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Original Communication

Fungi and straminipilous organisms growing on some aquarium fish species in water from different water bodies

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ABSTRACT

The authors investigated aquatic mycota growing on the muscles of 8 aquarium fish species in the water from five limnologically and trophically different water bodies (one spring, two rivers, one pond and one lake). All of these water bodies are strongly loaded in spring and in autumn. 50 species of aquatic mycota were found on the muscles of investigated aquarium fish. In autumn, 43 species were noted, while only 33 were observed in spring. In all the five reservoirs, oxidability, all nitrogen forms, phosphates and sulphates showed considerably higher values in spring, compared to autumn. Four such mycotic species *Monoblepharis sphaerica, Aphanomyces invadans, Aphanomyces piscicida* and *Saprolegnia polymorpha* were recorded for the first time from Poland.

KEYWORDS: mycota, aquatic fungi, straminipilous organisms, aquarium fish, hydrochemistry

INTRODUCTION

Apart from bacterial ailments affecting freshwater fish [1], diseases induced by lower aquatic fungi are also very common [2-6]. Fish epizooties caused by mycota, especially by species of the genera *Achlya* and *Saprolegnia* [7] have been observed since the middle of the 19th century. Mass fish deaths have been reported lately from farms of consumptionally important fish species [8-11]. No wonder that the subject has appeared in numerous surveys [7].

In the monographs on mycotic diseases of fish [2-4, 7] there are virtually no data concerning fungus species growing on aquarium fish specimens, except for *Carassius auratus* and *Poecilia reticulata* [12, 13].

In 1884 Bennett [14] reported on the occurrence of *Saprolegnia* sp. on *Carassius auratus* specimens, *Saprolegnia ferax* was observed by Clinton [15], *Saprolegnia parasitica* by Tiffney [16] and *Achlya* sp. by Yousuf [17]. As for *Poecilia reticulata*, only *Achlya bisexualis* was found to grow on this species [18].

In the present study we decided to examine several aquarium fish species with regard to the occurrence of aquatic fungus species and straminipilous organisms in order to supplement the already existing knowledge in this field.

MATERIAL AND METHODS

The study was performed on 8 aquarium fish species, including 1) *Carassius auratus* (L.), 2) *Corydorus paleatus* Jenyns, 3) *Gymnocorymbus ternetzi* Jordan, 4) *Labeo bicolor* Smith, 5) *Mollienisia sphenops* (Cuvier et Valenc.), 6) *Paracheirodon axelrodi* Schultz, 7) *Poecilia velifera* Regan and 8) *Xiphophorus maculatus* Gunther.

Water samples for the experiment were collected in spring (May) and autumn (September 2006) from spring Jaroszówka, rivers Biała and Supraśl, pond Fosa and lake Komosa:

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- Spring Jaroszówka, localized in the north part of Białystok. Limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 l/s, surrounding without trees. The spring is surrounded by cultivated fields. The bed is covered with sand.

- River Biała, lengh 9.8 km, a left - bank tributary of the Supraśl river flowing through Białystok City. The samples were collected in the upper course of the Biała river, the water was the least polluted.

- River Supraśl, right- bank tributary of the middle part of the Narew river flowing through the Knyszyńska Forest. Lenght 106.6 km. The samples were collected from the site above the municipal swimming pool at the sluice of an arm of the Supraśl river flowing just through the town Supraśl. The sampling site is surrounded by meadows. The bed is muddy.

- Pond Fosa, localized in the Palace Park of Białystok. Area 2.5 ha, max. depth 1.75 m. Pond with wild ducks and breeding swans as well as crucian carp and tench bred, used by anglers. The pond is surrounded by meadows with linden (*Tilia cordata* Mill.) and elm (*Ulnus carpinifolia* Gled.).

- Lake Komosa, localized in the Knyszyńska Forest. Area 12.1 ha, max. depth 2.25 m. The lake is surrounded by extensive coniferous woods.

Water samples (2.0 dm^3 in volume) for physico-chemical and mycotic investigations were collected at approximately 0.20 m under water surface using a Ruttner's apparatus. Hydrochemical analysis of the water included determination of temperature, pH, oxidability, carbon dioxide, general alkalinity, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphates, total iron, sulphates, chlorides, calcium, magnesium, dry residue, dissolved solids and suspended solids. The physico-chemical analysis was performed using methods recommended by Standard Methods [19]. The bait method [20], using fragments of muscles (10-20 fragments from 5-10 specimens (females and males) of respective species of 8 aquarium fish species, was applied to isolate fungi. The baits were placed in one litre-containers, poured with water from a respective reservoir (altogether fifteen containers for each species in one season), stored for approximately one month in a room with thermal and light conditions resembling the natural environment and observed consecutively under an optic microscope every few days starting from the third day of the culture. Then, several microscopic preparations were done from each sample and observed under an optic microscope at a magnification of 100x and 400x. At the same time an ocular micrometer was used to determine the developmental stages of the fungi. To identify the fungi, vegetative organs (shape and size of hypha), asexual reproduction organs (shape of sporangium) and sexual organs (oogonia, including oospores and antheridia) were assessed, using the keys of Batko [21], Johnson [22], Seymour [23] and Plaats- Niterink [24]. For isolation of the *Aphanomyces* fungal pathogen was performed using methods recommended by Willoughby and Roberts [25]. The systematics of straminipilous organisms was used according to Dick [26] and Johnson *et al*. [27, 28] of fungi according to Blackwell *et al*. [29], of Chytridiomycota according to James *et al*. [30], and Spizellomycetales according to Barr [31].

RESULTS

As shown by chemical analysis of water samples used for the experiment (Table 1), such parameters as oxidability, total amount of nitrogen and phosphate had the highest values in the river Biała and pond Fosa. This could suggest that the water in these reservoirs was at that time the richest in biogenes. In all the five reservoirs, oxidability, all nitrogen forms, phosphates and sulphates showed considerably higher values in spring, compared to autumn.

Fifty mycotic species were found to grow on the specimens of aquarium fish species (Table 2-3). In autumn, 43 species were noted, while only 33 were observed in spring. Worthy of note is the finding of four mycotic species new to Polish waters, namely *Monoblepharis sphaerica*, *Aphanomyces invadans*, *Aphanomyces piscicida* and *Saprolegnia polymorpha*. Such fungus species as *Saprolegnia ferax* and *Saprolegnia parasitica* were found to grow in spring and autumn on the specimens of all fish species examined in the water from all five reservoirs. Both in spring and in autumn the largest number of species occurred on the muscles of *Labeo bicolor*.

Both in spring and in autumn the fewest species were observed on the material in water collected

Table 1. Chemical composition (in mg l^{-1}) of water from the different water bodies (n=3).

Specification	Water bodies				
	Spring Jaroszówka s* - a**	River Supraśl s - a	River Biała s - a	Pond Fosa s - a	Lake Komosa s - a
Temperature (^oC)	4.2-6.6	6.2-10.8	7.6-11.8	8.2-13.4	7.8-12.2
pH	7.21-7.92	7.14-7.21	8.12-7.21	8.10-7.28	7.92-7.31
O_2	11.82-12.94	12.08-14.6	10.12-11.84	5.2-7.4	12.21-13.06
BOD_5	3.8-3.2	5.2-4.8	4.8-4.0	7.4-7.0	5.4-5.0
COD (Oxidability)	4.8-2.8	9.5-6.4	14.8-9.6	15.6-12.2	9.4-5.8
CO_2	15.4-19.8	25.2-22.0	24.6-30.8	63.8-30.8	15.4-17.6
Alkalinity in $CaCO_3$ (mval l^{-1})	3.9-3.6	3.6-4.2	3.4-4.7	5.2-5.8	2.8-4.2
$N\text{-}NH_3$	0.12-0.0	0.27-0.19	0.28-0.20	0.97-0.12	0.23-0.04
$N\text{-}NO_2$	0.013-0.0	0.015-0.009	0.031-0.018	0.004-0.003	0.011-0.003
$N\text{-}NO_3$	0.602-0.082	0.640-0.180	0.224-0.046	0.014-0.008	0.185-0.150
$P\text{-}PO_4$	2.972-0.280	1.854-0.460	3.105-0.302	3.500-0.340	1.382-0.170
Sulphates	121.0-56.0	84.0-62.0	122.0-110.0	85.0-75.0	124.0-110.0
Chlorides	22.0-32.0	16.0-40.0	31.0-29.0	32.0-40.0	17.0-22.0
Total hardness in Ca	101.5-101.2	88.56-87.84	72.0-66.92	102.24-120.64	70.56-97.2
Total hardness in Mg	14.62-18.06	12.04-20.64	17.63-37.84	21.07-23.22	10.32-22.36
Fe (total)	0.0-0.30	0.96-0.40	1.10-0.48	0.29-0.18	0.30-0.40
Dry residue	327.0-336.0	133.0-190.0	210.0-352.0	370.0-346.0	160.0-187.0
Dissolved solids	323.0-322.0	118.0-120.0	205.0-260.0	337.0-326.0	140.0-138.0
Suspended solids	4.0-14.0	15.0-70.0	5.0-92.0	33.0-20.0	26.0-49.0

* s - spring
** a - autumn

Table 2. Fungi and straminipilous organims found on aquarium fish species in spring.

Taxa	Spring Jaroszówka	River Biała	River Supraśl	Lake Komosa	Pond Fosa
Fungi					
Chytridiomycota					
Spizellomycetales					
Rozella septigena Cornu				1,6**	
Straminipila					
Peronosporomycetes					
Saprolegniales					
Achlya androgyna (W. Archer) T. W. Johnson et R. L. Seym	2		8		
Achlya apiculata de Bary	8				
Achlya colorata Pringsh.				3,4,7	
**Achlya diffusa* J. V. Harv. ex T. W. Johnson	2,4		1,4		1
**Achlya klebsiana* Pieters			4		
**Achlya orion* Coker et Couch			2		
**Achlya polyandra* Hildebr.		4	1	1,2	8
**Achlya racemosa* Hildebr.				1,4,5	1
Achlya rodrigueziana F. T. Wolf			2		
Aphanomyces irregularis W. W. Scott		6			
**Aphanomyces laevis* de Bary	1,6		2,8		1,2,4,8
**Aphanomyces piscicida* Hatai	4				
**Aphanomyces stellatus* de Bary			8	8	
Calyptralegnia basraensis Muhsin				2	
**Dictyuchus monosporus* Leitg.	2				
**Dictyuchus sterile* Coker		2,6,7			

Table 2 continued..

**Isoachlya anisospora* (de Bary) Coker			4		
**Isoachlya torulosa* (de Bary) Cejp				3,5	
**Leptolegnia caudata* de Bary			7		
**Saprolegnia australis* R.F. Elliott	4		4		4
**Saprolegnia diclina* Humphrey	1		1		
**Saprolegnia ferax* (Gruith.) Thur.	1-8	1-8	1-8	1-8	1-8
**Saprolegnia parasitica* Coker	1-8	1-8	1-8	1-8	1-8
**Saprolegnia polymorpha* Willoughby					1
**Saprolegnia shikotsuensis* Hatai *et al.*	4,6				2
**Saprolegnia unispora* (Coker et Couch) R. L. Seym				3,5	
**Scoliolegnia asterophora* (de Bary) M. W. Dick	7			4	
**Thraustotheca clavata* (de Bary) Humphrey	1,2,7		3		
Leptomitales					
Apodachlya brachynema (Hildebr.) Pringsh.	7				
**Leptomitus lacteus* (Roth) Agardh		1-5		1-5	
Pythiales					
Pythium hydnosporum (Mont.) J. Schröt.		1	7	4	
**Pythium middletonii* Sparrow		2		7	5
**Pythium ultimum* Trow		1	5	8	
Total number of species	14	9	17	15	10

* Know in the literature as parasites or necrotrophs of fish
** Number of fish - see Material and Methods

Table 3. Fungi and straminipilous organisms found on aquarium fish species in autumn.

Taxa	Spring Jaroszówka	River Biała	River Supraśl	Lake Komosa	Pond Fosa
Fungi					
Chytridiomycota					
Spizellomycetales					
Rozella septigena Cornu		4,7**		1,4,6	
Monoblepharidiales					
Monoblepharis sphaerica Cornu sen. Perrott	2				
Straminipila					
Peronosporomycetes					
Saprolegniales					
**Achlya ambisexualis* J. R. Raper				1	
**Achlya androgyna* (W. Archer) T. W. Johnson et R. L. Seym	2,4		7,8		
**Achlya bisexualis* Coker et Couch	8			8	8
**Achlya caroliniana* Coker	3				
Achlya colorata Pringsh.				3,4,5,7	
**Achlya diffusa* J. V. Harv. ex T. W. Johnson			1		1,2
**Achlya dubia* Coker				4	
**Achlya flagellata* Coker			7		
**Achlya klebsiana* Pieters		6	6		6
**Achlya orion* Coker et Couch			2		
**Achlya polyandra* Hildebr.			6		
**Achlya racemosa* Hildebr.		7			
Achlya treleaseana (Humphrey) Kauffman	6				

Table 3 continued..

**Aphanomyces invadans* Willoughby *et al.*					4
Aphanomyces irregularis W.W. Scott		1,3	6	1,4	5
**Aphanomyces laevis* de Bary	1,3,6,8	2,8	2,3,8	5	1-4,8
**Aphanomyces stellatus* de Bary				8	
**Calyptralegnia achlyoides* (Coker et Couch) Coker				4	
**Dictyuchus monosporus* Leitg.	2				
**Dictyuchus sterile* Coker		6,7	3,4,6,8	2,3,6	6,8
**Isoachlya anisospora* (de Bary) Coker			4		
**Leptolegnia caudata* de Bary		6,7	7	7	2
**Protoachlya paradoxa* (Coker) Coker				4	
**Pythiopsis cymosa* de Bary	2				
Saprolegnia anomala Gand. & Kurne				7	
**Saprolegnia australis* R.F. Elliott	4			4	
**Saprolegnia diclina* Humphrey			1		
**Saprolegnia ferax* (Gruith.) Thur.	1-8	1-8	1-8	1-8	1-8
**Saprolegnia megasperma* Coker				7	
**Saprolegnia monoica* Pringsh.	1				
**Saprolegnia parasitica Coker*	1-8	1-8	1-8	1-8	1-8
**Saprolegnia shikotsuensis* Hatai *et al.*	4,6	4,6		2,7,8	
**Saprolegnia subterranea* Dissmann			4,5		2
**Thraustotheca clavata* (de Bary) Humphrey	1,2,7				
Leptomitales					
Apodachlya brachynema (Hildebr.) Pringsh.	7				

Table 3 continued..

**Leptomitus lacteus* (Roth) Agardh		1,2,4,7,8		3,4,6,7	1-3,5
Lagenidiales					
Lagenidium humanum Karling		1,3			
Pythiales					
**Pythium debaryanum* Hesse					1
Pythium hydnosporum (Mont.) J. Schröt.	4		5	1	5
**Pythium middletonii* Sparrow		1			
**Pythium ultimum* Trow	5		7		
	18	13	17	20	14

* Know in the literature as parasites or necrotrophs of fish
** Number of fish - see Material and Methods

from the river Biała. Of the 46 mycotic species found on the aquarium fish species examined, 36 are known as fish parasites in natural conditions.

DISCUSSION

As revealed by the present study, the number of mycotic species growing on the muscles of the aquarium fish specimens examined is relatively high. Approximately the same number of zoosporic mycota occurred on the muscles of 4 fish species of the plantaginaceous family recently introduced to Polish waters [32]. The total number of mycota species as well as the number of mycota growing on the muscles of the respective fish species in autumn was higher compared to the spring period. This may be related to different amounts of chemical components in the water in these two periods. In spring the water was more abundant in biogenes, the phenomenon being already observed in the study on mycota growing on the muscles of fish introduced to Polish waters [32] and on the eggs of other fish species [33-40]. Considerably fewer species were found in the water richer in biogenes. The fewest species were observed on aquarium fish specimens both in spring and in autumn in the water from river Biała and pond Fosa, where the water was of eutrophic nature.

Susceptibility of living fish specimens to microbial infections depends to a large extent on stress factors, of which the most common are sudden changes in temperature and oxygen dissolved in water, as well as overcongestion of fish specimens and access to food [41]. These factors cause attenuation of the immune system and thus greater susceptibility of fish to various infections [4], which is especially common in the conditions of aquarium breeding.

Monoblepharis sphaerica, a zoosporic mycota new to Polish waters, was observed only in autumn in the water from spring Jaroszówka on the muscles of *Corydorus paleatus*. It is known in literature as a plant saprophyte growing on twigs found in water [21]. This species was first described in the 70s of the 19th century by Cornu [42], its biology was studied by Perrott [43]. *Aphanomyces invadans* was found to grow on the muscles of *Labeo bicolor* specimens in the water from pond Fosa in autumn, when the water showed low oxidability compared to other reservoirs and low content of biogenes, although higher values of sulphates and chlorides in comparison to the spring period. *Aphanomyces invadans* was first described as *Aphanomyces invaderis* by [44] as a pathogen of freshwater trophical fish causing epizooties including of *Labeo rohita* species. *Aphanomyces piscicida* was also found to grow on the muscles of *Labeo bicolor* specimens in the water collected from spring Jaroszówka, which in comparison to the water of other reservoirs had the lowest oxidability, the fewest biogenes of nitrogen type but a relatively high content of sulphates. Iron was not detected in this reservoir. *Aphanomyces piscicida* was described by Hatai *et al*. [45] in Japanese water bodies as a parasite of a fish species *Plecoglossus altivelis*. *Saprolegnia polymorpha*, the fourth species new to Polish waters, was first described by Willoughby [46] from waters on the British Isles as a parasite of koi carp *Cyprinus carpio*. In our study, this species was found to grow on the muscles of *Carassius auratus* in the water from lake Komosa in the spring period, when its water was the poorest in phosphates, calcium, magnesium and its alkalinity was the lowest, but it had the highest content of oxygen and sulphates.

Of the mycotic species found on the aquarium fish specimens and known in the literature of the subject as fish parasites in natural conditions worthy of special note are *Achlya dubia, Achlya flagellata, Achlya racemosa, Aphanomyces laevis, Dictyuchus monosporus, Isoachlya torulosa Leptomitus lacteus, Saprolegnia ferax, Saprolegnia parasitica* and *Saprolegnia shikotsuensis*. These species are known to cause mass death of fish, especially in hatcheries and in breeding ponds [2, 5, 47]. *Achlya dubia* was described as a parasite of freshwater fish in India [16], attacking mostly branchiae, fins and skin, like in Egypt where it caused great losses in the breeding of *Tilapia* [48]. In India, *Achlya flagellata* infected the whole eggs in one of the farms of *Tor tor*, a popular fish in this country [8]. *Achlya racemosa* attacked a culture of Japanese eel [49]. Substantial losses were caused by *Aphanomyces laevis* during the spawning time of trout in Taiwan [50]. *Dictyuchus monosporus* is known to cause the loss of eggs of the acipenserids in hatcheries near Astrachan in

Russia [51]. *Isoachlya torulosa* has been encountered on a few fish species introduced to Polish waters in recent years, namely on *Neogobius fluviatilis, Neogobius gymnotrachelus, Perccottus glenii* and *Pseudorasbora parva* [32]. *Leptomitus lacteus*, called the sewage fungus, attacks both young specimens of fish in lakes [52] and eggs in hatcheries [47]. However, the most pathogenic mycota growing on fish are *Saprolegnia ferax* and *Saprolegnia parasitica* [7], causing great losses in hatcheries [53], breeding ponds [10, 54] and in lakes [55]. *Saprolegnia shikotsuensis* is known as a parasite of Pacific salmon *Oncorhynchus nerka* var. *vernalis* [56], It should be emphasized that all the fungus species mentioned here have been observed on the eggs of fish inhabiting Polish waters [33-40, 57].

In conlusion, it should be stated that the present study on the mycotic species growing on the muscles of fish species bred in aquaria has revealed no species-related differences (apart from the new species), compared to the studies on fish living in natural reservoirs. On the aquarium fish specimens we observed the growth of *Saprolegnia ferax* and *Saprolegnia parasitica*, two parasitic species known to induce mass fish death in natural conditions [9, 58, 59].

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