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Original Communication

Zoosporic fungi and fungus-like organisms growing on the eggs of four species of sturgeonid fish (Acipenseriformes)

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ABSTRACT

The fungi and fungus-like (straminipilous) organisms growing on the eggs of *Acipenser sturio*, *A. persicus colchicus*, *A. oxyrhynchus* and *Polyodon spathula* in water of different trophy have been investigated. A total of 61 species including 56 fungus-like organisms and 5 fungal species were recorded. The largest number of species occurred on eggs in water from river Biała (more biogenic), the smallest in water from river Supraśl (poor in biogenes). *Achlya polyandra*, *Aphanomyces irregularis*, *Saprolegnia ferax*, *S. parasitica*, *Leptomitus lacteus* and *Pythium diclinum* were found on the eggs of all investigated species of sturgeon. *Aphanomyces pisci*, *Dictyuchus pisci*, *Saprolegnia bhargavae* and *Scoliolegnia subeccentrica* were noticed for the first time in Polish waters.

KEYWORDS: sturgeons, eggs, fungi, fungus-like (straminipilous) organisms, hydrochemistry

INTRODUCTION

Sturgeonid fish species which live mainly in the basins of the Black Sea and the Caspian Sea provide valuable meat and eggs, which are commonly known as caviar. Sturgeon is a migratory two-environmental fish, which occasionally lives in one environment - river or lake.

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Water pollutants and river dams form an obstacle for migratory species to reach their natural spawning grounds. Therefore, in order to maintain the proper stock of economically valuable species, breeding of juveniles has been commenced in hatcheries [1]. The literature devoted to artificial reproduction of sturgeonid fishes reports considerable mortality rate of eggs due to saprolegnia fungus infection [2-4]. Frequently, this loss reaches the amounts up to 70-90% of the incubated eggs [5, 6].

Nowadays, in some countries (including Poland), there is interest in sturgeonid fishes, which is evident from importing of eggs and hatching and breeding activities of a variety of sturgeonid fish species [7].

Thus, we have decided to publish the data concerning the development of zoosporic fungi on the eggs of four species of sturgeonid fishes from our inland waters. The zoosporic fungi growing on the eggs of six other sturgeonid species have already been published [8].

MATERIAL AND METHODS

The investigations included the eggs of the following fish species: Atlantic sturgeon (*Acipenser sturio* L.), Kolkhida sturgeon (*Acipenser persicus colchicus* V. Marti), paddlefish (*Polyodon spathula* Walbaum) and shortnose sturgeon (*Acipenser oxyrhynchus* Mitch.). The eggs of Atlantic sturgeon and Kolkhida sturgeon were obtained from hatcheries of the Georgia Fisheries and Ecology of Black Sea Institute in Bathumi, Georgia; the eggs of paddlefish came from hatcheries from the Ikrianskij Ekspierimentalnyj Osetrovyj Zavod “Goriachij Kluch”, Russia. The eggs of shortnose sturgeon were obtained from hatcheries of the Acadian Sturgeon and Caviar Inc., St. John, Canada, and have been incubated in the Institute of Inland Fisheries, Poland. The eggs were transported by air mail in thermos flask in physiological solution. Names of the investigated species were used according to Kottelat and Freyhof [9].

Water for the experiments was collected from four different water bodies: River Biała and Supraśl, Dojlidy Pond and Komosa Pond:

- River Biała, length 9.8 km, 3.7 m wide, 0.85 m depth, a left-bank tributary of the Supraśl River flowing through Białystok City.
- River Supraśl, length 93.1 km, 6.6 m wide, 1.1 m deep, the largest right-bank tributary of the middle Narew River flowing through the Knyszyńska Forest.
- Pond Dojlidy, near Białystok City, area 34.2 ha, maximum depth 2.85 m. Its south shores border with coniferous woods and its western part with the Białystok City.
- Pond Komosa, area 12.1 ha, maximum depth 2.25 m, surrounded densely by coniferous trees of the Knyszyńska Forest.

Nineteen parameters of these water samples were determined (Table 1) according to the generally accepted methods [10].

The following procedure was used while determining the presence of fungus species on the investigated eggs of particular sturgeon species. Water samples (800 ml each) were placed in 1000 ml vessel. 15-20 eggs of each sturgeon species were transferred to each vessel in accordance to general principles of culture [11]. All vessels were enclosed in Petri scales with the bed turned upside down to prevent possible airborne contamination

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

Specification	River Biała	River Supraśl	Pond Dojlidy	Pond Komosa
Temperature (°C)	17.8	17.0	18.0	17.4
pH	7.1	7.82	7.94	7.6
DO	6.4	11.2	9.4	12.8
BOD_5	7.2	2.8	9.7	7.4
Oxidability (COD)	15.82	6.60	15.74	13.2
CO_2	26.90	6.60	8.83	8.3
Alkalinity in $CaCO_3$ (mval l^{-1})	4.3	4.5	3.2	3.9
$N\text{-}NH_3$	0.621	0.142	0.168	0.161
$N\text{-}NO_2$	0.132	0.006	0.008	0.009
$N\text{-}NO_3$	0.473	0.014	0.050	0.034
$P\text{-}PO_4$	1.824	0.158	0.242	0.255
Sulphates (SO_4)	73.24	32.38	45.65	42.75
Chlorides (Cl)	66.44	17.12	25.04	23.51
Total hardness in Ca	98.26	73.42	61.87	68.40
Total hardness in Mg	17.42	11.58	12.18	28.81
Fe	0.92	0.48	0.84	1.54
Dry residue	434.0	97.0	257.0	375.0
Dissolved solids	324.0	179.0	210.0	312.0
Suspended solids	110.0	18.0	47.0	63.0

with fungal spores. The vessels were stored at 15 ± 2°C, with access to daylight resembling natural conditions and following the recommended instructions [12]. Water analyses and experiments were carried out in three parallel repetitions.

The eggs covered with fungal mycelia (from each vessel) were observed every 3-4 days under a light-microscope and the presence of morphological structures (zoospores, antheridia and oogonia) of aquatic fungi was recorded. These experiments were carried out for one month.

The fungi species were identified using the keys of Johnson [13], Seymour [14], Batko [15], Karling [16], Pystina [17] and the authors of respective studies.

In our study, the systematics of straminipilous species used were according to Dick [18], true fungi according to Blackwell *et al.* [19], and of saprolegniaceae according to Johnson *et al.* [20, 21]. The results were tested for significance with ANOVA and the results evaluated by the S-Scheffe test [22].

RESULTS

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

Sixty-one zoosporic fungus species, including 56 belonging to the Straminipila and five to the Fungi, were found growing on the eggs of investigated sturgeon species (Table 2). The fewest fungus species were isolated from the eggs of sturgeon from the Supraśl River (18 species) and the largest number from the water of Biała River (31 species). The largest number of fungus species occurred on eggs of Kolkhida sturgeon (31 species) and the fewest on shortnose sturgeon (26 species). The most common were *Achlya polyandra*, *Aphanomyces irregularis*, *Saprolegnia ferax*, *S. parasitica*, *Leptomitus lacteus* and *Pythium diclinum*. Four species *Aphanomyces pisci*, *Dictyuchus pisci*, *Saprolegnia bhargavae* and *Scoliolegnia subeccentrica* have been recorded in Poland for the first time and have grown on the eggs in the water from River Biała. Several rare species such as *Aphanomyces frigidophilus*, *Aphanomyces invadans*, *Saprolegnia polymorpha* and *Pythium hydnosporum* have also been found. The largest number of species were found in the water from River Biała and fewer in the water from the River Supraśl (Table 3).

DISCUSSION

As a result of water pollution and intensive fishing, the occurrence areas of different sturgeon species have been reduced very much during last years. Reproduction areas have been rapidly reduced. A good example can be the situation of Atlantic sturgeon. In the middle of the 20th century it was present in all European rivers for spawning and now it occurs just in two rivers in France and in river Rioni in Georgia [23-25]. Kolkhida sturgeon which occurs in whole kaukasian coast of Black Sea enters river Rioni for spawning [26, 27]. Paddlefish is now present in the Missisipi river, USA and in its tributary, whereas the shortnose sturgeon lives in the waters along the Canadian coast and along the north parts of the USA [28-30]. They are both being bred in Poland [7].

Present study has proved that growth of the respective aquatic fungus species on the sturgeon eggs depended on the water body from which the water has been collected. The largest number of species developed on eggs from water of River Biała and the fewest in River Supraśl. The water collected from those two water bodies differed in the content of the chemical compounds. Water from River Biała had considerably more biogene compounds, mainly phosphorus, whereas water from River Supraśl was very poor in this substrate. It confirms our earlier assumptions on other sturgeon species [8].

The resistance of the fish eggs to viral, bacterial and fungal infections [31-33] depends on many factors, such as the structure of the egg wall, health condition of females and carotenoid content in the eggs. Carotenoids, especially β-carotene and

Table 2. Aquatic fungi and fungus-like organisms found on the eggs of sturgeon fishes.

Taxa	Eggs of sturgeon			
	Atlantic	Kolkhida	Paddlefish	Shortnose
Straminipila (fungus-like organisms)				
Peronosporomycetes				
Saprolegniales				
1. *Achlya ambisexualis* Raper	x			x
2. *A. americana* Humphrey		x	x	
3. *A. androgyna* (W. Archer) T. W. Johnson & R .L. Seym.	x			
4. *A. bisexualis* Coker et Couch		x	x	
5. *A. caroliniana* Coker	x			x
6. *A. colorata* Pringsh.	x	x		
7. *A. debaryana* Humphrey			x	x
8. *A. diffusa* J. V. Harv. ex J. W. Johnson		x		x
9. *A. dubia* Coker	x		x	
10. *A. glomerata* Coker		x	x	x
11. *A. hypogyna* Coker et Pemberton	x	x	x	
12. *A. klebsiana* Pieters		x		x
13. *A. megasperma* Humphrey	x		x	
14. *A. oligocantha* de Bary	x	x	x	
15. *A. orion* Coker et Couch		x		x
16. *A. polyandra* Hildebr.	x	x	x	x
17. *A. racemosa* Hildebr.		x	x	
18. *A. radiosa* Maurizio	x			x
19. *A. treleaseana* (Humphrey) Kauffman		x	x	
20. *Aphanomyces frigidophilus* Kitanch. & Hatai			x	
21. *A. invadans* Willoughby *et al.*	x			
22. *A. irregularis* W.W.Scott	x	x	x	x
23. *A. keratinophilus* (M. Ookubo & Kobayashi) R. L. Seym. & T. W. Johnson			x	
24. *A. pisci* R. C. Srivast.		x		

Table 2 continued..

25. *A. piscicida* Hatai		x		
26. *Calyptralegnia achlyoides* (Coker et Couch) Coker	x			x
27. *Dictyuchus monosporus* Leitg.		x	x	
28. *D. pisci* Khulbe & Sati	x			
29. *D sterilis* Coker	x		x	
30. *Isoachlya toruloides* Kauffman et Coker	x	x		
31. *Leptolegnia caudata* de Bary			x	x
32. *Protoachlya paradoxa* Coker		x	x	
33. *P. polysporus* (Lindst.) Apinis	x			x
34. *Pythiopsis cymosa* de Bary	x	x		
35. *Saprolegnia anisospora* de Bary	x			x
36. *S. australis* R. F. Elliott	x			
37. *S. bhargavae* Khulbe & B. L. Verma		x		
38. *S. diclina* Humphrey		x		
39. *S. ferax* (Gruith.) Thur.	x	x	x	x
40. *S. monilifera* de Bary			x	
41. *S. monoica* Pringsh.				x
42. *S irregularis* T. W. Johson & R. L. Seym.	x			
43. *S. parasitica* Coker	x	x	x	x
44. *S. polymorpha* Willeughby				x
45. *S. shikotsuensis* Hatai *et al.*		x		
46. *S. unispora* (Coker et Couch) R. L. Seym.		x	x	
47. *Scoliolegnia asterophora* (de Bary) M. W. Dick	x			x
48. *S. subeccentrica* M. W. Dick			x	
Leptomitales				
49. *Apodachlya pyrifera* Zopf			x	
50. *Leptomitus lacteus* (Roth) Agar.	x	x	x	x
Pythiales				
51. *Pythium debaryanum* R. Hesse	x			x
52. *P. diclinum* Tokun.	x	x	x	x
53. *P. hydnosporum* (Mont.) J. Schröt.		x		

Table 2 continued..

54. *P. intermedium* de Bary		x		x
55. *P. middletonii* Sparrow	x	x		
56. *P. ultimum* Trow			x	x
Fungi (true)				
Ascomycota				
Saccharomycete				
Saccharomycetales				
57. *Candida albicans* (Robin) Berkhout				x
Blastocladiomycota				
Blastocladiomycetes				
Blastocladiales				
58. *Allomyces anomalus* R. Emers.				x
59. *A. arbuscula* E. J. Butler	x			
60. *Blastocladiopsis parva* (Whiffen) Sparrow		x		
Zygomycota				
Zygomycetes				
Zoopagales				
61. *Zoopage phanera* Drechsler	x		x	
Total number of species	30	31	28	26

Table 3. Number of species found on eggs at the water bodies analyzed.

Water body	Fungi and fungus-like organisms (see Table 2)	Total number of species	Exclusive of one of the water bodies	Number of species
River Biała	1,4,6,7,10,11,12,14,16,18,21,22,23, 24,25,28,29,34,35,37,38,42,43,46, 48,49,51,53,56,60,61	31[a]	1,7,14,23,24,25,28,29,34,37, 46,48,53,56,60,61	16[a]
River Supraśl	5,8,9,10,11,16,22,26,31,38,42,44, 47,49,51,52,54,57	18[b]	8,9,26,31,44,47,52,54,57	9[b]
Pond Dojlidy	2,3,4,6,10,11,13,15,16,17,18,19,20, 22,27,33,36,38,39,42,49,51,58	23[c]	2,17,20,27,33,36,39,58	8[b]
Pond Komosa	3,4,5,10,13,15,16,19,21,22,30,32,35, 38,40,41,42,43,45,49,50,51,55,59	24[c]	15,30,32,40,41,45,50,55,59	9[b]

Data in the same column (third and fifth) with the same letter index (a, b, or c) do not differ between each other significantly ($p \leq 0.05$).

astaxanthin retard the development of cancer [34]. Additionally, carotenoids play important antioxidative role [35, 36]. Hydroxycarotenoids (yellow carotenoids) and ketocarotenoids (red carotenoids) are transformed into all three forms of the vitamin A and both belong to the most common carotenoids not only among fish but also among aquatic animals. In sturgeonids eggs [37, 38], in juveniles [39], and in adult fishes [40], as well as in the currently studied fishes, they comprise a significant role among all the carotenoids and are the main source of Vitamin A in sturgeonids. As commonly known, invertebrates from Black Sea, which are the sturgeons food, contain many carotenoids (especially hydroxy-carotenoids and ketocarotenoids) [41-44]. This could explain the small amount of fungi species growing and developing on the eggs, for example, of Kolkhida sturgeon.

Parasitic and necrotroph *Aphanomyces pisci* [45] has been newly recorded in Poland in the water from the Biała River on the eggs of Kolkhida sturgeon. The water from Biała River was comparatively richest in biogenic compounds. A second representative of Straminipila, *Dictyuchus pisci,* which also grew on the eggs of the Atlantic sturgeon in water from the Biała River was first described in India [46]. *Saprolegnia bhargavae* grew on the eggs of Kolkhida sturgeon in water from the Dojlidy Pond and has been noticed as three new species in Poland. It was also first described in India [47]. The saprotroph species of *Scoliolegnia subeccentrica* [48] grew on the eggs of the paddlefish in the water from the Supraśl River, which was comparatively poor in biogenic compounds.

Besides the species which are new in Polish waters, other five were also found. *Aphanomyces frigidophilus* is a rare straminipilous species, which was observed on the eggs of the paddlefish sturgeon only in water of Dojlidy Pond. We have already found it in Polish waters on the eggs of salmonid [49, 50] and coregonid fishes [51, 52]. It has been also detected on the eggs of the Japanese charr *Salvelinus leucomaenis* [53]. *Aphanomyces invadans* has been found on the eggs of the Atlantic sturgeon in the water from River Biała and was first described by the Willoughby *et al.* [54] as a tropical freshwater fish pathogen causing epizooties. The latter was reported by Czeczuga *et al.* [55] in the muscles of the aquarium fish *Labeo bicolor*. *Aphanomyces piscicida*, a rare species, was described by Hatai *et al.* [56] in Japanese water bodies as a parasite of fish *Plecoglossus altivelis* and was also found on the muscles of *Labeo bicolor* [55]. In our study it was found growing on the eggs of Kolkhida sturgeon in the water from River Biała. *Saprolegnia polymorpha* has been encountered on the shortnose sturgeon eggs in water from the Komosa Pond and was first described by Willoughby *et al.* [57] from the water of British Isles as a parasite of koi carp

Cyprinus carpio. We have already noticed it out during studies on zoosporic fungi of the aquarium fishes [55]. We found *Pythium hydnosporum* on the eggs of Kolkhida sturgeon in the water from Supraśl River and have already found it also on the eggs of the white fish, vendace, and picke [33, 58, 59]. It was first described in Germany as a saprotroph [60].

It is worth emphasizing that two rare fungi species were present on the eggs of examined sturgeon. *Aphanomyces keratinophilus* was found growing on the eggs of paddlefish sturgeon in water from River Biała. This species was first described in Japan by Ookubo and Kobayashi [61] as a saprotroph *Phlyctidium keratinophilum* on keratinized materials. These authors renamed it later to *Phizophydium ellipsoidium*. Johnson and Seymour [62] translocated it to *Aphanomyces* genus. *Candida albicans* occurred on the eggs of shortnose sturgeon in water from River Biała. In fish, this fungus was observed in the: European white fish eggs (*Coregonus albula*) in hatchery [63], on the eel fry montee (*Anguilla anguilla*) [64] and on the eggs of sturgeon *Huso huso* [8]. Fungus species from *Candida* genus were also observed on the eggs of *Salmo gairdneri* in ponds of Ukraine by Nagornaya *et al.* [65] and on juveniles from this species [66].

Present study has proved that growth of the respective aquatic fungus species on sturgeonid eggs depends on water body from which the water has been collected (Table 3). The largest number of species have developed on the eggs in water from the river Biała and fewest in water from river Supraśl. The chemical analysis of water collected from those rivers showed water differences with regard to the content of chemical compounds. Samples from the river Biała had considerably more biogenic compounds, mainly phosphorus, whereas the water from river Supraśl was very poor in biogenes. This could confirm our earlier assumptions that the infection level of fish eggs in hatcheries [63] and in our experiment (also of eggs of other six species of sturgeonid fish) [8] definitely depends on the water trophicity state. Therefore, water trophicity in the hatcheries during artificial incubation of sturgeonid eggs must be taken into consideration.

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