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Moina macrocopa (Straus): A Plankton Crustacean as a Vector for Fungus-Like Fish Parasites

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Abstract: The authors investigated the occurrence of fungi and fungus-like organisms (chromistan organisms) growing on dead *Moina macrocopa* specimens in 3 bodies of water of varied trophicity. Dead specimens of the crustacean *Moina macrocopa*, fish feed in aquaculture in countries of the Mediterranean basin, were used as bait. In all, 55 species were identified on the *Moina macrocopa* specimens, including 42 chromistan organisms and 13 fungus species. Of the 42 chromistan organisms, 13 are known as parasites or necrotrophs of fish species. They included *Achlya americana, Achlya debaryana, Achlya klebsiana, Saprolegnia ferax, Saprolegnia monoica,* and *Saprolegnia parasitica*; all are known to frequently cause mycotic diseases in economically important fish populations. Dead specimens of *Moina macrocopa* should thus be regarded as vectors of aquatic chromistan organisms that induce mycosis in fish.

Key Words: *Moina macrocopa*, fungi, fungus-like organisms, mycosis in fish

Introduction

Studies conducted on aquatic fungi, whose mass invasion of crustaceans occurs in European waters (Czeczuga et al., 1998, 1999, 2000, 2002), have shown the involvement of non-chitinophilic species, apart from purely chitinophilic fungi, in the process of decay. The former include a number of new species to Polish waters as well as those having been referred to as plant saprophytes.

Moina macrocopa, a plankton crustacean that invades the waters of the Mediterranean Sea basin and southern Asia (Hutchinson, 1976; Gündüz, 2002), has sometimes been used in feeds for aquaculture. In order to gather complete evidence of fungi growing on crustaceans we decided to focus this time on *Moina macrocopa*, which turned out to be involved in the mineralization of this type of substrate. We also identified some known fungus-like fish parasites growing on specimens of this crustacean. Thus, for certain fish parasites, dead *Moina macrocopa* specimens are a potential vector.

Materials and Methods

The study was performed on dead and dried *Moina macrocopa* (Straus), which is used in the aquacultures of India and countries of the Mediterranean basin as feed for many species of fish fry and adult specimens, and ornamental fish in particular (Shariff et al., 1992). The water for the experiments was collected from 3 different sources: 2 running (Cypisek Spring and Suprasl River) and 1 stagnant (Dojlidy Pond).

Cypisek Spring: North part of Bialystok, limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 l/s. The spring is surrounded by cultivated fields, but no trees. The bed is covered with sand.

Suprasl River: Right bank tributary of the middle part of the Narew River flowing through the Knyszynska Forest, length 106.6 km. The samples were collected from the site above the municipal swimming pool at the sluice of an arm of the Suprasl River, flowing just through the town of Suprasl. The sampling site is surrounded by meadows. The bed is muddy.

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Dojlidy Pond: Near Bialystok, area 34.2 ha, maximum depth 2.85m. Its south shores border with coniferous woods and its western part with the town of Bialystok. The samples were collected from the western part of this pond, which is used by the inhabitants of the town as a beach.

In all, 19 water parameters of the above sampling sites were determined (Table 1) according to the methods recommended by the Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1995).

For the determination of the presence of aquatic chromistan organisms and fungal species on the crustacean specimens the following procedure was employed: A certain number of pieces of crustacean (about 2 mg) were transferred to 2 samples of water representing each site in a 1.0-dm^3 vessel (all together 6 vessels for this species) and placed in the laboratory at ambient temperature. Some of the pieces from each vessel were observed under a light microscope and the mycelium of aquatic fungi on the pieces of crustacean was recorded. The methods are described in detail by Seymour and Fuller (1987). The pieces of the crustacean specimens were observed under a microscope for 1.5 weeks. The duration of the experiments was 4 weeks. Identification of fungi species was based on morphology and biometric data of antheridia and oogonia of the fungi and fungus-like organisms.

Identification of the fungi was aided by the following keys: Johnson (1956), Seymour (1970), Batko (1975), Karling (1977), Plaats-Niterink (1981), Dick (1990), and Pystina (1998).

The systematics of aquatic fungi and fungus-like organisms (chromistan organisms) were used according to the methods recommended by McLaughlin et al. (2001). The chemical parameters of the water and the fungal flora were investigated by statistical analysis (McGarical et al., 2000).

Results

Samples used for analysis were obtained from 3 limnologically different water reservoirs (Table 1), varying in physical and chemical parameters. The Cypisek

Table 1. Chemical composition (in mg l^{-1}) of water from different sampling sites.

Specification	Cypisek Spring	Suprasl River	Dojlidy Pond
Temperature (°C)	13.8	17.0	16.0
pH	7.26	7.82	7.94
O_2	7.6	11.2	9.4
BOD_5	2.8	2.8	8.7
Oxidability	5.80	6.60	15.74
CO_2	11.0	6.6	8.8
Alkalinity in $CaCO_3$ (mval l^{-1})	4.3	4.5	3.2
N-NH_3	0.400	0.140	0.165
N-NO_2	0.027	0.008	0.006
N-NO_3	0.036	0.014	0.050
P-PO_4	1.400	0.500	0.045
Sulfates	56.77	32.38	45.66
Chlorides	30.0	17.0	25.0
Total hardness in Ca	96.48	73.44	61.92
Total hardness in Mg	16.77	11.61	12.04
Fe	0.85	0.50 1	0.80
Dry residue	361	97	257
Dissolved solids	211	179	210
Suspended solids	150	18	47

Spring contained the most biogenes, while the fewest were present in the Suprasl River.

In all, 55 species, including 13 fungi and 42 fungus-like organisms, were found to develop on the dead *Moina macrocopa* specimens (Table 2, Figure 1), most in the Suprasl River water (41) and the fewest in Cypisek Spring water (20). Of the fungus species found in the study, *Catenophlyctis variabilis, Olpidiopsis saprolegniae, Olpidiopsis varians, Olpidiopsis vexans, Pythium helicoides*, and *Pythium salinum* have not previously reported on dead crustaceans. Moreover, 3 rare fungus species, namely *Achlya crenulata, Myiophagus ucrainica*,

Table 2. Aquatic fungi and fungus-like organisms found on the *Moina macrocopa* (Straus) specimens.

Fungi	Cypisek Spring	Suprasl River	Dojlidy Pond
Chromista (fungus-like organisms)			
Oomycetes			
Lagenidiales			
Lagenidium giganteum Couch		x	x
Olpidiopsis saprolegniae (Braun) Cornu		x	
Olpidiopsis varians Shanor			x
Olpidiopsis vexans Barret		x	x
Myzocytium microsporum (Karling) Sparrow		x	x
Myzocytium zoophthorum Sparrow	x	x	x
Saprolegniales			
**Achlya americana* Humphrey		x	x
Achlya crenulata Ziegler		x	
**Achlya debaryana* Humphrey		x	x
**Achlya klebsiana* Pieters	x	x	x
Achlya oblongata de Bary			x
Achlya treleaseana (Humphrey) Kauffman			x
Aphanomyces bosminae W. W. Scott	x		
Aphanomyces daphniae Prowse		x	x
Aplanes androgynus (W. A. Archer) Humphrey	x	x	
**Dictyuchus sterile* Coker		x	
**Isoachlya monilifera* (de Bary) Kauffman			x
**Leptolegnia caudata* de Bary			x
Saprolegnia anisospora de Bary			x
Saprolegnia asterophora de Bary	x	x	
**Saprolegnia delica* Coker		x	x
**Saprolegnia ferax* (Gruith.) Thuret	x	x	x
Saprolegnia glomerata (Tiesenhausen) A. Lund		x	x
**Saprolegnia monoica* Pringsh.		x	x
**Saprolegnia parasitica* Coker	x	x	x
Saprolegnia pseudocrustosa A. Lund	x	x	x
**Saprolegnia torulosa* de Bary	x	x	x
Peronosporales			
Pythium afertile Kanouse et Humphrey	x	x	x
Pythium aquatile Höhnk		x	
Pythium butleri Subraman.		x	
**Pythium debaryanum* R. Hesse		x	x
Pythium elongatum V. D. Matthews	x		

Table 2. (continued)

Pythium graminicola Subraman	x		
Pythium helicandrum Drechsler		x	
Pythium helicoides Drechsler	x	x	x
Pythium imperfectum Höhnk		x	
Pythium inflatum V. D.Matthews		x	
Pythium intermedium de Bary		x	
Pythium myriotylum Drechsler	x	x	x
Pythium perniciosum Serbinow		x	x
Pythium salinum Höhnk		x	
**Pythium ultimum* Trow		x	x
Fungi			
Chytridiomycetes			
Olpidiales			
Blastulidium paedophthorum Perez	x		
Myiophagus ucrainica (Wize) Sparrow	x	x	x
Olpidium granulatum Karling	x		
Olpidium rotiferum Karling	x		
Chytridiales			
Chytriomyces hyalinus Karling			x
Karlingia chitinophila Karling			x
Phlyctochytrium aureliae Ajello		x	
Podochytrium chitinophilum Willoughby	x	x	x
Rhizophlyctis ingoldii Sparrow			x
Rhizidiomyces bivellatus Nabel		x	
Blastocladiales			
Blastocladiella britanica Horenst. et Cant.		x	x
Catenaria anguillulae Sorokin	x	x	x
Catenophlyctis variabilis (Karling) Karling		x	
Total number	20	41	33

*Known in the literature as parasites or necrotrophs of fish.

and *Rhizophlyctis ingoldii*, were isolated from dead *Moina macrocopa* specimens. Of the 42 fungus-like organisms (Chromista), 13 are known as parasites or necrotrophs of many fish species, including those bred in aquaculture.

The statistical method showed that the factors that had a big influence on the amount of fungal species were chlorides in Dojlidy Pond water and sulfates in the water of Cypisek Spring and Suprasl River. Analysis showed that the mycoflora of Dojlidy Pond was affected by the considerable level of chlorides, showing a negative correlation (–0.8209, level of significance 0.04). In the water of Cypisek Spring and Suprasl River, a negative correlation was revealed in the case of sulfates concentration (respectively, –0.8315 and –0.7217, level of significance 0.04).

Discussion

Plankton crustaceans forming zooplankton aggregations grow in water reservoirs of various types, especially during the summer. The descent of dead specimens to the bottom of reservoirs, the so-called "rain of corpses", contributes to the formation of bottom deposits. Chitin carapaces are relatively durable and can remain in the sediment as long as several thousand years. Apart from chitin-decomposing bacteria, lower fungi belonging to the so-called chitinophilic group are involved in decay (Sparrow, 1960).

The list of fungi involved in the mineralization of dead specimens of plankton crustaceans described in a previous study (Czeczuga et al., 2002) has been supplemented with 6 new species. *Catenophlyctis variabilis* is known in

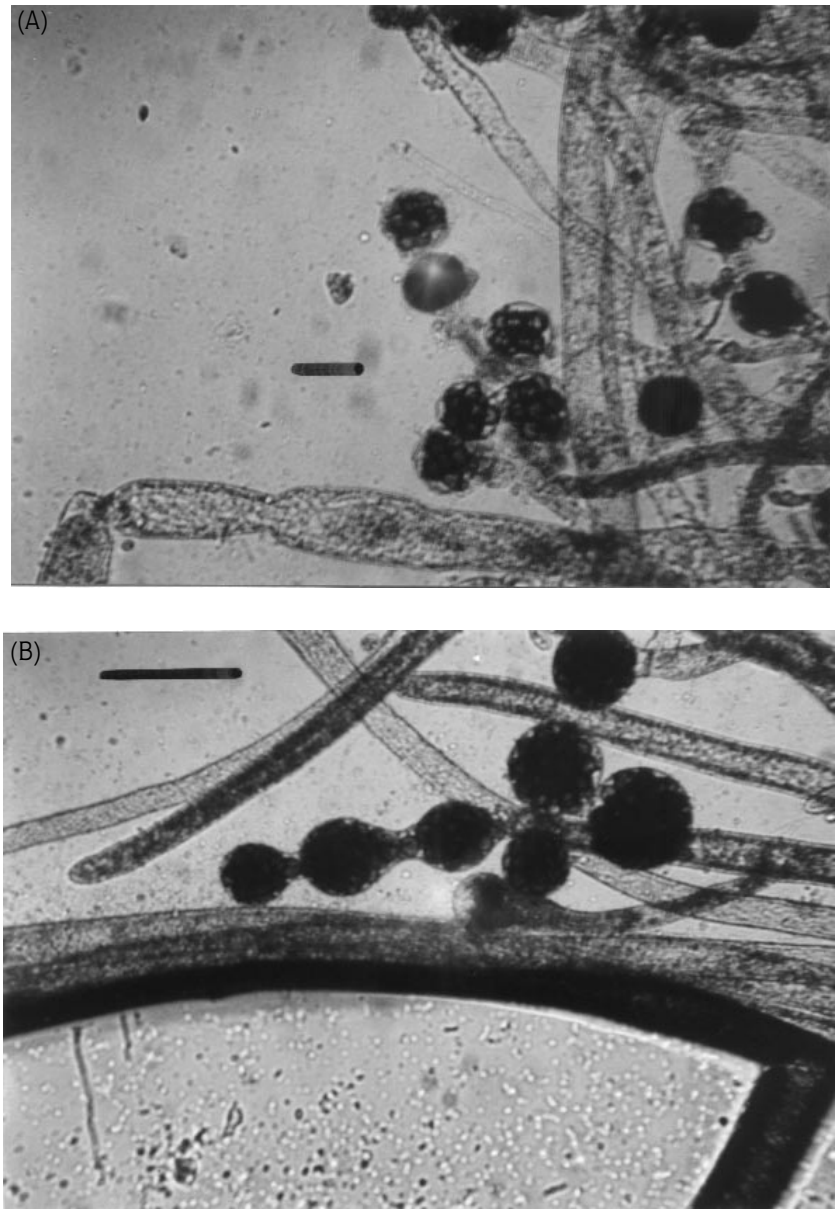

Figure 1. Some fungus-like organisms growing on the *Moina macrocopa* specimens.
A. *Saprolegnia ferax*, hyphae from oogonium.
B. *Saprolegnia torulosa*, hyphae from oogonium.
Bar scale = 25 µm.

the literature as aquatic and a soil saprophyte (Batko, 1975) and was first described from human hair (Karling, 1947). We found this species relatively frequently on such animal substrates as dragonflies (Czeczuga et al., 1999). The other 3 species of the genus *Olpidiopsis* have been referred to as parasites of other fungi of the *Achlya* and *Saprolegnia* genera (Batko, 1975). *Olpidiopsis saprolegniae* also grows on fish eggs (Czeczuga and Muszynska, 1999); however, both species of *Pythium*, new to this type of substrate, are known as plant root parasites. *Pythium helicoides* was described from Florida, USA, as a parasite of *Phaseolus vulgaris* L. roots

(Drechsler, 1930), while *Pythium salinum*, as a plant saprophyte, was isolated from an estuary near Kiel in Germany (Höhnk, 1953). A rare species, *Achlya crenulata,* is known from the waters of Florida (Ziegler, 1958) and from fish eggs in northeastern Poland (Czeczuga and Muszynska, 1999). We observed its growth on *Moina macrocopa* carapaces in the water of the Suprasl River, as well as on several aquatic insects (Czeczuga and Godlewska, 2001, 2006) and on dead specimens of the plankton crustacean *Mesocyclops leuckarti* (Claus) (Czeczuga et al., 2002). A second rare species, *Myiophagus ucrainica* was isolated from some species of aquatic insects (Czeczuga and Godlewska, 2001). Another rare species, *Rhizophlyctis ingoldii*, was found to grow in the water of Dojlidy Pond. This species was first described as a chitinophilic fungus (Sparrow, 1957) and then it was isolated from bottom deposits of some water reservoirs in the British Isles (Willoughby, 1961).

Due to the intensive development of aquaculture in south Asian countries in recent years, especially in India (Stirling, 1985; Ray and Das, 1992), and in the basin of the Mediterranean Sea, *Moina macrocopa* is used to feed the hatch of numerous fish species. Of particular importance is the finding of 13 species of fungus-like organisms (chromista) on dead specimens of this crustacean, which repeatedly causes great losses at different latitudes. These species include *Achlya americana*, *Achlya debaryana, Achlya klebsiana, Saprolegnia ferax, Saprolegnia monoica*, and *Saprolegnia parasitica*. Two species of the genus *Pythium*, namely *Pythium debaryanum* and *Pythium ultimum*, and *Achlya americana* are known to cause great losses in the breeding of *Tilapia* in Africa (El-Sharouny and Badran, 1995), the basic source of animal protein on that continent. *Achlya debaryana* sometimes causes epizootic infection on the Indian subcontinent (Khulbe et al., 1994). *Achlya klebsiana* causes substantial damage at various latitudes (Neish and Hughes, 1980); however, the most dangerous fish parasites, both in aquacultures and in natural conditions, are the species of the genus *Saprolegnia* (Hussein et al., 2001). *Saprolegnia ferax* is frequently found in ponds in masses (Frick and Reinhold, 1987) and *Saprolegnia monoica* grows on eggs (Osipian et al., 1988), while *Saprolegnia parasitica* causes mass death of fish fry (Sati et al., 1982) and adult fish specimens (Hatai and Hoshiai, 1992).

Pythium debaryanum and *Pythium ultimum* were isolated from 2 species of *Tilapia* (El-Sharouny and Badran, 1995). The frequency of occurrence of *Pythium debaryanum* and *Pythium ultimum* was moderate on the gills, and low to rare on fins, skin, and intestine. These authors classified *Pythium* among the common fungus-like organisms on *Tilapia* samples. *Pythium* species have also been isolated from the eggs of some fish species of Cyprinidae (Czeczuga, 1996).

Among the experimental containers with water from 3 water reservoirs, the largest group of fungi species to appear on dead *Moina macrocopa* specimens was chitinophilic fungi: *Achlya bosminae, Achlya daphniae, Karlingia chitinophila, Lagenidium giganteum, Myzocytium zoophthorum*, and *Podochytrium chitinophilum.* As the experiment progressed, other species were also identified, some of which have been previously described as fish parasites or necrotrophs, followed by animal saprophytes and eventually plant saprophytes. A similar phenomenon was observed in a previous study of fungi blooming on chitin coats of dragonflies (Czeczuga et al., 1999). This also refers to the fungi involved in the mineralization of other chemically different substrates, such as bird feathers (Czeczuga et al., 2004) or animal hair (Czeczuga and Muszynska, 2001). At the beginning of the experiment, the keratin substrate was attacked by typically keratinophilic species, but when the decay process continued, other non-keratinophilic species, and even plant saprophytes, became involved.

The statistical method showed that chlorides (pond) and sulfates (spring and river) influenced the amount of species growing on the *Moina macrocopa* specimens. As is known (Häkanson, 1999), chlorides and sulfates are pollution indicators for some water basins. Both of them increase with the eutrophication of basins. It is well known that in waters rich with biogenes the amount of fungi and fungus-like organisms is less. This phenomenon was observed earlier (Czeczuga et al., 1999, 2000, 2002) and in the present investigation.

As shown in the current study, dead *Moina macrocopa* specimens used as feed for fish fry in aquaculture can be the source of infection in fish farms of southern Asia, particularly on the Indian subcontinent, and in countries of the Mediterranean basin. Fungi and fungus-like organisms growing on this crustacean do not lose their vitality, even when not in aquatic conditions (Czeczuga,

1992), which has been confirmed in the climatic conditions of India (Sridhar and Kaveriappa, 1988; Hood and Robinson, 1989). Hyphae found on land in dry air conditions were still vital when transported to aquatic conditions 1 year later. Uneaten dead *Moina macrocopa* specimens can serve as vectors for the many species of fungus-like organisms, fish parasites, or necrotrophs. Thus, not only should antimycotic agents be used against fungi in fish specimens, but also fungus growth on the crustaceans they consume should be prevented.

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