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Original Research

Water Fungi and Fungus-like Organisms Isolated from Surface Waters Situated in the Białowieża Primeval Forest Using the Liver Fluke *Fasciola Hepatica* L. of European *Bison Bonasus* L. as Bait

B. Kiziewicz

Department of General Biology, Medical University of Białystok, 15-089 Białystok, Kilińskiego 1, Poland

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Abstract

Water fungi and fungus-like organisms as a biological factor of ecological water systems have significant influence on the environment and its modification. They decompose necrosis substrates found in water bodies. Fungi also can act as facultative parasites and then frequently occur on their hosts. The main aim of the present study is to investigate which of the fungi and straminipiles can grow on the liver fluke *Fasciola hepatica* in the River Narewka and in natural ponds of Białowieża village.

In order to isolate fungi and fungus-like organisms, fragments of adults and eggs from liver flukes were introduced as bait into samples from each water body. The occurrence and growth of aquatic fungi and fungus-like organisms on *Fasciola hepatica* was investigated in laboratory conditions.

The liver fluke *F. hepatica* used in this study was collected from free-living European bisons, *Bison bonasus*, which were killed in Białowieża Primeval Forest.

The adult liver fluke *F. hepatica* turned into the habitat for seventeen species of fungi and straminipiles. They were *Achlya polyandra*, *A. prolifera*, *Apodachlya brachynema*, *Aphanomyces laevis*, *Ap. irregularis*, *Catenaria anguillulae*, *Dictyuchus monosporus*, *Leptolegnia caudata*, *Leptomitus lacteus*, *Pythium debaryanum*, *Sirolpidium zoophtorum*, *Saprolegnia diclina*, *S. glomerata*, *S. hypogyna*, *S. monoica*, *S. ferax* and *S. parasitica*.

Six fungal species such as *Achlya prolifera*, *Aphanomyces irregularis*, *Myzocytium zoophthorum*, *Catenaria anguillulae*, *Saprolegnia ferax* and *S. parasitica* were found on *F. hepatica* eggs**.**

The majority of fungi which were marked on *F. hepatica* were animal parasites or necrotrophs (13).

Keywords: fungi, Straminipila, liver fluke *Fasciola hepatica*, bison European *Bison bonasus*, the River Narewka, natural ponds of Białowieża village, Białowieża Primeval Forest, physical and chemical analysis

Introduction

Water fungi and fungus-like organisms as biological factors of ecological water systems influence the environment significantly. They decompose together with bacteria necrosis substrates occuring in water bodies. Moreover, they can optionally act as parasites, too**.** Animal parasites constitute a large group and are often found on invertebrates and vertebrates [1, 2, 3]. The liver fluke *Fasciola hepatica* occurs in the common bile ducts of mammal hosts such as wild and farm black rats, ostriches, sheep, cattle, pigs, donkeys, hares, bisons and humans [4, 5, 6, 7]. Adult liver flukes which live in bile ducts, produce eggs which are passed in faeces. When the eggs separate from faecal mate-

*e-mail: bkizbiol@amb.edu.pl

rial in wet areas, under optimal conditions they will hatch, releasing the larvae or miracidia. The miracidia invade the lymnaeid snails in which they develop and multiply as sporocyst, rediae and cercariae. The tadpole-like cercariae leave the snails and swim until they locate on water plants and form metacercariae, which are the mammal-infective stage of the fluke. If ingested by hosts, including humans, the metacercariae settle in the small intestine and the released immature flukes penetrate the intestinal wall and enter the abdominal cavity. The young flukes penetrate the liver capsule and migrate through the liver tissue for 6 to 7 weeks before entering the bile ducts prior to becoming adult flukes. The flukes reach sexual maturity and commence egg production [8].

The main aim of the present study was to determine species composition of the fungi and fungus-like organisms growing on the liver fluke *F. hepatica* in the River Narewka and in natural ponds situated in Bałowieża village of Białowieża Primeval Forest in the years 2002-03 as well as the effect of ecological factors, including certain physical and chemical parameters of water and composition of the substratum from which the fungi were isolated.

Materials and Methods

The water samples were collected from the following water reservoirs: Narewka River and natural ponds of Białowieża village of Białowieża Primeval Forest.

1. The Narewka River on the Polish side is 39.4 kilometers long. The upper stretch of the river flows across the area of Białowieża Primeval Forest. The samples were collected in the upper course of the river near Białowieża village.
2. Natural ponds in Białowieża village, an area of 2 hectares, are sites of crucian carps being bred for fishing. The ponds in Białowieża village are natural bodies supplied by water from the Narewka. They are overgrown by plants.

The liver fluke *F. hepatica* used in this study was collected from free-living European *Bison bonasus* L., from Białowieża Primeval Forest which were killed during natural selection. Nowadays, the population of wild European *Bison bonasus* in the Białowieża Primeval Forest on the Polish side consists of up to 349 individuals and on the Belarusian side of about 275 individuals in 2002 [9].

The bait method using adults and egg liver fluke *F. hepatica* was applied to isolate fungi [10]. The liver fluke isolates were placed in one-liter containers with water samples collected from the respective sites. The containers were covered with glass plates, at least partly to protect the water from penetration by bacteria. The samples were stored for approximately one month in a laboratory at the same temperature as that measured in the respective water reservoir, river or pond. Light and temperature were regulated to resemble natural thermal and light conditions [11]. Microscopically determined mycelia were removed from the baits and transferred to sterilized Petry plates containing distilled water. The microscopic examination of the mycelia was repeated after a few days. Several microscopic preparations were carried out each time. Identification of the aquatic fungi and fungus-like organisms involved measurements and determination of the vegetative organs – shape and size of the hyphae, asexual reproductive organs – shape of sporangium and spores, and generative organs – the structure of the oogonium, oosporangium and antheridium. Fungi were identified according to the works of Batko [10], Dick [12] and Pystina [13]. The results were subjected to statistical analysis using *t*-test to determine the significance of differences in the number of fungi and fungus-like organisms on eggs and on adult liver flukes in particular sites of water.

Physical and chemical investigations were performed using commonly applied hydro- biological methods [14].

Table 1. Physical and chemical parameters of water from the different sites (mean from 6 samples).

Specification	Water bodies	
	River Narewka	Ponds Białowieża village
Temperature (^{o}C)	8.4	12.5
pH	7.2	7.9
Oxidability (mg l^{-1})	15.4	12.6
N-NH_4 (mg l^{-1})	0.610	0.450
N-NO_2 (mg l^{-1})	0.014	0.010
N-NO_3 (mg l^{-1})	0.17	0.22
P-PO_4 (mg l^{-1})	0.83	0.40

Results

As revealed by physical and chemical analysis of the River Narewka and ponds of Białowieża village collected from particular sites in 2002 and 2003, the water at the site on the Narewka had the highest number of biogenes (N-NH_4, P-PO_4) and oxidability. In natural ponds of Białowieża village the highest level of nitrate nitrogen was marked (Table 1).

Eighteen fungi and fungus-like organisms were isolated from adult liver flukes. Only five species of fungus were marked on *F. hepatica* eggs. The greatest number of fungi was found on adults (17), the lowest number on eggs of liver flukes (6). The results obtained may be important to parasitologists, because 18 of the recorded fungi are known as necrotrophs or animal parasites (Table 2, Fig. 1).

Table 2. Fungi and fungus-like organisms found on eggs (1) and adults (2) of liver fluke *Fasciola hepatica* in particular sites of the River Narewka and of ponds in Białowieża village.

Taxa	Site			
	River Narewka (1)	River Narewka (2)	Ponds Białowieża village (1)	Ponds Białowieża village (2)
Straminipila				
Peronosporomycetes				
Leptomitales				
Apodachlya brachynema (Hildebr.) Pringsh.		x		x
**Leptomitus lacteus* (Roth) Agardh				x
Pythiales				
**Myzocytium zoophthorum* Sparrow	x		x	
Pythium debaryanum R. Hesse		x		x
**Sirolpidium zoophthorum* Vishniac		x		
Saprolegniales				
**Achlya polyandra* Hildebr.				x
**A. prolifera* Nees.	x			x
Aphanomyces irregularis W.W. Scott	x	x	**x**	
**Ap. laevis* de Bary		x		x
**Dictyuchus monosporus* Leitg.		x		x
**Leptolegnia caudata* de Bary				x
**Saprolegnia diclina* Humphrey		x		x
S. glomerata (Tiesenhausen) Lund		x		
**S. ferax* (Gruith.) Thur.	x	x	x	**x**
**S. hypogyna* (Pringhs.) de Bary		x		x
**S. monoica* Pringsh.				x
**S. parasitica* Coker	x	x	x	x
FUNGI				
Chytridiomycetes				
Blastocladiales				
Catenaria anguillulae Sorokin	x		x	**x**
Total number	6	11	5	14

*Fungi known in literature as parasites or necrotrophs of animals

There was not any difference between the number of fungi and straminipiles which were marked on eggs and adult liver flukes and particular sites of water during the study.

Discussion

Eighteen fungus species were collected during this experiment. The genera, namely *Saprolegnia ferax* and *S. parasitica*, were of high frequency of occurrence on eggs and adult liver flukes in the River Narewka and in ponds of Białowieża village.

Representatives of the class Peronosporomycetes were the most numerous group of fungus-like organisms found on liver flukes.

The eggs and fragments of adults liver flukes examined were inhabited by a number of fungus-like organism species which frequently cause significant losses in fish farming, including *Achlya polyandra*, *A. prolifera*, *Apha-*

nomyces irregularis, *A. laevis*, *Leptolegnia caudata*, *Saprolegnia diclina*, *S. ferax*, *S. hypogyna*, *S. monoica* and *S. parasitica*. These straminipiles are commonly encountered both on dead vegetation and animal material. However, in favourable conditions they parasitize on weakened and mechanically damaged animals, mainly spawn and fish in their different developmental periods [15, 16, 17].

Aphanomyces laevis attacks both eggs and adult individuals of many economically valuable fish species [18]. In our study *Pythium debaryanum* found on eggs of *Fasciola hepatica* was isolated from the River Narewka and from ponds of Białowieża village. *Pythium debaryanum* has been known since the middle of the previous century as phytopatogenic fungus-like organism and has been encountered in various water reservoirs [13, 19].

Interestingly, the fungus-like organisms *Leptolegnia caudata* on *Fasciola hepatica* were found in the ponds of Białowieża village. This species was encountered as a parasite of predatory crustaceans *Leptodora kindtii* [10]. *Leptolegnia caudata* was observed to be an aquatic and soil saprotroph found on crustaceans, dragonflies, snake skin and spawn of various fish species [18, 20, 21].

A nitrophilic fungus-like organism, *Leptomitus lacteus*, commonly found in sewage, was isolated only once on an adult liver fluke in the ponds of Białowieża village. This taxon allows detection of the inflow of industrial effluents and was investigated by Staniak [22]. *Leptomitus lacteus* is a parasite and necrotroph fungus which was marked from the spawn of certain fish [17, 23] and from perch [24]. Two species, *Myzocytium zoophthorum* and *Sirolpidium zoophthorum*, were isolated on eggs and adult liver flukes from the River Narewka and ponds of Białowieża village. These fungus-like organisms are described as parasites of rotifers and larvae of mollusces, while *Myzocytium megastomum* was found on aquatic insects in lakes and ponds in Poland by Czeczuga and Godlewska [25]. It was described by Batko [10] as a parasite of algae.

Of the Chytridiomyces class isolated in the study, *Catenaria anguillulae*, which was found in the River Narewka and in ponds of Białowieża village on eggs *Fasciola hepatica*, was the most frequently encountered fungus. This fungus, known as a phytosaprotroph and zoosaprotroph, was previously isolated from chitin and keratin-containing substrates [26-30].

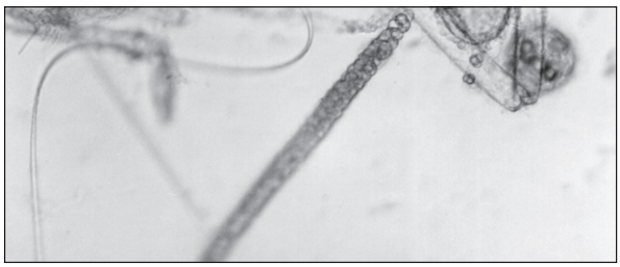

Fig. 1. Dictyuchus monosporus -sporangium growing on the liver fluke *Fasciola hepatica*.

The majority of species of fungi which were marked on *Fasciola hepatica* are animal parasites or necrotrophs [1, 10, 31].

In conclusion, the liver fluke *Fasciola hepatica* is connected with an aquatic water environment, in which part of its development is spent, i.e. egg development, miracidium stage, intramoluscal stage, cercaria and metacercariae. In this stage fungi participate in food-competition which as decomposers, parasites and predators can play a part in stopping parasite development. Particularly mycosis infection can occur on eggs and larvae stages of *Fasciola hepatica* in water such as: miracidia, cercariae and metacercariae, which is probably why the flukes do not always reach sexual maturity in their definitive hosts.

The fungi which were marked on eggs of the liver fluke *Fasciola hepatica* can lead to stopping the development of the parasite in aquatic water environment. They can contribute to the elimination of *Fasciola hepatica* at an initial stage of its development. This study on fungus growth on parasites is an important element to broaden knowledge on mycology, epidemiology and parasitology.

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