

Species of *Pythium* isolated from eggs of fresh-water fish

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In the present study species from the genus *Pythium* were isolated from eggs of fresh water fish. Altogether sixteen species of fungi were found on fish eggs including 13 species noted for the first time from fish.

Key words: fungi, *Pythium*, fish eggs.

INTRODUCTION

Species of *Pythium* are found in various habitats. A large number of these species are recognized as saprophytes and parasites of higher plants. Not only are they parasites of terrestrial plants and aquatic alga-type plants (S p a r r o w, 1931), but are also found on eggs of crabs (A t k i n s, 1955) and fish (S c o t t, O' B i e r, 1962). In fish, only *Pythium ultimum* and a few unidentified species were observed on eggs, which questioned (S c o t t, W a r r e n, 1964; S t u a r t, F u l l e r, 1968) their parasitic nature. These data were confirmed by other authors (W i l s o n, 1976; N e i s h, H u g h e s, 1980; S r i v a s t a v a, 1980; D u d k a et al., 1989). F l o r i n s k a j a (1969), who found *Pythium proliferum* on fish eggs in hatcheries, while C z e c z u g a and W o r o n o w i c z (1993) noted *Pythium artotrogus* on eggs of *Coregonus lavaretus*, *C. albula* and *Esox lucius*. The above data as well as very scant evidence of the occurrence of species of *Pythium* on fish eggs inclined us to carry out detailed investigations, which would provide more information about the biological properties of some of these fungi species.

MATERIALS AND METHODS

Water samples were taken from three bodies of water: River Supraśl, pond-moat in Branicki Park and Lake Komosa. In water samples eighteen parameters were determined (Table 1) according to the generally accepted methods (G o l t e r m a n, C l y m o, 1969).

Table 1

Chemical composition (in mg l⁻¹) of the different water (September, 1995)

Specification	Water from		
	Pond-moat	Lake Komosa	River Supraśl
Temperature (°C)	11.6	13.8	12.4
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 (mval dm ⁻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
P (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
S (SO ₄)	28.38	12.75	19.75
Fe	0.524	1.542	0.512
Dry residue	358	375	317
Dissolved solids	282	312	305
Suspended solids	76	61	12

In order to determine the presence of *Pythium* species on eggs, the following procedure was applied: fish eggs (100-200) were transferred to two for each water a 1.0 litre vessel (together for each species was six vessel) and kept in laboratory at a temperature approaching that of the given hatchery. Some eggs from each vessel was observed under a microscope and the mycelium (form zoospore and oogonia) of aquatic fungi growing on the eggs was recorded. The methods were described in more detail in the paper of S m i t h et al. (1985) and F u l l e r, J a w o r s k i (1986). The eggs of the various fish species were examined for 1-1.5 weeks. The eggs were mostly live but sometimes dead.

The following keys were used to determine the species of *Pythium*: S p a r r o w (1960), P l a a t s - N i t e r i n k (1981) and D i c k (1990).

RESULTS

The data indicated a range trophicity in the bodies of water investigated defined by the concentration of phosphate and various forms of nitrogen.

We observed the growth of sixteen fungi species of the genus *Pythium* on the eggs of fresh-water fish (Table 2 and Fig. 1). Some of them were observed sporadically, while such species as *P. artotrogus* var. *macranthum*, *P. middletonii*, or *P. ultimum* occurred commonly. In addition thirteen species of *Pythium* had never been observed on fish eggs before.

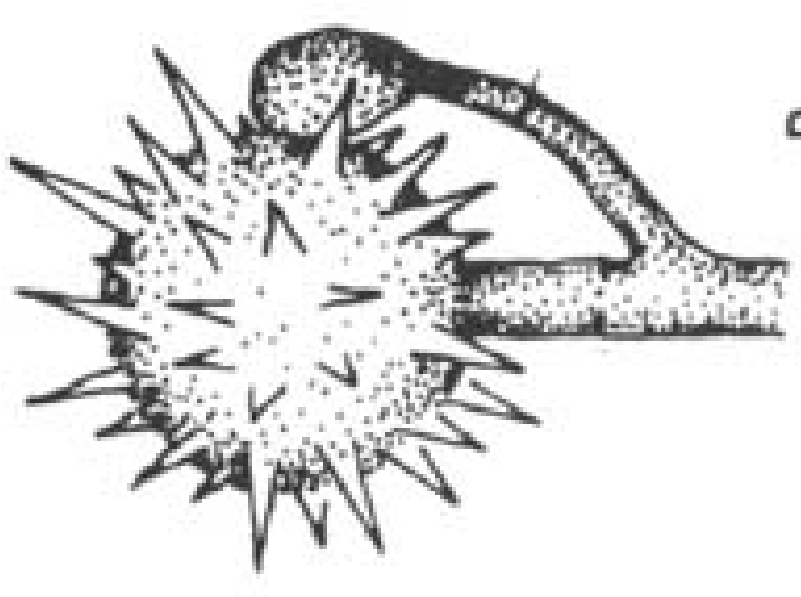
Table 2

Fungi of the genus *Pythium* were found on the eggs of fish in the different water

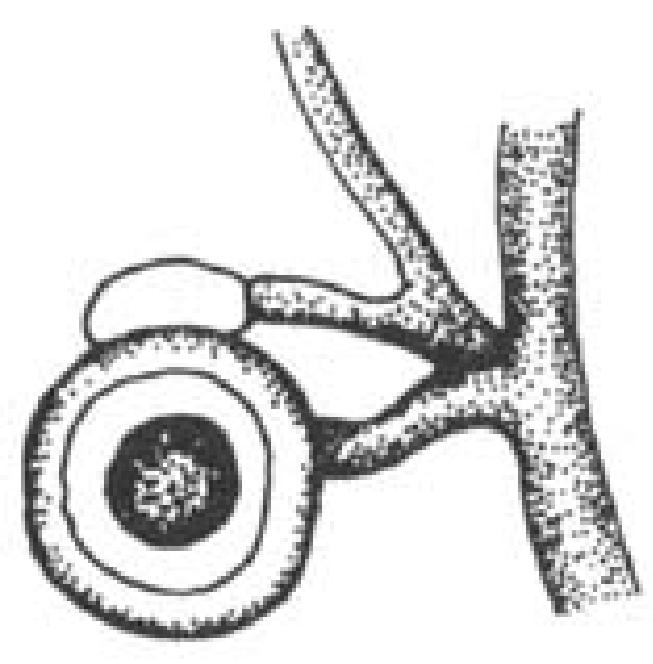
Species	Water from		
	pond	lake	river
<i>P. aristosporum</i> Vanterpool		x	
<i>P. artotrogus</i> var. <i>macranthum</i> Sideris	x	x	x
<i>P. debaryanum</i> Hesse	x	x	x
<i>P. echinulatum</i> Matthews		x	
<i>P. inflatum</i> Matthews			x
<i>P. intermedium</i> de Bary	x	x	x
<i>P. mamillatum</i> Meurs	x	x	
<i>P. marsipium</i> Drechsler	x	x	
<i>P. middletonii</i> Sparrow	x	x	x
<i>P. oligandrum</i> Drechsler	x		
<i>P. pulchrum</i> Minden in Falck		x	
<i>P. rostratum</i> Butler	x		x
<i>P. spinosa</i> Sawada	x		
<i>P. sylvaticum</i> Campbell et Hendrix		x	x
<i>P. torulosum</i> Coker et Patterson	x	x	x
<i>P. ultimum</i> Trow	x	x	x
Number of species	11	12	9



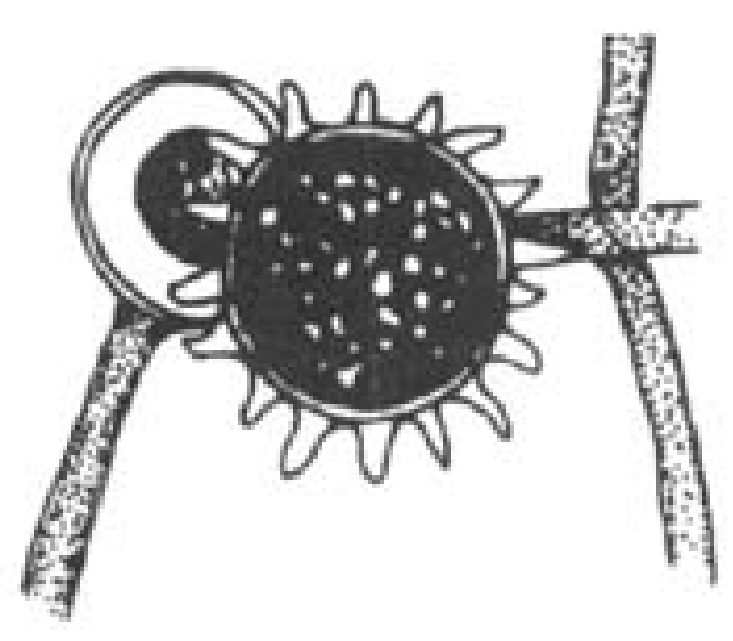
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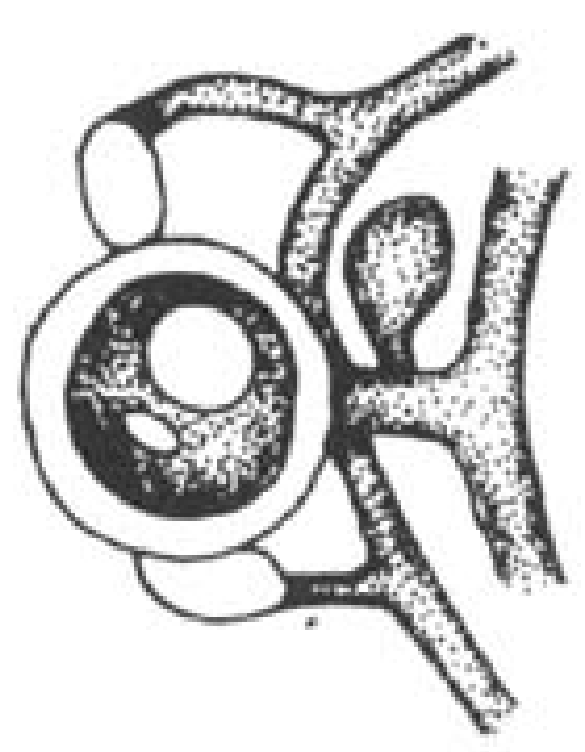
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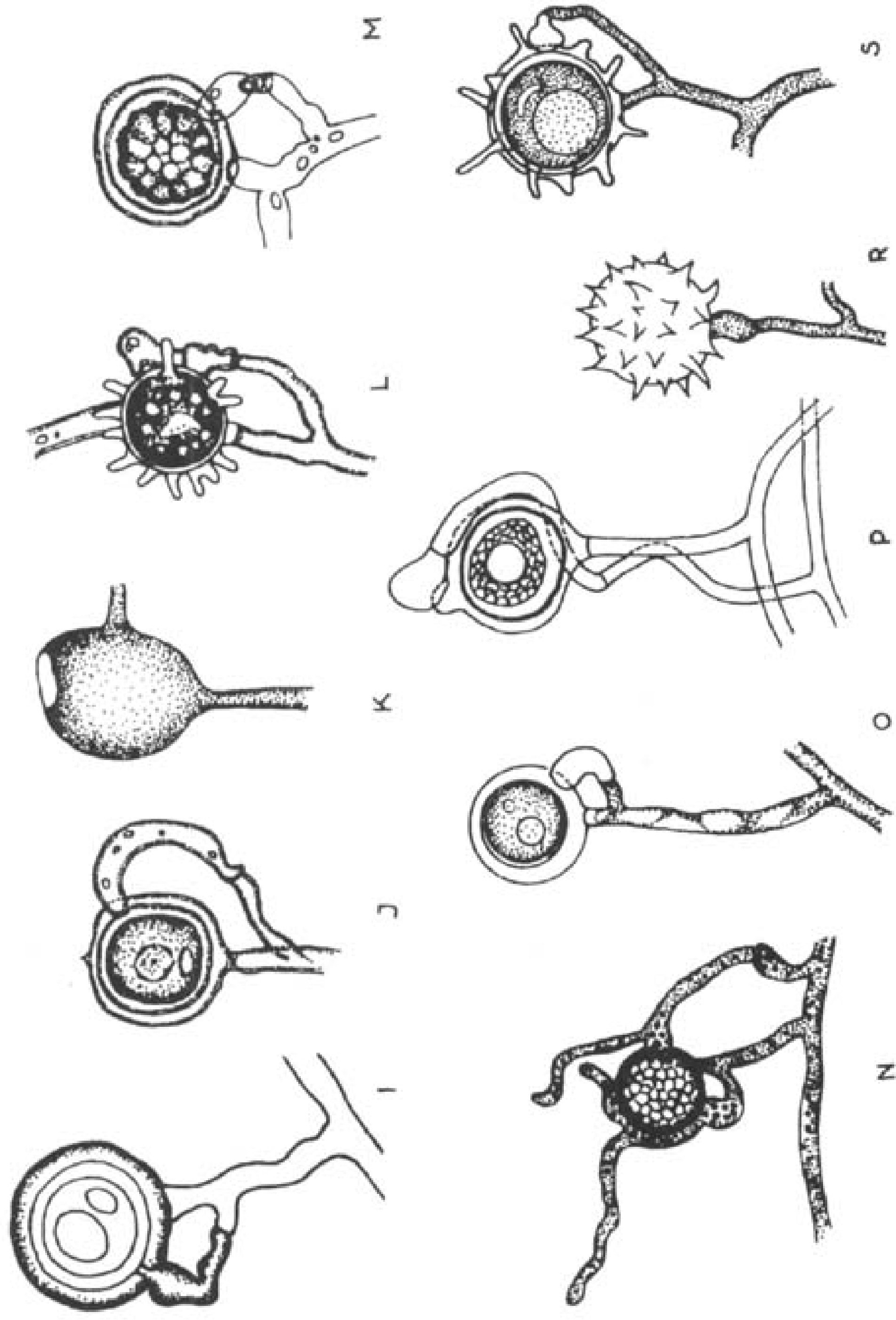


Fig. 1. Species of *Pythium* growing on fish eggs

A — *P. aristosporum* (oospore — 35 μm diamet.), B — *P. artotrogus* var. *macranthum* (oogonium — 25 μm), C — *P. debaryanum* (oogonium — 22 μm), D — *P. inflatum* (oogonium — 22 μm), E — *P. intermedium* (sporangium — 20 μm), F — *P. marsipium* (proliferating sporangia), G — *P. middletonii* (oogonium — 22 μm), H — *P. oligandrum* (oogonium — 27 μm and antheridium), I — *P. pulchrum* (oogonium — 19-23 μm and antheridium), J — *P. rostratum* (oogonium — 20 μm and antheridium), K — *P. rostratum* (sporangium — 30 μm), L — *P. spinosum* (oogonium — 21.8 μm and antheridium), M — *P. torulosum* (mature oogonium — 15 μm and antheridium), N — *P. torulosum* (young oogonium with two antheridia), O — *P. ultimum* (oogonium — 21.5 μm), P — *P. sylvaticum* (oogonium — 20 μm with aplerotic oospores and declivous antheridium), R — *P. echinulatum* (oogonium — 13.8-24.2 μm), S — *P. mamillatum* (oogonium — 13.5-18.5 μm and antheridium)

* *P. aristosporum* Vanterpool 1938; it was reported for the first time from Canada in infected wheat roots. In Japan it caused root decay of various plants, and was noted in rice fields (I w a i z a k o et al., 1976; I c h i t a n i, K a n g, 1988; I c h i t a n i, K i n o s h i t a, 1990). We observed the growth of this species on the eggs of *Leuciscus cephalus* only in the lake.

P. artotrogus var. *macranthum* Sideris 1932; it was described as a saprophyte. It is mainly recognized as a saprophyte living on vegetative parts as a water phyto-saprophyte of plants and on dead insects in water (S k i r g i e ł ł o, 1954), and as water phytosaprophyte (C z e c z u g a, 1995 c). We observed it on eggs of *Coregonus lavaretus*, *Coregonus albula* and *Esox lucius* in hatcheries in north-eastern Poland (C z e c z u g a, W o r o n o w i c z, 1993), on eggs of *Acipenser nudiventris* (C z e c z u g a et al., 1995) and other fish species.

* *P. debaryanum* Hesse 1874. Since the second half of XIX century it has been recognized as a phytopathogenic fungus as well as a soil (J o h n s o n, 1971) and aquatic (B a t k o, 1975) saprophyte. In the aquatic environment it occurs in springs (C z e c z u g a et al., 1989), running waters (M i l k o, 1965; C z e c z u g a, 1991 a, 1995 b) and stagnant lake-type waters of various trophicity (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970; C z e c z u g a, W o r o n o w i c z, 1993) in spring, autumn and winter (C z e c z u g a, 1991 b, 1995 a). In fish, it was found to grow on the eggs of *Leuciscus cephalus*.

* *P. echinulatum* Matthews 1931 was described as a saprophyte from soil in the United States. It was found to be a saprophyte of waters in Ukraine (M e s h c h e r y a k o v a, 1970) and Iceland (J o h n s o n, 1971). We observed it on eggs of *Leucaspius delineatus* in the lake.

* *P. inflatum* Matthews 1931 was described as a soil saprophyte. It has been hitherto encountered as an aquatic saprophyte (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970). We observed it on eggs of *Cobitis taenia* and *Ictalurus nebulosus*.

* *P. intermedium* de Bary 1881. The species was noted for the first time in soil. It is recognized as a soil saprophyte (A l i - S h t a y e h et al., 1986; H a e d n a n, D i c k, 1987), parasitizing on prothalia of horsetail and fern, and on seedlings of cross plants (S k i r g i e ł ł o, 1954; B a t k o, 1975). It also occurs on in water (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970). We observed it on eggs of *Ctenopharyngodon idella*.

* *P. mamillatum* Meurs 1928. It was first described from the Netherlands. M e s h c h e r y a k o v a and L o g v i n e n k o (1970) noted this species in a body of water in Ukraine; J o h n s o n (1971) isolated it from soil in Iceland. We found it on eggs of *Alburnoides bipunctatus* in the pond and lake.

* noted for the first time from fish

* *P. marsipium* Drechsler 1941, was first described as *Pythium carolianum* from Kyoto in Japan (I t o, 1936). The above author (I t o, 1942, 1944) recognized it as the species formerly described by Drechsler. This relatively rare species was noted in Japan in the 1940's in water purification devices in the former Soviet Union (M e s h c h e r y a k o v a, 1970) and in Taiwan (H s i e h, C h a n g, 1976; H s i e h, 1978). It has been recently isolated from a pond in Osaka, Japan (A b d e l z a h e r et al., 1993, 1994 a) and from lakes in Ukraine (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970). We observed this species on eggs of *Rhodeus sericeus amarus*.

P. middletonii Sparrow. It has been recognized as *Pythium proliferum* de Bary since XIX century (de B a r y, 1860). S p a r r o w (1960) initiated a new name, *Pythium middletonii*. The fungus was found to be a saprophyte of ditch water (S z w a n k e, 1938), in lakes (J o h n s o n, 1971), decaying algae and dead insects in water (N a u m o v, 1954; K a r l i n g, 1967). In fish, it was observed for the first time by F l o r i n s k a y a (1969) in a hatchery on eggs of several fish species. We observed this species on eggs of many fish species in north-eastern Poland. It has been hitherto recognized as an aquatic saprophyte of rivers and large lakes (C z e c z u g a, 1991 c, 1994 b).

* *P. oligandrum* Drechsler 1930, was first described as a saprophyte and is also recognized as an aggressive mycoparasite (D e a c o n, 1976; M a d s e n et al., 1995). It occurs in the soil of vegetable fields (K i n o s h i t a et al., 1994). We observed its growth on eggs of *Pungitius pungitius*.

* *P. pulchrum* Minden 1916. This species was isolated for the first time from soil samples in Germany. It was also found to occur in bodies of waters in Ukraine (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970) and soil samples in Iceland (J o h n s o n, 1971). We observed the growth of this species on eggs of *Noemacheilus barbatulus* in the pond.

* *P. rostratum* Butler 1907. It is a very common aquatic and soil fungus (L u n d, 1934; S k i r g i e l l o, 1954; I c h i t a n i et al., 1992). It was isolated from soil samples in India. This species occurs in various types of water bodies ranging from sunk wells (C z e c z u g a et al., 1987), rivers (S t p i c z y Ń s k a - T o b e r, 1965; C z e c z u g a, 1995 b), post-peat pits (S t p i c z y Ń s k a, 1962), ponds (M e s h c h e r y a k o v a, 1970) to lakes of various trophicity (C z e c z u g a, 1991 c, 1994 a, 1995 a). We found it on eggs of *Gobio gobio*.

* *P. spinosum* Sawada. It was noted for the first time in Taiwan (S a w a d a, C h e n, 1926) on the damping-off of *Antirrhinum majus*. It is also known to occur in root systems of other plants (I c h i t a n i et al., 1989 a). We found it only on eggs of *Cottus gobio* in the pond.

* *P. sylvaticum* Campbell ex Hendrix 1976. The fungus was found to grow on eggs of *Alosa sapidissima*. It was reported for the first time from the south of the United States, and since then it has been recognized as a saprophyte. Recently it has

been recognized in Japan as a pathogen of barley causing browning root rot in these plants (Kusonoki, Ichitani, 1994). We observed its growth on eggs of *Pelecus cultratus*.

* *P. torulosum* Coker et Patterson 1927. Described for the first time as a saprophyte and recognized as a free-living fungus of different types of grass (Ichitani et al., 1989 b). It also occurs in aquatic habitats (Meshcheryakova, Logvinenko, 1970; Johnson, 1971; Hallet, Dick, 1981). In fish, it was found to grow on gonads in the 3rd stage of *Anguilla anguilla*.

P. ultimum Trow 1901, described as a soil saprophyte. The species is a parasite of tulip bulbs (Moore, Buddin, 1937; Moore, 1940, 1979; Humphreys-Jones, de Rooy, 1975) causing great damage (Ichitani et al., 1991) in crops, and of other plants (Mazen et al., 1985; Tojo et al., 1992, 1993; Kusonoki, Ichitani, 1994). It tolerates high levels of salinity (Hassan, Fadl-Allah, 1993). In the aquatic environment this saprophyte occurs in a variety of bodies of water ranging from rivers (Czeczuga, Próba, 1987) to lakes of various trophicity (Czeczuga, 1991 c, 1994 b, 1995 a; Czeczuga, Woronowicz, 1992). In fish, it was observed on eggs of *Lepomis macrochirus* (Scott, O'Bier, 1962) and *Acipenser nudiventris* (Czeczuga et al., 1995). In our studies *Pythium ultimum* was found on eggs of many fish species.

DISCUSSION

Most species of *Pythium* developed on fish eggs in Lake Komosa (12); lowest number of these species was noted in the River Supraśl (9) (Table 2). The water of Lake Komosa had the lowest alkalinity and concentration of CO₂, N-NH₃, PO₄, chloride, calcium, manganese and sulphate and the highest concentrations of N-NO₂ and iron.

The water of the River Supraśl was characterized by a considerably high concentration of oxygen and N-NO₃, and low of oxydability.

Such species as *P. artotrogus* var. *macranthum*, *P. debaryanum*, *P. intermedium*, *P. middletonii*, *P. torulosum* and *P. ultimum* were noted on fish eggs in all the bodies of water investigated. *P. aristosporum* was found to grow on fish eggs only in water the lake whereas *P. inflatum* occurred only in the river. *Pythium spinosum* was only observed on fish eggs from the pond. The water of this pond did not contain detectable amounts of N-NO₂ and was characterized by high of oxidability, alkalinity and CO₂, N-NH₃, PO₄, chloride, calcium, manganese and sulphate concentrations.

Species of the genus *Pythium* have been generally recognized as soil parasites of grass and cultivated. The present study showed that many species of *Pythium* colonize eggs of fresh-water fish. This is particularly important for those fish species which have lost their reproduction ability under natural conditions due to eutrophication and pollution of water, and thus have to be reproduced under controlled

conditions in hatcheries. Consequently this may lead to the mass-development of parasitic fungi which are responsible for the damage of a considerable number of incubated eggs (Lartseva, Altufiev, 1987; Hatai, Hoshiai, 1992) or sometimes even all eggs are lost (Sati, Kuhlbe, 1981; Dudka et al., 1989). It should be assumed that species of *Pythium*, except for *Saprolegnia* and *Achlya*, contribute significantly to mycosis.

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