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Review

Effect of trophic status in lakes on fungal species diversity and abundance

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ABSTRACT

The objective of this study was to determine the species diversity and abundance of fungi in relation to the hydrochemical conditions, with special emphasis on the trophic status and degree of pollution of lakes. The study was conducted in 14 lakes of the Augustów Lakeland (central Europe, NE Poland) with different hydrological conditions, type of stratification and trophic status. The analyses were performed in the hydrological year 2013. In the waters of the studied lakes, the mean abundance of fungi was 5600 ± 3600 CFU/mL. The minimum value (800 CFU/mL) was recorded for the mesotrophic Płaskie Lake, and the maximum value (14,000 CFU/mL) was recorded for the eutrophic Pobojno Lake. A total of 38 species of fungi were identified, including 11 belonging to the aquatic hyphomycetes; up to 14 species were potentially pathogenic fungi. The potentially pathogenic fungi, particularly *Candida albicans* and *Scopulariopsis fusca*, were found in lakes with increased concentrations of chloride and sulphate(VI) ions and may thus serve as indicators of the degree of water pollution. This paper illustrates that the species diversity and abundance of fungi in limnic waters depend on the concentration of organic matter, chlorophyll *a* concentration, and the degree of water pollution. The results suggest that aquatic fungi can be a valuable indicator of the degree of pollution and the sanitary quality of the water.

Contents

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1. Introduction

Aquatic fungi are a phylogenetically diverse group of organisms. Their occurrence has been recorded in almost all types of aquatic environments throughout the world. Aquatic fungi can be of autochthonic or allochthonous origin. Microfungi directly enter water through the surface runoff of soils or are of anthropogenic origin. The role of fungi in aquatic ecosystems mainly involves their participation in the decomposition of organic matter, particularly of plant origin (Krauss et al., 2011; Pascoal et al., 2005). Romani et al. (2006) suggest that fungi supply bacteria with the organic resources necessary for their survival and functioning, which they could not obtain by themselves. Due to the secretion of enzymes from the oxidase group, fungi are able to decompose phenol compounds which are less available to other microorganisms, such as humic substances, and various xenobiotics (Augustin et al., 2006; Baldrian, 2006; Jain et al., 2005; Junghanns et al., 2005; Steinberg, 2008). Moreover, fungi inhabiting water ecosystems actively participate in the synthesis of autochthonic humic substances (Damare and Raghukumar, 2008). The content of organic matter in water, which as suggest by Dunalska (2011) contributes to eutrophication can also considerably affect the species structure and abundance of mycoplankton. In addition, decomposition of organic matter may result in the release of phosphorus and nitrogen, which intensifies the eutrophication process and may result in algal blooms (Carlson, 1977).

Research the role of fungi in the waters have not been conducted for large-scale. Knowledge on the role of fungi in waters is fragmentary. After several years of intensive research on leaves decomposing in water, a fair amount is known concerning the taxonomic diversity of aquatic hyphomycetes (Krauss et al., 2011; Orłowska et al., 2009; Solé et al., 2008; Sridhar et al., 2001; Wurzbacher et al., 2010), commonly recognised as predominant in many water ecosystems. In addition, the astonishing species diversity of fungi belonging to other groups (often typical pathogens) has been demonstrated, but their ecology remains largely unknown (Nikolcheva and Bärlocher, 2005). It is estimated that only approximately 7 percent of the total number of species of aquatic fungi have been identified and described thus far. Analyses on the taxonomic diversity of aquatic hyphomycetes, yeast-like and zoosporic fungi in the waters of Poland and throughout the world have thus far been performed using microscopic methods (Cressa and Smith, 2007; Jobard et al., 2010; Orłowska et al., 2009). It is increasingly emphasised that only the application of molecular methods, such as the fingerprinting (restriction fragment length polymorphism—RFLP) of internal non-coding regions of rDNA (ITS fragments), which show high interspecific variability, permits the determination of the species diversity of fungi and their ecological function in various types of waters.

The objective of this study was to determine the species diversity and abundance of aquatic fungi in relation to the hydrochemical conditions, type of stratification, trophic status, and degree of pollution of the lakes of the Augustów Lakeland (central Europe, NE Poland). Monitoring in water ecosystems, particularly those used for economic or tourist purposes, which focuses on the abundance and species diversity of microfungi appears to be highly desirable.

2. Materials and methods

2.1. Study area

The study area covered a group of 14 natural lakes located in central Europe in the northeastern area of Poland, the Augustów Lakeland (Fig. 1). The monitoring of limnic waters was performed in the hydrological year 2013 during the occurrence of favourable meteorological conditions to facilitate reliable data collection. The studied lakes had varied hydrological conditions.

2.2. Methods of analysis

2.2.1. Collection of material for analysis

Water samples were collected four times in different hydrological seasons in January, May, August, and October of the hydrological year 2013 using a Limnos sampler from a depth of 0.5 m (epilimnion). Water temperature, conductivity (EC), oxygen saturation, dissolved oxygen concentration and pH were measured in the field using a HQ40D Hach Lange meter.

2.2.2. Water chemistry

Analysis of the physical and chemical water parameters was conducted immediately after sample collection, determining sulphate(VI), and chloride ion concentrations (APHA, 1992). Chlorophyll *a* concentration was determined according to Polish Standard (1986) (PN-86/C-05560/02), the total phosphorus (TP) and dissolved reactive phosphorus (DRP) concentrations were determined using the molybdenian spectrophotometric method (Standard methods for the examination of water and waste water, 1999). The concentration of dissolved organic carbon (DOC) was determined by the high temperature catalytic method of incineration in a TOC-5050A analyser (Shimadzu) and particulate organic carbon (POC) was determined by the chromate method (Bowman, 1998).

2.2.3. Fungal cultures

To estimate fungal abundance, 250 μL of unfiltered water, diluted to ratios of 1:10 and 1:100, were placed on Sabouraud agar plates enriched in chloramphenicol (0.5 g/L) and incubated for 5 days at either 37 °C, 25 °C or 5 °C. Each time, this analysis were conducted in 3 replicates. After incubation, the numbers of colonies and different morphotypes of fungal colonies were determined (Descals, 2007). Fungal abundance was expressed as CFU/mL.

2.2.4. DNA extraction

A representative colony of each morphotype was subcultured, and its DNA was isolated using Genomic Mini AX Yeast and Bead-Beat Micro Gravity DNA Isolation Kit according to the manufacturer's instructions. Three hundred and seven DNA samples were isolated, representing 38 morphotypes of fungi.

2.2.5. PCR procedure

Following Gaitanis et al. (2002) with modifications, the RFLP bands were used to identify the isolates. PCR reactions were performed in 0.2 mL Eppendorf tubes in a reaction mixture containing 2 μL of isolated DNA, 10 pmol of ITS1 primer (5′-TCCGTAGGTGAACCTGCGG-3′), 10 pmol of ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′) (Gupta et al., 2000), 11.75 μL of nuclease-free water (A&A Biotechnology, Poland), and 12.5 μL of PCR Master MixPlus (A&A Biotechnology, Poland). The PCR mixtures were first incubated for 3 min at 95 °C, followed by 40 cycles at 95 °C, 52 °C and 72 °C for 1 min at each temperature. The last cycle was performed for

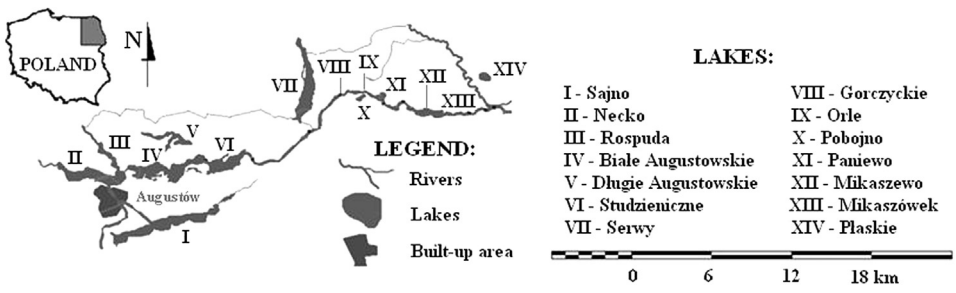

Fig. 1. Map of the distribution of the study sites in the Augustów Lakeland in Polish territory in the northern part of the Podlasie region.