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Aquatic fungi developing on eggs of six sturgeon species from Far East

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ABSTRACT

In this study, straminipilous (fungus-like) organisms growing on the eggs of *Acipenser schrenckii*, *Acipenser sinensis*, *Acipenser medirostris*, *Huso dauricus*, *Acipenser mikadoi* and *Acipenser baerii*, in water bodies of different trophic levels, were investigated. A total of 54 species were recorded, with the largest number of species occurring on eggs in the water from the Biała River (very biogenic), and the smallest number on eggs in the water from the Supraśl River (poorly biogenic). *Achlya polyandra*, *Aphanomyces irregularis*, *Saprolegnia ferax*, *Saprolegnia parasitica*, *Leptomitus lacteus* and *Pythium diclinum* were found on the eggs of all of the investigated species of sturgeon fish. However, *Aphanomyces frigidophilus*, *Aphanomyces invadans*, *Aphanomyces piscicida*, *Dictyuchus pisci*, *Saprolegnia bhargavae*, *Saprolegnia polymorpha* and *Saprolegnia salmonis* were observed rarely in Polish waters. Amino acid, carbohydrate and urease tests were performed on these particular genera of fungi.

KEYWORDS: sturgeons, eggs, aquatic fungi, hydrochemistry

INTRODUCTION

Water pollution and intensive fishing are the main causes leading to a drop in the populations of many fish species, including anadromous sturgeons. The natural reproduction areas of these species are shrinking, and therefore fish farms, where artificial reproduction takes place, are gaining importance. The economically significant fish species are the ones that are mainly vulnerable to this situation. In many countries, saprolegniasis is the most problematic fungal infection of sturgeonids cultured in freshwater [1]. Some of the species of straminipilous organisms occurring most often in mature sturgeonids and their eggs (wild and farmed) have broad economic impacts. These organisms have also been observed on the eggs of many other economically important fish species, such as salmonids [2]. Sturgeons are related to this group of fishes. Straminipilous organisms have been isolated from the eggs of sturgeonid species from the Caspian Sea [3] and Black Sea basins [4], and are already described in the literature. This paper describes the fungal diseases of the eggs of six species of sturgeons from the Far East.

MATERIALS AND METHODS

This investigation included the eggs of the following sturgeon species, during their spawning period:

- Amur sturgeon, *Acipenser schrenckii* (Brandt, 1869). Collected from the Amur River near Khabarovsk (Khabarovsk krai, Russia). The Amur sturgeon is an anadromous and endemic species of the Amur River system. It occurs in two morphs: brown and grey. The adults occur in stretches of the river with a sandy or stony bottom, and feed on benthic organisms. The maximum length is 300 cm, maximum

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weight is 190 kg and the length at maturity ranges from 96-125 cm [5, 6].

- Chinese sturgeon, *Acipenser sinensis* (Gray, 1835). Collected from the Pearl River. This is an anadromous species, occurring in the Northwestern Pacific in China and Japan (Sagami Sea), Korea and the Chinese Sea. The maximum length is 130 cm and the maximum weight is 600 kg [6, 7].
- Green sturgeon, *Acipenser medirostris* (Ayres, 1854). Collected from the Komandory Islands (Russia) near the Aleutian Islands (USA). This is an anadromous species, occurring in North America from the Aleutian Islands and the Gulf of Alaska to Ensenada, Mexico. It is considered to be vulnerable in Canada, and is found in estuaries, the lower reaches of large rivers and in salty or brackish waters at the mouths of rivers. The maximum length is 250 cm, the maximum weight is 159 kg and the length at maturity is 100 cm [6, 8].
- Kaluga, *Huso dauricus* (Georgi, 1775). Collected from Amur River near Khabarovsk (Khabarovsk krai, Russia). The kaluga is an anadromous species, distributed throughout the Amur basin, ascending as far as Argun, Shilka and Onon. This species ranges from the Amur Liman to the Amur estuary in the Sea of Okhotsk. The adults also inhabit some lakes, such as the Orel and Sungari in China. Four populations have been recognized in the Amur River basin: the first is found on the leaves of the Sea of Okhotsk and Sea of Japan, the second is found in the lower Amur, the third is found in the middle Amur and the fourth is found in the lower reaches of the Zeya and Bareya rivers. The kaluga feeds on adult fishes, and its maximum length is 560 cm, maximum weight is 1000 kg and its length at maturity ranges from 170 to 190 cm [6, 9, 10].
- Sakhalin sturgeon, *Acipenser mikadoi* (Hilgendorf, 1892). Collected from the Amur River near Khabarovsk (Khabarovsk krai, Russia). It is an anadromous species, occurring in the Northwestern Pacific in the Bering Sea, Tumnin (Data) River, northern Japan and Korea. It is also commercially cultured in Japan. From April until May, adults feed in freshwater and then return to the ocean during the fall. The maximum length is 150 cm [6, 7, 11].
- Siberian sturgeon, *Acipenser baerii* (Brandt, 1869). This species was collected from the Lena River near Yakutsk (Sakha Republic, Russia). It occurs in Asia in Siberia, the Ob, Irtysh and Yenisei Rivers, Lena and Kolyma. Some non-migratory populations exist in the Irtysh River system. Spawning occurs in the main river channel over a stone-gravel or gravel-sand bottom, with a strong current [6, 9, 10, 12].

Water for these experiments was collected from three different bodies of water: the Supraśl River, Biała River and Komosa Lake. The characteristics of these bodies of water were mentioned in our previous papers [4]. According to generally accepted methods [13], 19 parameters of these water samples were determined (Table 1). The procedures used for determining the presence of fungal species on the investigated eggs were the same as those identified in previous papers [14]. The eggs were used as bait, and the fungi were isolated according to Kitancharoen *et al.* [15]. Carbohydrate, urease and amino acid tests were performed on the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Saprolegnia* and *Pythium* genera, according to Yuasa and Hatai [16] and Kitancharoen and Hatai [17]. The details of those methods are described in our previous paper [18]. *Saprolegnia parasitica* was identified from the bundles of long hairs on the secondary cysts, and during their indirect germination. The systematic identification of the straminipilous organisms was used, according to Dick [19].

RESULTS

The chemical characteristic of the water used in our experiments varied considerably in biogenic compounds (Table 1). The most abundant concentrations of all of the forms of nitrogen and phosphate were found in the water from the Biała River, and the lowest amounts were in the water from the Supraśl River. The samples also contained CO_2, sulphates, chlorides, calcium, dry residue, dissolved solids and suspended solids. Water from the Supraśl River also contained the lowest levels of BOD_5, COD, CO_2, sulphates, chlorides, magnesium, iron, dry residue, dissolved solids and suspended solids.

Fifty-two Straminipilous species, including 43 belonging to the Saprolegniales, 2 to the

Table 1. Chemical and physical properties of water in particular water bodies (in mg l^{-1}).

Specification	River Biała	River Supraśl	Pond Komosa
Temperature (°C)	17.8	17.0	17.4
pH	7.1	7.82	7.6
DO	6.4	11.2	12.8
BOD_5	7.2	2.8	7.4
Oxidability(COD)	15.82	6.60	13.2
CO_2	26.90	6.60	8.3
Alkalinity in $CaCO_3$ (mval l^{-1})	4.3	4.5	3.9
$N\text{-}NH_3$	0.621	0.142	0.161
$N\text{-}NO_2$	0.132	0.006	0.009
$N\text{-}NO_3$	0.473	0.014	0.034
$P\text{-}PO_4$	1.824	0.158	0.255
Sulphates (SO_4)	73.24	32.38	42.75
Chlorides (Cl)	66.44	17.12	23.51
Total hardness in Ca	98.26	73.42	68.40
Total hardness in Mg	17.42	11.58	28.81
Fe	0.92	0.48	1.54
Dry residue	434.0	197.0	375.0
Dissolved solids	324.0	179.0	312.0
Suspended solids	110.0	18.0	63.0

Leptomitales and 6 to Pythiales, were found growing on the investigated eggs of six sturgeon species (Table 2). Two were species belonging to the true fungi. The fewest number of species were observed on the eggs of the kaluga (7), but the remaining 5 species had twice as many zoosporic fungi (15-17 species). *Achlya polyandra*, *Aphanomyces irregularis*, *Saprolegnia ferax*, *Saprolegnia parasitica*, *Leptomitus lacteus* and *Pythium diclinum* were found on the eggs of all of the investigated sturgeonid species. Of special note was the observation of a few straminipilous species: *Aphanomyces frigidophilus*, *Aphanomyces piscicida* (both species on the eggs of the Amur sturgeon), *Aphanomyces invadans* (green sturgeon), *Dictyuchus pisci*, *Saprolegnia bhargavae* (Chinese sturgeon), *Saprolegnia polymorpha* (Siberian sturgeon) and *Saprolegnia salmonis* (Sakhalin sturgeon). These species are seen rarely in Polish hydromycology. The highest number of species of straminipilous organisms was observed on the eggs placed in the water from the Biala River (28), while the lowest number occurred in the water from the Supraśl River (15 species) (Table 3). Only six amino acids were determined to be assimilated by the investigated fungi: alanine, arginine, asparagine, cysteine, glutamine and histidine. All stated specimens of the species from the *Achlya*, *Aphanomyces*, *Leptolegni*a, *Pythium* and *Saprolegnia* genera assimilated glucose and starch, whereas the specimens from *Leptolegnia*, *Pythium* and *Saprolegnia* did not (Table 4).

DISCUSSION

Sturgeon is a migratory two- environmental fish, which only occasionally lives in one environment [9, 10]. Water pollutants and river dams form an obstacle for migratory sturgeon to reach their natural spawning grounds. Therefore, in order to maintain the proper stock of economically valuable species, including also sturgeonid species, breeding of the young was recommended in hatcheries. The literature devoted to artificial propagation of sturgeonid fishes reports considerable mortality rate of the eggs due to saprolegnia fungus infection.

Table 2. Aquatic fungi found on the eggs of sturgeon fishes.

Taxa	**Sturgeon species**					
	Amur sturgeon	**Chinese sturgeon**	**Green sturgeon**	**Kaluga**	**Sakhalin sturgeon**	**Siberian sturgeon**
Fungi						
Zygomycota						
Zygomycetes						
Zoopagales						
1. *Zoopage phanera* Drechsler						x
2. *Zoophagus insidians* Sommerst.	x					
Straminipila						
Peronosporomycetes						
Saprolegniales						
3. *Achlya ambisexualis* Raper	x					
4. *A. americana* Humphrey			x			
5. *A. androgyna* (W.Archer) T.W. Johnson & R.L. Seymour					x	
6. *A. bisexualis* Coker et Couch	x					
7. *A. caroliniana* Coker	x					
8. *A. debaryana* Humphrey					x	x
9. *A. diffusa* J.V. Harv. ex J.W. Johnson		x				
10. *A. dubia* Coker					x	
11. *A. glomerata* Coker					x	x
12. *A. hypogyna* Coker et Pemberton	x				x	
13. *A. klebsiana* Pieters						x
14. *A. megasperma* Humphrey		x				
15. *A. oligocantha* Coker						x
16. *A. orion* Coker et Couch						x
17. *A. polyandra* Hildebr.	x	x	x	x	x	x
18. *A racemosa* Hildebr.						x
19. *A. radiosa* Maurizio						x
20. *A. treleaseana* (Humphrey) Kauffman						x
21. *Aphanomyces frigidophilus* Kitan. et Hatai	x					
22. *A. invadans* Willoughby *et al.*			x			
23. *A irregularis* W.W. Scott	x	x	x	x	x	x
24. *A. piscicida* Hatai	x					
25. *Dictyuchus monosporus* Leitgeb						
26. *D. pisci* Khulbe & Sati		x				
27. *Isoachlya toruloides* Kauf. et Coker					x	
28. *Leptolegnia caudata* de Bary	x					
29. *Protoachlya paradoxa* Coker		x				
30. *P. polyspora* (Lindst.) Apinis		x				

Table 2 continued..

31. *Pythiopsis cymosa* de Bary		x				
32. *Saprolegnia anisospora* de Bary	x		x			
33. *S. australis* R. F. Elliott			x			
34. *S. bhargavae* Khulbe & B. L. Verma						x
35. *S. diclina* Humphrey	x					
36. *S. ferax* (Gruith.) Thur.	x	x	x	x	x	x
37. *S. monilifera* de Bary			x			
38. *S. monoica* Pringsh.			x			
39. *S. irregularis* T. W. Johnson & R. L.Seymour			x			
40. *S. parasitica* Coker	x	x	x	x	x	x
41. *S. polymorpha* Willoughby						x
42. *S. salmonis* Hussein et Hatai					x	
43. *S. shikotsuensis* Hatai *et al.*					x	x
44. *S. unispora* (Coker et Couch) R. L.Seymour	x					
45. *Scoliolegnia asterophora* (de Bary) M. W. Dick			x			
46. *Traustotheca clavata* (de Bary) Humphrey		x				
Leptomitales						
47. *Apodachlya pyrifera* Zopf					x	
48. *Leptomitus lacteus* (Roth.) C.Agardh	x	x	x	x	x	x
Pythiales						
49. *Pythium artotrogus* de Bary		x				
50. *P.debaryanum* R. Hesse				x		
51. *P. diclinum* Tokun.	x	x	x	x	x	x
52. *P. intermedium* de Bary			x			
53. *P. middletonii* Sparrow	x					
54. *P. ultimum* Trow		x				
Total number	18	15	15	7	15	18

Frequently, this loss amounts to 70-90% of the incubated eggs [20-22]. The literature concerning the occurrence of fungus species on the eggs of sturgeonid fishes is poor. Clinton [23] first reported the occurrence of *Saprolegnia ferax* fungus on the eggs of *Acipenser sturio*. In the past years this subject was not investigated intensively. Only, after the intensive breeding of sturgeonid fishes in artificial conditions started, and the process was affected by a great loss of eggs due to the aquatic fungi infection, the interests on saprolegniosis in the sturgeonids increased. A number of reports on the occurrence of zoosporic fungi on the incubated eggs in the basin of the Caspian Sea, referring mainly to such sturgeons as *Huso huso*, *Acipenser güldenstädti*, and *Acipenser stellatus* [cf. 20-22] have been lately issued. The fungus species occurring on eggs were investigated by Czeczuga *et al.* [3] in those species as well as in *Acipenser güldenstädti persicus*, *Acipenser mudiventris*, and *Acipenser ruthenus*. The latest investigations included the

Table 3. Aquatic fungi found on eggs in different water bodies.

Water from	Aquatic fungi (see Table 2)	Total number of species
River Biała	2,3,4,8,10,12,13,16,17,19,23,24,25,26,28,29,32,33,34,36,37,39,40,46,49,51,53,54	28*
River Supraśl	2,5,6,8,12,15,20,22,25,31,36,40,41,45,52	15*
Pond Komosa	1,7,8,9,11,12,14,16,18,21,23,25,27,30,35,36,38,40,42,43,44,48,50	23*

*Differences significant at the ≤ 0.05 level

Table 4. Amino acids, carbohydrate and urease assimilation by aquatic fungi isolated from sturgeon 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, Sal	+
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 – Froctose, Gal – Galactose, Glu – Glucose, Man – Mannose, Raf – Raffinose, Suc – Sucrose, Mal – Maltose, Lac – Lactose, Mel – Melibiose, Cel – Cellobiose, Tre – Trehalose, Sta – Starch, Dex – Dextrin, Rha – Rhamnose, Gly – Glycerol, Sal – Salicin.
+ positive; - negative.

eggs of *Acipenser oxyrhynchus*, *Acipenser persicus colchicus*, *Acipenser sturio* and *Polyodon spathula* [4].

The present study has revealed the fewest number of fungus species on the eggs of *Huso dauricus*. It was observed also in *Huso huso* species. Lartzeva [20], while studying the growth of saprolegnia fungi on the eggs of three acipenserid species, observed similar results. Similar results were observed also by Czeczuga *et al.* [3] while investigating fungi on the eggs of *Huso huso*. We observed only 7 aquatic fungus species on the eggs of *Huso dauricus*, while on the eggs of other acipenserid species 15 to 18 species of saprolegnia fungi were found. This observation was interpretated as the result of the occurrence of relatively thick sheets on the eggs in representatives of the genus *Huso* [21].

The most common fungus species found on all investigated eggs of the six acipenserid species studied include: *Achlya polyandra*, *Aphanomyces irregularis*, two species of *Saprolegnia* genus (*S. ferax*, *S. parasitica*), *Leptomitus lacteus* and *Pythium diclinum*. We observed similar results while investigating fungus species occurring on eggs of four species of acipenserid fishes from Black Sea basin [4]. Those fungus species were also common on the eggs of the acipenserid fishes from the Caspian Sea basin [3]. Those species are common on the eggs of salmonid species [24] and cyprinid taxa [25]. *Pythium diclinum* (syn. *P. gracile*) has been observed to grow as a parasite on the eggs of few fish species in India [26] and on the skin of piranha [27].

The present study has revealed the growth of some fungus species that are rarely observed on the eggs of acipenserid fishes. This group includes three species of *Aphanomyces* genus (*A. frigidophilus*, *A. invadans*, *A. piscicida*), *Dictyuchus pisci* and three species of *Saprolegnia* genus (*S. bhargavae*, *S. polymorpha*, *S. salmonis*). The species of *Aphanomyces frigidophilus* was first observed on the eggs of the Japanese charr [28], and later found on the eggs of coregonid fishes [29, 30] and salmonids [31, 32]. This straminipilous species grew also on the eggs of the paddlefish sturgeon [4]. *Aphanomyces invadans* was first described by Willoughby *et al.* [33] on freshwater tropical fishes and was later reported on the aquaticus fish *Labeo bicolor* [34] and on the eggs of Atlantic sturgeon [4]. A third rare species- *Aphanomyces piscicida* was first described by Hatai *et al.* [35] in Japan as a parasite of *Plecoglossus altivelis* fish species. We have observed this species on the muscles of *Labeo bicolor* [34] and on the eggs of Kolkhida sturgeon [4]. *Dictyuchus pisci* was first described in India [36] and was also found on the eggs of the Atlantic sturgeon [4]. *Sprolegnia bhargavae* was also first described in India [37] and was also found on the eggs of Kolkhida sturgeon [4]. *Saprolegnia polymorpha* was described for the first time by Willoughby [38] in the waters of British Isles as a parasite of koi carp. *Saprolegnia salmonis* is new to sturgeonid fishes.

It was first described in cultured sockeye salmon raised in Hokkaido, Japan [39]. Later, this fungus species was found on immature stages of rainbow trout, masu salmon, brown trout and Japanese charr [40]. In our study *Saprolegnia salmonis* was found on the eggs of *Coregonus lavaretus* [31], *Salmo salar* [32] and on muscles of the piranha [27].

Peronosporomycetes of the order Saprolegniales, especially *Saprolegnia*, *Achlya* and *Aphanomyces* species are responsible for fish infection in natural environment [41, 42], aquaculture in farms [43, 44], and hobby fish tanks [34] including all freshwater fish and in all their developmental forms. Fungal infection on fish eggs is a major economic problem regarding many freshwater species, especially economically important farmed fishes [2, 32, 45] including sturgeonid species [1, 3, 4, 20, 21].

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