Original Research

Zoosporic Fungi Growing on the Eggs of Sea Trout (*Salmo trutta* m. *trutta* L.) in River Water of Varied Trophicity

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Abstract

We investigated the growth of hydromycoflora on the eggs of sea trout (*Salmo trutta* morpha *trutta* L.) in river water of different eutrophication levels. The eggs investigated were collected from 42 females caught during their spawning migration in the Parsęta River (west Pomerania), in Świbno on the Vistula River, and from those bred in fresh water in hatcheries at Miastko. The water for the experiments was collected from three different rivers: Biała (most), Krasna (middle) and Supraśl (low eutrophication). Fifty-three species of fungi were identified on the eggs of forty-two females of sea trout. *Achlya polyandra, Saprolegnia ferax* and *Saprolegnia parasitica* were found on the eggs of all females. In the water from the Biała we observed 25 zoosporic fungus species on eggs, 34 in water from the Krasna, and 43 in water from the Supraśl.

Forty zoosporic fungus species were found on the eggs of females from Świbno and 34 from Miastko, while only 25 were observed on the eggs of females from Parsęta. The following rare fungi were found: *Achlya inflata, Aphanomyces frigidophilus* and *Saprolegnia salmonis*.

Keywords: sea trout, *Salmo trutta* m. *trutta*, eggs, zoosporic aquatic fungi, rivers, hydrochemistry

Introduction

Aquaculture of many economically valuable fish species carried out on a mass scale results in an increasing number of viral, bacterial and mycotic diseases [1]. The pathogenic organisms belong to a group of cosmopolitan species and thus this is the problem observed on various continents [2-4]. Mycotic infections are a particularly serious threat in salmonid fishing [5-7].

Two species in the genus *Salmo*, namely Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta* m. *trutta*) enter the inland waters of Poland for spawning time [8,9]. In the inland waters of Poland, *Salmo trutta* occurs as three biological subspecies living in different habitats and varying in shape, colour and biology [9]. These are brown trout (*Salmo trutta* m. *fario* L.), lake trout (*Salmo trutta* m. *lacustris* L.) and sea trout (*Salmo trutta* m. *trutta* L).

In the literature relating to mycotic infections in trout specimens, almost all reports refer to brown trout (*Salmo trutta* m. *fario*). Hine [10] was the first to report *Achlya polyandra* and *Achlya racemosa* on the eggs of trout as a species. *Achlya racemosa* was also observed by Humphrey [11]. Brown trout were found to be colonized by *Saprolegnia monoica* [12], by *Saprolegnia delica* and *Pythium* sp. [13], and by *Saprolegnia parasitica* [14]. On the eggs of all three subspecies of *Salmo trutta*, Czeczuga et al. [15] detected several zoosporic fungus species of the genus *Achlya, Aphanomyces, Dictyuchus* and *Saproleg-*

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nia. The growth of *Saprolegnia ferax* [16,17], *Saprolegnia invaderis* [18], *Saprolegnia parasitica* [19], *Saprolegnia* type I [20] and *Saprolegnia salmonis* was noted on the skin and muscles of brown trout [7]. On adult specimens of sea trout Willoughby [20] found *Saprolegnia* type I, while Czeczuga et al. [15] observed the growth of 10 species, mainly representatives, *Achlya* and *Saprolegnia* of the genera.

Mass loss of salmonid eggs due to these fungi or even death of fry [21-24] and adult fish, including *Salmo trutta* specimens [25], have been quite common.

Therefore, we decided to examine the eggs of a few tens of trout females entering the rivers in Pomerania for spawning and those cultured in ponds to detect zoosporic fungal growing on the eggs in the water from three rivers of varied trophicity.

Material and Methods

The eggs investigated were collected at the end of October and in the first half of November 2003 from 42 female sea trout, *Salmo trutta* L., caught during their spawning migration in Parsęta on the Parsęta River and in Świbno on the Vistula River, and from those bred in fresh water in hatcheries at Miastko.

Water samples for the experiments were collected from three different rivers:

1. River Biała, 9.8 km, 2.7 m wide, 0.85 m deep, a left-bank tributary of the Supraśl River flowing through Białystok City. The samples were collected in the upper course of the Biała River, this water was the least polluted.
2. River Krasna, 7.5 km, 3.1 m wide, 0.7 m deep, in its upper copurse down to Ciasne village, through Knyszyn Forest, in the vicinity of Ciasne among fields and below this village through the Knyszyn Forest, exclusively. It flows through a lake called Komosa, through fish ponds in Krasne, and then west of this place falls into the Supraśl as its left tributary. The section below lake Komosa is sometimes regarded as a separate river called Pilica.
3. The Supraśl, 93.1 km, 6.6 m wide, 1.1 m deep, the longest right-bank tributary of the middle Narew, rises in rather low hills in the south-east of Zabłudów. It takes the Sokołda waters and flows as far as the mouth of the River Narew, via a vide post-glacial valley, continous with the River Narew valley. In this section, the Supraśl takes some major left-bank tributaries - the rivers Płoska and Biała that carry all kinds of impurities from Białystok.

Nineteen parameters of these water samples were determinated (Table l), according to generally accepted methods [26].

The following procedure was followed while determining the presence of aquatic fungus species on the eggs. The eggs (5-7) were transferred to thirty 1.0- dm^{-3} vessels (all together a total of 126 vessels) and placed in the laboratory at a temperature approaching that of a respective body of water. The subsamples from each vessel were observed under a microscope and the presence of mycelium (forming zoospores, antheridia and oogonia) of aquatic fungi growing on eggs was recorded. The methods used are described in detail by Seymour and Fuller [27]. The samples of eggs were examined through one (or one and a half) weeks. The experiments were carried out for three weeks.

The fungi were identified using the following keys: Johnson [28], Seymour [29] and Batko [30] and in the works of the authors who were the first to describe a respective species.

Results

Water samples used for the experiment differed in trophicity (Table 1). Samples from the river Biała were the most abundant in biogenes, chlorides and sulphates, less abundant was the river Krasna and the least – the Supraśl River. Moreover, water in the Supraśl had the highest content of dissolved oxygen and the lowest oxidability.

Fifty-three zoosporic fungus species, including 50 belonging to the Saprolegniales, two to Peronosporales and one to the Leptomitales, were found to grow on the eggs of *Salmo trutta* m. *trutta* in the water collected for analysis from three rivers in Podlasie (Table 2, Fig. 1). The fewest fungus species were isolated on the eggs of trout from the Biała (25), the most in the Supraśl (43 species). Such species as *Achlya apiculata, Achlya debaryana, Achlya diffusa, Achlya oblongata, Achlya papillosa, Achlya polyandra, Achlya prolifera, Aphanomyces frigidophilus, Aphanomyces laevis, Aphanomyces parasiticus, Saprolegnia delica, Saprolegnia diclina, Saprolegnia ferax, Saprolegnia glomerata, Saprolegnia monoica, Saprolegnia parasitica, Saprolegnia torulosa, Saprolegnia unispora, Thraustotheca clavata* and *Leptomitus lacteus* were found to grow on trout eggs in the water of all three rivers.

The most commonly encountered species on the trout eggs were *Achlya polyandra, Saprolegnia ferax, Saprolegnia parasitica* and *Leptomitus lacteus*. Such species as *Achlya apiculata, Achlya debaryana*, *Achlya dubia, Achlya flagellata, Achlya polyandra, Aphanomyces frigidophilus, Aphanomyces parasiticus, Dictyuchus sterile, Saprolegnia delica, Saprolegnia diclina, Saprolegnia ferax, Saprolegnia glomerata, Saprolegnia torulosa, Saprolegnia unispora* and *Thraustotheca clavata* were observed on the eggs of trout entering the water of all three spawning sites.

Discussion

In Poland, sea trout are more common than Atlantic salmon. In the rivers of Pomerania, trout constitute 99.5 to 100% of the total salmonid catch [8,9]. Baltic trout usu-

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

Specification	River		
	Biała	Krasna	Supraśl
Temperature, °C	1.0	0.5	0.3
pH	7.1	7.8	8.0
O_2	9.20	15.04	18.12
BOD_5	7.86	5.92	4.14
COD	15.82	7.85	6.50
CO_2	26.90	18.15	11.15
Alkalinity in $CaCO_3$ (mval l^{-1})	4.30	3.92	3.80
$N\text{-}NH_3$	0.62	0.29	0.12
$N\text{-}NO_2$	0.13	0.01	0.01
$N\text{-}NO_3$	0.47	0.06	0.02
$P\text{-}PO_4$	1.82	0.85	0.71
Sulphates	73.24	43.60	29.20
Chlorides	66.44	16.00	12.42
Total hardness in Ca	98.26	64.80	59.80
Total hardness in Mg	17.40	13.84	11.20
Fe	0.92	0.30	0.24
Dry residue	434.0	356.0	301.0
Dissolved solids	324.0	335.0	254.0
Suspended solids	110.0	21.0	47.0

ally after 2-3 years of life in the sea enter Polish rivers, most frequently choosing the place where they were born or to which they were let in, the phenomenon referred to as "homing instinct." After laying eggs, a female covers them with gravel and stones to form a 30 cm high mound, or nest. The eggs remain in the nest for a few months, and the hatch usually occurs in March, depending on water temperature. The trout eggs, like those of other salmonid fish species, are particularly threatened by mycotic infections, mainly for two reasons. First, this is the result of salmonid reproduction biology itself; second, it is due to the ecodevelopment of aquatic zoosporic fungi. As for salmonid reproduction, the eggs stay on the bottom of a water reservoir covered with gravel throughout the time between egg-laying in the autumn and development in the spring. As for the other reason, most zoosporic fungi, especially those belonging to the Saprolegniales, which are common fish parasites, prefer lower temperatures of approximately a few degrees above 0ºC.

The zoosporic fungus species that have been encountered on fish eggs are mostly representatives of the Saprolegniales [31-36], especially species of the genus *Achlya, Saprolegnia* and *Aphanomyces*. In the present study, the most frequently encountered fungi on the eggs of *Salmo trutta* m. *trutta* included such species in the genus *Achlya* as *Achlya polyandra, Achlya apiculata* and *Achlya debaryana*, and of the genus *Saprolegnia* – *Saprolegnia parasitica* and *Saprolegnia ferax.* The most common fungi were: *Achlya polyandra, Saprolegnia parasitica, Saprolegnia ferax* (on the eggs of all 42 females) and *Leptomitus lacteus* on 19. Both *Saprolegnia parasitica* and *Saprolegnia ferax* are commonly encountered in salmonids [37-41] and in other fish species [42, 43], while *Achlya polyandra* has been rarely detected in our studies [31, 32, 42, 43]. This fungus has been quite frequently observed only on the eggs of all the examined species of lampreys and dragon-flies, as well as numerous aquatic insects [44-46]. We have found this species also on the fur of a few animal species and on feathers of several bird species in the waters of Podlasie [47, 48]. Moreover, the growth of *Achlya polyandra* was observed by Osipian et al. [49] on the eggs of lavaret and trout from Lake Sevan (Armenia). The most common fungus species found on the eggs of the sea trout include *Leptomitus lacteus*. In our study this fungus was found to grow on the eggs of 19 females from Parsęta and Świbno sites in the water of the three rivers. *Leptomitus lacteus*, commonly known as a sew-

Table 2. Zoosporic fungi recorded on eggs of *Salmo trutta* m. *trutta* L.

Taxa fungi	Females from			On eggs of how many females	In water from river		
	Miastko	Parsęta	Świbno		Biała	Krasna	Supraśl
Saprolegniales							
Achlya americana Humphrey		x	x	7		x	x
Achlya apiculata de Bary	x	x	x	11	x	x	x
Achlya caroliniana Coker	x			1	x		
Achlya colorata Pringsheim	x			5			x
Achlya crenulata Ziegler	x			1			x
Achlya debaryana Humphrey	x	x	x	12	x	x	x
Achlya diffusa Harvey ex Johnson		x	x	8	x	x	x
Achlya dubia Coker	x	x	x	5			x
Achlya flagellata Coker	x	x	x	6	x	x	
Achlya glomerata Coker			x	3			x
Achlya inflata Coker			x	1			x
Achlya megasperma Humphrey			x	1		x	
Achlya oblongata de Bary	x		x	7	x	x	x
Achlya oligocantha de Bary		x		2			x
Achlya papillosa Humphrey	x		x	5	x	x	x
Achlya polyandra Hildebrand	x	x	x	42	x	x	x
Achlya prolifera Nees	x		x	7	x	x	x
Achlya proliferoides Coker	x		x	4	x		
Achlya radiosa Maurizio	x		x	3		x	x
Achlya rodrigueziana F.T. Wolf			x	1			x
Achlya treleaseana (Humphrey) Kauffman	x			1			x
Aphanomyces frigidophilus Kitancharoen et Hatai	x	x	x	13	x	x	x
Aphanomyces irregularis Scott	x		x	4		x	
Aphanomyces laevis de Bary			x	8	x	x	x
Aphanomyces parasiticus Coker	x	x	x	11	x	x	x
Aplanes androgynus (Archer) Humphrey	x			2			x
Cladolegnia eccentrica (Coker) Johannes	x			1		x	
Dictyuchus monosporus Leitgeb	x		x	1			x
Dictyuchus sterile Coker	x	x	x	7		x	x
Isoachlya anisospora (de Bary) Coker	x			1		x	
Saprolegnia crustosa Maurizio		x		1			x
Saprolegnia delica Coker	x	x	x	11	x	x	x
Saprolegnia diclina Humphrey	x	x	x	10	x	x	x
Saprolegnia ferax (Gruith.) Thuret	x	x	x	42	x	x	x
Saprolegnia furcata Maurizio	x		x	4		x	x

Table 2 continues on next page...

Saprolegnia glomerata (Tiesenh.) Lund	x	x	x	15	x	x	x
Saprolegnia hypogyna (Pringsheim) de Bary			x	5			x
Saprolegnia megasperma Coker	x		x	1			x
Saprolegnia mixta de Bary		x		2		x	x
Saprolegnia monoica Pringsheim			x	5	x	x	x
Saprolegnia paradoxa Maurizio		x		2	x		
Saprolegnia parasitica Coker	x	x	x	42	x	x	x
Saprolegnia salmonis Hussein et Hatai			x	4	x	x	
Saprolegnia shikotsuensis Hatai et al.	x		x	5		x	x
Saprolegnia terrestris Cookson ex Seymour	x			1			x
Saprolegnia torulosa de Bary	x	x	x	8	x	x	x
Saprolegnia turfosa (Minden) Gaumann		x		1			x
Saprolegnia uliginosa Johannes		x	x	2		x	x
Saprolegnia unispora Coker et Couch	x	x	x	10	x	x	x
Thraustotheca clavata (de Bary) Humphrey	x	x	x	16	x	x	x
Leptomitales							
Leptomitus lacteus (Roth) Agardh		x	x	19	x	x	x
Peronosporales							
Pythium gracile Schenk			x	1		x	
Pythium rostratum Butler			x	1			x
Total number of fungus	34	25	40		25	34	43

age fungus, the growth of this fungus was observed on eggs of coregonid [31] and other salmonid species [34]. This fungus was found also on eggs of cyprinid taxa [42] and other fish families [43], and on lamprey eggs [44]. Also worth noting is the occurrence on the eggs of *Salmo trutta* m. *trutta* of such zoosporic fungus species as *Achlya papillosa*, *Aphanomyces frigidophilus, Saprolegnia salmonis* and *Pythium gracilis*. *Achlya papillosa* was found to grow on the eggs of 5 females in the water of the three rivers examined. In literature of the subject, *Achlya papillosa* is known as a phytosaprophyte [28,30]. Up to now, we have observed this fungus on the eggs of gudgeon *Gobio albipinnatus* in lake Komosa, through which flows the Krasna river [42]. Moreover, *Achlya papillosa* has been observed on feathers of such birds as pigeon, magpie and green woodpecker in the waters of northeastern Poland [48]. As revealed by Izvekova [50], aquatic isolates of *Achlya papillosa* contain enzymes of the protease group involved in animal protein decomposition.

Aphanomyces frigidophilus, like *Saprolegnia salmonis*, has hitherto been reported only from Japan. *Aphanomyces frigidophilus* was detected on the eggs of Japanese charr (*Salvelinus leucomaenis*) [51] and *Saprolegnia salmonis* on the eggs of sockeye salmon *Oncorhynchus nerka* [52]. In our study, *Aphanomyces frigidophilus* was found to grow on the eggs of 13 females from all three sites and in the water of the three rivers. However, *Saprolegnia salmonis* was found to grow on the eggs of 4 females from Świbno only and in the water from rivers Biała and Krasna. We recently observed the growth of these two species on the eggs of lavaret (*Coregonus lavaretus*) collected from lake Gołdopiwo in Mazury [53] and from lake Wdzydze in Kaszuby [54]. In the literature, *Pythium gracile* is known as a parasite of aquatic algae, especially green algae [30]. In our study, it was found on the eggs of only one female from Świbno in the water collected from the river Krasna. We have hitherto observed its growth on the eggs of crucian carp *Carassius carassius* in e.g. lake Komosa, through which the River Krasna

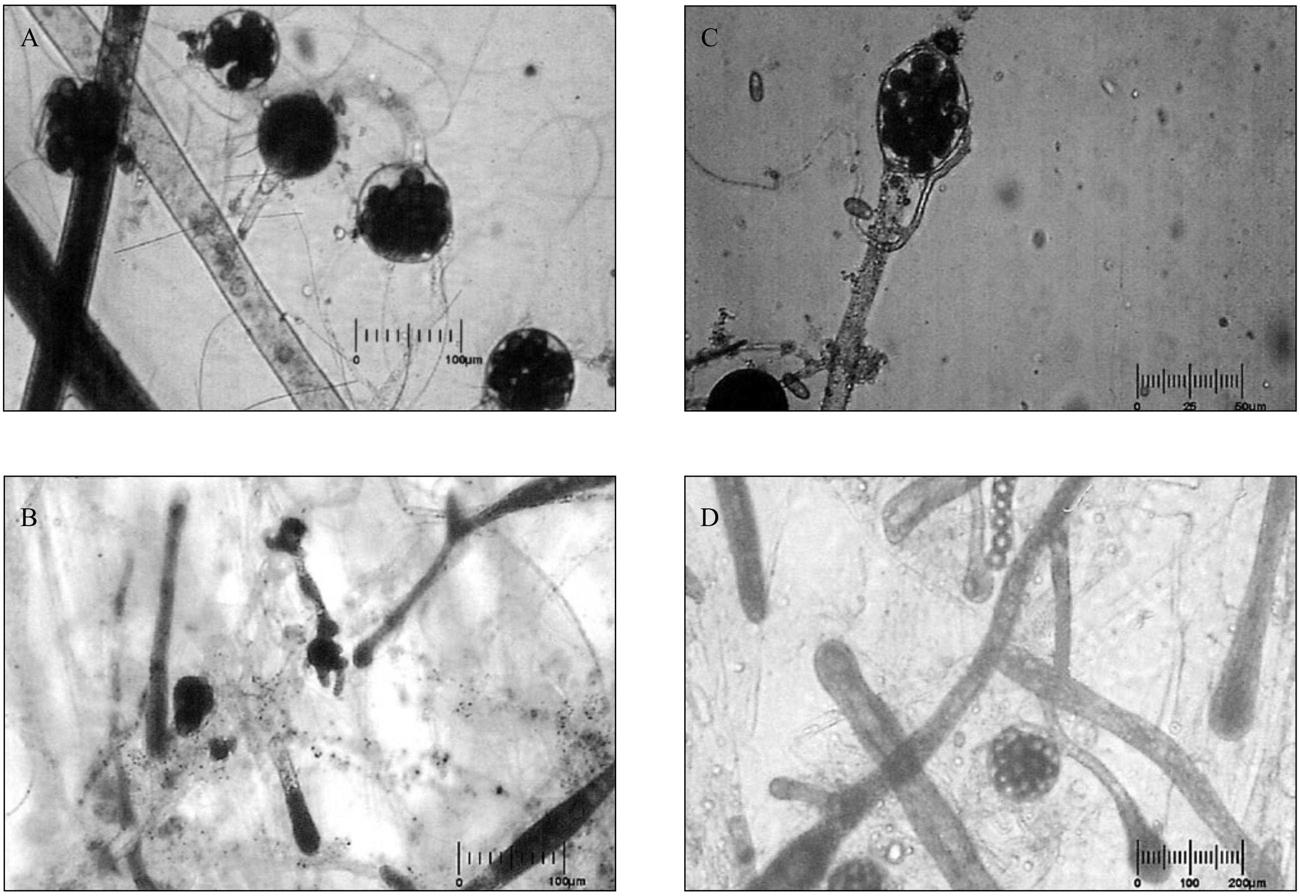

Fig. 1. Some species of fungi growing on the eggs of *Salmo trutta* m. *trutta*: A - *Achlya polyandra* - hyphae from oogonia; B - *Saprolegnia parasitica* - gemmae; C - *Saprolegnia salmonis* - oogonium from diclinous antheridium; D - *Saprolegnia turfosa* - gemmae and oogonia.

passes [42]. It should be noted that Sati and Khulbe [55] observed the growth of *Pythium gracile* as a parasite on the gills of a few fish species. In open waters of the lake- and river-type *Pythium gracile* is quite frequently encountered as a phytosaprophyte [56].

As already mentioned, in the River Biała, the most eutrophic of the rivers examined, we found the fewest fungus species on the eggs of trout, while in the river Supraśl, the least abundant in biogenes, the number of isolated species was the highest. We observed this kind of phenomenon while studying the growth of fungi on the eggs of certain cyprinid species [57,58] and coregonid species [34,53,54]. It should be emphasized here that in oligotrophic lakes in Switzerland more fungi were found on the respective fish species than in eutrophic lakes [59]. However, in acipenserid fish species belonging to the genus *Acipenser* and *Huso* we observed a reverse phenomenon – most fungi were found to grow on eggs in eutrophic waters [60].

Of great significance are the environmental conditions of a respective watercourse during the spawning period and growth of salmonid juveniles before they flow to the Baltic Sea, and whether and to what degree stressogenic factors [61] affect both reproduction and growth of fish [62]. For salmonid species the stressogenic factors include first of all drops in oxygen concentration, and food availability and quality [63]. Therefore, in fish aquacultures, especially those of salmonid species, most valuable are the populations that tolerate stressogenic factors well [64] and are immune to viral and bacterial infections [65], including mycotic ones [66]. If we additionally take into consideration that both mycelia and spores of zoosporic fungi are characterized by the phenomenon of chemotaxis [67,68], the threat to these fish species becomes completely clear.

Zoospores of fungi included in the Saprolegniales occur in water reservoirs in well oxygenated layers. The oxygen optimum for the genus *Achlya* ranges from 12.0 to 15.0, while for the genus *Saprolegnia* the range is 8.0-12.0 mg O_2 l^{-1} [69]. Moreover, the optimum temperatures for these genera oscillate between 0ºC and 10ºC. Such thermal and oxygen conditions can be found at the spawning sites of salmonid species. Chemotaxis-exhibiting zoospores found in water [67,68] are particularly "sensitive" to asparagine and glutamine [14], two amino acids known to occur in fish tissues. In the biology of fungi, especially in their nutrition, the enzymes they produce play a significant role [70]. Most of these enzymes are the same for the species of the genus *Achlya* and *Saprolegnia*, but some groups of enzymes differ according to ive species [71]. In phytosaprophytic species, the enzymes of the celulase and pectinase groups known to break down vegetable cell walls predominate [72, 73], while in zoosaprophytic and parasitic species, proteolitic enzymes of the proteinase group which decompose animal cells are present [50]. In this context, it appears comprehensible that the same fungus species of the *Achlya, Saprolegnia, Pythium* and other genera are found to grow both on vegetable and animal substrates. Lipases and alkaline phosphates have been found in the mycelium of *Saprolegnia diclina* isolated from the sheet of brook charr eggs (*Salvelinus fontinalis*) [74]. Within one and the same fungus species, not only morphological but first of all physiological and biochemical isolates are present [10, 40, 75, 76], absorbing the respective organic compounds [41, 77]. Certain fish-pathogenic strains of *Saprolegnia* exhibit chymotripsin-like activity and this enzymatic activity has been claimed to be a likely contributing factor to the pathogenesis of saprolegniasis [78]. As revealed by Dieguez-Uribeondo et al. [79] studying the parasite of crayfish *Aphanomyces astaci*, zoosporic fungi show a number of morphological, physiological and biochemical actions to adopt to environmental factors, which result in some alternations in the fungus genome [80]. Changes of the same nature lead to the production of strains of varied virulence within one species.

As fungi can be found all over the world and mycotic infections cause losses in the fishing industry, effective methods are necessary to protect eggs, fry and adult specimens against saprolegniosis, which is especially important with regard to economically valuable species. Considerable attention has been paid recently to the methods that are to strengthen resistance of aquaculture species to mycotic infections [81]. Great expectations are associated with biotechnology that will enable the production of stress- and mycosis-resistant sub-species [66, 82].

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