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AQUATIC FUNGI GROWING ON THE EGGS ON NINE SALMONID SPECIES OF THE GENUS *HUCHO*, *SALMO*, AND *SALVELINUS*

GRZYBY WODNE ROZWIJAJĄCE SIĘ NA IKRZE DZIEWIĘCIU GATUNKÓW RYB ŁOSOSIOWATYCH Z RODZAJU *HUCHO*, *SALMO* I *SALVELINUS*

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The authors investigated of the mycoflora developing on the eggs of nine species of salmonids of the genus *Hucho*, *Salmo*, and *Salvelinus*.

INTRODUCTION

The salmonids play an important role in sea- and fresh-water fisheries. No wonder the factors decreasing populations of these fish species in their natural environments attracted interest as early as in the previous century. Fungal diseases outbreaks many times have caused great damage to salmonids in fresh water. In the years 1877–1881, an epizootic of the Atlantic salmon caused by *Saprolegnia* sp. decimated a population of this species (Buckland et al. 1880; Stirling 1880; Huxley 1882). Fungus-induced losses in the *Salmo salar* population occur every now and then. A great one started in 1964 in the rivers of Ireland, then shifted to inland waters of Great Britain (Munro 1970) and finally to continental waters of Europe (de Kinkelin and Turdu 1971; Neish and Hughes 1980). At first, it was thought that the epizootic of *Salmo salar* was caused by *Saprolegnia ferax*. However, many years of studies have demonstrated that *Saprolegnia diclina* type 1 is the fungus responsible (Willoughby 1969, 1972, 1977, 1978, 1986).

The occurrence of *Saprolegnia parasitica* on *Salmo salar* individuals was investigated by Stuart and Fuller (1968), Willoughby (1968) and Wood et al. (1988). Florinskaya (1969) revealed the presence of *Saprolegnia delica*. Moreover, the growth of *Saprolegnia delica* was observed on *Salmo clarkii* individuals (Scott and O'Bier 1962), while *Saprolegnia ferax* (cf. Murray 1885) and *Saprolegnia parasitica* (cf. Tiffney 1939a, b) on individuals of *Salmo sebago* Girard. The development of aquatic fungi has also been observed on *Salvelinus fontinalis* individuals. Schnetzler (1887) reported the occurence of *Saprolegnia*

ferax on *Salvelinus fontinalis*, which was confirmed in later studies (Agersborg 1933; Scott 1964). Tiffney and Wolf (1937) found *Achlya flagellata* and Scott and O'Bier (1962)—*Saprolegnia parasitica* on *Salvelinus fontinalis*. *Saprolegnia parasitica* was also observed on *Salmo trutta* m. *fario* (cf. Tiffney 1939a, b). Davies and Lazar (1940) described a new fungal species *Saprolegnia invaderis* from sea trout individuals, the species thought to be *Saprolegnia ferax* (cf. Seymour 1970). Moreover, Willoughby (1972), Willoughby and Pickering (1977), and Richards and Pickering (1979) observed *Saprolegnia parasitica* growth on *Salmo trutta* and *Salvelinus alpinus* individuals.

Considering the eggs of the salmonid representatives investigated in our study, *Saprolegnia delica*, *Saprolegnia monoica* and *Pythium* sp. have been hitherto observed on the eggs of *Salmo trutta*, while *Saprolegnia ferax* and *Pythium* sp. on the eggs of *Salvelinus fontinalis* (cf. Scott and O'Bier 1962; Scott 1964). Except for *Saprolegnia ferax*, also *Saprolegnia diclina* has been found on both dead and living eggs of sea trout (Smith et al. 1985).

MATERIAL AND METHODS

The present study was based on the eggs (non fertilised) of the following fish species:

1. *Hucho hucho* L.—from the Fish Culture Station PAU in Łopuszna, Poland.
2. *Salmo penshinensis* Pallas—from the Kamchatka River in Russia.
3. *Salmo salar* L.—from an ocean fish farm in Iceland.
4. *Salmo trutta* m. *fario* L.—from the Fish Culture Station PAU in Łopuszna, Poland.
5. *Salmo trutta* m. *lacustris* L.—from A. Lityński Stock Centre of PAU in Gawrych Ruda near Suwałki, Poland.
6. *Salmo trutta* m. *trutta* L.—from the Drawa River, Poland.
7. *Salvelinus alpinus* (L.)—from the Thingvallavatn Lake in Iceland.
8. *Salvelinus alpinus salvelinus* L.—from Constance Lake in Germany.
9. *Salvelinus fontinalis* (Mitchill)—from the Fish Culture Station PAU in Wielka Puszcza near Porąbka, Poland.

The materials were transported in thermos flask in physiological solution (for species 2, 3, 7, and 8 transported by air mail).

The water for experiments was collected from three different water bodies: Supraśl river, moat pond in the Branicki Park and Komosa Lake. Eighteen parameters of these water samples were determined according to the generally accepted methods (Golterman and Clymo 1969).

For the determinations of the presence of aquatic fungus species on the eggs, the following procedure was followed: certain amounts of eggs (100–200) of each fish species were transferred to two (for each water) 1.0-l vessels (in total six vessel were used for each

species) and placed in the laboratory at the temperature approaching that of the respective hatchery. The part of the eggs from each vessel was observed under a microscope and the mycelium (form zoospore, oogonia, and conidia) of aquatic fungi growing on the eggs was recorded. The methods were described in detail in the papers of Smith et al. (1985) and Fuller and Jaworski (1986). The eggs of various fish species were examined for one to one and a half weeks. The eggs were predominately alive (some were dead). The duration of the experiments was three weeks.

For identification of the fungi the following keys were used: Johnson (1956), Sparrow (1960), Seymour (1970), Batko (1975), and Dick (1990).

RESULTS

Table 1

Chemical composition (in mg/dm^3) of the different water (May, 1995)

Specification	Moat pond	Lake Komosa	River Supraśl
Temperature °C	14.2	12.4	10.8
pH	7.0	7.6	7.5
O_2	8.2	12.8	16.7
Oxidability	13.5	7.4	7.2
CO_2	22.0	13.2	17.6
Alkalinity in $CaCO_3$*	4.4	3.9	4.1
N (NH_3)	0.442	0.101	0.218
N (NO_2)	0.0	0.012	0.005
N (NO_3)	0.008	0.034	0.041
PO_4	1.912	0.255	0.311
Cl	70.0	23.5	26.2
Total hardness in Ca	88.56	68.40	83.52
Total hardness in Mg	40.85	28.81	31.82
SO_4	28.38	12.75	19.75
Fe	0.524	1.542	0.512
Dry residue	358.0	373.0	317.0
Dissolved solids	282.0	312.0	305.0
Suspended solids	76.0	61.0	12.0

* in mval/dm^3.

The results of chemical studies of water are presented in Tab. 1. The data reveal vide range of trophicity in the water body studied, defined by the content of phosphorus and various forms of nitrogen.

Forty-six aquatic fungus species were found on the eggs of nine taxa of salmonids, including *Zoopage phanera* as a representative of Zygomycetes (Tab. 2). *Saprolegnia parasitica* was observed on the eggs of all the taxa examined, while such fungus species as *Dictyuchus sterilis*, *Leptomitus lacteus*, *Saprolegnia ferax*, and *Saprolegnia shikotsuensis* were present in most of them. Worth special noting is the finding of *Achlya treleaseana*, *Blastocladiopsis parva*, *Isoachlya torulosa*, *Olpidiopsis aphanomycis*, *Pythium middletonii*, *Pythium torulosum*, *Saprolegnia asterophora*, *Saprolegnia eccentrica*, and *Saprolegnia irregularis* on the eggs of the taxa investigated. The most fungus species were found to grow on the eggs of *Salmo trutta* m. *lacustris* (20), the fewest on the eggs of *Salvelinus fontinalis* (8) (Tab. 3). Most of them developed on the eggs in the water of lake Komosa (34), the fewest in the water of a moat pond (29) (Tab. 4). In the water of all three basins the majority of fungi were found on the eggs of *Salmo trutta* m. *lacustris* (Tab. 5), while in the moat pond the fewest fungi were

present on the eggs of *Salvelinus fontinalis* (4), in the water of lake Komosa—on the eggs of *Salmo penshinensis* and *Salvelinus alpinus salvelinus* (4 on each), and in the river Supraśl on the eggs of *Salmo penshinensis* (4).

Table 2

Aquatic fungi found on the eggs of salmonid species

Species of fungi	Salmonid species (see Materials and Methods)
1. *Achlya ambisexualis* Raper	5
2. *Achlya americana* Humphrey	3, 4
3. *Achlya bisexualis* Coker et Couch	1, 3, 6
4. *Achlya caroliniana* Coker	4, 7
5. *Achlya diffusa* Harvey et Johnson	1, 6
6. *Achlya dubia* Coker	1, 2, 5, 8
7. *Achlya klebsiana* Pieters	5, 6, 8
8. *Achlya orion* Coker et Couch	7, 9
9. *Achlya polyandra* Hildebrand	5
10. *Achlya prolifera* Nees	5, 9
11. *Achlya racemosa* Hildebrand	3, 8
12. *Achlya radiosa* Maurizio	5
13. *Achlya treleaseana* (Humphrey) Kauffman	3, 5
14. *Aphanomyces laevis* de Bary	7, 9
15. *Aphanomyces stellatus* de Bary	6
16. *Aplanes androgynus* (Archer) Humphrey	3, 7
17. *Blastocladiopsis parva* (Whiffen) Sparrow	5
18. *Calyptralegnia achlyoides* (Cocker et Couch) Cocker	5
19. *Dictyuchus anomalus* Nagai	1, 3
20. *Dictyuchus monosporus* Leitgeb	5
21. *Dictyuchus sterilis* Coker	1, 2, 4, 6, 7, 8, 9
22. *Isoachlya anisospora* (de Bary) Coker	5
23. *Isoachlya torulosa* (de Bary) Cejp	5
24. *Leptolegnia caudata* de Bary	4, 5, 7, 8
25. *Leptomitus lacteus* (Roth) Agardh	1, 2, 3, 4, 5, 7, 8, 9
26. *Olpidiopsis aphanomycis* Cornu	7
27. *Protoachlya paradoxa* (Coker) Coker	1, 5
28. *Pythiopsis cymosa* de Bary	7
29. *Pythium artotrogus* de Bary	7, 8
30. *Pythium middletonii* Sparrow	3
31. *Pythium torulosum* Cocker et Patterson	7
32. *Pythium ultimum* Trow	1
33. *Saprolegnia asterophora* de Bary	4
34. *Saprolegnia australis* Elliott	2, 3, 6
35. *Saprolegnia delica* Coker	2, 4, 6
36. *Saprolegnia diclina* Humphrey	5
37. *Saprolegnia eccentrica* Coker	1, 3
38. *Saprolegnia ferax* (Gruith) Thurnet	3, 5, 6, 7, 9
39. *Saprolegnia hypogyna* (Pringsheim) de Bary	2, 5, 8
40. *Saprolegnia irregularis* Johnson et Seymour	3
41. *Saprolegnia megasperma* Coker	8
42. *Saprolegnia mixta* de Bary	2, 7, 8
43. *Saprolegnia monoica* Pringsheim	1, 2, 5
44. *Saprolegnia parasitica* Coker	1, 2, 3, 4, 5, 6, 7, 8, 9
45. *Saprolegnia shikotsuensis* Hatai et al.	1, 3, 4, 6, 9
46. *Zoopage phanera* Drechsler	3, 8

Table 3

Aquatic fungi found on the eggs of particular salmonid species

Salmonid species	Fungi (see Table 2)	Total number
Hucho hucho	3, 5, 6, 19, 21, 25, 27, 32, 37, 43, 44, 45	12
Salmo penshinensis	6, 21, 25, 34, 35, 39, 42, 43, 44	9
Salmo salar	2, 3, 11, 13, 16, 19, 25, 30, 33, 37, 38, 40, 44, 45, 46	15
Salmo trutta m. *fario*	2, 4, 21, 24, 25, 33, 35, 44, 45	9
Salmo trutta m. *lacustris*	1, 6, 7, 9, 10, 12, 13, 17, 18, 20, 22, 23, 24. 25, 27, 36, 38, 39, 43, 44	20
Salmo trutta m. *trutta*	3, 5, 7, 15, 21, 34, 35, 38, 44, 45	10
Salvelinus alpinus	4, 8, 14, 16, 21, 24, 25, 26, 28, 29, 31, 38, 42, 44	14
Salvelinus alpinus salvelinus	6, 7, 11, 21, 24, 25, 29, 39, 41, 42, 44, 46	12
Salvelinus fontinalis	8, 10, 14, 21, 25, 38, 44, 45	8

Table 4

Aquatic fungi found on the eggs of salmonid species in different water

Water from	Fungi (see Table 2)	Total number
Moat pond	1, 2, 3, 4, 5, 6, 13, 14, 16, 17, 18, 19, 21, 24, 25, 26, 27, 29, 33, 34, 35, 38 ,39, 40, 41, 42, 43, 44, 45	29
Lake Komosa	3, 4, 5, 6, 7, 8, 9, 10 11, 12, 14, 15, 16, 19, 20, 21, 22, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 38, 43, 44, 45, 46	34
River Supraśl	2, 3, 4, 6, 7, 10, 11, 13, 14, 15, 16, 19, 21, 22, 23, 24, 25, 31, 32, 33, 34, 36, 37, 38, 39, 40, 42, 43, 44, 45	30

DISCUSSION

According to literature survey, the most frequently found fungi in the salmonids are the species of the genus *Saprolegnia* – *Saprolegnia ferax* and *Saprolegnia parasitica.* The present study has revealed the occurrence of many other species of the salmonid eggs also those not yet encountered. *Saprolegnia ferax* and *Saprolegnia parasitica* are the most common species found on the salmonid eggs. This fungal group also includes *Dictyuchus sterilis*, *Leptomitus lacteus* and *Saprolegnia shikotsuensis*. *Dictyuchus sterilis* was previously found on the skin of some fish species, although not salmonids, in India (Srivastava and Srivastava 1977, 1978). In our case, this is the first finding of this fungus on fish eggs. *Dictyuchus sterilis* is relatively frequently encountered in water, particularly on plant remains (Batko 1975). *Leptomitus lacteus* was observed by Lennon (1954), then by Scott and O'Bier (1962) on the eggs of rainbow trout and pike, by Florinskaya (1969) on dead and living fish individuals, and on perch individuals in Lake Windermere (Willoughby 1970; Pickering and Willoughby 1977; Willoughby and Roberts 1991). We found *Leptomitus lacteus* on the eggs of *Coregonus albula* in a hatchery in Węgorzewo in Masuria (Czeczuga

and Woronowicz 1993). *Saprolegnia shikotsuensis* was first described on the Pacific salmon individuals *Oncorhynchus nerka* var. *adonis*, from Lake Shikotsu on the island of Hokkaido in Japan (Hatai et al. 1977). It was also found to grow on the eggs of some acipenserid species (Czeczuga et al. 1995) and of some North Pacific salmon species (Czeczuga and Muszyńska 1996). *Achlya treleaseana* has been hitherto encountered only on the eggs of two acipenserid species, *Acipenser güldenstädti* and *Acipenser nudiventris* Czeczuga et al. 1995), thus it is new to salmonids. *Blastocladiopsis parva*, whose growth we observed on the eggs of lake trout, is an interesting species. This fungus is quite common in water basins of various types in northeastern Poland (Czeczuga 1995b), grows on keratinophil substrates (Czeczuga and Muszyńska 1994) and chitin-containing substrates (Czeczuga and Godlewska 1994). We found it on the eggs of sturgeon *Acipenser nudiventris* (Czeczuga et al. 1995). *Isoachlya torulosa*, described as *Saprolegnia torulosa* in the previous century (Seymour 1970) and later included by Cejp (1959) in the genus *Isoachlya*, was found to grow only on the eggs of lake trout. This is the first finding of this fungus in fish. *Olpidiopsis aphanomycis*, known as a parasite of *Aphanomyces* fungi, is also a species new to salmonids and fish in general (Fig. 1). In our study, it was found on the eggs of *Salvelinus alpinus*. *Pythium torulosum*, known as an aquatic or rather soil saprophyte (Ichitani et al. 1989), also appeared new to fish. Fungi of the genus *Pythium* are known mainly as soil parasites of plants (Plaats-Niterink 1981; Yu and Ma 1989). However, in 1962 Scott and O'Bier reported on the occurrence of *Pythium ultimum* mycelium on *Lepomis macruchirus* individuals. Later, on the eggs of several species of *Coregonus lavaretus*, incubated in a hatchery Florinskaya (1969) observed *Pythium proliferum* mycelium. Czeczuga and Woronowicz (1993) revealed the development of *Pythium artotrogus* on the eggs of European white-fish (*Coregonus albula*), Baltic white-fish (*Coregonus lavaretus*) and pike (*Esox lucius*). Its mycelium and that of *Pythium ultimum* were found on the eggs of sturgeon *Acipenser nudiventris* (Czeczuga et al. 1995) and on the eggs of the others fishes. *Saprolegnia asterophora* is known as an aquatic and soil saprophyte (Seymour 1970), particularly in the acidified environment. It has been encountered on dead fishes (Hayren 1928) and on *Leuciscus* sp. individuals (Petersen 1910); yet it is not mentioned in surveys concerning mycosis in fish (Neish and Hughes 1980; Srivastava 1980; Dudka et al. 1989). In our study, it was found on the eggs of *Salmo trutta* m. *fario*. *Saprolegnia eccenrica*, most frequently found in soil and seldom lakes (Seymour 1970), was observed in present study on the eggs of *Hucho hucho* and *Salmo salar*. *Saprolegnia irregularis*, first described in culture conditions and in water of various sources of Iceland (Johnson and Seymour 1975), was observed by us on the eggs of *Salmo salar*, which was its second finding in fish. In the study of Czeczuga and Muszyńska (1996) it has been encountered on the eggs of *Oncorhynchus nerka* var *adonis*.

Table 5

Aquatic fungi found on the eggs of particular salmonid species in different water (in parenthesis number of fungi)

Salmonid species	Fungi (see Table 2) in water from					
	Moat pond		Lake Komosa		River Supraśl	
Hucho hucho	6, 19, 21, 25, 27, 43, 44, 45	(8)	3, 5, 26, 32, 43, 45	(6)	3, 19, 32, 44	(5)
Salmo penshinensis	21, 25, 34, 35, 42, 44	(6)	6, 21, 43, 44	(4)	6, 34, 39, 44	(4)
Salmo salar	2, 3, 16, 34, 38, 40, 44	(7)	11, 16, 19, 30, 37, 44, 46	(8)	13, 18, 25, 34, 40, 44, 45	(7)
Salmo trutta m. *fario*	4, 21, 33, 44, 45	(5)	4, 21, 33, 35, 44, 45	(6)	2, 4, 21, 24, 25, 33, 44, 45	(8)
Salmo trutta m. *lacustris*	1, 13, 17, 18, 24, 38, 39, 44	(8)	6, 7, 9, 10, 12, 20, 22, 27	(10)	10, 22, 23, 24, 25, 36, 43, 44	(8)
Salmo trutta m. *trutta*	5, 6, 21, 38, 44	(5)	5, 7, 15, 21, 34, 35, 38	(7)	15, 21, 38, 44, 45	(5)
Salvelinus alpinus	14, 25, 26, 38, 44	(5)	4, 8, 14, 24, 28, 31, 44	(7)	16, 21, 31, 42, 44	(5)
Salvelinus alpinus salvelinus	6, 21, 24, 29, 41	(5)	6, 25, 29, 46	(4)	6, 7, 11, 21, 25, 39, 42	(7)
Salvelinus fontinalis	38, 44, 45	(3)	8, 10, 14, 21, 25, 38, 44	(7)	14, 25, 38, 44, 45	(5)

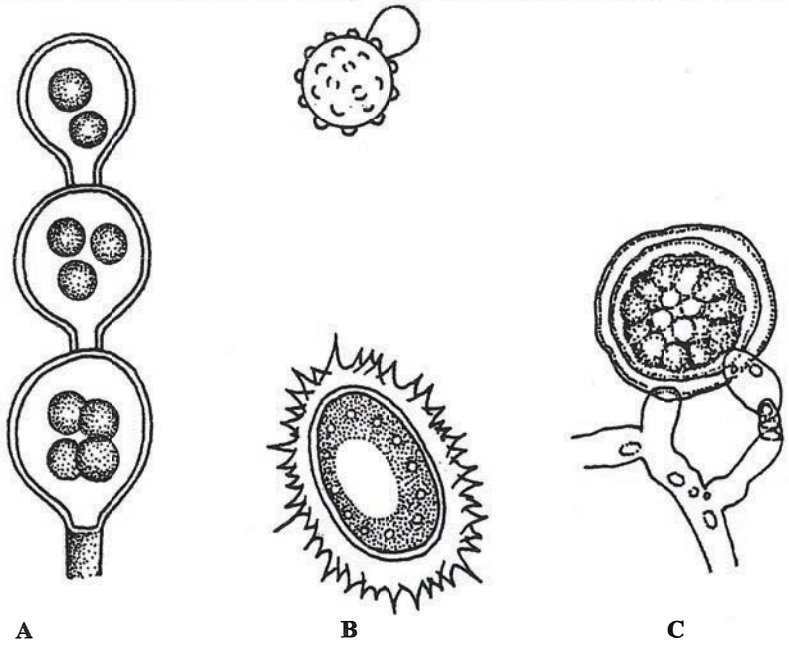

Fig. 1. Aquatic fungi new to fishes. A—*Isoachlya toruloides* (oogonia from oospores – 12–30 μm); B—*Olpidiopsis amhanomycis* (spores – 25–50 μm); C—*Pythium torulosum* (oogonia – 13–18 μm, and antheridia).

The present study has shown different numbers of aquatic fungus species growing on the eggs of various salmonid species in the same experimental conditions. This may be explained by the nature of the eggs itself and by the quality of water being the eggs habitat. Considering the former, the structure of the internal membrane of the eggs plays a significant role (Lartseva and Altufiev 1987). The eggs of *Huso huso* could be an example—it hosted the smallest number of fungi, compared to other acipenserid fishes in the same conditions (Lartseva 1986). The observations of Lartseva have been confirmed by Czeczuga et al. (1985). Condition of a female of a given species the incubated eggs come from has also an effect (Lartseva and Dudka 1985, 1990; Czeczuga 1994a). A role of water quality seems undoubtful, since certain fungus species develop on the eggs only in specific water (Tab. 6). For instance, the eggs of *Salvelinus alpinus* and *Salvelinus fontinalis* was infected by the mycelium of *Achlya orion* only in Lake Komosa. *Blastocladiopsis parva*, quite common in various water bodies, was found nowhere but in moat pond

Table 6

Fungus species developing on eggs only in specific water

Water from	Fungi (see Table 2)
Moat pond	1, 17, 18, 26
Lake Komosa	8, 12, 20, 28, 30, 46
River Supraśl	9, 23, 36

water and only on the eggs of lake trout. *Achlya polyandra*, however, quite commonly found in the waters of north-eastern Poland (Czeczuga 1994b, 1995a), grew only on the eggs of lake trout in river water.

It would, therefore, seem that the presence of a host is not the only factor playing a role, probably hydrochemical. When comparing the hydrochemical data of these water body, it can be seen that only the water of the moat pond did not contain detectable amounts of $N\text{-}NO_2$. Water of this pond is characterised by a comparatively highest oxidability, alkalinity and content of CO_2, $N\text{-}NH_3$, PO_4, chloride, calcium, manganese, and sulphates (Tab. 1). However, the water of lake Komosa had the lowest alkalinity and content of CO_2, $N\text{-}NH_3$, PO_4, chloride, calcium, manganese, and sulphates. Water of this lake had the highest $N\text{-}NO_2$ and iron concentrations. The water of the river Supraśl is characterised by a comparatively highest content of oxygen and $N\text{-}NO_3$, and this river was also characterised by the lowest oxidability of all water body studied.

Fish eggs mycosis is the result of environmental conditions, the structure of the egg capsule and the thickness of mucous layer covering it. Stress factor affecting the female in the spawning season may be important.

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GRZYBY WODNE ROZWIJAJĄCE SIĘ NA IKRZE DZIEWIĘCIU GATUNKÓW RYB ŁOSOSIOWATYCH Z RODZAJU *HUCHO*, *SALMO* I *SALVELINUS*

STRESZCZENIE

Badano w warunkach laboratoryjnych występowanie grzybów wodnych na ikrze 9 gatunków ryb łososiowatych należących do rodzaju *Hucho, Salmo* oraz *Salvelinus*. Badaniami objęto ikrę *Hucho hucho, Salmo penshinensis, Salmo salar, Salmo trutta* m. *fario, Salmo trutta* m. *lacustris, Salmo trutta* m. *trutta, Salvelinus alpinus, Salvelinus alpinus salvelinus* oraz *Salvelinus fontinalis*.

Do doświadczeń używano wody ze stawu, jeziora i rzeki, uwzględniając w niej poszczególne parametry hydrochemiczne.

Ogólnie stwierdzono na ikrze rozwój 46 gatunków grzybów zoosporowych, w tym jednego przedstawiciela Zygomycetes (*Zoopage phanera*). Najmniej gatunków rozwijało się na ikrze *Salvelinus fontinalis* (8), najwięcej zaś — na ikrze *Salmo trutta* m. *lacustris* (20). Spośród stwierdzonych grzybów 3 gatunki: *Isoachlya toruloides, Olpidiopsis aphanomycis* oraz *Pythium torulosum* okazały się nowymi dla ryb.

Najwięcej gatunków grzybów rozwijało się na ikrze badanych ryb łososiowatych w wodzie z jeziora (34), a najmniej w wodzie ze stawu (29), która okazała się najzasobniejszą w związki biogenne.

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