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Stimulatory activity of four green freshwater sponges on aquatic mycotal communities

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The influence of the four species of green sponges (*Ephydatia muelleri*, *Heteromeyenia stepanowii*, *Spongilla fluviatilis*, and *Spongilla lacustris*) on the occurrence of aquatic mycotal species in the water of three four water bodies of different trophy was investigated in this study. Seeds and snake exuviae were used as baits. For the measurement of the primary and extracellular production by symbiotic algae of green sponges and assimilation of those products by mycota, radioactive carbon (^{14}C) was used. A total of 75 mycotal species were found to be growing on the baits. The fewest mycota were noted in the containers in water from oligotrophic Lake Hańcza; the most in the containers with eutrophic water from River Supraśl. More mycota were found to grow in the containers with green sponges (Sp) than in the controls (Co) in water from all water bodies. The mean ratio of Sp/Co in green sponges ranged from 2.30 (*E. muelleri*) to 4.80 (*H. stepanowii*); in brown colonies (without symbiotic algae) it was 0.90. Mean value of ^{14}C fixation (primary production) in symbiotic algae of *Spogilla fluviatilis* was 5.67 mg C g^{-1} dry weight sponge per hour. The effect of green sponges on the abundance of aquatic mycotal species is caused by dissolved organic matter produced during photosynthesis by symbiotic zoochlorellae, a symbionts of green sponges and excreted into the water environment (*S. fluviatilis* excreted mean 12.8% of carbon fixation). Those excreted organic substances serve as nutrients for aquatic mycota. The mean value of extracellular products assimilated by mycota was 4.96 mg C g^{-1} dry weight mycelium per hour.

Key words: Sponges, symbiotic zoochlorellae, aquatic mycota, interactions, hydrochemistry.

INTRODUCTION

The distribution of plant and animal hydrobionts in water ecosystems of a lake type is very variable. Independently of the limnological lake type, we observe the highest amount of hydrobionts species in litoral. So, that is why in such lake types the most variable interactions between the species occur (Carpenter, 1998). A dominating form of such interactions is allelopathic activity of some algae species- mainly of some submerged macrophytes (Gross et al., 1996). Besides, important feeding interactions of a type predator-victim, such ones in which the extracellular

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products of the representatives of one species can be a medium for the representatives of the second species. Such type of a feeding interaction occurs mainly between plant hydrobionts. During the lakes fitolittoral among numerous hydrobionts we can also observe green species of sponges living (Hutchinson, 1993) (as it is well known) in symbiosis with unicellular green algae from symbiotic zoochlorellae species. Our preliminary examinations on the *Ephydatia fragilis* showed the influence of the colonies of this sponge on the growth of some species of mycota (Czeczuga et al., 2011a). This paper relates to the interactions between water mycota and colonies of four other species of green sponges which are quite popular in the waters of North-Eastern Poland.

MATERIALS AND METHODS

The colonies of four green species of sponges were collected for experiments from the following water bodies:

1. *Ephydatia muelleri* (Lieberkühn, 1855) - litho littoral of lake Hańcza, area 291.5 ha, max. depth 108.5 m, located in Suwałki Landscape Park (54°16'N, 22°48'E, altitude 229 m). It is a typical oligo- mesotrophic lake. The sampling site was on the south-eastern side of the lake near Blaskowizna village. The site is surrounded by meadows with alder. The bed is covered with stones.
2. *Heteromeyenia stepanowii* (Dybowski, 1884) syn. *Carterius stepanowii* (Dybowski, 1884) - phytolittoral of lake Blizno, located in the Augustów Forest (53°57.6'N, 23°04'E, altitude 133.2 m). Area 238.5 ha, max. depth 28.8 m. it is a typical eutrophic lake. South-western shores are surrounded by coniferous woods, while the northern shores of the lake border with the village Ateny. The sampling site was on the northern site of the lake. The bed is muddy.
3. *Spongilla fluviatilis* (Linnaeus, 1759) - river Supraśl, length 106.6 km, the right bank tributary of the river Narew, flowing through the Knyszyńska Forest. The sampling site was in the town Supraśl (53°13'N, 23°20'E).
4. *Spongilla lacustris* (Linnaeus, 1759)- pond Dojlidy, located near Białystok City (53°07'N, 23°10'E), area 34.2 ha, max. depth 2.85 m, its south shore borders 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. We investigated the green and brown colonies without symbiotic algae for this species.

For determination of species of investigated sponges the key of Van Soest (2002) was used and the taxonomic status was used according to World Porifera Database (Van Soest et al., 2009). In this paper we have used the name of autotrophic sponges symbiotic zoochlorellae as *Zoochlorella* sp. as Brandt (1892) first did and as it was lately used in Algae Base (Guiry and Guiry, 2015). We also used some papers in which authors have used the synonym zooxanthellae (Yonge, 1944) and *Chlorella* sp. (Reisser and Wiessner, 1984). Water samples for chemical analysis and experiments were collected at depth of 15 to 30 cm and at a distance of 1 m from bank. Twenty-one (21) environmental parameters (Table 1) were determined using standard methods (APHA, 2005). 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 used collected. The fourth container served as a control containing only baits without sponge.

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 sponge and controls) in accordance to the general principles of culture (Watanabe, 2002). All containers were enclosed in Petri dishes with the bed turned upside down to prevent possible airborne contamination in the containers with mycotal spores. The containers were stored at 18 ± 2°C, with access to daylight resembling natural conditions and following recommended instructions (Seymour and Fuller, 1987). 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 (zoospore, antheridia and oogonia) of aquatic mycota growing in particular containers were recorded. The baits were observed under a microscope every 3 to 4 days. The size of the mycotal structures was measured using light-microscopy at 600×. Determinations of particular species of mycota have been performed according to the following keys: Seymour (1970), Batko (1975), Plaats-Niterink (1981), Pystina (1998), Watanabe (2002). Systematics of straminipiles has succeeded according to Dick (2001), of fungi according to Blackwell et al. (2006), and of Chytridiomycota according to James et al. (2000), and of Saprolegniaceae according to Johnson et al. (2002; 2005). The quantities of substances formed during the process of photosynthesis both inside and outside the cells of symbiotic zoochlorellae were measured using radioactive carbon (^{14}C). The method was similar to that described by Steemann-Nielsen (1952) for the study of photosynthesis of phytoplankton in accordance with the general principles of the techniques. We have described it in detail in our previous paper (Czeczuga et al., 2011b).

S. fluviatilis material was obtained by cutting off 5 cm long terminal sections (fragments) of sponge branch. Samples of water from River Supraśl were filtered through a gauze with 50 µm meshes and with three 5 cm long fragments of green sponge poured always into two 250 mL bottles (one light and one dark) to which 4.5 ml of $Na_2{}^{14}CO_3$ solution (ca 10 µCi) was then added after which they were sealed carefully. In the light bottle cellular and extracellular production by symbiotic zoochlorellae was determined and the dark bottle was used for determination of the assimilation of the carbon in the dark. To second light bottle in which mycotal species were growing on seeds and baits we have added three 5 cm long fragments of green sponge which was before this in a light bottle with $Na_2{}^{14}CO_3$ for determination of the radioactivity in mycelium as assimilation of extracellular products excreted by symbiotic zoochlorellae sponge. All three bottles were lowered to aquarium (1 × 0.5 × 0.5 m) in which the water was stored at 18 ± 2°C, with access to daylight resembling natural conditions and following the recommendations of Gilbert and Allen (1973). The effect of sponges on the number of aquatic mycotal species is presented as a ratio of the number of cases where the species were found in the containers with sponge (Sp) to those in the control containers (Co) (Magurran, 1988). Some results were tested for significance using the S-Scheffe test, which is considered to be one of the most conservative post-hoc tests (Winer, 1997).

RESULTS

Chemical analysis of the water used for the experiment showed that the water was the most eutrophic in the Pond Dojlidy, the least eutrophic in the Lake Hańcza and middle in the Lake Blizno and River Supraśl. This is expressed by such parameters of chemical analysis of the water as dissolved oxygen content (DO), COD of all three forms of nitrogen, phosphates (Table 1). In the

Table 1. Chemical and physical parameters of water in particular water bodies.

Specification (Water parameter)	River Supraśl	Pond Dojlidy	Lake Blizno	Lake Hańcza
Temperature (°C)	22.0	23.2	22.6	20.3
Transparency (m)	Bed	0.8	2.1	8.5
pH	7.52	7.84	8.02	8.08
DO (mg L^{-1}) - dissolwed oxygen	13.40	9.60	12.42	16.65
BOD_5 (mg L^{-1}) - biological oxygen demand	7.20	5.40	2.81	2.86
COD (mg L^{-1}) - oxidation	7.0	8.03	4.12	3.92
CO_2 (mg L^{-1})	8.82	13.25	5.32	2.12
Alkalinity in $CaCO_3$ (mval L^{-1})	4.34	4.60	2.45	2.07
$N\text{-}NH_3$ (mg L^{-1})	0.232	0.321	0.213	0.249
$N\text{-}NO_2$ (mg L^{-1})	0.008	0.013	0.003	0.001
$N\text{-}NO_3$ (mg L^{-1})	0.025	0.036	0.025	0.016
$P\text{-}PO_4$ (mg L^{-1})	0.124	0.450	0.140	0.015
Sulphates (mg L^{-1})	34.97	23.41	14.08	12.74
Chlorides (mg L^{-1})	21.02	18.07	14.04	13.36
SiO_2 (mg L^{-1})	0.61	0.43	0.25	0.81
Total hardness (mg Ca L^{-1})	70.56	88.56	40.89	35.52
Total hardness (mg Mg L^{-1})	12.47	16.32	11.42	9.75
Fe (mg L^{-1})	0.50	0.72	0.12	0.09
Dry residue (mg L^{-1})	166.0	296.0	182.0	102.0
Dissolved solids (mg L^{-1})	141.0	280.0	140.0	77.0
Suspended solids (mg L^{-1})	15.0	16.0	42.0	25.0

present experiment, 75 types of mycotal species-including 21 species of true Fungi: 1 belonging to Ascomycota, 5 to Blastocladiomycota, 13 to Chytridiomycota, 2 to Zygomycota. 54 species belonging to Straminipila including 3 species of Hyphochytriomycota, 50 to Peronosporomycota and 1 to Plasmodiophoromycota (Table 2) were found to grow on baits. Total number of mycotal species in particular water bodies were different, the largest number of mycotal species were found in water from eutrophic River Supraśl (38), and smallest in water from oligotrophic Lake Hańcza (15 species). *Zoopage pachyblasta* is the new one in polish hydromycology. Whereas, *Blyttiomyces laevis*, *Dangeardia laevis*, *Rozellopsis uliginosa* and *Sommerstorffia spinosa* are rare species. In the waters of all four aquatic bodies there was a considerably larger number of mycota developed in containers with sponges that is in controls. 14 species were found to grow only in control containers, 36 species in containers with sponges and 25 species were isolated both in controls and sponges-containing containers and 9 species in containers with brown colonies (Table 3). Fewer mycotal species were observed in control containers in comparison to the containers with green sponges (Table 4). This referred to the all types of examined green sponges in all types of examined water. The mean Sp/Co ratio oscillated between 2.3 (*E. muelleri*) and 4.8 (*H. stepanowii*). In containers with brown colonies of *Spongilla lacustris* the mean Sp/Co ratio were found 0.90. The mean of photo synthetically fixed ^{14}C in symbiotic zoochlorellae cells was 5.67 mg C g^{-1} dry weight sponge h^{-1} and extracellular production is 12.8% of cellular production (Table 5). The dark fixation of carbon was 3.94% of light fixation. The value of mycotal carbon assimilation was 4.96 mg C g^{-1} dry weight mycelium h^{-1}.

DISCUSSION

All four investigated fresh water species of sponges contained large number of presumably intracellular symbiotic zoochlorellae (Czeczuga, 1974). In the present investigation, the symbiotic green algae have been identified as *Chlorella* genus (Kessler, 1982) and this name is currently regarded as a taxonomic synonym of *Zoochlorella* (ITIS- Report, 2002). From the sponge *S. fluviatilis* Lewin in Massachusetts, USA isolated *Chlorella sorokiniana* Shihira and Krause (Douglas and Huss, 1986). This species of green alga occurred also as free-living strans (Reisser, 1984). *C. sorokiniana* discussed by Reisser (1984) and Douglas and Huss (1986) was confirmed to be *Choricystis parasitica* (K. Brandt) Pröschold and Darienko (Pröschold et al., 2011) and belongs to Trebouxiophyceae family of Chlorophyta. The basionym for this species is *Zoochlorella parasitica* described by Brandt in Germany (1881) for the first time. Handa et al. (2006) reported that *Choricystis minor* (Skuja, Fott) appears as a symbiont of freshwater

Table 2. Aquatic mycota found in particular water bodies.

Fungi	Ascomycota	Eurotiales	Lake Blizno	Lake Hańcza	Pond Dojlidy	River Supraśl
1. *Aspergillus niger* Thieg.	Blastocladiomycota	Blastocladiales			x	
2. *Blastocladiella britanica* Horenst et Cantino						x
3. *Blastocladiopsis parva* (Whiffen) Sparrow						x
4. *Catenaria anguillulae* Sorokin	Chytridiomycota	Chytridiales		x		x
5. *Catenaria sphaerocarpa* Karling					x	x
6. *Catenophlyctis variabilis* Karling			x		x	
7. *Blyttiomyces laevis* Sparrow						x
8. *Dangeardia laevis* Sparrow						x
9. *Polychytrium aggregatum* Ajello		Cladochytriales		x	x	x
10. *Septochytrium variabile* Berdan					x	
11. *Truitella setifera* Karling						x
12. *Nowakowskiella macrospora* Karling		Rhizophydiales		x	x	x
13. *Rhizophydium apiculatum* Karling						x
14. *Rhizophydium chitinophilum* Atik.		Monoblepharidales				x
15. *Rhizophydium nodulosum* Karling						x
16. *Septochytrium verrucosum* Cejp						x
17. *Monoblepharis hypogyna* Perrott		Spizellomycetales		x		
18. *Karlingia polonica* Hassan	Zygomycota	Mucorales				x
19. *Rhizophlyctis rosea* (de Berg et Woronin) A. Fisch.					x	
20. *Mucor hiemalis* Wehm. Zoopagales		Zoopagales			x	
21. *Zoopage pachyblasta* Drechsler	Hyphochytriomycota	Hyphochytriales		x		
22. *Rhizidiomyces bivellatus* Nabel		Olpidiopsidales				x
23. *Olpidiopsis aphanomyces* Cornu	Peronosporomycota	Leptomitales		x		
24. *Olpidiopsis saprolegniae* (Braun) Coker				x		x
25. *Apodachlya pyrifera* Zopf		Pythiales			x	
26. *Leptomitus lacteus* (Roth) Agardh						x
27. *Lagenidium humanum* Karling						x
28. *Myzocytium zoophthorum* Sparrow					x	
29. *Pythiogeton nigricans* A. Batko						x
30. *Pythiogeton uniformae* A. Lund						x
31. *Pythium aristosporum* Vanterp.					x	
32. *Pythium butleri* Subraman.					x	
33. *Pythium debaryanum* R. Hesse				x	x	
34. *Pythium helicandrum* Drechsler					x	
35. *Pythium hemmianum* Takahashi					x	
36. *Pythium inflatum* V. D. Matthews			x			
37. *Pythium middletonii* Sparrow				x		
38. *Pythium rostratum* E. J. Buttler				x		x
39. *Pythium ultimum* Trow						x
40. *Rozellopsis uliginosa* A. Batko		Saprolegniales		x		
41. *Achlya americana* Humphrey			x		x	
42. *Achlya androgyna* (W.A. Archer) T. W. Johnson et R.L. Seym.						x
43. *Achlya debaryana* Humphrey			x			
44. *Achlya diffusa* J.V. Harv. ex T.W. Johnson					x	
45. *Achlya dubia* Coker			x			
46. *Achlya klebsiana* Pieters					x	
47. *Achlya oblongata* de Bary					x	
48. *Achlya oligocantha* de Bary						x
49. *Achlya orion* Coker et Couch			x			

Table 2. Contd.

50. *Achlya papillosa* Humphrey			x			x
51. *Achlya polyandra* Hildebr.			x	x	x	x
52. *Achlya prolifera* Nees			x			
53. *Achlya treleaseana* (Humphrey) Kauffm.					x	
54. *Aphanodictyon papillatum* Huneyc.						x
55. *Aphanomyces amphigynus* Cutter						x
56. *Aphanomyces bosminae* W. W. Scott					x	
57. *Aphanomyces irregularis* W.W. Scott			x			x
58. *Aphanomyces laevis* de Bary					x	
59. *Dictyuchus monosporus* Leitgeb.			x	x		x
60. *Isoachlya monilifera* (de Bary) Kauffm.						x
61. *Isoachlya toruloides* Kauffm. et Coker			x			x
62. *Leptolegnia caudata* de Bary						x
63. *Leptolegniella keratinophila* Huneyc.						x
64. *Leptolegniella piligena* Ookubo et Kabayashi						x
65. *Saprolegnia anisospora* de Bary			x		x	
66. *Saprolegnia diclina* Humphrey			x			
67. *Saprolegnia ferax* (Gruith.) Thur.			x	x	x	x
68. *Saprolegnia glomerata* (Tiesenh.) A. Lund			x		x	
69. *Saprolegnia lapponica* Gäun.			x			
70. *Saprolegnia litoralis* Coker					x	
71. *Saprolegnia parasitica* Coker			x	x	x	x
72. *Scoliolegnia asterophora* (de Bary) M.W. Dick					x	
73. *Sommerstorffia spinosa* Arnaudov			x			
74. *Thraustotheca clavata* (de Bary) Humphrey	Plasmodiophoromycota	Plasmodiophorales			x	x
75. *Woronina polycystis* Cornu			x			
Total number of species			20^a	15^b	30^c	38^d

The different letters indicate the values differ significantly ($p \leq 0.05$).

Table 3. Aquatic mycota found in particular containers.

Specification	Mycotal species (see Table 2)	Number of species
Only with green sponges	2,5,8,11,12,13,17,18,21,22,25,26,32,33,35,36,37, 40,42,44,46,47,48,49,50,52,53,56,60,62,66,68,69,70,72,73	36^a
Only control	3,9,14,16,15,20,27,31,34,38,43,54,63,64	14^b
With sponges and control	1,4,6,7,10,19,23,24,28,29,30,39,41,45,51,55,57, 58,59,61,65,67,71,74,75	25^c
Brown colonies of *S. lacustris*	6,19,37,42,51,58,59, 67,71	9^d

The different letters indicate the values differ significantly ($p \leq 0.05$).

Table 4. Mean number of mycotal species in containers with sponges (Sp) and control containers (Co) (n = 9 for particular species).

Species of sponge	Sp ± SD	Co ± SD	Ratio Sp/Co
Ephydatia muelleri	*18.2 ± 1.73	7.9 ±1.54	2.30
Heteromeyenia stepanowii	*12.5 ± 1.48	2.6 ± 1.02	4.80
Spongilla fluviatilis	*16.9 ± 1.24	4.7 ± 0.95	3.59
Spongilla lacustris	*13.6 ± 0.98	3.1 ± 0.68	4.38
Spongilla lacustris-brown (without algae)	4.7 ± 0.92	5.2 ± 0.74	0.90

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

sponge. This species become a synonym of *C. parasitica* (identical in SSU rDNA sequence) latter (Pröschold et al. 2011) and nowadays occurs as symbiotic algae of *S. fluviatilis* and *S. lacustris*. *C. parasitica* is a heterotypic

Table 5. ^{14}C fixation, excretion by symbiotic algae zoochlorellae of *Spongilla fluviatilis* and assimilation of extracellular products by fungi mycota (n = 9).

Specification	Mean value	Range
Light ^{14}C fixation by symbiotic algae zoochlorellae of *Spongilla fluviatilis* in mg C g^{-1} dry weight of sponge in one hour	5.67	3.12 - 8.25
Excretion of fixed carbon (extracellular production) in % of fixated carbon by symbiotic algae zoochlorellae	12.80	7.50 - 15.90
Dark fixation by green sponge in % of light fixation	3.94	2.07 - 7.82
Assimilation by mycota of the extracellular products in mg C g^{-1} dry weight of mycelium in one hour	4.96	1.92 - 7.84

synonym of Coccomyxa minor described by Skuja in Sweden (1948), and C. minor described as a free-living species for the first time by Fott in Czech (1976). The cells of *C. parasitica* as a *Spongilla endosymbionts*, were broadly ellipsoid to slightly curved in shape, were 1.5 to 3.0 µm in length, and 1.0 to 1.5 µm in width, and had thin cell wall, and contained a parietal chloroplast without pyrenoid. They reproduced by formation of two autospores (Pröschold et al., 2011).

Within some species of marine sponges are also found symbiont algae belonging to the cyanobacteria (Wilkinson, 1979). The green hydrosulphuric bacteria, both unicellular and multicellular algae of all systematic types (Fogg, 1971) including nanoplankton and higher aquatic plants (Wetzel, 1969) excrete some products of photosynthesis into the aquatic environment. It is so called extracellular production (Fogg, 1966). This production may rich up to 50% of photosynthate in free-living cells of *Chlorella vulgaris* and changes according to the age of the cells and light conditions (Czeczuga et al., 2015). The chemical composition of extracellular production has been known. Dissolved organic compound released to the aquatic environment consists of sugars (glucose, maltose) (Gocke et al., 1981), free amino acids (Bengtsson, 1982; Carlucci et al., 1984) and polymeric substances (Hoagland et al., 1993). Also, the algae as symbionts of sponges excrete soluble extracellular products (Muscatine, 1963; Muscatine et al., 1967; Gilbert and Allen, 1973). In *S. fluviatilis* main is the glucose (Muscatine et al., 1967; Reisser, 1984) with trace amount of the amino acids- alanine, glutamine acid and a compound resembling erythrose-4-phosphate (Wilkinson, 1980). It has been showed previously that the values of extracted extracellular products in sponges are different. *Chlorella*- like alga from *Spongilla* sp. excrete 3 to 4.4% of totally fixed carbon (Muscatine et al., 1967). Whereas, the symbiotic alga from *S. lacustris* excreted only about 1% of net primary production (Gilbert and Allen, 1973), the *Chlorella* symbionts of *S. fluviatilis* release extracellular products to the water in proportions between 9 and 17% of the total photosynthetically fixed carbon (Wilkinson, 1980; Reisser, 1984). Our examinations showed similar values for the symbiont of *S. fluviatilis*. Dissolved organic matter (extracellular products) which was produced during photosynthesis by symbiotic zoochlorellae of the green hydra (*Chlorohydra viridissima*), was excreted into water environment from 1.42 to 7.08% (mean 3.12%) of fixated carbon (^{14}C) (Czeczuga et al., 2011b). These extracellular products are a medium for a water mycota what confirm the examinations of radioactive assimilation of those products by mycelia (Table 5).

The results of the experiment revealed that mean Sp/Co ratio for four investigated species of green sponges ranged from 2.30 to 4.80, which means that there were more of the mycotal species in containers with green sponges than in control containers. In all containers with green sponges grew 36 mycotal species, whereas, in control containers 14 and in containers with brown colonies *S. lacustris* (without symbiotic algae) only 9 mycotal species. The symbiotic zoochlorellae excretes the photosynthesis substances in water (extracellular products) which composed a medium for mycotal species with stimulatory effect of green sponges. The mean Sp/Co ratio in containers with brown colonies of sponge was less than 1 (0.90, ranged from 0.81 to 0.99) that means the inhibitory effect of brown sponge on some mycotal species. One of those mechanisms is inhibiting the substances excretion which reduces the hydrobionts growth and is called, according to Erhard (2006), allelopathy. There are many types of compounds involved in allelopathy, e.g., alkaloids, phenols, alcohols, enzymes, glycosides, ketones, lactones, terpens etc. The phenols, lactones and alkaloids belongs to commonly inhibiting compounds in water organisms.

Also secondary compounds, that is, polyphenolic compounds, lactones or alkaloids are excreted into the aquatic environment by some species of algae (Richmond, 1973) and water plants (Gross et al., 1996) inhibiting the growth of hydrobionts. The growth of aquatic mycota is suppressed by the cyanobacteria, the charales algae (Czeczuga et al., 2010b) containing polyphenolic compounds (allin, allicin, diallyl disulfide) and the representatives of Ranunculaceae family excrete protoanemonin and anemonin (lactones) which also inhibit the growth of mycota (Czeczuga et al., 2010a). As our previous experiments have shown, the influence of macrophytes on the growth of mycotal species depends

on the seasons (Czeczuga et al., 2008) and varies in different parts of the aquatic plant stemp (Czeczuga et al., 2013; 2015). So, there are 3 main types of interactions between plant organisms and mycota in water bodies. The most types of water plants excrete into the water the products of photosynthesis which are the food for the mycota and cause their growth, but some other species of plants excrete some substances which inhibit mycota development. Also, such water plants neither stimulate nor inhibit the growth of water mycota.

As already revealed, the mean ratio of Sp/Co ranged from 2.30 (*E. muelleri*) to 4.80 (*H. stepanowii*). In the green sponge *E. fragilis* this mean ratio were found 3.5 (Czeczuga et al., 2011a), and in green hydra *C. viridissima* 2.06 (Czeczuga et al., 2011b). The new species in polish hydromycology *Zoopage pachyblasta* grew inside the containers with sponge in water of Lake Hańcza. This species has firstly been described by Drechsler (1935) as a destructive to terricolous amoebae. In Great Britain it was found in the clay of river and in the bog (Peach, 1954). Apart from the species which is new in polish waters, some other rare species were also found. *Blyttiomyces laevis* was observed on baits in containers with sponges in the water from River Supraśl. It has been firstly describe in America by Sparrow (1952) on algae of *Zygonema* genus. The second rare species in polish waters- *Dangeardia laevis* occurred only inside the control containers also with water from River Supraśl. This species has firstly been described by Sparrow and Barr (1955) as a parasitize colonial algae of Douglas Lake, USA. Both those species were found in some polish water bodies of seeds and fruits of some species of plants.

The third rare species- *Rozellopsis uliginosa* occurred on baits in containers with sponges in the water from Lake Hańcza. This species has firstly been described in Poland by Batko (1977) as a parasite in mycelium of *Nellymyces megaceros*. This is the second site where this mycotal species occurs in Poland. *Sommerstorffia spinosa* a predacious species catching rotifers occurs in soil and as aquatic epiphyte. This species was found to grow on baits in control containers with water from Lake Blizno. *S. spinosa* has firstly been described by Arnaudov (1923) in Bulgaria. Till nowadays it has been encountered in a few countries including northeastern Poland. In Poland, it has been found in spring, in polytrophic pond, in river Biała, in mesotrophic lake Wigry and oligomesotrophic lake Hańcza.

Conclusion

A total of 75 mycotal species, including 21 Fungi, 53 Straminipila and 1 Plasmodiophoromycota species were retrieved from all water samples. The largest number of mycotal species was found in water from eutrophic River Supraśl (38) and smallest in water from oligomesotrophic lake Hańcza (15). 14 species of the mycota were found to grow only in control containers, 36 species in containers with green sponges, and 25 species were isolated in both - controls and sponges containing containers. In containers with brown colonies (without symbiotic algae) only 9 species were found. Fewer mycotal species was recorded in control containers in comparison to containers including green sponges. The mean ratio of mycota found in water samples with green sponges versus those without sponges (control) (Sp/Co) ranged from 2.30 (*E. muelleri*) to 4.80 (*H. stepanowii*). The Sp/Co ratio in containers with brown colonies (without symbiotic zoochlorellae) of *S. lacustris* was 0.90. Green sponges revealed stimulatory and brown (without symbiotic algae) inhibitory effect (allelopathy) on growth of some mycotal species. The mean of photosynthetically fixed ^{14}C in symbiotic zoochlorellae cells reached 5.67 mg C g^{-1} dry weight sponge h^{-1} and extracellular production constituted 12.8% of cellular production. The dark fixation of carbon came to 3.94% of light fixation. The value of mycotal carbon assimilation amounted to 4.96 mg C g^{-1} dry weight mycelium h^{-1}. The extracellular products of symbiotic zoochlorellae of green sponges are being utilized by mycotal species as their nutriens.

Conflict of interests

The authors have not declared any conflict of interest.

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