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Zoosporic Fungi Growing on Freshwater Molluscs

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Abstract

Mycoflora developing on the living and dead specimens of eleven species of molluscs were investigated under laboratory conditions. Sixty zoosporic fungus species were found to grow on the specimens investigated, including 2 chytridiomycetes, 57 oomycetes and 1 zygomycetes fungus.

Out of these 60 species, 32 are known as parasites or necrotrophs of fish. On the living specimens of investigated molluscs were found to grow 25 species of fungus. Two fungus species were recorded for the first time from Poland.

Keywords: molluscs, Gastropoda, Bivalvia, hydrochemistry, aquatic fungi, zoosporic fungi

Introduction

Zoosporic fungi found in abundance in inland waters take an important part in the organic matter cycle in various water basins. They include mainly plant or animal saprophytes [2, 29]. Some species of animal saprophytes became specialized in the mineralization of keratin-containing animal remains [6] while others - of chitin-containing substrates [4]. There are also zoosporic fungi which lead a parasitic life on water organisms. Investigators have been mainly concerned with parasitic fungus species growing on fish used for consumption [23, 31]. Fungi of other aquatic animals have not been mentioned in the literature of the subject, apart from rare references to *Saprolegnia parasitica* found on injured newts or frogs [2, 9]. There are no literature data concerning other aquatic animals, including molluscs. Only Minden [22], when studying fungi of Germany, accidentally found the occurrence of one zoosporic fungus, *Saprolegnia curvata,* on the mass eggs of a snail, *Bunynia* sp. The aim of our present study was to investigate the zoosporic fungi growing on molluscs in the waters of northeastern Poland.

Material and Methods

The study was conducted on species of freshwater molluscs found in the waters of northeastern Poland (Table 1).

The water for experiments was collected in April 1996 from three different water bodies: oligotrophic Spring Cypisek (limnokrenic type, with 0.36 m, depth 0.16 m, discharge 0.9 1/sek.), eutrophic River Supraśl (length 106.6 km, this is the right-bank tributary of the middle part of the Narew River, flowing through the Knyszynska Forest), and mesotrophic Lake Komosa (12.1 ha, max. depth of 2.25 m, is surrouded by extensive coniferous woods of Knyszynska Forest). Physico-chemical parameters of these water samples were determined according to the standard methods [12].

For determining the presence of aquatic fungi species on the molluscs, the following procedure was employed: some living specimens and a certain amount (10-15 little pieces) of particular body parts of each species of mollusc were transfcred to two for each water a 4.0 litre vessel (together for each of species was six vessels) and placed in the laboratory at a temperature approaching the ambient outside one (about 7°C).

When white-greyish spots resembling tiny cotton-wool floes appeared on the material examined, some of them were collected with sterile pipettes and were observed under a microscope and the mycelium (zoosporic, antheridia and oogonia forms) of aquatic fungi growing on the living specimens and on the pieces of molluscs was recorded. The methods, were described in detail for fishes in Smith et al. [27]. The specimens and the pieces of the various molluscs species were examined for one and a half weeks. Length on the incubations was four weeks.

Table 1. Mollusc species investigated, and origin of their specimens.

Species of mollusc	Sign for Table 3	Collected from
Gastropoda		
Bithynia leachi Sheppard	*B.l.*	Pond Dojlidy, 21 ha, max. depth 2,5 m
Galba palustris O.F. Müll.	*G.p.*	Pond Dojlidy, 21 ha, max. depth 2.5 m
Limnaea stagnalis L.	*L.s.*	Lake Komosa, 12.1 ha, max. depth 2.25 m
Planorbarius corneus L.	*P.c.*	River Supraśl, length 106.6 km, depth 1.75 m
Planorbis planorbis L.	*P.p.*	Pond Fosa, 2.5 ha, max. depth 1.75 m
Radix ovata L.	*R.o.*	Pond Fosa, 2.5 ha, max. depth 1.75 m
Theodoxus fluviatilis L.	*T.f.*	River Supraśl, length 106.6 km, depth 1.75 m
Viviparus viviparus L.	*V.v.*	River Supraśl, length 106.6 km, depth 1.75 m
Bivalvia		
Anodonta cygnea L.	*A.c.*	Lake Komosa, 12.1 ha, max. depth 2.25 m
Dreissena polymorpha (Pall.)	*D.p.*	Lake Necko, 400 ha, max. depth 25.2 m
Unio pictorum L.	*U.p.*	River Supraśl, length 106.6 km, depth 1.75 m

The molluscs were identified using the key by Piechocki [24] and for identifications of the fungi the following keys were used: Johnson [17], Sparrow [29], Seymour [26], Batko [2], Karling [18] and Dick [10].

Results

Hydrochemical data of water used for the experiment are presented in Table 2. The highest values of oxydability, ammonium nitrogen, nitrate and nitrite nitrogen and phosphates were found in the River Suprasl. Spring water appeared to be the least of these parameters.

Table 2. Mean chemical composition (in mg l^{-1}) of the different water samples (n-5).

Specification	Water bodies		
	Spring Cypisek	River Supraśl	Lake Komosa
Temperature (°C)	0.2	2.7	4.7
pH	7.05	7.14	7.11
O_2	12.4	6.8	10.6
BOD (Oxydability)	3.5	10.0	8.7
CO_2	24.2	22.0	17.6
Alkalinity in $CaCO_3$ (mval l^{-1})	5.4	4.1	3.6
$N\text{-}NH_3$	0.08	0.16	0.12
$N\text{-}NO_2$	0.005	0.010	0.006
$N\text{-}NO_3$	0.078	0.162	0.082
$P\text{-}PO_4$	0.214	0.356	0.300
Cl	33.0	24.0	18.2
Total hardness in Ca	116.64	66.24	61.92
Total hardness in Mg	18.92	22.36	19.78
SO_4	64.18	17.27	23.44
Fe	0.0	0.48	0.98
Dry residue	256.0	260.0	262.0
Dissolved solids	198.0	207.0	245.0
Suspended solids	58.0	53.0	17.0

Sixty species of zoosporic fungi were found to grow on 11 species of freshwater molluscs (Table 3). *Dreissena polymorpha* individuals were the least attractive for the fungi (10 fungus species), while *Viviparus viviparus* the most attractive (21 species). Moreover, such species as *Olpidiopsis major* and *Achlya papillosa* appeared new to Polish hydromycoflora. The fungus species found included 32 already encountered in fish as necrophiles or parasites. Only 7 fungus species already observed in fish were present on *Dreissena polymorpha, Planorbarius corneus* and *Theodoxus fluviatilis.* The largest number of fungus species [16] were observed on *Galba palustris* (Table 4).

The largest number of fungus species developed on molluscs in water from the Spring Cypisek [46], the smallest in the Suprasl River [34] (Table 5).

Discussion

The present study revealed a varied number of aquatic fungus species on the particular molluscs species examined. This may be associated with several factors among which, as demonstrated on fish [16, 28, 34], a significant role can be ascribed to mucous cover, which protects the organism against microbe invasion. Also stressogenic factors, such as water chemism, temperature or nutrition have an effect, reducing the organism resistance to microbe infections.

The data obtained indicate that the largest numbers of aquatic fungus species in general and those encountered only on fish or those observed only in one water reservoir were found on the molluscs examined in the water of the Spring Cypisek, the smallest numbers in the River Suprasl. Chemical analysis of water used for experiments revealed that of these three reservoirs, the water of the River Suprasl resembled the eutrophic type, while the Spring Cypisek the oligotrophic type. Lake Komosa water was between those two. A number of aquatic fungus species were found on molluscs in all three water types. Some were known fish parasites, like *Achlya polyandra, Dictyuchus sterilis, Saprolegnia ferax,*

Table 3. Aquatic fungi found on the specimens of molluscs in the different water samples (species known in literature as parasites or necrotrophs of fish are marked with asterisk; 1-living, d-dead).

Classis, Ordo and species of fungi (according to Batko 1975)	Specimens of mollusc	Mollusc (see Table 1)	Number of mollusc
1	2	3	4
Chytridiomycetes			
Olpidiales			
1. *Rozella septigenum* Cornu	d	*G.p.*	1
Blastocladiales			
2. *Blastocladiopsis parva* (Whiffen) Sparrow	d	*A.c.,T.f.*	2
Oomycetes			
Lagenidiales			
3. *Olpidiopsis major* Maurizio	d	*V.v.*	1
4. *Olpidiopsis saprolegniae* (Braun) Coker	d	*G.p.,P.p.*	2
Saprolegniales			
5.* *Achlya americana* Humphrey	l	*D.p.,G.p.,P.p.,T.f.*	4
6. *Achlya apiculata* de Bary	d	*P.p.,T.f., Vv.*	3
7.* *Achlya bisexualis* Coker et Couch	l	*L.s*	1
8.* *Achlya caroliniana* Coker	l	*G.p.,V.v.*	2
9. *Achlya colorata* Pringsheim	d	*P.c.,P.p.,R.o.,T.f.,U.p.*	5
10.* *Achlya diffusa* Harvey ex Johnson	l,d	*B.l.,D.p.,G.p.,L.s.,R.o.*	5
11.* *Achlya dubia* Coker	l	*A.c.,B.l.,P.p.,V.v.*	4
12.* *Achlya flagellata* Coker	l	*G.p.,P.c.*	2
13.* *Achlya klebsiana* Pieters	l	*A.c.*	1
14. *Achlya oligacantha* de Bary	d	*L.s.,P.c*	2
15.* *Achlya orion* Coker et Couch	l	*B.l.,G.p.,P.c.,R.o.*	4
16. *Achlya papillosa* Humphrey	d	*T.f.*	1
17. *Achlya polyandra* Hildebrand	l,d	*A.c.,B.l.,G.p.,L.s.,P.c.,P.p.,T.f.,U.p.,V.v.*	9
18.* *Achlya prolifera* Nees	l,d	*G.p.,U.p.,V.v.*	3
19.* *Achlya racemosa* Hildebrand	d	*B.l.,V.v*	2
20. *Achlya radiosa* Maurizio	d	*B.l.,L.s.*	2
21. *Achlya rodrigueziana* F.T. Wolf	d	*T.f.*	1
22. *Achlya stellata* de Bary	d	*T.f.*	1
23. *Achlya treleaseana* (Humphrey) Kauffman	l,d	*A.c.,D.p.,L.s.,P.c.,P.p.,U.p.*	6
24. *Allomyces anomalus* Emerson	d	*L.s.*	1
25.* *Aphanomyces laevis* de Bary	l,d	*D.p.,G.p.,L.s.,P.p.,R.o.,T.f.,V.v*	7
26. *Aphanomyces parasiticus* Coker	d	*D.p.,U.p.*	2
27.* *Aphanomyces stellatus* de Bary	l,d	*L.s.,U.p.*	2
28.* *Aplanes androgynus* (Archer) Humphrey	d	*B.l.,V.v.*	2
29.* *Calyptralegnia achlyoides* (Coker et Couch) Coker	d	*P.p.*	1
30. *Cladolegnia unispora* (Coker et Couch) Johanes	d	*P.p.*	1
31.* *Dictyuchus monosporus* Leitgeb	d	*G.p.*	1
32.* *Dictyuchus sterilis* Coker	l,d	*A.c.,B.l.,D.p.,G.p.,L.s.,P.c.,R.o.,T.f.,V.v.*	9
33.* *Isoachlya anisospora* (de Bary) Coker	d	*L.s.,P.c.,R.o*	3
34.* *Isoachlya monilifera* (de Bary) Kauffman	l,d	*B.l.,G.p.,P.p.*	3
35.* *Leptolegnia caudata* de Bary	d	*A.c.,B.l.,G.p.*	3
36.* *Pythiopsis cymosa* de Bary	d	*V.v.*	1
37. *Saprolegnia anisospora* de Bary	d	*T.f.,U.p.*	2
38. *Saprolegnia asterophora* de Bary	d	*A.c.,P.p.*	2
39.* *Saprolegnia australis* Elliott	l,d	*B.l.,V.v.*	2
40.* *Saprolegnia delica* Coker	d	*B.l.,G.p.*	2
41. *Saprolegnia eccentrica* Coker	d	*B.l., V.v*	2
42.* *Saprolegnia ferax* (Gruith.) Thurnet	l,d	*A.c.,D.p.,G.p.,L.s.,P.c.,P.p.,R.o.,T.f.,U.p.,V.v.*	10
43. *Saprolegnia hypogyna* (Pringsheim) de Bary	d	*B.l.,V.v.*	2
44. *Saprolegnia litoralis* Coker	d	*A.c.,R.o.*	2
45.**Saprolegnia megasperma* Coker	d	*U.p.*	1
46.**Saprolegnia mixta* de Bary	l,d	*B.l.,G.p.,R.o.,V.v.*	4
47.**Saprolegnia monoica* Pringsheim	l,d	*L.s.,R.o.,U.p.,V.v*	
48.**Saprolegnia parasitica* Coker	l,d	*A.c.,B.l.,D.p.,G.p.,L.s.,P.c.,P.p.,R.o.,T.f.,U.p.*	10
49.**Saprolegnia shikotsuensis* Hatai et al.	l,d	*B.l.,P.c.,R.o.,T.f.*	4
50.**Saprolegnia subterranea* Dissmann	d	*P.p.*	1
51. *Saprolegnia unisospora* (Coker et Couch) Seymour	d	*D.p.*	1

Continued of Table 3

1	2	3	4
52. *Saprolegnia terrestris* Cookson ex Seymour	d	*R.o.*	1
53. *Saprolegnia torulosa* de Bary	d	*T.f.*	1
54.**Thraustotheca clavata* (de Bary) Humphrey	l,d	*D.p.,P.p.,U.p.*	3
Leptomitales			
55.**Leptomitus lacteus* (Roth) Agardh	l,d	*A.c.,R.o.,T.f.,U.p.*	4
Peronosporales			
56.**Pythium artotrogus* de Bary	l,d	*A.c.*	1
57. *Pythium middletonii* Sparrow	d	*V.v.*	1
58.* *Pythium proliferum* de Bary	d	*V.v.*	1
59. *Zoophagus insidians* Sommerstorff	d	*A.c.,U.p.,V.v.*	3
Zygomycetes			
60. *Zoopage phanera* Drechsler	l,d	*A.c.,B.l.,L.s.,V.v.*	4
Total number	l – 25 d – 53		

Table 4. Aquatic fungi on the particular species of molluscs.

Species of mollusc	Fungi (see Table 3)	Total number
Gastropoda		
Bithynia leachi Sheppard	10,11,15,17,19,20,28,32,34,35,39,40,41,43,46,48,49,60	18(12)*
Galba palustris O.F. Mull.	1,4,5,8,10,12,15,17,18,25,31,32,34,35,40,42,46,48	18(16)
Limnaea stagnalis L.	7,10,14,17,20,23,24,25,27,32,33,42,47,48,60	15(9)
Planorbarius corneus L.	9,12,14,15,17,23,32,33,42,48,49	11(7)
Planorbis planorbis L.	4,5,6,9,11,17,23,25,29,30,34,38,42,48,50,54	16(9)
Radix ovata L.	9,10,15,25,32,33,42,44,46,47,48,49,52,55	14(11)
Theodoxus fluviatilis L.	2,5,6,9,16,17,21,22,25,32,37,42,48,49,53,55	16(7)
Viviparus viviparus L.	3,6,8,11,17,18,19,25,28,32,36,39,41,42,43,46,47,57,58,59,60	21(12)
Bivalvia		
Anodonta cygnea L.	2,11,13,17,23,32,35,38,42,44,48,55,56,59,60	15(8)
Dreissena polymorpha (Pall.)	5,10,23,25,26,32,42,48,51,54	10(7)
Unio pictorum L.	9,17,18,23,26,27,37,42,45,47,48,54,55,59	14(8)

* numbers in parenthesis designated total number of parasites or necrotrophs of fishes

Saprolegnia parasitica and *Leptomitus lacteus.* We also observed a large number of fungi growing in spring water while studying fungi on lamprey eggs [3], anadromic herring species [7] and on the eggs of crucian *carp-Carassius carassius* [5].

The increased incidence of aquatic fungi on the molluscs examined can be explained by the chemism of the Spring Cypisek water, poor in nitrogen and phosphorus compounds. The character of the water reservoir itself promotes mycoflora species diversity. It is known in hydromycology that shallow coast zone and soil in the washed area provide the best conditions for lower aquatic fungi [2, 30, 35]. Shallow and small water basins, like the Spring Cypisek, from limnobiological point of view consist only of coast zone and thus abundance of lower aquatic fungus is not surprising.

Out of 60 species growing on individuals of the molluscs examined, 2 have never been found in Polish waters. One of them, *Olpidiopsis major,* was first described at the end of the previous century by Maurizio [20] as a parasite of other fungus species of the genus *Saprolegiiia.*

In our study, it was found on *Viviparus vivipanis* individuals only in water from Lake Komosa. *Achlya papillosa,* the other new species, was first observed by Humphrey [15] also at the end of the previous century as an aquatic plant saprophyte. In the thirties of our century Apinis [1] included it in the genus *Saprolegnia.* Johnson [17] and Seymour [26], however, suggested that fungus corresponded better to the genus *Achlya* than *Saprolegnia.* We found it on *Theodoxus fluviatilis* individuals only in water from Spring Cypisek.

Two species, *Saprolegnia ferax* and *Saprolegnia parasitica,* are worth noting as the main perpetrators of losses in fish industry. In our study, *Saprolegnia ferax* was found on all molluscs, except *Bithynia leachi.* Also *Saprolegnia parasitica* occurred on all the molluscs examined, except *Viviparus vivipanis. Saprolegnia ferax* has been known since the previous century for great losses it made in fish industry. Well known is the Atlantic salmon epizooty due to *Saprolegnia ferax* in British rivers in the years 1877-1881 [32]. The relevant literature of later years have reported on losses made by *Saprolegnia ferax* [11, 33]. Also *Saprolegnia parasitica* has been the cause of serious disasters on fish farms, such as the death of 50% of fry of

Table 5. Aquatic fungi found on the molluscs species from different waters.

Water from	Fungi (see Table 3)	Only in one water	Total number
Spring Cypisek	1,2,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,23,26,27,28,31,32,	1,4,5,7,16,31,40,41,45,	
	34,35,38,39,40,41,42,43,45,46,47,48,49,51,52,53,54,55,56,57,59	51,52,53	46(26)*
River Supraśl	5,6,8,9,10,11,13,15,17,19,21,22,23,25,27,29,30,32,33,34,35,38,39,42,	21,22,29,30,44	
	43,44,46,47,48,49,54,55,59,60		34(22)
Lake Komosa	2,3,9,10,11,12,14,15,17,18,19,20,23,24,25,26,27,28,32,33,34,35,36,	3,24,36,37,50,58	
	37,38,42,43,46,47,48,49,50,55,58,59,60		36(21)
Growing on molluscs in all waters	9,10,11,15,17,19,23,27,32,34,35,38,42,43,46,47,48,49,55,59		20(13)

* numbers in parenthesis designated total number of parasites or necrotrophs of fishes

the Pacific salmon *Oncorhynchus kisutch* in Miyagi Prefecture in Japan [13, 14]. Moreover, these two species of aquatic fungi are known to be the cause of mycotic infections in 40% of adult population of perch in certain Swiss lakes [21]. Together with other fungi (mainly of the genus *Achlya)* they lead to 90% losses of incubated eggs of acipenserid fish in hatcheries [19]. In the case of other fish species they can induce total destruction of eggs in some hatcheries [11, 25]. The zoosporic fungus species known as fish parasites can cause certain losses in the populations of freshwater molluscs species examined.

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