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Growth of mycotal species on the eggs of *Cyprinus carpio* in limnologically and trophically different water bodies

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The study investigated the growth of hydromycoflora on the eggs of common carp (*Cyprinus carpio* L.) in five water bodies of different eutrophication levels. Thirty-three (33) species of mycotal organisms, including 28 species belonging to Peronosporomycota, 2 to anamorphic fungi and 1 each to Ascomycota, Blastocladiomycota and Zygomycota were identified on the eggs. The water from River Biala and Pond Fosa, which are more biogenic, had the largest number of mycotal species (20 and 19, respectively) on the eggs of common carp. The smallest number of those species was found on the eggs of common carp in water from Spring Cypisek, Pond Komosa and River Suprasl, which are low in biogenes (12, 11 and 11, respectively). *Achlya diffusa*, *Aphanomyces laevis*, *Saprolegnia ferax*, *Saprolegnia parasitica* and *Pythium ultimum* belong to the species that were most frequently found on *C. carpio* eggs. The following rare mycotal species were also found: *Allomyces arbuscula*, *Aphanomyces frigidophilus*, *Candida albicans*, *Fusarium aquaeductum*, *F. culmorum* and *Zoopage phanera.* Amino acid, carbohydrate and urease tests were used. Of the *C. carpio* eggs that were investigated, 12.7% were found to be infected with mycotal species.

Key words: *Cyprinus carpio*, common carp, eggs, mycotal species, infections, hydrochemistry.

INTRODUCTION

The occurrence of the carp in the inland waters of Europe was first recorded in the works of Aristotle (284-322 B.C.). This was later confirmed by Plinius (23-79 A.D.) (Wolny, 1974). The origins of the domestication of carp probably took place in the estuary of the Danube River (Dacia) during the 5th and 6th centuries B.C. Monk Albert Magnus (1230-1310) reported on carp cultures in ponds in Bavaria, Germany, and the first mention of the breeding of this species in France dates back to 1313. Carp was known to have been held in ponds in Bohemia-Moravia in medieval times. In 1547, the Moravian Bishop of Olomun-Dubravius wrote the first European manual about carp farming in ponds. In terms of Germanic lands, there were descriptions in 1551 by Gessner, and for France in 1555 by Belon then in 1558 by Rondelet. These manuals contributed greatly to the dissemination

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of information about the culturing of this species. In Asia (China and Japan), the carp was domesticated much earlier for ornamental purposes (*Cyprinus carpio* koi). Carp is now grown on all continents except antarctic.

C. carpio is distributed across Europe and Asia, in the Black, Caspian and Aral sea basins, and has been introduced to waters throughout the world. However, wild stocks only occur naturally in rivers draining to the Black, Caspian and Aral seas (Kottelat and Freyhof, 2007; ITIS, 2010). A rheophilic wild population in the Danube River is assumed to be the origin of the European species, but this population is now under threat (Kottelat, 1997; ITIS, 2010).

Adults inhabit warm, deep, slow-moving and standing waters, such as lowland rivers and large, well-vegetated lakes. They are hardy and tolerate a wide variety of conditions, but generally favour large water bodies with slow-moving or standing water and soft bottom sediments (ITIS, 2010). Both adults and juveniles feed on a variety of benthic organisms and plant material (Bryliński, 2000; ITIS, 2010).

Today, with overharvest of wild fisheries, the intensity of aquafarming is increasing, especially in developing countries (Food and Agricultural Organization of the United Nations (FAO), 2012). This applies mainly to freshwater aquaculture and shrimp production, and to a lesser extent mariculture. In freshwater aquaculture, farming of common carp species (the earliest domesticated carp species in Europe) is of great economic importance.

The growth of fungal species on the specimen eggs of the common carp was first described in 1885 by Walentowicz, in Kaniow, Austria. Walentowicz referred to the presence of *Achlya nowicki* Raciborski (doubtful taxa) and *Saprolegnia monoica*. Observations and experiments on the *Saprolegnia ferax* that infected fish, including common carp specimens, have been described by Clinton (1894). Graff (1928) has also reported on the presence of *Saprolegnia parasitica* on specimens of common carp in ponds in Western Montana, U.S.A., and Chinapelli (1933) has looked at the experimental infection of the *Saprolegnia* species in carp specimens in water bodies of Italy.

Hörter (1960) investigated the lethal mycosis of carp skin that was caused by *Fusarium culmorum*. Srivastava and Srivastava (1976) have also mentioned the infection of common carp eggs on the Indian subcontinent, while Czeczuga and Muszynska (1999) have described the straminipilous and fungal species occurring on carp eggs in middle and east Europe. In addition, Froelich and Engelhardt (1996) have investigated the effects of different antifungal agents on the successful hatching koi carp eggs, and Padmakumar et al. (1985) and Khodabandeh and Abtahi (2006) have looked at the effects of such agents on the development of common carp eggs. In this context, we decided to investigate the growth of mycotal species on the eggs of common carp in water from limnologically and trophically different water bodies.

MATERIALS AND METHODS

The eggs of *C. carpio* Linnaeus, 1758 (syn. *Cyprinus regius* Lesniewski, 1837; *Cyprinus hungaricus* Heckel, Taczanowski, 1877; *C. carpio hungaricus* Heckel, Wałecki, 1889) (English name: common carp, carp) were investigated.

The water for these experiments was collected from five different water bodies located in the northeastern region of Poland: Spring Cypisek (53°07´N, 23°10´E): located in the south part of the Knyszynska Forest; width of 0.41 metres (m); depth of 0.17 m; discharge of 0.6 dm^3/s; the limnokrenic type: River Biala (53°15´N, 22°20´E): length of 9.8 km; left-bank tributary of the River Suprasl, flowing through the city of Bialystok; River Suprasl (51°10´N, 21°10´E): length of 106.6 km, right-bank tributary of the middle part of the River Narew, flowing through the Knyszynska Forest; Pond Fosa (53°18´N, 23°15´E): in the Palace Park (Bialystok); an area of 2.5 hectares (ha); depth of 1.75 m; a breeding area for wild ducks; a culture of crucian carp for anglers; Pond Komosa (52°08´N, 21°12´E): an area of 12.1 ha, depth of 2.25 m; surrounded by coniferous trees of the dense Knyszynska Forest

Nineteen (19) parameters of those water samples were measured (Table 1) according to generally accepted methods (APHA, 2005). Water samples for the analysis and for the experiments were collected from each reservoir at depth of 15-30 cm at a distance of 1.5 m from the bank (except spring). In the laboratory, the samples were filtered through gauze and then poured into 1000 ml vessels.

Eggs were collected (after fertilization) at the end of April from the hatchery at the Knyszyn Farm.

Water samples from specific water bodies (800 ml each) were placed into 1000 ml vessels, and 30 eggs were transferred to each vessel in accordance with the general principles of culture (Watanabe, 2000). Before transfer, eggs were washed with distilled water to remove fungal spores attached to their surface. All vessels were enclosed in Petri scales with the bed turned upside down to prevent possible airborne contamination with fungal spores. The vessels were stored at temperature of 17± 0.5°C, with access to daylight that resembled natural conditions and following the recommended instructions (Seymour and Fuller, 1987). The pH of the water was analysed separately for every vessel (Peterson and Bridge, 1994). The water analyses and experiments were carried out in three parallel repetitions. Eggs were taken from each vessel, and the eggs that were covered with fungal mycelia were observed every 3-to-4 days under a light-microscope. The presence of morphological structures such as zoospores, antheridia and oogonia that belong to aquatic fungi was recorded. Fungal species were identified using the keys of Johnson et al. (2005), Pystina (1998) and Petrini and Petrini (2013). The systematics of straminipiles species according to Dick (2001) were used in this experiment. The experiments were carried out for one month, and the results were then tested for significance using ANOVA and evaluated by the Scheffe test (Winer, 1997).

Amino acid, carbohydrate and urease tests were performed on the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera, based on Yuasa and Hatai (1996). For the carbohydrate utilization test, yeast nitrogen base agar was the medium used for the cultures of the fungal isolates. Glucose yeast (GY) agar was used for the urease test. The basal medium used in the amino acid assimilation test was the same as that used for the carbohydrate assimilation test. Bromo thymol blue and phenol red that was added to the yeast nitrogen-based broth and GY broth, respectively, were used as indicators. These methods are described in detail in our previous paper (Czeczuga et al., 2011).

RESULTS

Thirty three (33) mycotal species were found to be

Table 1. Chemical and physical properties of water in particular water bodies (in mg l^{-1}) (April, 2012).

Property	Spring	River		Pond	
	Cypisek	Supraśl	Biała	Fosa	Komosa
Temperature (°C)	14.5	16.8	15.2	18.0	14.9
pH	7.2	7.1	7.3	7.1	7.2
DO	16.4	8.2	18.6	6.4	14.8
BOD_5	2.6	10.8	4.2	12.8	5.1
COD (Oxidability)	9.9	16.8	11.2	20.2	9.0
CO_2	37.4	26.9	29.4	22.4	18.6
Alkalinity ($CaCO_3$ mval l^{-1})	4.6	4.3	3.6	5.8	3.8
$N\text{-}NH_3$	0.254	0.662	0.315	0.864	0.196
$N\text{-}NO_2$	0.015	0.128	0.037	0.114	0.018
$N\text{-}NO_3$	0.245	0.470	0.317	0.552	0.072
$P\text{-}PO_4$	0.920	1.820	1.02	3.598	0.706
Sulphates (SO_4)	36.8	73.2	50.4	85.1	40.6
Chlorides (Cl)	40.5	66.4	50.2	79.3	35.4
Total hardness (in Ca)	110.9	98.2	93.6	24.2	76.6
Total hardness (in Mg)	21.8	17.4	17.2	20.6	18.4
Fe	0.38	0.92	0.47	1.06	0.26
Dry residue	174.2	434.0	412.0	429.0	220.0
Dissolved solids	150.0	324.0	384.0	370.0	198.0
Suspended solids	24.2	110.0	28.0	59.0	22.0

growing on the eggs of the *Cyprinus carpio*, including 29 belonging to the Peronosporomycete (Oomycetes) class and 1 to the Zygomycete, 1 to the Endomycete and 2 to the Hyphomycete classes (Table 2). Analysis of water samples from 5 different water bodies in Northeastern Poland found the greatest number of mycotal species on the eggs of common carp from the waters of River Biala and Pond Fosa (20 and 19, respectively) and the smallest number of mycotal species from the waters of Spring Cypisek, Pond Komosa and River Suprasl (12, 11 and 11, respectively). Species such as *A. diffusa*, *A. laevis*, *S. ferax*, *S. parasitica* and *P. ultimum* were found to be growing on the carp eggs in the water from all five water bodies. It is worth making a special note about the discovery of *A. arbuscula*, *A. frigidophilus*, *C. albicans*, *F. aquaeductum*, *F. culmorum* and *Zoopage phanera*. Table 3 shows the assimilation tests for amino acids, carbohydrates and urease for species from the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera.

DISCUSSION

The most eutrophic water came from River Biala and Pond Fosa, while the water from Spring Cypisek, River Suprasl and Pond Komosa contained the lowest amounts of biogenic compounds.

The eggs of the common carp living in the water from River Biala and Pond Fosa, the most eutrophic of the water bodies examined, had the greatest number of mycotal species on them. The eggs of the common carp in the water from River Suprasl, Pond Komosa and Spring Cypisek, which had the least abundance of biogenes, had the smallest number of mycotal species. We also observed this kind of phenomenon while studying the growth of fungi on the eggs of certain cyprinid species (Czeczuga and Muszynska, 1999) and coregonid species (Czeczuga and Muszynska, 1998). However, a reverse phenomenon has been observed in Switzerland where more fungi were found on the pike (*Esox lucius* L.) and perch (*Perca fluviatilis* L.) species in oligotrophic lakes than on those in eutrophic lakes (Meng, 1980). It is important to emphasize that in acipenserid fish species, with the greatest amount of fungi were found to be growing on the eggs of eutrophic waters (Czeczuga et al., 1995). There has been an extensive search for effective measures to prevent or limit mycotic infections of eggs that are in hatcheries, as well as of fish fry and adult specimens in aquacultures. According to Marking et al. (1994), iodiphors and formalin are being commonly used as antifungal agents in fish cultures.

Mycotal species, such as *A. diffusa*, *A. klebsiana*, *A. orion*, *Aphanomyces stellatus*, *A. laevis*, *P. ultimum* and *S. parasitica*, were frequently found on eggs in the water from all five water bodies. These species all belong to the group of opportunistic pathogens that are sapro and necrotrophic (Bruno and Wood, 1999).

Butler (1911) was the first to describe *Allomyces arboscula*, a species found in the water and soil on

Table 2. Fungi and straminipiles from the reservoirs analysed.

Taxa	Spring Cypisek	River Biala	River Suprasl	Pond Fosa	Pond Komosa
Fungi					
Ascomycota					
Saccharomycetales					
1. *Candida albicans* (Robin) Berrk.		x			
Blastocladiomycota					
Blastocladiales					
2. *Allomyces arbuscula* Butler	x		x		x
Zygomycota					
Zoopagales					
3. *Zoopage phanera* Dreschsler				x	x
Straminipila					
Peronosporomycota					
Leptomitales					
4.*Leptomitus lacteus* (Roth) Agar	x			x	x
Pythiales					
5. *Pythium pulchrum* Minden	x	x	x	x	
6. *P. ultimum* Trow	x				x
Saprolegniales					
7. *Achlya diffusa* J. V. Harv. et J. W. Johnson	x		x	x	
8. *A. dubia* Coker		x			
9. *A. klebsiana* Pieters		x	x	x	
10. *A. megasperma* Humphrey	x				x
11. *A. orion* Coker et Couch		x	x	x	
12. *A. prolifera* Nees	x			x	
13. *A. proliferoides* Coker				x	x
14. *Aphanomyces frigidophilus* Kitauch. et Hatai	x				x
15. *A. irregularis* W. W. Scott	x	x	x	x	
16. *A. laevis* de Bary	x	x	x		
17. *A. parasiticus* Coker		x	x		
18. *A. stellatus* de Bary				x	
19. *Dictyuchus anomalus* Nagai		x		x	x
20. *D. monosporus* Leitgeb		x			x
21. *monilifera* (de Bary) Kauf		x		x	
22. *Leptolegnia caudata* de Bary		x			
23. *Protoachlya polyandra* (Lindst.) Apinis			x	x	
24. *Saprolegnia anisospora* de Bary		x		x	
25. *S. australis* Elliot		x		x	
26. *S. ferax* (Gruith) Thurnet	x	x	x	x	x
27. *S. litoralis* Coker		x			
28. *S. parasitica* Coker	x	x			
29. *S. shikotsuensis* Hatai et al.			x	x	
30. *S. terrestris* Cookson et Seymour		x			
31. *Scololegnia asterophora* (de Bary) M. W. Dick		x		x	

Table 2. Contd

Anamorphic fungi						
Hyphomycetes						
Moniliales						
32. *Fusarium aquaeductum* (Radlk et Rabh) Saccardo						x
33. *F. culmorum* (W. G. Smith) Saccardo	x					
Total species (the different letters indicate significant difference, p≤0.05)	12a	20b	11a	19b		11a

Table 3. Amino acids, carbohydrate and urease assimilation by straminipiles isolated from *Cyprinus carpio*.

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	+

Amino acids = Ala, alanine; Arg, arginine; Asp, aspargine; Cys, cysteine; Glu, glutamine; His, histidine; Carbohydrate = Fru, froctose; Gal, galactose; Glu, glucose; Man, mannose; Mal, maltose; Mel, melibiose; Cel, cellobiose; Dex, dextrin; Gly, glycerol; Lac, lactose; Rha, rhamnose; Raf, raffinose; Sta, starch; Suc, sucrose; Tre, trehalose.

substrates of plant and animal origin. Our previous study discussed our findings of *Allomyces arbuscula* growing on the eggs of cyprinid species such as *Chondrostoma nasus*, *Gobio albipinatus* and *Scardinus erythrophthalmus* (Czeczuga and Muszynska, 1999). *A. arbuscula* has also been isolated from the eggs of *C. carpio* on a fish farm in Thailand (Chukanhom and Hatai, 2004). Other species from the *Allomyces* genus, such as *A. anomalus* (Sati, 1983; Khulbe et al., 1995; Czeczuga and Muszynska, 1999) and *A. macrogynus* (Czeczuga and Muszynska, 1999), have also been reported on Cyprinidae eggs, including carp eggs.

A. frigidophilus was first described in Poland and in Europe by Czeczuga et al. (2004) in salmonid eggs. Kiziewicz et al. (2013) then reported the presence of *A. frigidophilus* in water from Polish springs.

In the present study, *C. albicans* was found on the eggs of common carp in water from River Biala. This fungus had previously been observed on the eggs of *Coregonus albula* in the hatchery in Wegorzewo, Poland and on the fry of eel montee *Anguilla anguilla*, as well as on the eggs of the rainbow trout (Czeczuga and Woronowicz, 1993). It should be mentioned that Hatai and Egusa (1975) isolated *Candida sake* cells from the gastro-tympanites of amago salmon. Findings of *Fusarium aquaeductum* and *F. culmorum* on common carp eggs is also worth noting because these belong to an abundant genus comprising saprophitic and phytopathogenic species and the facultative parasites of animals, mainly invertebrates (Booth, 1971). In our study, *Fusarium aquaeductum* grew on the eggs in water from Pond Komosa and also in the bodies of certain invertebrate species (Alton, 1985) and on the coregonidae eggs incubated in hatcheries (Czeczuga and Woronowicz, 1993). *F. culmorum*, which developed on the eggs of *Cyprinus carpio* in water from Spring Cypisek, is known to cause lethal mycosis of carp skin (Hörter, 1960). *F. culmorum* also afflicts the eggs of coregonids (Czeczuga and Muszynska, 1998). According to Marchenko (1988), *Fusarium avenaceum* var. *herbarium* has been found to cause mycosis of the swim bladders of salmonids in areas in the Far East. Some *Fusarium* species have also been isolated from *Clarias* species on the African continent (Easa et al., 1984; Refai et al., 2010). In our study, the *Zoopage phanera* species, which is rarely found in fishes, was found on eggs in water from Pond Fosa and Pond Komosa. These predacious fungi attack terricolous amoebae (Drechsler, 1935), and we found them in organic debris in the coastal area of bog-springs and in mesotrophic-type lakes. They also occur on the eggs of rainbow trout (Czeczuga and Muszyńska, 1999). The information about the amino acid, carbohydrate and urease assimilation by these species that occur on the eggs of the common carp did not differ from data obtained from fungal species that developed on the eggs of other fish species in the waters of Japan (Yuasa and Hatai, 1996). Some species of *Achlya*, *Aphanomyces*, *Pythium* and *Saprolegnia* genera assimilated some other amino acids and carbohydrates on the eggs of salmonid species from *Oncorhynchus* genus (Kitancharoen and Hatai, 1998; Czeczuga et al., 2011). Perhaps it is combined with biological variety of those fungal species.

Conclusion

Examination of the growth of fungi and straminipiles organisms on the eggs of common carp (*Cyprinus carpio* L.), in five trophically different water bodies was performed. Thirty-three (33) of mycotal organisms, developing and growing on the eggs of common carp (25 belonging to the Saprolegniales, two to the Pythiales, two to Moniliales and one species belonging to the Saccharomyces, Blastocladiales, Zoopagales and Leptomitales, respectively) were found. The greatest number of mycotal species was found in water from most eutrophic Biała River and Pond Fosa (20 and 19 species, respectively), and the lowest was identified in water from the less eutrophic River Supraśl and Pond Komosa (11 species respectively each other). *Achlya* and *Saprolegnia* were the most prevalent genera. The most commonly encountered species were *Phytium pulchrum* and *Saprolegnia ferax*. *Allomyces arbuscula, Aphanomyces frigidophilus, Candida albicans, Fusarium aquaeductum, F. culmorum* and *Zoopage phanera* were rarely found. Species of *Achlya, Aphanomyces, Leptolegnia, Pythium* and *Saprolegnia* genera assimilated six amino acids, 16 carbohydrate and urease (only *Leptolegnia, Pythium* and *Saprolegnia*). All species of *Achlya, Aphanomyces, Leptolegnia, Pythium* and *Saprolegnia* assimilated alanine, glucose and starch.

Our investigations show, that limnologically different water bodies do not increase the number of mycotal species on investigated eggs and that the greatest amount of fungi was found to be growing on the fish eggs in eutrophic waters (statistical significance showed in Table 2).

Conflict of interests

The authors did not declare any conflict of interest.

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