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Vol. 7(20), pp. 2378-2384, 14 May, 2013
DOI: 10.5897/AJMR2013.5625
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Straminipiles (Oomycota) developing on the eggs of an African catfish, *Clarias gariepinus* Burchell in water bodies of Poland

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Accepted 3 May, 2013

The straminipiles (Oomycota), lineage of fungus-like eukaryotic microorganism, growing on the eggs of an African catfish, *Clarias gariepinus*, in water of different trophicity in Poland have been investigated. Twenty-one (21) species were recorded. The largest number of species occurred on eggs in water from River Biala and Pond Fosa (more biogenic), the smallest in water from River Suprasl and Pond Dojlidy (poor in biogenes). The most commonly encountered species on the *C. gariepinus* eggs were: *Saprolegnia parasitica, Achlya polyandra, A. oblongata, A. prolifera, Aphanomyces frigidophilus, S. ferax* and *Leptomitus lacteus*. Amino-acid, carbohydrate and urease tests were used. 11.4% of *C. gariepinus* eggs investigated were found to be infected by Straminipiles species.

Key words: *Clarias gariepinus*, eggs, straminipiles (Oomycota), infections, hydrochemistry.

INTRODUCTION

In some countries, including those from the African continent, fish meat owes its nutrient value not only to protein and fats, but also to other biologically active substances, which have played an important role in humans' lives (Czeczuga et al., 2013). The world has relied greatly on two natural systems: oceanic fisheries and cattle farming (Brown, 2000). With wild fish production stagnating, growth in overall fish production has come almost entirely from the global boom in aquaculture, especially in developing countries. Over a recent 10 year period, aquaculture production increased by an average of 11% per year (van West, 2006). In coming years, aquaculture will represent more than 30% of total fish available for consumption (and it increases all the way up to 41% in 2012) (Delgado et al., 2003). In all hatcheries, one of the more important problems comes from aquatic straminipilous fungi growing on the fishes' eggs (Woo and Bruno, 1999).

Straminipiles species are responsible for infections on fish in aquaculture, fish farms and hobby fish tanks. In some countries (including Poland), there is interest in introduction of certain exotic fish species for aquaculture, presently evident in importing eggs and in hatching and breeding activities (Perez et al., 2003). *Clarias gariepinus* (known as sharptooth catfish or African catfish) is one such species and is highly recommended as a food fish in Africa and beyond (Okeyo, 2003).

MATERIALS AND METHODS

Description of host species

Clarias gariepinus Burchell, 1822 (syn. *C. lazera* Cuvier et Valenc., 1840); *C. mossambicus* Peters, 1852 (English name: sharptooth catfish African catfish).

C. gariepinus is distributed in Africa, almost Pan-African; absent

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

Property	River			Pond		
	Biała	Krasna	Supraśl	Dojlidy	Fosa	Komosa
Temperature (°C)	17.8	18.1	17.0	18.0	19.2	17.4
pH	7.1	7.8	7.8	7.9	7.0	7.6
DO	6.4	15.1	11.2	9.4	0.5	12.8
BOD_5	7.2	5.9	2.8	9.7	9.2	7.4
Oxidability(COD)	15.82	7.85	6.60	15.80	20.20	13.20
CO_2	26.9	18.5	6.6	8.8	22.4	8.3
Alkalinity in $CaCO_3$ (mval l^{-1})	4.3	3.9	4.5	3.2	5.8	3.9
$N\text{-}NH_3$	0.621	0.290	0.142	0.168	0.864	0.161
$N\text{-}NO_2$	0.132	0.015	0.006	0.008	0.114	0.009
$N\text{-}NO_3$	0.473	0.612	0.014	0.050	0.552	0.034
$P\text{-}PO_4$	1.824	0.854	0.158	0.242	3.598	0.255
Sulphates (SO_4)	73.24	43.60	32.38	45.65	25.06	42.75
Chlorides (Cl)	66.44	16.02	17.12	25.04	45.21	23.51
Total hardness in Ca	98.26	64.80	73.42	61.84	79.27	68.40
Total hardness in Mg	17.42	18.84	11.58	12.18	24.2	28.81
Fe	0.92	0.30	0.48	0.84	1.06	1.54
Dry residue	434.0	356.0	197.0	257.0	429.0	375.0
Dissolved solids	324.0	335.0	179.0	210.0	370.0	312.0
Suspended solids	110.0	21.0	18.0	47.0	59.0	63.0

from the Maghreb, upper and lower Guinea and Cape Province, and probably Natal Province. Present also in Southwest Asia, Jordan, Lebanon, Israel, Syria and southern Turkey (Skelton, 2002), and widely introduced to other parts of Africa, Europe and Asia. Several countries reported on the adverse ecological impact after their introduction (ITIS, 2010).

The species occurs mainly in quiet waters, lakes and pools but may also occur in fast flowing rivers and in rapids (Allanson, 2004). They are tolerant of extreme environmental conditions (Teugels, 1986). They feed on insects, plankton, invertebrates and fish, but also take young birds, rotting flesh and plants (de Moor and Bruton, 1988). They migrate to rivers and temporary streams to spawn (Witte and de Winter, 1995). Marketed fresh and frozen, they are eaten broiled, fried and baked (Frimodt, 1995).

Characteristics of water bodies

Three rivers and three ponds located in the North-eastern region of Poland (53°07' N, 23°10' E to 53°13' N, 23°20' E) were chosen for the study: 1) River Biala, length 9.8 km, width 3.7 m, depth 0.85 m, a left-bank tributary of the River Suprasl flowing through Bialystok City. 2) River Suprasl, length 93.1 km, width 6.0 m, depth 1.1 m, the largest right-bank tributary of the middle River Narew flowing through the Knyszynska Forest. 3) River Krasna, length 7.5 km, width 3.1 m, depth 0.7 m, a left-bank tributary of the River Suprasl, flowing through the Knyszynska forest. 4) Pond Dojlidy, near Bialystok City, the area 34.2 ha; maximum depth, 2.85 m. Its south shores border coniferous woods and its western part borders Bialystok City. 5) Pond Fosa occurs in Branicki Park of Bialystok City, area 2.5 ha, maximum depth 1.75 m, in which swans breed and wild duck colonies stay in addition to crucian carp. 6) Pond Komosa, area 3.2 ha, maximum depth 1.25 m, surrounded densely by coniferous trees of the Knyszynska forest.

Nineteen (19) parameters of those waters were determined (Table 1) according to generally accepted methods (APHA, 2005).

Isolating and identifying the straminipiles species

The investigated eggs (720), covered with mycelia, were collected at the end of May from the hatchery of the Institute of Ichtyobiology and Aquaculture, Polish Academy of Sciences in Golysz, Poland. The eggs were washed three times in sterilized distilled water before the experiment (Paterson and Bridge, 1994).

The following procedures were used while determining the presence of straminipiles Oomycota species on the investigated eggs (after fertilization) of *C. gariepinus.* Water samples (800 ml each) from the different studied sites were placed in 1000 ml vessels. Twenty (20) to 30 eggs were transferred to each vessel in accordance to general principles of culture procedures (Watanabe, 2002). All vessels were enclosed in Petri dishes with the bed turned upside down to prevent possible airborne contamination with mycotal spores. The vessels were stored at 15 ± 2°C, with access to daylight resembling natural conditions and following the recommended instructions (Seymour and Fuller, 1987). Water analyses and experiments were carried out in three parallel repetitions.

The eggs covered by mycelia (from each vessel) were observed every three to four days under a light-microscope and the presence of morphological structures (zoospores, antheridia and oogonia) of aquatic fungi were recorded. Those experiments were carried out for one month. The taxa of straminipiles species were identified using the keys of Johnson et al. (2005), Pystina (1998) and Dick (2001). Amino acids, carbohydrate and urease tests were performed on the genera: *Achlya, Aphanomyces, Leptolegnia, Pythium* and *Saprolegnia* according to Yuasa and Hatai (1996) and Kitancharoen and Hatai (1998). For the carbohydrate utilization test, the medium used to culture the mycotal isolates was yeast nitrogen based agar (Difco) and glucose-yeast (GY) extract agar was used for the urease test.

Table 2. Straminipiles organisms recorded on the eggs of *C. gariepinus.*

Taxa	Water bodies
Straminipiles	
Peronosporomycetes	
Saprolegniales	
1. *Achlya caroliniana* Coker	B, D, F
2. *A. flagellata* Coker	B, F, Ko
3. *A. oblongata* de Bary	B, F, Ko, Kr, S
4. *A. polyandra* Hildebr.	B, D, Ko, Kr, S
5. *A. prolifera* Nees	B, F, Ko, Kr, S
6. *Aphanomyces frigidophilus* Kitanch. et Hatai	B, F, Ko, Kr, S
7. *A. laevis* de Bary	B, D, Ko
8. *Dictyuchus pisci* Khulbe and Sato	B, F, Ko
9. *Isoachlya monilifera* (de Bary) Kauffman	B, D, Ko
10. *Leptolegnia caudata* de Bary	B, F
11. *Saprolegnia anisospora* de Bary	B, F, Kr
12. *S. eccentrica* (Coker) R.L. Seym.	B, F
13. *S. diclina* Humphrey	B, D, Ko, Kr, S
14. *S. ferax* (Gruith) Thur.	B, D, Ko, Kr, S
15. *S. litoralis* Coker	B, F
16. *S. mixta* de Bary	B, F
17.*S. parasitica* Coker	B, D, Ko, Kr, S
18. *S. shikotsuensis* Hatai and co-authors	Ko, Kr
Leptomitales	
19. Leptomitus *lacteus* (Roth) C. Agardh	B, D, Ko, Kr, S
Pythiales	
20. *Pythium diclinum* Tokun	B, F, Ko, Kr, S
21. *P. proliferum* de Bary	B, F, Kr
Number of straminipilous organisms in water from River Biała (B)	20[a]
Number of straminipilous organisms in water from River Krasna (Kr)	12[b]
Number of straminipilous organisms in water from River Supraśl (S)	9[c]
Number of straminipilous organisms in water from Pond Dojlidy (D)	8[c]
Number of straminipilous organisms in water from Pond Fosa (F)	21[d]
Number of straminipilous organisms in water from Pond Komosa (Ko)	14[e]
Number of straminipilous organisms in water from rivers	13.7[a]
Number of straminipilous organisms in water from ponds	14.3[a]

Means with the same letter are not significantly different (p>0.05).

To prevent the growth of bacteria, ampicillin and streptomycin were applied to selected dilations. A basal medium was used in amino acid assimilation test. Medium preparation and indicator were the same as for the carbohydrate assimilation test. Bromothymol blue and phenol red were used as indicators, added into yeast nitrogen base broth and GY broth, respectively. A positive result was determined by the change in the colour of the medium to pink or purple, and a change to orange and yellow was considered to be a negative result. Those methods were described in detail in our previous paper (Czeczuga et al., 2011b). The results were tested for significance with analysis of variance (ANOVA) and evaluated by the S-Scheffe test (Winer, 1997).

RESULTS

Hydrochemical parameters of the water used in the experiment are shown in the Table 1. The most eutrophic was the water from River Biala and Pond Fosa. Water from River Suprasl had the lowest content of all forms of nitrogen and phosphates. The highest levels of the COD, CO_2, chlorides, sulphates and calcium were found in the River Biala. Water from the Komosa Pond contained the highest levels of magnesium and iron. Eighty-two (11.4%) of the 720 *C. gariepinus* eggs examined had been infected with straminipiles organisms.

Twenty-one (21) straminipiles species, including 18 belonging to the Saprolegniales, 2 to Pythiales and 1 to the Leptomitales were found to be growing on the eggs of *C. gariepinus* (Table 2). The species from *Achlya* and *Saprolegnia* genera were the most common. *Saprolegnia*

Table 3. Amino acid, carbohydrate and urease assimilation by straminipiles isolated from eggs of *C. gariepinus*.

Species of genus	Amino acid	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, asparagine; Cys, cysteine; Glu, glutamine; His, histidine. Carbohydrate: Fru, Fructose; Gal, galactose; Glu, glucose; Man, mannose; Mal, maltose; Mel, melibiose; Cel, cellobiose; Dex, dextrin; Gly, glycerol; Lac, lactose; Rha, rhamnose; Sal, salicin; Raf, rafinose; Sta, starch; Suc, sucrose; Tre, trehalose. +, Positive; -, negative.

parasitica (28) specimens, *Achlya polyandra* (27), *A. oblongata* (25), *A. prolifera* (22), *Aphanomyces frigidophilus* (20), *Saprolegnia ferax* (18) and *Leptomitus lacteus* (15) belonged to the most commonly encountered species on the examined eggs. On many of the eggs examined, only some straminipiles species were encountered. The highest number of straminipiles species was observed to grow in the water from River Biala and Pond Fosa (both the most eutrophic), while the lowest number occurred on the eggs in water from River Suprasl and Pond Dojlidy (both low of eutrophication). Species from *Achlya*, *Aphanomyces*, *Leptolegnia* and *Saprolegnia* genera assimilated alanine, but did not assimilate methionine, lysine, ornithine or leucine and glycine (Table 3). All isolated species from *Achlya, Aphanomyces*, *Leptolegnia* and *Saprolegnia* genera assimilated glucose and starch, but they did not assimilate arabinose and salicin. Urease was only assimilated by the species from *Leptolegnia*, *Pythium* and *Saprolegnia* genera.

DISCUSSION

Species found in this study have been reported previously (Czeczuga et al., 2011b). Twenty-one (21) straminipiles species belonging to eight genera were found growing on the eggs of *C. gariepinus* in the water from six limnologically and trophically different water bodies of North-eastern Poland. The *Achlya* and *Saprolegnia* genera were the most prevalent. In the water bodies of the African continent, in the area of the natural distribution of the species, there are also such straminipiles species belonging to *Achlya, Aphanomyces, Dictyuchus, Pythium* and *Saprolegnia* genera (El-Sharouny and Badram, 1995; El-Nagdy and Nasser, 2000; El-Hissy et al., 1982, 2004; Ali and Abdel-Raheem, 2003; Ali, 2007). The most commonly encountered species found in African water bodies belonged to *Saprolegnia, Pythium, Phytophthora* and *Achlya* genera (El-Hissy and Khallil, 1989; Khallil et al., 1993; El-Hissy et al., 2004). The most commonly encountered from *Saprolegnia* genus has been the *S. ferax*, less so *S. parasitica* and *Leptomitus lacteus* species has not been registered previously in those water bodies.

El-Sharouny and Badram (1995), while investigating zoosporic fungi on different parts of the body of *Tilapia nilotica* and *T. galileae* from the waters of the River Nile, have recorded 17 species belonging to *Saprolegnia*, *Achlya*, *Dictyuchus*, *Pythium*, *Allomyces* and *Aphanomyces* genera. The most common were: *Saprolegnia ferax*, *S. diclina*, *Achlya dubia*, *A. americana*, *A. racemosa*, *A. flagellata*, *Dictyuchus sterilis*, *Pythium undulatum* and *Aphanomyces sp.* As already mentioned, in the River Biala and Pond Fosa, the most eutrophic water body examined, we found the fewest number straminipiles species on the eggs of *C. gariepinus,* while in the River Suprasl and Pond Dojlidy (the least abundant in biogenes) the number of isolated species has been the highest. We observed such phenomenon while studying the growth of mycobiota on the eggs of certain cyprinid species (Czeczuga and Muszynska, 1999) and coregonid species (Czeczuga and Muszynska, 1998). It should be emphasized that in oligotrophic lakes in Switzerland more mycobiota species have been found on the respective fish species than in eutrophic lakes (Meng, 1980). However, in acipenseridae fish species, we have observed a reverse phenomenon – less species have been found to grow on the eggs in eutrophic waters (Czeczuga et al., 2011c). Only two species belonging to genus *Pythium* have been detected on the eggs of *C. gariepinus*. They were the common species of the water bodies and of the eggs of fresh-water polish fishes (Czeczuga, 1996).

As this investigation shows, *S. parasitica* was the most commonly encountered mycotal habitat on *C. gariepinus* eggs. Similar phenomenon has been observed during the investigations on the development of mycobiota species on the eggs of coregonid (Czeczuga and Muszynska, 1998), salmonid (Czeczuga et al., 2005, 2011a) and sturgeonid fish species (Czeczuga et al., 2011c). According to Fregeneda-Grandes et al. (2007) the most frequent aquatic straminipiles species on the eggs of *Salmo trutta L.* belonged to the *S. diclina.* Secondary cysts (taxonomically important) of the *S. parasitica* specimens from the *C. gariepinus* eggs had different number of bundles per cyst, hairs per bundle and their length differed. Studies on the morphology of the secondary cysts of pathogenic *Saprolegnia* species from different geographical regions (Hatai et al., 1990; Fregeneda-Grandes et al., 2000; Diéguez-Uribeondo et al., 2007) have shown that the number of bundles, the number of hairs and their length are adaptive features associated with different environmental conditions and surface structure of water hosts. Also the chemical composition of the zoospores and cysts of saprotrophic and fish pathogenic *Saprolegnia* species are different (Burr and Beakes, 1994). The salinity significantly reduced the total free amino-acids and protein content of fish pathogen *Saprolegnia parasitica* mycelia (Ali, 2005). Also the investigations of *Saprolegnia diclina-S. parasitica* complex from different geographical regions on ITS rDNA sequence have shown morphological and physiological differences and variations in their pathogenicity (Diéguez-Uribeondo et al., 2007). Adaptation to parasitism in *Saprolegnia* species also might have occurred at the spore level by the development of the long-hooked hairs to facilitate the host attachment. The differences in the number of morphological and physiological straminipiles forms and the results of the phylogenetic analysis of the *Saprolegnia* species based on ITS of rDNA sequences were observed. It all causes the taxonomical problems in *Saprolegniales.* There are several reports of true Fungi as primary infection agents of adult fishes species of the *Clarias* genus from the African continent (Easa et al., 1984; Refai, 1987; Refai et al., 2010). Isolated fungal species belonged to such genera as: *Aspergillus, Fusarium, Mucor, Penicillum, Rhizopus, Scopulariopsis, Paeciliomyces* and *Curvularia.* Yeasts from *Candida, Rhodotorula* and *Torulopsis* have also been isolated. The most predominant fungi were those species such as: *Aspergillus flavus*, *Fusarium* species and *Candida albicans* (Refai et al., 2010). Therefore, in wild specimens of *C. gariepinus* the most important fish parasites are protozoans (Dykova, 2006), monogeneas (Buchmann and Bresciani, 2006) and straminipiles – especially from the *Saprolegnia* genus. According to Refai et al. (2010) *Saprolegnia* species were isolated only in a few present of species encountered. In farmed specimens of *C. gariepinus*, mainly the ruptured intensive syndrome (RIS), disease of an open abdomen and broken head disease (BHD) have occurred (Chybowski, 2000). The formation of the injury in both diseases represents the infestational snout for pathogenic mycotal species occurring in water bodies. In the biology of mycobiota, especially in their nutrition, the enzymes they produce play a significant role. Most of those enzymes are the same for the species from *Achlya* and *Saprolegnia* genera, but some groups of enzymes differ in particular species (Denis, 1985). In phytosaprotrophic species, the enzymes from the cellulase and pectinase group, known to break down the vegetable cell wall, predominate (Chamier, 1985), while in zoosaprotrophic and parasitic species, the proteolitic enzymes from the proteinase group, which decompose the animal cells, are present (Izvekova, 1985). Lipases and alkaline phosphatases have been found in mycelium of *Saprolegnia diclina* isolated from the sheet of *Salvelinus fontinalis* eggs (Rand and Munden, 1992). Within one and the same straminipiles species, not only morphological but first of all physiological and biochemical isolates are present (Yuasa and Hatai, 1994) absorbing the respective organic compounds (Griscenko and Rudikov, 1985). Certain fish-pathogenic strains of *Saprolegnia* exhibit chymotripsin-like activity and this enzymatic activity has been claimed to be likely contributing factor to the pathogenesis of saprolegniasis (Peduzzi and Bizzozero, 1977). Studying the parasite of crayfish – *Aphanomyces astaci,* the straminipiles species have shown (Diéguez-Uribeondo et al., 1995) a number of morphological, physiological and biochemical actions to adapt to environmental factors, which result in some alternations in the fungus genome (Huang et al., 1994). Changes of the same nature lead to production of strains of varied virulence within one species.

As shown, the investigated species from *Saprolegnia* and *Achlya* genera have assimilated the most amino acids, while the lowest amount has been assimilated by *Pythium* species. Both species of *Pythium* and all species of *Achlya* have assimilated the highest number of the carbohydrates (16 and 14, respectively). *Aphanomyces* species only assimilated glucose and starch. A similar phenomenon has been observed previously during the investigations of the mycobiota species on the eggs of *Oncorhynchus* species (Czeczuga et al., 2011b). The species, recorded on the *C. gariepinus* eggs (belonging to Saprolegniales and Leptomitales) has been classified as an opportunistic pathogen, which are sapro- and necrotrophic (van West, 2006; Czeczuga et al., 2011). In hyphae of opportunistic species both proteolitic (animals) and cellulase/ pectinase enzymes have occurred. In phytosaprophitic species, in which the order of *Pythiales* belongs to Peronosporales (Oomycota), the enzymes which are known to break down vegetable cell walls predominate (Bodemann et al., 1985). This could suggest that the *Pythium* species occurred on the *C. gariepinus* eggs preferred mostly plant substances such as carbohydrates.

Our present study has proven that in comparison with other fish species, the *C. gariepinus* eggs are very resistant (only 11.4% of infected eggs). Our investigations that have been devoted to artificial reproduction of the salmonid fishes reported the considerable mortality rate of the eggs due to *Saprolegnia* infection: up to 29.4% in *Salmo trutta* m. *trutta* (Czeczuga et al., 2005), 31.8% of *S. salar* (Czeczuga et al., 2011a), up to 27.1% in *Oncorhynchus gorbuscha* (Czeczuga et al., 2011b).

In sturgeonid fishes this loss reached 70 to 90% of all incubated eggs (Lartzeva, 1986).

Great fertility from 5 x 10^3 to 193 x 10^3 eggs (Skelton, 2002), higher resistance to *C. gariepinus* by the eggs as well as wide tolerance to extreme environmental conditions are the main factors supporting the population densities of *C. gariepinus* in diverse water bodies.

ACKNOWLEDGEMENT

The authors wish to thank to anonymous reviewers for their valuable corrections of the manuscript.

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