). Studies on mycotic infections in adult individuals of the North Pacific salmon were conducted prior to those conducted in the young. Rucker [41] found *Saprolegnia* sp. in *Oncorhynchus* sp. individuals, now included in the species *Saprolegnia parasitica* [42]. McKay [43] observed *Saprolegnia diclina* parasitizing coho salmon (*Oncorhynchus kisutch*) individuals. Neish [44, 45] investigated saprolegniasis in adult kokanee salmon (*Oncorhynchus nerka* var. *adonis*) individuals, and those studies were linked with the occurrence of *Saprolegnia parasitica*. Hatai and Hoshiai [46] studied saprolegniasis in coho salmon, demonstrating heavy losses caused by *Saprolegnia parasitica* in the breeding of this species, up to 50%. Also, in northwest USA, *Saprolegnia parasitica* is a major cause of dwindling wild Chinook and steelhead salmon (*Salmo gairdneri*)

populations [47]. Hatai *et al.* [48] revealed the growth of *Saprolegnia australis* on rainbow trout individuals and a new species - *Saprolegnia shikotsuensis* - on kokanee salmon individuals [24]. Marchenko [49] investigated anamorphic fungi that caused mycosis of pink salmon (*Oncorhynchus gorbuscha*) and chum salmon on fish farms in Sakhalin. Data regarding mycosis of the float in individuals of certain species of the genus *Oncorhynchus* can be found in the works of Hatai and Egusa [24], Miyazaki *et al.* [50], Tashiro *et al.* [51], and Lartzeva [52]. Hussein and Hatai [23] isolated *Saprolegnia salmonis* as a new species from cultured kokanee salmon in Hokkaido, Japan. Hatai and Egusa [53] isolated *Candida sake* from amago salmon specimens, and Czeczuga and Muszyńska [8] revealed the presence of *Candida albicans* on the eggs of masu salmon.

Of great significance are the environmental conditions of a respective watercourse during the spawning period and growth of salmonid juveniles before they migrate to the sea, and the degree to which stressogenic factors [54] affect both reproduction and the growth of fish. For Chinook salmon species, the stressogenic factors include the influence of hydrology and waterway distance in a large river [55], bioaccumulation of polychlorinated biphenyls [56, 57], food availability and quality [58], bacterial infections [59, 60], amoebic organism representatives of the *Cochliopodia* spp. genus [61], and myxosporean parasite *Ceratomyxa shasta* [62, 63]. Stressogenic factors in representatives of *Oncorhynchus* species not only cause changes in cortisol concentration [64], lower body weight growth [65], and flesh color [66], but they also have an influence on the quality of the gametes. According to Campbell *et al.* [67], stress reduces the quality of gametes produced by rainbow trout. The stressed representatives produce fewer gametes, which are smaller and contain fewer carotenoids [68]. Therefore, in fish aquacultures, especially those of the *Oncorhynchus* species, the most valuable are the populations that tolerate stressogenic factors well [54] and are immune to viral and bacterial infections [69, 70], as well as mycotic ones [71].

Anthropogenic pollution of the waters, especially chloro-organic compounds, also causes reproductive disturbances in salmon fish species. The first reported observation of mass mortality in Atlantic salmon larvae during the transition period from yolk sac feeding to active feeding (called M74 syndrome) was made in 1974 at the Miljö hatchery, located on the Mörum River in southern Sweden. Those specimens were gray in color due to skin discoloration, their livers had numerous vacuoles and low glycogen levels, and many other histopathological symptoms were noted [72]. Both the eggs and muscles of salmon females with M74 syndrome have low levels of astaxanthin [73]. In addition, the eggs of females with M74 syndrome are yellow in color, due to elevated levels of yellow carotenoids and reduced levels of red carotenoids [68, 74]. Yolk sac fry mortality syndrome, which is similar to M74, has also been described in coho salmon, Chinook salmon, rainbow trout, brown trout, and lake trout (*Salvelinus namaycush*) in the Great Lakes in North America [75]. This is known in the literature as early mortality syndrome (EMS).

The biochemical characteristics of fungal species belonging to Saprolegniaceae were reported for the first time by Wolf [76]. He found that *Achlya bisexualis* has a strong ability to hydrolyze peptone, which was comparable to *Saprolegnia ferax*. The effect of various carbon sources on the growth and sexual reproduction of *Aphanomyces euteiches* was investigated by Papavizas and Ayer [77]. Gleason *et al.* [78] tested carbohydrates for Saprolegniales and described that *Achlya ambisexualis* exhibited more rapid growth on the medium with sucrose than the species of the *Saprolegnia*, *Dictyuchus*, and *Leptolegnia* genera did. Biochemical tests have been used to identify and classify groups of fungi [79, 80], especially oomycetes [81], which specifically utilize carbohydrates or amino acids, and in that manner, produce sources (such as carbon or nitrogen) for enzymatic activity [82]. As reported by Schreurs *et al.* [83], amino acids such as phenylalanine, methionine, arginine, and glutamine induce growth and branching of the mycelium in *Achlya* species. The chemotaxis-exhibiting zoospores found in water [84] are especially sensitive to asparagine and glutamine [85]; these two amino acids are predominant in fish tissues and eggs.

In the biology of fungi, especially in the nutrition process, the enzymes that they produce play a

significant role [86]. Most of these enzymes are the same for the species of the genera *Achlya*, *Dictyuchus*, and *Saprolegnia*, but some groups are different for some species [87]. In phytosaprophytic species, the enzymes of the cellulase and pectinase groups mainly break down vegetable cell walls [88], while in zoosaprophytic and parasitic species, proteolytic enzymes of the proteinase group decompose animal cells [89]. In this context, it appears likely that the same fungus species of *Achlya*, *Saprolegnia*, *Leptolegnia*, *Dictyuchus*, *Pythium*, and other genera can grow on both vegetable and animal substrates [90, 91]. Lipases and alkaline phosphatases have been found in the mycelia of *Saprolegnia diclina* isolated from the sheet of brook char eggs (*Salvelinus fontinalis*) [82]. In addition, all species of fungi found on Chinook salmon eggs were often found on vegetable substrates [92, 93].

Our experimental data of the biochemical characteristics of fungi isolated from Chinook salmon eggs are similar to data for the other species of the *Oncorhynchus* genus [9, 16, 17].

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