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# Interactions between colonies of the sponge *Eunapius fragilis* and aquatic fungi

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## ABSTRACT

The authors investigated the influence of the sponge *Eunapius fragilis* occurred on dead reed *Phragmites australis* (reed-sponge) on occurrence of aquatic fungi species in the water of five water bodies of different trophic levels of trophism. Seeds and snake exuviae were used as baits. The fewest fungi were noted in the containers with reed-sponge and control in water from Pond Fosa (most trophic), the most with containers in water from Lake Blizno (fewest trophic). More fungi were found to grow in the containers with reed-sponge (Rs) than in the controls (Co) in water from all water bodies. The mean ratio of Rs/Co ranged from 2.3 (Lake Blizno) to 4.5 (Pond Fosa). The effect of reed-sponge on the abundance of aquatic fungi species is probably caused by dissolved organic matter produced during photosynthesis by *Zoochlorella* sp., a symbiont of the sponge, and excreted into the environment. This excreted organic substance serve as a nutrient for aquatic fungi. *Aphanomyces volgensis* was recorded in Poland for the first time.

**KEYWORDS:** aquatic fungi, hydrochemistry, interactions, Poland, sponge

## INTRODUCTION

Water reservoirs, especially lakes, are characterized, both in horizontal and vertical layers, by variable abiotic conditions and thus by a variety of organisms. Lakes show a complex of interactions between groups of hydrobionts inhabiting a respective lake [1]. This variety of organisms and interactions are especially abundants in the littoral zone [2], whose specificity is shaped, as far as the higher plants are concerned, by littoral reed [3]. This plant does not only influence different light and temperature conditions in this zone, but its last year's dead stalks serve as a substrate for numerous species of periphyton [4] and animals including this sponge species [5].

Conducting studies on the interactions between various groups of littoral plants and zoosporic fungi observed in various types of water reservoirs [4, 6-9], we decided to find out the relationship between zoosporic fungi and colonies of the sponge *Eunapius fragilis* which is known to live in a symbiotic association with autotrophic cells of green alga belonging to *Zoochlorella* genus. *Eunapius fragilis* occurs on reeds in the shallow water of the lakes in the phytolittoral zone [10, 11].

## MATERIAL AND METHODS

Colonies of *Eunapius fragilis* (Leidy,1851) (syn. *Spongilla fragilis*, Leidy, 1851) were collected for experiments from the phytolittoral zone of the eutrophic lake Blizno in Northeast Poland, together with fragments of last year's stalks of the reed *Phragmites australis* (Cav.) Trin. ex Stendel, which in this lake are frequently intensively overgrown by reed sponge, thus assuming dark

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green colour. For determination of species of the investigated sponge, the key of Van Soest [12] was used and the taxonomic status was according to Brands [13].

Therefore, 15 cm long fragments of stalks obtained from three plants were placed in 1-litre containers and spilled with water collected from the relevant water bodies. Before that, the stalk fragments were fastened with a thread to a glass slide, which prevented their floating onto the surface. All 15 cm long fragments of reed stalks devoid of sponge were rewashed several times with distilled water to remove from their surface bacteria and fungi.

Water samples were collected from water bodies at five locations:

• River Biała, length 9.8 km, the left-bank tributary of Supraśl River, flowing through Białystok City;

• River Supraśl, length 106.6 km, the right-bank tributary of the Narew River, flowing through the Knyszyńska Forest;

• Pond Dojlidy, located near Białystok City, area 34.2 ha, maximal depth 2.85 m, its south shore with coniferous woods and its western part with the town of Białystok. This part has been used by the inhabitants of the town as a beach;

• Pond Fosa, covering an area of 2.5 ha, maximal depth 1.75 m. Pond with wild ducks and breeding swans as well as crucian carp. The pond is surrounded by meadows with linden and elm;

• Lake Blizno, located in Augustów Forest, covers an area of 238.5 ha, maximal depth 28.8 m. Its south-western shores are surrounded by extensive coniferous woods, the northern shores with the village Ateny. The sampling site was on the northern side of the lake.

Water samples for analysis and experiments were collected from each water bodies at the depth of 15-30 cm at a distance of 0.5 m from the bank. The water was filtered though a gauze and then poured to the containers. Nineteen parameters were determined for physical and chemical characteristics of the water using standard methods [14].

Water samples (800 ml each) were placed in 1,000 ml containers. For each location, three containers with water from that particular water body were collected. The fourth container served as a control containing three 15 cm long fragments of stalks obtained from three old reeds without sponges.

The seeds of buckwheat (*Fagopyrum sagittatum* Gilib.), hemp (*Cannabis sativum* L.), Persian clover (*Trifolium resupinatum* L.), white clover (*Trifolium repens* L.) and snake exuviae (*Natrix natrix* L.) were used as baits (in containers with reed-sponge and controls) in accordance to the general principles of culture [15].

All containers were enclosed in Petri dishes with the bed turned upside to prevent possible airborne contamination in the containers with fungal spores. The containers were stored at 15 ± 2°C, with access to daylight resembling natural conditions and following recommended instructions [16]. The analyses of water and experiments were carried out in three parallel repetitions.

After one month of exposure, clusters from the containers' bottom and side walls, as well as the surface of baits were examined under a light-microscope. Morphological structures (zoospores, antheridia and oogonia) of aquatic fungi growing in particular containers were recorded. The baits were observed under a microscope every 3-4 days. The size of the fungal structures was measured using light-microscopy at 600x. For determinations of particular species of fungi, the following keys were used: Seymour [17], Batko [18], Plaats-Niterink [19], Pystina [20], Watanabe [15]. The original species description were additionally consulted. The taxonomy of straminipilous organisms was based on Dick [21] and Johnson *et al*. [22, 23], zoosporic fungi on Barr [24] and James *et al*. [25] and higher Fungi on Blackwell *et al*. [26].

The effect of reed- sponge on the number of aquatic fungal species was presented as a ratio of the number of cases where a species were found in the containers with reed-sponge (Rs) to those in the control (Co) [27].

Results were subjected to statistical analysis [28].

## RESULTS

The water used in our experiment varied with respect to the abundance in biogenic compounds

(Table 1). Pond Fosa appeared the most abundant in all three forms of nitrogen and phosphates, the water from Lake Blizno, had the smallest amounts of these substances. This also referred to $BOD_5$, COD, $CO_2$, alkalinity, chlorides, magnesium and iron.

During the experiment, 30 species of the fungi grew on the baits, including 25 species of Straminipila, and 5-Fungi (Table 2). The species that were new to Polish aquatic micobiota included *Aphanomyces volgensis* and rare-*Saprolegnia multispora*. The most species of the fungi grew on the seeds of the clovers as baits (Table 3). Three species of straminipilous organisms were noted on the baits only in the control containers, and 17 species in containers with the reed-sponge, whereas 10-in the control containers and with reed- sponge (Table 4). Fewer species of fungi were observed in control containers, as compared to the containers with reed-sponge (Table 5). This referred to the water of all five water bodies studied. The mean Rs/Co ratio oscillated between 2.3 (Lake Blizno) and 4.5 (Pond Fosa).

## DISCUSSION

In the terminal part of the vegetation period, aquatic fungi contribute greatly to the mineralization of dead fragments of plants in the littoral zone of lakes [29, 30]. A few hundred fungal species are found to grow in this zone. Reed is one of the most common plants inhabiting the littoral parts of lowland rivers and lakes [2]. Reed leaves are the habitat for over 50 aquatic

**Table 1.** Chemical and physical properties of water in particular water bodies.

| Specification | River | | Pond | | Lake |
|---|---|---|---|---|---|
| | Biała | Supraśl | Dojlidy | Fosa | Blizno |
| Temperature ($^oC$) | 12.8 | 12.0 | 13.2 | 13.8 | 12.6 |
| pH | 7.31 | 7.52 | 7.84 | 7.02 | 8.02 |
| DO (mg $l^{-1}$) | 12.08 | 13.40 | 9.60 | 1.84 | 16.42 |
| $BOD_5$ (mg $l^{-1}$) | 4.82 | 7.20 | 5.40 | 9.22 | 2.81 |
| COD (mg $l^{-1}$) | 9.02 | 7.00 | 7.03 | 15.12 | 4.12 |
| $CO_2$ (mg $l^{-1}$) | 15.83 | 8.82 | 13.25 | 22.43 | 5.32 |
| Alkalinity in $CaCO_3$ (mval $l^{-1}$) | 4.71 | 4.34 | 4.60 | 5.78 | 2.45 |
| N-$NH_3$ (mg $l^{-1}$) | 0.642 | 0.232 | 0.321 | 0.865 | 0.213 |
| N-$NO_2$ (mg $l^{-1}$) | 0.011 | 0.008 | 0.013 | 0.115 | 0.003 |
| N-$NO_3$ (mg $l^{-1}$) | 0.050 | 0.025 | 0.036 | 0.054 | 0.025 |
| P-$PO_4$ (mg $l^{-1}$) | 1.504 | 1.024 | 0.450 | 3.625 | 0.140 |
| Sulphates (mg $l^{-1}$) | 68.11 | 34.97 | 23.41 | 28.07 | 14.08 |
| Chlorides (mg $l^{-1}$) | 40.02 | 21.02 | 18.07 | 45.24 | 14.04 |
| Total hardness (mg Ca $l^{-1}$) | 92.16 | 70.56 | 88.56 | 79.34 | 40.89 |
| Total hardness (mg Mg $l^{-1}$) | 22.34 | 12.47 | 16.34 | 26.31 | 11.32 |
| Fe (mg $l^{-1}$) | 0.90 | 0.50 | 0.72 | 1.08 | 0.12 |
| Dry residue (mg $l^{-1}$) | 532.0 | 166.0 | 296.0 | 430.0 | 182.0 |
| Dissolved solids (mg $l^{-1}$) | 496.0 | 141.0 | 280.0 | 369.0 | 140.0 |
| Suspended solids (mg $l^{-1}$) | 36.0 | 15.0 | 16.0 | 61.0 | 42.0 |

**Table 2.** Aquatic fungi found in water from particular water bodies.

| Taxa | River | | Pond | | Lake |
|---|---|---|---|---|---|
| | Biała | Supraśl | Dojlidy | Fosa | Blizno |
| Straminipila | | | | | |
| Peronosporomycetes | | | | | |
| Saprolegniales | | | | | |
| 1. *Achlya americana* Humphrey | x | x | x | x | x |
| 2. *A. androgyna* (W. Archer) T. W. Johnson & R. L. Seym. | | x | | | |
| 3. *A. debaryana* Humphrey | | | x | | x |
| 4. *A. dubia* Coker | | x | x | | x |
| 5. *A. klebsiana* Pieters | x | | | | |
| 6. *A. papillosa* Humphrey | | | | | x |
| 7. *A. polyandra* Hildebr. | x | x | x | x | x |
| 8. *A. prolifera* Nees | | | | | x |
| 9. *A. proliferoides* Coker | | x | | | |
| 10. *A. treleaseana* ( Humphrey) Kauffman | x | x | | | |
| 11. *Aphanomyces irregularis* W. W. Scott | x | x | x | x | x |
| 12. *A. volgensis* Domashova | | x | | | |
| 13. *Dictyuchus monosporus* Leitg. | | | | x | |
| 14. *Isoachlya torulosa* ( de Bary ) Cejp | | | x | | x |
| 15. *Saprolegnia anisospora* de Bary | | x | | | x |
| 16. *S. diclina* Humphrey | x | | | | x |
| 17. *S. ferax* (Gruith.) Thur. | x | x | x | x | x |
| 18. *S. glomerata* (Tiesenh.) A. Lund | | | x | x | x |
| 19. *S. lapponica* Gäun. | | | | | x |
| 20. *S. litoralis* Coker | | | | x | x |
| 21. *S. multispora* B. Paul & Steciow | | x | | | |
| 22. *S. parasitica* Coker | x | | x | x | x |
| 23. *Thraustotheca calvata* (de Bary) Humphrey | x | | | | |
| Pythiales | | | | | |
| 24. *Pythium inflatum* V. D. Matthews | | | x | | x |
| 25. *P. rostratum* E. J. Butler | | | x | | |

Table 2 continued..

| | | | | | |
|---|---|---|---|---|---|
| Fungi | | | | | |
| Chytridiomycota | | | | | |
| Chytridiales | | | | | |
| 26. *Achlyogeton entophytum* Schenk | | x | | | |
| 27. *Phlyctochytrium aureliae* Ajello | x | | | | |
| Blastocladiomycota | | | | | |
| Blastocladiales | | | | | |
| 28. *Blastocladiopsis parva* (Whif.) Sparrow | | x | | | |
| 29. *Catenophlyctis variabilis* (Karling) Karling | x | | x | x | x |
| Zygomycota | | | | | |
| Zoopagales | | | | | |
| 30. *Zoopagus insidians* Sommerst. | | x | | | |
| Total species | 11 | 14 | 12 | 9 | 17 |

**Table 3.** Aquatic fungi found on particular baits.

| Bait | Straminipilous organisms and fungi (see Table 2) | Only on one bait | Total number of species |
|---|---|---|---|
| Seeds of buckwheat | 4, 7, 10, 11, 13, 17, 22 | 13 | 7 |
| Seeds of hemp | 1, 3, 5, 7, 9, 16, 17, 22, 26 | 9, 26 | 9 |
| Seeds of Persian clover | 1, 2, 4, 6, 7, 10, 14, 16, 17, 19, 21, 22, 23, 25, 28 | 6, 19, 21, 25, 28 | 15 |
| Seeds of white clover | 1, 2, 3, 5, 7, 8, 10, 12, 14, 15, 16, 17, 18, 22, 24 | 8, 12, 15, 20, 24 | 15 |
| Exuviae of snake | 1, 5, 7, 11, 16, 18, 27, 29, 30 | 27, 29, 30 | 9 |

fungal species [29], whereas its stalks remain undecomposed until the following vegetation season, constituting a substrate for the growth of *Eunapius fragilis* colonies. In lake Blizno, where the material was collected for the experiment, almost every second last year's reed stalk contains smaller or larger colonies of this sponge. In shallow places the sponge is less green, in deeper sites - dark green, due to a higher content of chlorophylls in the cells of its symbiotic partner *Zoochlorella* sp. Carotenoid- type pigments that facilitate photosynthesis also undergo changes dependent from environmental conditions [5, 31], the phenomenon being called chromatic adaptation that takes place in all algae, aquatic and terrestrial plants [32]. Chromatographic

**Table 4.** Aquatic fungi found in particular containers.

| Specification | Straminipilous organisms and fungi (see Table 2) | Number of species |
|---|---|---|
| Only with reed - sponge | 2, 5, 6, 8, 9, 10, 16, 17, 20, 22, 23, 25, 26, 27, 28, 29, 30 | 17* |
| Only control | 12, 13, 21 | 3* |
| With reed - sponge and control | 1, 3, 4, 7, 11, 14, 15, 18, 19, 24 | 10* |

*Differences significant at the ≤ 0.05 level.

**Table 5.** Mean number of fungal species in containers with reed-sponge (Rs) and control containers (Co), (n = 9 for particular water bodies).

| Water bodies | Rs ± SD | Co ± SD | Ratio Rs/Co |
|---|---|---|---|
| River Biała | *12.0 ± 1.98 | 3.0 ± 1.52 | 4.0 |
| River Supraśl | *13.5 ± 1.76 | 4.5 ± 1.68 | 3.0 |
| Pond Dojlidy | *8.4 ± 2.02 | 2.3 ± 1.92 | 3.7 |
| Pond Fosa | *9.0 ± 1.92 | 2.0 ± 1.43 | 4.5 |
| Lake Blizno | *10.5 ± 1.46 | 4.5 ± 1.18 | 2.3 |

*Differences significant at the ≤ 0.05 level in respect to Co.

analysis of photosynthesizing pigments in the zoochlorellae of the sponge and in free-living cells of *Chlorella* sp. has shown that they are the same. Both unicellular and multicellular algae of all systematic types excrete some products of photosynthesis into the aquatic environment, the so called extracellular production [33]. This extracellular production, specific to all photosynthesizing bacteria [34, 35] and to higher aquatic plants [36], may reach from 50% in *Chlorella vulgaris* [37] to 70% in the photosynthesizing bacterium *Chlorobium limicola* [38]. Such levels of extracellular production can also be observed in the phytoplankton as a whole, including picophytoplancton [39]. The soluble extracellular products of the symbiotic algae of sponges were investigated [40]. The chemical composition of the extracellular production has been known. Tolbert & Zell [41] were the first to reveal glycolic acid release to the aquatic environment in algae. The dissolved organic compounds released to the aquatic environment consist of polymeric substances [42], free glucose [43] and free amino acids [44]. These substances are absorbed by other organisms, especially by the heterotrophic ones [45]. For instance, free amino acid is absorbed not only by bacteria [46] or aquatic fungi [47] but also by some phytoplankton species [48]. In this context, it can be assumed that *Zoochlorella* sp. cells found in the *Eunapius fragilis* colony release the extracellular production that can serve as a substrate for aquatic mycoflora, similarly to the one secreted by algae, mosses or macrophytes [6, 7]. However, the stimulatory effect excreted by the reed- sponge is substantially greater as compared to algae, mosses or macrophytes. It may be that the cells of this sponge itself excrete a substance that acts as a bait on certain aquatic fungal species. This might explain the fact that in our study as many as 17 fungal species were found to grow in containers with reed- sponge as compared to only 3 species in control samples (Table 4).

Reed-sponges found in the water collected from pond Fosa had the highest stimulatory effect on fungi (ratio 4.5). It should be mentioned that the water in pond Fosa was the most eutrophic of all the waters used in the experiment. The growth of fungal species in the containers with reed-sponge was the lowest in the case of the least eutrophic water from lake Blizno (ratio 2.3). This synergistic effect has been observed for macrophytes on bacterioplankton and fungi in trophically different water bodies [7, 49].

The stimulatory effect of *Eunapius fragilis* on the growth of fungi can be observed till the end of autumn. At the beginning of winter, the colony itself dies releasing the so-called gemmules protected by two membranes enclosing air-filled chambers necessary for the sponge cells to survive the winter [50]. Young sponges begin to grow from the gemmules from the early spring. In lake Blizno, green colonies of *Eunapius fragilis* begin to cover reed stalks at the end of April/beginning of May.

Also secondary compounds, i.e. polyphenolic compounds, lactones or alkaloids are excreted into the aquatic environment by water plants, inhabiting the growth of hydrobionts, especially phytoplankton [51, 52], bacteria [49, 53], as well as aquatic fungi [6-9]. The growth of aquatic fungi is also suppressed by algae of the order Charales containing polyphenolic compounds [8], as well as plankton cyanobacteria [54, 55] and periphyton cyanobacteria [4]. The interactions between aquatic macrophytes and aquatic fungi undergo changes during the vegetation period. In the first half of this period, macrophytes stimulate the growth of aquatic mycoflora, whereas in the second half, particularly in its terminal part, their inhibitory effect can be observed [7]. This may be associated with an accumulation of secondary compounds at the end of the vegetation period, which after passing to the aquatic environment inhibit the growth of hydromycoflora. Moreover, two lactone compounds, protoanemonin and anemonin, found in representatives of Ranunculaceae considerably suppress the growth of many aquatic fungal species [8].

Almost all the species identified in the current study have been frequently encountered in various water reservoirs in Poland, except for *Aphanomyces volgensis* which appears new to the Polish waters and *Saprolegnia multispora* as rare species. *Aphanomyces volgensis* was described for the first time in Russia by Domashova [56] from the River Volga. In our study, it was found to grow in control containers on the seeds of white clover in the water collected from the River Supraśl. *Saprolegnia multispora* was also isolated from the water in the River Supraśl on the seeds of Persian clover in control containers. This species was first described by Paul & Steciow [57] in the Burgundian region (France) from water samples. Previously we have found this species in the spring Cypisek [58] and in the river Supraśl [59]. Worthy of note is also the finding of *Achlyogeton entophytum*, a relatively rare chytridial fungus in Poland, in water samples from the River Supraśl on the *Cannabis* seeds in containers with reed- sponges. This species was first reported from Germany as a parasite of algae of the genus *Cladophora* [60].

## CONCLUSIONS

In the littoral zone of lakes, colonies of the reed-sponge *Eunapius fragilis*, of green colour due to a symbiotic relationship with *Zoochlorella* sp., are found to grow on last year's dead reed stalks. In laboratory conditions, the presence of this sponge was observed to cause a higher increase in the number of fungal species growing on baits in waters of various trophicity. This type of interaction is presumably connected with extracellular production of autotrophic zoochlorellae, providing substrates for aquatic fungi. Also other algae and a number of macrophytic species exert a stimulatory effect on the growth of fungi in the littoral zone. Cyanobacteria, some algal species (Charales) and some macrophytes (member of Ranunculaceae) by excreting a number of secondary compounds are known to inhibit the growth of aquatic fungi. Thus, the number of aquatic fungus species in the lake littoral zone is not only dependent on abiotic factors but also to a large extent on interactions in the biotic sphere.

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