

Massive occurrence of the alien invasive species *Pleodorina indica* (Volvocales, Chlorophyta) in a reservoir located in urban areas of Central Poland

by

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Abstract

Pleodorina indica (Iyengar) H. Nozaki is a rare species from the Volvocaceae family (Chlorophyta, Volvocales), which naturally occurs in aquatic ecosystems of the tropical climate zones. This is the first record of this species in Poland in a flow-through man-made reservoir on the Olechówka River in Łódź. The sampling site of *P. indica* in Łódź is the northernmost site in Europe. Among other records, the species has been also reported from Austria in 1996 and the Czech Republic in 2003. During the summer of 2015, abundant occurrence of this species was observed at the sampling site, at extremely high air and water temperatures. Physical parameters in the reservoir and the duration of the *P. indica* bloom were measured. In addition, morphological features of the species were observed with the use of light and confocal microscopy.

Key words: *Pleodorina*, phytoplankton, alien species, algal bloom, Chlorophyta

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Introduction

The *Pleodorina* Shaw 1894 genus belongs to the Volvocaceae family (Chlorophyta, Volvocales) (Coleman 2012) and includes six species: *P. californica* Shaw, *P. indica* (Iyengar) Nozaki, *P. japonica* Nozaki (Nozaki et al. 1989), *P. sphaerica* Iyengar, *P. thompsonii* F. D. Ott et al. and *P. starii* Nozaki et al. (Nozaki et al. 2006; Dembowska 2013). Among these species, *P. indica* is the only one with potentially expansive and invasive properties for Central Europe (Kaštovský 2010). Primarily, *P. indica* was described as an endemic species for the subtropical and tropical climate zones in India (Znachor, Jezberová 2005). Later, however, the species was also found in Central America (Mexico) and South America (Argentina) (Zalocar 1993). At the beginning of the 21st century, *P. indica* was identified in Central Europe (Austria, the Czech Republic). It occurs mostly in wetlands, slow-flowing rivers or stagnant water.

The first record of *P. indica* forming large-scale blooms comes from the Central European region, the downstream of the Malše River in the Czech Republic, August 2003. In Central Poland, the species was identified at the sampling site on the Olechówka River in the course of the research on the biodiversity of phytoplankton algae in urban eutrophic water ponds being under a strong human impact.

The aim of this study is to complete the ecological and morphological characteristics of the first record from Poland of a thermophilic, expansive species of the green algae – *Pleodorina indica* (Iyengar) Nozaki.

Study area

In 2015, a study of the biodiversity of planktonic algae in urban eutrophic water reservoirs under anthropogenic impact was conducted in the flow-through man-made reservoir located on the Olechówka River (Łódź, Central Poland, Central Europe).

The sampling site was located near Tomaszowska Street in Łódź (51°43'48.2"N; 19°31'47.1"E). The surface of this reservoir is 1 ha, a capacity of 17,500 m³ and an average depth of 1.75 m (Fig. 1). The Olechówka River is a tributary of the Jasień River and its course is limited to the city of Łódź. The Olechówka riverbed is open along the entire course with engineered banks. Shores of the reservoir at the sampling site were stabilized by concrete slabs (Diehl 1997).

The reservoir is located in the industrial area of the Łódź agglomeration with heavy traffic nearby, including heavy road transport. The number of farms and households is limited in the immediate vicinity



Figure 1

Location of the flow-through reservoir with *Pleodorina indica* (Iyengar) Nozaki

of the sampling site, while the nearest housing estates are located within a radius of 800 m. The sampling site is surrounded by lush vascular vegetation, shrubs and trees, which are a source of allochthonous matter in the reservoir. In order to maintain the continuous flow of the river, a hydro-technical installation was built with another reservoir in this particular area, upstream of the studied reservoir. However, its performance was limited during the study due to the low water level of the Olechówka River.

Materials and methods

Samples were collected in the summer of 2015 from 5 June to 16 September, with maximum weekly intervals. After the first observation of the bloom at the sampling site, samples were collected every 2-3 days. Samples were collected with the use of a plankton net into 125 ml plastic containers. Each time, physical parameters such as pH, conductivity (EC), water and air temperature were measured at the same time of day, between 10 and 11 A.M. All phytoplankton samples (N = 20) were collected and immediately transported to the laboratory of the Department of Algology and Mycology at the University of Łódź, in heat-insulating containers. Observations were conducted with the use of a Nikon Eclipse i50 light microscope and Leica SP-8 confocal microscope in cooperation with the Laboratory of Microscopy Imaging & Specialist Biological Techniques at the Faculty of Biology & Environmental Protection. A Canon D450 camera and a camera built-in in a confocal microscope (Leica SP-8) were used for the photographic documentation. Samples were then fixed with a mixture

of formaldehyde and 96% ethanol (ratio of mixture 1:1). The following publications were used for the taxonomic identification and analysis of variation in morphological features: Nozaki et al. 1989; Starmach 1989; Znachor, Jezberová 2005; Nozaki et al. 2006; Dembowska 2013.

Results

Abundant occurrence of a rare species of Volvocaceae (Chlorophyta) *Pleodorina indica* (Iyengar) Nozaki was recorded in the reservoir on the Olechówka River (Łódź, Central Poland).

Observations of water blooms caused by the abundant occurrence of *Pleodorina indica* in relation to hydro-meteorological conditions

The study of phytoplankton assemblages was conducted from 5 June to 16 September. From 3 to 7 July, there was a significant increase in air temperature by over 10°C, with the maximum air temperature reaching 33.2°C. The presence of *Pleodorina indica* colonies was observed for the first time in a sample collected on 7 July (air temperature = 32.9°C; water

temp. = 25.7°C; pH 8.2; EC = 295.7 $\mu\text{S cm}^{-1}$) (Fig. 2). The average percentage occurrence of *Pleodorina indica* in the sample was 95% (Fig. 3A). Mostly only mature colonies were observed in the samples. They were also found in the bloom formed at the same time on the surface of the studied reservoir. However, the bloom disappeared within the next 48 hours. There was a continuous rainfall on 8-10 July and the air temperature dropped to 15-20°C. In the sample collected on 10 July (air temp. 14.5°C; water temp. 17.5°C; pH 7.7; EC = 163.7 $\mu\text{S cm}^{-1}$) (Fig. 2), colonies of *P. indica* started to disintegrate, while the average percentage occurrence decreased to 5%. The regeneration of the *P. indica* population was observed in a sample from 13 July (air temp. 20.2°C; water temp. 20.3°C; pH 7.05; EC = 213.5 $\mu\text{S cm}^{-1}$) (Fig. 2) and the average percentage occurrence was 80%. Next, only single colonies were recorded in the samples from 19, 21 and 30 of July and 7, and 16 of August. At that time, there were significant variations in air temperature in the following days, with an amplitude above 10°C, from 32.1°C on 19 July 2015 to 19.3°C on 30 July 2015. *Pleodorina indica* colonies were no longer present in the samples collected after 17 August 2015.

Morphological characteristics of *Pleodorina indica* (Iyengar) Nozaki

Phylum: Chlorophyta Pascher, 1914

Class: Chlorophyceae Wille, 1884

Ordo: Volvocales Oltmanns, 1904

Family: Volvocaceae Ehrenberg, 1834

Genus: *Pleodorina* W.R. Shaw, 1894

Pleodorina indica (Iyengar) Nozaki 1989 = *Eudorina indica* Iyengar 1933

Pleodorina indica forms spherical or oval shaped colonies. The width of the observed colonies fluctuated within the range of 40-236 μm , while the length varied from 50 to 215 μm (Fig. 3, Fig. 4). The cells are spread radially on the edges of gelatinous colonies in the sets of 32, 64 or 128 cells, with two flagella in each cell, a stigma and contractile vacuoles. They also have a cup-shaped chloroplast with one or more pyrenoids. Somatic cells are located in front of the reproductive cells in the gelatinous matrix of the colony, gradually increasing their size (Fig. 4). Each cell of the cenobial colony is enclosed in individual sheaths (Fig. 4A). Peripheries of the colonies have

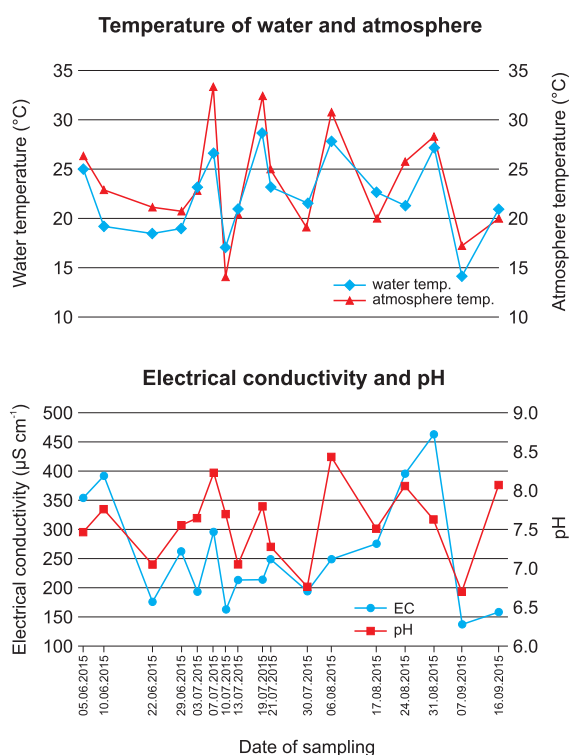


Figure 2

Air and water temperature, pH reaction and conductivity at the sampling site during the study

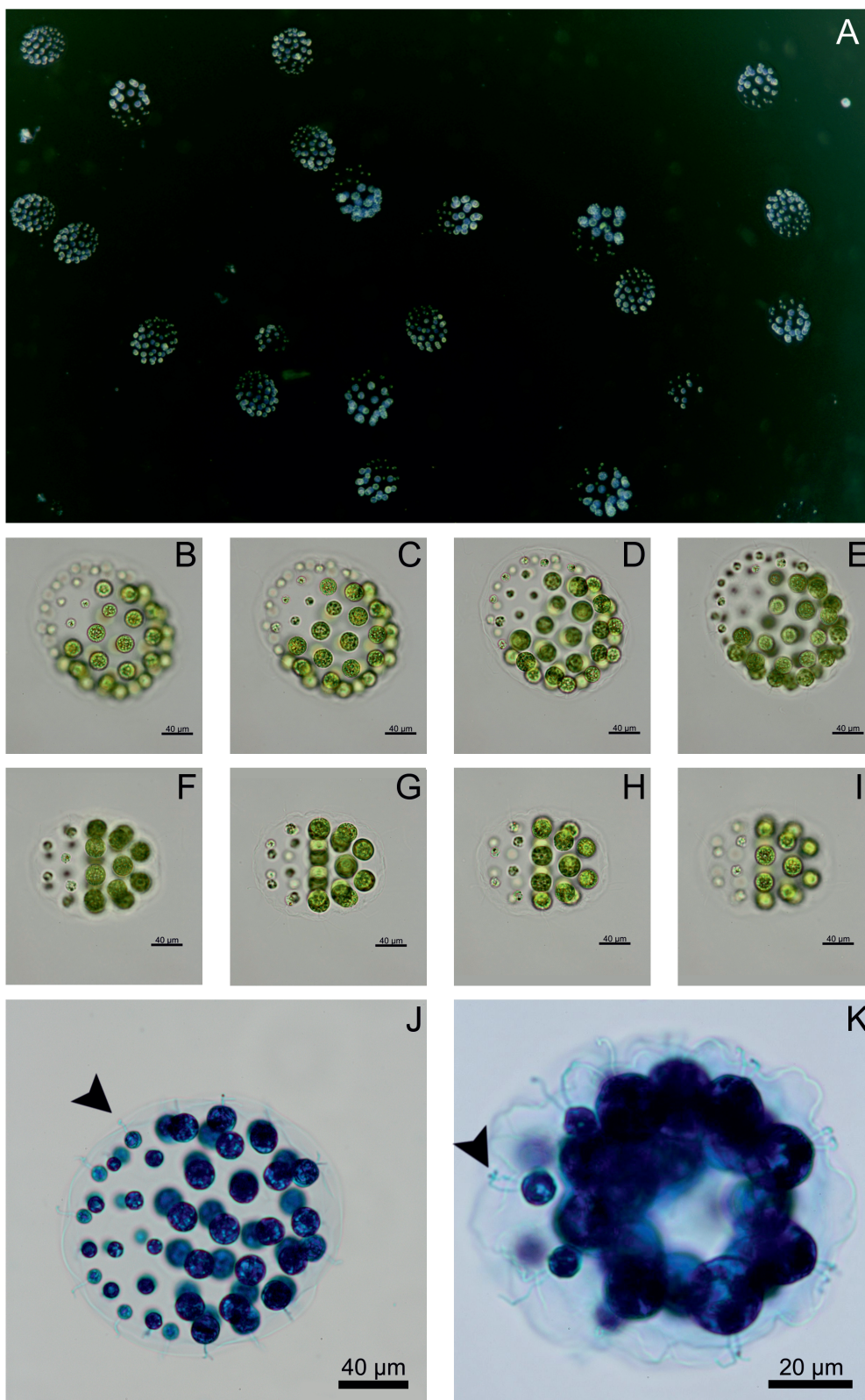
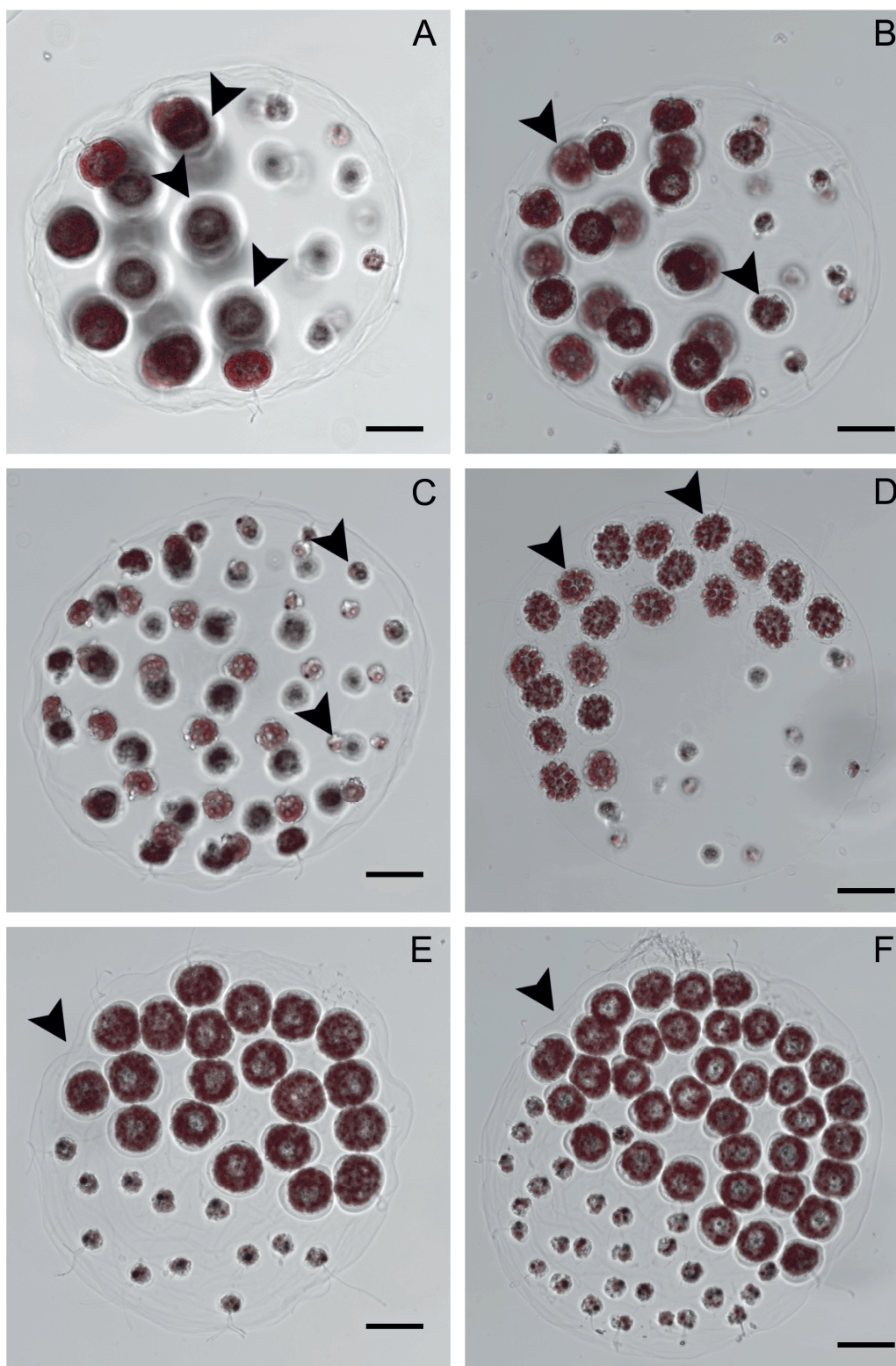


Figure 3

Pleodorina indica (Iyengar) H. Nozaki (A-K) Light microscope, Nikon Eclipse 50i

(A) Dark field view, monoculture colony, magnification 10x4; (B-I) magnification 10x40; (J, K) arrows indicate flagellum, colony stained with blue methylene, magnification 10x40, 10x100

**Figure 4**

Pleodorina indica (A-F) Confocal microscope, Leica SP-8. Scale bar 20 μm

(A) arrows indicate individual sheaths in the gelatinous matrix; (D, E) Surface of the 32-cells colony; (F) 64-cells colony; (B) arrows indicate vegetative cells; (C) arrows indicate somatic cells; (D) arrows indicate vegetative cells during division (asexual reproduction); (E, F) arrows indicate undulating peripheries of the colony

an undulating ending (Fig. 3J-K). The ongoing colony degradation observed under a light microscope resulted in an increase in the average length and width of the observed specimens and blunting of the undulating edges of the colony. However, in samples from 19 July to 17 August 2015, the occurrence of individual, morphologically well-developed colonies (shape and number of cells) was observed, even though they were smaller than the colonies in previous samples.

Discussion

This cosmopolitan genus, typical for freshwater ecosystems, currently includes 6 species, of which *P. californica* Shaw is the most common and most often recorded taxon (Dembowska 2013). The species was described for the first time in 1894 as *Eudorina californica*. Reports on the occurrence of *P. californica* come mainly from the United States of America where it is a rather common species (Dembowska 2013). The species was also reported from Asia, Africa and Canada (Smith 1916; Duthie, Socha 1976; Nozaki 2003; Coleman 2012), Australia, New Zealand and Venezuela (Day et al. 1995; Dembowska 2013). In Europe, it was recorded on the Iberian Peninsula, in Ukraine and Romania (Dembowska 2013).

The *Pleodorina* genus functioned in the taxonomic nomenclature as a synonym of the *Eudorina* Ehrenberg genus (Goldstein 1964). In 1992, Nozaki & Kuroiwa (1992) isolated a new genus from the Volvoceae family named *Pleodorina* and recent molecular phylogenetic studies have shown its polyphyletic nature. Moreover, the nature of the *Eudorina* genus was defined as paraphyletic with reference to the *Pleodorina*, *Volvox* and *Platydorina* genera (Nozaki et al. 2006). In Poland, the *Pleodorina* genus was recorded for the first time in the dam reservoir in Włocławek on 7 August 1998. In further studies, reports on the *Pleodorina* genus related mainly to the oxbows of the Vistula River in Toruń, during the low water level of the river. The species was identified as *Pleodorina californica* Shaw (Dembowska 2013, Fig. 6).

Pleodorina indica (Iyengar) Nozaki noted in the sample from 7 July 2015 in the reservoir in Łódź (Fig. 1) is morphologically consistent with the characteristics of this species given by Znachor, Jezberová (2005) and by Nozaki et al. (1989). *Pleodorina indica* was originally described as a species endemic to India, where it occurs only in the marshes in the province of Mysore and in the area of Madras (Ettl 1983). Recently, however, its occurrence was also confirmed in Mexico and Argentina (Nozaki et al. 1989;

Znachor 1993). In the sample from the algae collection from the University of Vienna (Austria, Central Europe), collected in 1996 from the Danube River near Gießgang Greifenstein (Lower Austria), the strain of *Pleodorina indica* (strain ASW05153) was found during phylogenetic studies of the isolates of the *Pleodorina* and *Eudorina* genus (Angeler et al. 1999; Coleman 2002; Znachor, Jezberová 2005). This was the first record of this species in Central Europe. The next record of *P. indica* from Europe comes from the Malše River, České Budějovice in the Czech Republic during exceptionally high air temperatures in summer. The species on the surface of the river created a massive bloom with a visible foam-like scum. This is the first confirmation of *P. indica* tendency to occur in the form of massive blooms in the temperate climate zones. One year later, the species was recorded for the second time in the same area, but its biomass was significantly reduced (Znachor, Jezberová 2005). In the course of further investigation in the Czech Republic, the presence of *P. indica* was confirmed at 10 other sites, e.g. at a sampling site 50 km downstream from its original location, in Hněkovice and in an eutrophic pond nearby, however, with no records of blooms.

The water temperature at the time when *P. indica* blooms occurred on the Malše River was higher by 2-4°C on average, the air temperature exceeded 30°C. The maximum air temperature recorded was 36.8°C on 13 August 2003. The pH values were also higher (Znachor, Jezberová 2005).

The thermophilic green alga *P. indica* was observed under similar hydro-meteorological conditions in the reservoir on the Olechówka River (Fig. 1). The average air temperature exceeded 30°C and the pH value before the blooms was alkaline until reaching the maximum value along with the occurrence of blooms on 7 July, i.e. pH 8.23 (Fig. 2). The average monthly temperature in the summer of 2015 was approximately 2°C higher than in the previous two decades (1995-2014) (Fig. 5). Conductivity showed significant fluctuations, from 140 to 460 in the reservoir with abundant presence of *P. indica*. At the early stage of the bloom, conductivity reached 295.7 $\mu\text{S cm}^{-1}$. The most likely factor causing the occurrence of *P. indica* and bloom formation is the air temperature.

The first record of *Pleodorina indica* in the central part of Poland may indicate expansion of this species to the north. Further investigation is needed to confirm the permanent occurrence of *P. indica* in the reservoir on the Olechówka River and to verify its tendency to form blooms.

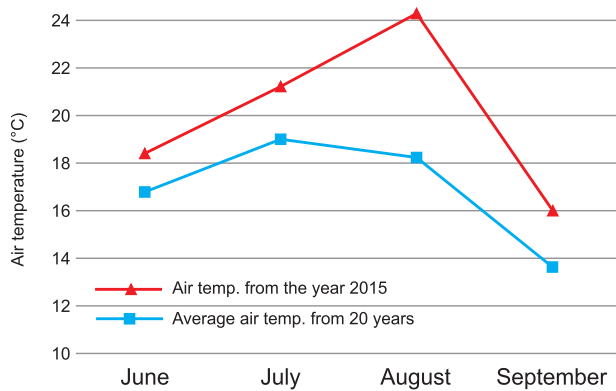


Figure 5

Average monthly temperatures from 1995-2014 in comparison with average monthly temperatures from 2015, in Łódź

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Authors' contributions

The following statement about the authors' contribution to the research has been made: research design, writing of the manuscript: JŻW; writing of the manuscript, experimental part, figures design: PK.

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