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Harmful effect of the straminipiles organisms on the reproduction of the Adriatic sturgeon

Bazyli Czeczuga[1], Ewa Czeczuga-Semeniuk[1], Nela Sarosiek[2] and Adrianna Semeniuk[1]

[1]Department of General Biology, Medical University, Mickiewicza 2C, 15-222 Bialystok, Poland;
[2]Department of Diagnostic Imaging, Wojewodzki Szpital Zespolony, Skłodowskiej-Curie 26,
15-950 Bialystok, Poland.

ABSTRACT

The authors investigated the growth of straminipiles fungi on the eggs of Adriatic sturgeon (*Acipenser naccarii*) in water from three trophically different rivers. 170 (32.7%) out of 520 investigated Adriatic sturgeon eggs were infected by straminipiles organisms from 27 species. The largest number of species occurred on the eggs in water from river Biala (more biogenic), and the smallest in water from river Suprasl (poor in biogenes). The most commonly encountered straminipiles species on the Adriatic sturgeon eggs were *Achlya polyandra*, *Aphanomyces irregularis*, *Saprolegnia ferax*, *S. parasitica*, *Leptomitus lacteus* and *Pythium diclinum*. Amino acid, carbohydrate and urease tests were used.

KEYWORDS: Adriatic sturgeon, *Acipenser naccarii*, reproduction, eggs, straminipiles infections, hydrochemistry

1. INTRODUCTION

Sturgeon is a migratory, two-environmental species, which occasionally lives in one-environment fresh or sea water. Water pollutants and river dams form an obstacle for migratory species to reach their natural spawning grounds [1]. Therefore, in order to maintain the proper stock of economically valuable species, breeding of juveniles has been commenced in hatcheries [2]. Adriatic sturgeon (sturgeonid fish) belongs to such species.

In all hatcheries, the most important problem is the aquatic fungi, especially straminipiles species, growing on the fish eggs [3]. In sturgeonid species, this loss often reaches the amounts up to 70% of the incubated eggs in some hatcheries [4]. Thus, we have decided to publish the data concerning the development of straminipiles fungi on the eggs of Adriatic sturgeon from our inland waters. The zoosporic fungi growing on the eggs of other sturgeonid species have already been published [5-9].

2. MATERIALS AND METHODS

2.1. Short description of species

Adriatic sturgeon (*Acipenser naccarii* Bonaparte, 1836) is distributed in the Adriatic Sea [10] and its tributaries between Po (Italy) [11] and Buna (Albania) [12, 13] drainages. Its appearance was recorded in Corfu [14] and in the lowermost part of Adriatic rivers from Soca to Drin. Records from Tyrrhenian slope of Italy [15, 16], Spain [17] and occasionally of France [18] were erroneous [19]. Only a single surviving, naturally-spawning population resides at confluence of Ticino and Po [19-21]. Nowadays it occasionally appears in Adriatic Sea and coastal rivers. The spawning population of Adriatic sturgeon survived in Albania, but has been extirpated. Now the status of this endemic species is critically endangered [22- 26]. It occurs in the sea close to the shore and estuaries, not entering pure marine waters. In freshwater, it inhabits large deep rivers and feeds on bottom-living invertebrates and small fishes.

It is threatened by habitat destruction, pollution and overfishing. The flesh is used for food but the eggs are not consumed as caviar [19]. We have decided to publish the data concerning the development of straminipiles fungi on the eggs of sharp tooth catfish from trophically different waters.

2.2. Characteristics of water bodies

Water for the experiments was collected from three different water bodies: River Biala, River Krasna and River Suprasl:

- River Biala - length 9.8 km, width 3.7 m, 0.85 m deep, a left-bank tributary of the Suprasl River flowing through Bialystok City.
- River Krasna - length 7.5 km, width 3.1 m, 0.7 m deep, a left-bank tributary of the Suprasl River, flowing through the Knyszynska Forest.
- River Suprasl - length 93.1 km, width 6.0 m, 1.1 m deep, the largest right-bank tributary of the middle part of the Narew River flowing through the Knyszynska Forest.

Nineteen parameters of these water samples were determined (Table 1) according to the generally accepted methods [27].

2.3. Determination of straminipiles species

The eggs for investigation were collected from Adriatic sturgeon (*Acipenser naccarii* Bonaparte, 1836) caught from Buna River (Albania). The eggs were transported by air in thermos flask in physiological solution.

The following procedure was used while determining the presence of straminipiles species on the investigated eggs of Adriatic sturgeon. Water samples (800 ml each) were placed in 1000 ml vessels. 20-30 eggs were transferred to each vessel in accordance with the general principles of culture. All vessels were enclosed in Petri scales with the bed turned upside down to prevent possible airborne contamination with fungal spores. The vessels were stored at $15 \pm 2\ ^{o}C$, with access to daylight resembling natural conditions and following the recommended instructions [28]. Water analyses and experiments were carried out in three parallel repetitions.

The eggs covered with fungal mycelia (from each vessel) were observed every 3-4 days under a light microscope and the presence of morphologic structures (zoospores, anteridia and oogonia) of aquatic fungi was recorded. Amino acids, carbohydrate and urease tests were performed on the *Achlya, Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera according to Yuasa and Hatai [29] and Kitancharoen and Hatai [30]. For the carbohydrate utilization test, the medium used to culture the fungal isolates was Yeast Nitrogen Base agar (Difco), and GY (glucose-yeast extract) agar was used for the urease test. To prevent the growth of bacteria, ampicillin and streptomycin were applied at a selected dilution. The basal medium was used in amino acid assimilation test. Medium preparation and indicator were the same as that for the carbohydrate assimilation test. Bromothymol blue and phenol red were used as indicators, and added to Yeast Nitrogen Base broth and GY broth, respectively. A positive result was evidenced by the color change of the medium to pink or purple, and a change to orange or yellow was considered to be a negative result. These methods have been described in detail in our previous paper [9]. The experiments were carried out for one month. Dicks' systematics of straminipiles species was used [31]. The results were tested for significance with ANOVA and evaluated by the S- Scheffe test [32].

3. RESULTS

Hydrochemical parameters of water used in the experiment are shown in Table 1. The most eutrophic was the water from River Biala, while the water of River Suprasl had the lowest content of all forms of nitrogen and phosphates. The highest levels of COD, CO_2, chlorides, sulphates and calcium were found in the River Biala. Water from River Krasna contained the highest levels of oxygen (DO) and magnesium.

170 (32.7%) out of 520 investigated Adriatic sturgeon eggs were infected by straminipiles organisms. Some species of the straminipiles organisms occurred on many eggs. Twenty seven straminipiles species including 22 belonging to the Saprolegniales, 4 to Pythiales and one to the Leptomitales were found to grow on the eggs of Adriatic sturgeon (Table 2). The largest number of species occurred on the eggs in water from River Biala (more biogenic), the smallest from

Table 1. Chemical and physical properties of water in investigated rivers (in mg $1/l^{-1}$).

Specification	River Biala	River Krasna	River Suprasl
Temperature (ºC)	17.8	17.4	17.0
pH	7.1	7.6	7.8
DO	6.4	12.8	11.2
BOD_5	7.2	7.4	2.8
Oxidability (COD)	15.8	13.2	6.6
CO_2	26.9	8.3	6.6
Alkalinity in $CaCO_3$ (mval $1/l^{-1}$)	4.3	3.9	4.5
$N\text{-}NH_3$	0.621	0.161	0.142
$N\text{-}NO_2$	0.132	0.009	0.006
$N\text{-}NO_3$	0.473	0.034	0.014
$P\text{-}PO_4$	1.824	0.255	0.158
Sulphates (SO_4)	73.24	42.75	32.38
Chlorides (Cl)	66.44	23.51	17.12
Total hardness in Ca	98.26	68.40	73.42
Total hardness in Mg	17.42	28.81	11.58
Fe	0.92	1.54	0.48
Dry residue	434	375	197
Dissolved solids	324	312	179
Suspended solids	110	63	18

River Suprasl (poor in biogenes). The most commonly encountered species on the Adriatic sturgeon eggs were: *Achlya polyandra*, *Aphanomyces irregularis*, *Saprolegnia ferax*, *S. parasitica*, *Leptomitus lacteus* and *Pythium diclinum*.

6 of the 11 amino acids tested, namely: methionine, lysine, ornithine, phenyloalanine, leucine and glycine could not be assimilated by the investigated fungi. Species from *Achlya*, *Aphanomyces, Leptolegnia* and *Saprolegnia* genera assimilated glucose and starch (excepting species from *Pythium* genus). Urease was assimilated by specimens from *Leptolegnia*, *Pythium* and *Saprolegnia* genera (Table 3).

4. DISCUSSION

The straminipiles species encountered on Adriatic sturgeon eggs were mostly representatives of the Saprolegniales. Similar observation was made in sturgeonid species belonging to *Huso* and *Polyodon* genera [8, 9]. We have already observed this in investigations on the straminipiles on sturgeonid eggs from the Caspian [7] and the Black Sea basin [8] and also from Siberia and Far East region [9]. Rare (for fish) straminipiles species such as *Aphanomyces invadans, Saprolegnia polymorpha* and *Pythium middletonii* were also found.

Aphanomyces invadans has been found on the eggs of the Adriatic sturgeon in the water from River Biala. This pathogen was first described by Willoughby *et al.* [33] as *Aphanomyces invaderis*, which has been causing the epizooties in a tropical freshwater fish. Now it is listed in the index of Fungi as *Aphanomyces invadans* [34]. The disease caused by *Aphanomyces invadans* is characterized by the presence of distinctive mycotic granulomas in skin, muscles and internal tissues [35-38]. Epizootic produced by *Aphanomyces invadans* is called epizootic ulcerative syndrome (EUS), which affects wild and farmed fish in Australia [39],

Table 2. Straminipiles organisms recorded on the eggs of *Acipenser naccarii* (number of investigated eggs - 520, infected - 170).

Taxa	Water bodies	On eggs number - %
Straminipila		
Peronosporomycetes		
Saprolegniales		
1. *Achlya ambisexualis* J. R. Raper	B, S	18-3.5
2. *A. americana* Humphrey	B	21-4.0
3. *A. caroliniana* Coker	B	8-1.5
4. *A. colorata* Pringsh.	B	24-4.6
5. *A. dubia* Coker	B, K	17-3.5
6. *A. glomerata* Coker	B	26-5.0
7. *A. hypogyna* Coker et Pemberton	B, K	12-2.3
8. *A. oligacantha* de Barry	B	10-1.9
9. *A. polyandra* Hildebr.	B, K, S	75-14.4
10. *A. racemosa* Hildebr.	K	21-4.0
11. *A. radiosa* Maurizio	B, K	18-3.5
12. *Aphanomyces frigidophilus* Kitan. et Hatai	B, K	9-1.7
13. *A. invadans* Willougby *et al.*	B	19-3.7
14. *A. irregularis* Scott	B, K, S	8-1.5
15. *Leptolegnia caudata* de Barry	K	62-11.9
16. *Saprolegnia anisospora* de Barry	B, K	9-1.7
17. *S. australis* R. F. Elliott	B	18-3.5
18. *S. ferax* (Gruith) Thur.	B, K, S	66-12.7
19. *S. irregularis* T. W. Johson & R. L. Seym.	K	9-1.7
20. *S. monilifera* de Barry	B	7-1.3
21. *S. parasitica* Coker	B, K, S	91-17.5
22. *S. unispora* (Coker et Couch) R. L. Seym.	B, K	11-2.1
Leptomitales		
23. *Leptomitus lacteus* (Roth) C. Agardh	B, K, S	54-10.4
Pythiales		
24. *Pythium debaryanum* Hesse	B	11-2.1
25. *P. diclinum* Tokun	B, K, S	48-9.2
26. *P. intermedium* de Barry	B, S	9-1.7
27. *P. ultimum* Trow	B, K	4-0.8

Number of species in water from River Biala (B) - 24[a]
Number of species in water from River Krasna (K) - 16[b]
Number of species in water from River Suprasl (S) - 8[c]
Different letters ([a, b] or [c]) indicate that the number of species in water from rivers differs significantly at ≤ 0.05 level.

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

Species of genus	Amino acid	Carbohydrate	Urease
Achlya	Asp, Glu, Arg, Ala	Fru, Glu, Man, Raf, Suc, Mal, 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, 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.
Carbohydrates: 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.

Asia (India, Indonesia [38], Philippines [40], Thailand [33]), North America [41], Africa [42] and Europe [43]. EUS is causing mass mortality of wild and farmed fishes and severe economic loss. According to Lilley *et al.* [38], the first report of the disease came from Japan in 1971, where it is known as mycotic granulomatosis (MG) [35]. In Australia, it was called as red spot disease (RSD) [36] and in USA as ulcerative mycosis (UM) [44]. In the early 1970s, the pathogenic isolate in Japan has been named *Aphanomyces piscicida* [45] and was firstly described in pond-raised goldfish *Carassius auratus* L. and ayu *Glecoglossius altivelis* Temminck & Schegel [35, 46]. Roberts *et al*. indicated that *Aphanomyces piscicida* affects (only in Asia) over 100 species of freshwater and estuarine fishes [37]. *Aphanomyces invadans* occurs in sea mullet (*Mugil cephalus* L.) [36] and in Atlantic menhaden (*Brevoortia tyrannus* [Latrobe]) along the East Coast of the United States [47] and in some inland areas (farm ponds in Georgia and Louisiana). Lilly *et al.* [38, 48, 49] performed the examinations of 20 isolates of *Aphanomyces invadans* from Australia, Bangladesh, Indonesia, Japan, Philippines and Thailand using random amplification of polymorphic DNA. The authors showed a high degree of genetic homogeneity using 14 random ten-mer primers. In addition, the DNA sequence characteristics are identical to pathogens from the East Coast of the United States [47]. Straminipiles organism *Aphanomyces invadans* in fish similarly as chytrid fungus *Batrachochytrium dendrobatidis* in amphibians [50] causes mass mortalities of wild and farmed fishes. Nowadays epizootic ulcerative syndrome (EUS), recorded already in Japan 45 years back [51] and later in the tropical regions [52], is present in all continents. *Saprolegnia polymorpha*, the second rare species in polish waters, has been first described by Willoughby [53] in waters of British Isles on the ornamental carp (*Cyprinus carpio* L. *coi*). In our previous study, this species has already been found on the *Carassius auratus* (L.) muscles [43] and on the *Acipenser oxyrhynchus* eggs [40] in the water from Lake Komosa. Five species from *Pythium* genus and about 20 species belonging to opportunistic pathogens [54-56] have been observed on Adriatic sturgeon eggs. We have already observed the growth of sixteen fungi species of the *Pythium* genus on the eggs of freshwater fish [56]. *Pythium middletonii*- the third species rare to freshwater fish was first described by de Bary as *P. proliferum* in 19th century. Sparrow [57] gave a new name- *P. middletonii*. The fungus has been found to be a saprophyte on algae and dead insects in ditch and lake water [58]. In fish, it has been observed for the first time by Florinskaya [54] in a hatchery, on the eggs of several fish species. We observed this species on the eggs of *Acipenser sturio* L. and *A. persicus colchicus* U. Marti in water from Pond Komosa [8]. It has been hitherto recognized as an aquatic saprophyte of rivers and lakes [59].

The majority of recorded fungal species on the eggs of Adriatic sturgeon belong to opportunistic pathogens [60]. Those species are saprotrophic and necrotrophic, grow on dead plant fragments and cause primary infections in fish. The most eutrophic water bodies (River Biala) are highly favorable environments for opportunistic fungal species, than the poor eutrophic water of River Suprasl. The opportunistic species can develop in eutrophic water bodies and reach the dead fragments of plants. As it is known, in the lifecycle of Saprolegniales species, different structures, sexual and asexual take part. The sexual structures- antheridia and oogonia can release some motile oospores. After germination, the oospore releases a new mycelium. The asexual spore or sporangium is formed at the end of hyphal cells and releases many motile zoospores [61]. They have been called “the primary zoospores”, which encyst to form “secondary zoospores”, which are motile for a longer period of time and form “secondary cysts”. These are the infective form for fishes. In opportunistic species, the secondary cysts are different in saprophytic and in fish pathogenic forms (contain hooked hairs) and their presence has been considered as an adaptation for attaching to a substrate, especially a fish surface, their eggs and other water organisms [62-64].

CONCLUSION

Examination of the growth of straminipiles organisms on the eggs of Adriatic sturgeon in three trophically different rivers was performed. Twenty-seven species of straminipiles organisms were found to develop and grow on the eggs of Adriatic sturgeon. The greatest number of straminipiles species was found in water from the most eutrophic Biala River (24 species). 170 (32.7%) out of 520 investigated Adriatic sturgeon eggs were infected by straminipiles organisms. Straminipiles infection of fish eggs is a major economic problem. It affects many freshwater species, especially economically important farmed fishes, including the sturgeonid species.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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