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Rapid monitoring of cyanobacteria in lakes – a case study in the Wel River catchment, Poland

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Abstract: The purpose of the study was to ascertain the effect of cyanobacterial abundance and its taxonomic structure on the results of measurements made by a fluorometric device designed to detect *in situ* chlorophyll *a* and phycocyanin. A multiparameter water quality probe was tested at 10 lakes located in the Wel River catchment. We found a strong correlation between the chlorophyll *a* concentration determined by laboratory procedure (CHL-*a*) and that obtained as a result of the probe measurements (YSI CHL-*a*) (R=0.78) as well as between the YSI CHL-*a* and the total phytoplankton biomass (R=0.73), whereas YSI CHL-*a* was not a good predictor of cyanobacterial biomass (R=0.24). The phycocyanin recorded by the probe was proportional to the total biomass of cyanobacteria (R=0.86); however, this cyanobacterial taxonomic structure influenced the fluorometric signal. Nevertheless, our study showed significant differentiation of phycocyanin measurement distribution at different levels of cyanobacterial abundance (<2 mg L^{-1}, between 2 and 10 mg L^{-1}, and >10 mg L^{-1}), which indicates that the PC-YSI measurements are valuable in the detection of increased risk of exceeding health alert thresholds recommended by the WHO.

Key words: cyanobacteria, phycocyanin, monitoring, fluorescence, YSI probe

Introduction

Cyanobacteria, floating freely in the water column, are usually a permanent constituent of phytoplankton in waters characterized by higher trophic level (e.g., Dokulil and Teubner 2000). Because of their sensitivity to increasing fertilization and temperature, extensive proliferation of cyanobacterial taxa in surface waters occurs and causes water quality problems all over the world. The mass development of cyanobacteria, known as cyanobacterial blooms, poses a major hazard to the environment and to human health, as their presence considerably influences other water organisms and the properties of water. In addition, what is particularly important, some of the most commonly occurring species have the ability to produce cyanotoxins (cytotoxins, neurotoxins, hepatotoxins and dermatotoxins) (Huisman et al. 2018). Therefore, observation of cyanobacteria appearance is a crucial element of different monitoring networks, i.e., monitoring for ecological status classification of waterbodies according to the Water Framework Directive (EC 2000), water sites of "Natura 2000" network of protected areas (Mróz 2012) and control procedures of bathing waters (MZ RP 2019).

Due to the constant escalation in the occurrence of cyanobacterial blooms, often including toxic species, public institutions responsible for maintaining good water quality and/or bathing sanitary conditions need monitoring tools that can signal a prior warning about the risk of potentially toxic prokaryotic algae appearance.

Currently, cyanotoxins are not routinely measured and the majority of assessments of health risk associated with cyanobacterial blooms in waterbodies is mainly based on the abundance of cyanobacteria, expressed more commonly as their biomass. The traditional method of monitoring freshwaters for phytoplankton, including cyanobacteria, involves the collection of water samples and laboratory analysis. In microscopic procedure, usu-

ally performed according to Utermöhl (Utermöhl 1958; Vollenweider 1969; CEN 2004; Brierley et al. 2007), samples are transferred to a settling chamber for counting under an inverted microscope. The biomass is estimated on the basis of phytoplankton number through the measurements of the species size, adaptation of each taxon to geometric shapes and the transformation to its biovolume. Next, biomass results can be applied to ecological status classification and/or to bloom risk assessment. This method allows the total phytoplankton biomass, the biomass and the percentage share of cyanobacteria and other phytoplankton groups to be estimated. It gives the detailed taxonomic structure of a pelagic algae assemblage. However, it is also quite a laborious and time-consuming method. A more convenient, quicker and less expertise-demanding procedure for planktic algae density estimation is the extraction of chlorophyll *a* (CHL-*a*), the photosynthetic pigment common in both eukaryotic and prokaryotic phytoplankton, using the spectrophotometric method (Nusch 1980). The CHL-*a* concentration in water samples carries information about the total phytoplankton density in water. However a serious weakness of both methods is a delay between sample collection and the analytical data becoming available for use.

A promising technique, becoming increasingly used for conducting advanced monitoring tests, is fluorometry. This technique allows the detection of the presence and concentration of cyanobacteria and several other groups of eukaryotic algae. It enables both *in situ* monitoring and online monitoring and real-time data collection. Laboratory spectrofluorometers make it possible to recognize the basic quantitative and qualitative structure of phytoplankton also in unfixed samples (Kring et al. 2014; Sobiechowska-Sasim 2014). Particularly popular are probes with a fluorometric sensor for measuring phycocyanin (PC) – a phycobiliprotein present in substantial amounts in cyanobacterial taxa beside CHL-*a* and carotenoids used to harvest light for photosynthesis (van den Hoek et al. 2002; Izydorczyk et al. 2005; Brient et al. 2008; Bastien et al. 2011). The ability of phycocyanin to fluoresce is the basis of all commercial fluorimeters for *in vivo* measurements. Such probes excite phycocyanin fluorescence by illuminating the sample with a light beam of a selected wavelength, and then measure the fluorescence excited at a greater wavelength (YSI Incorporated 2009).

This technique is a modern tool for immediate monitoring of water quality; measurement automation eliminates the need for tedious and time-consuming microscopic analyses, employing highly qualified staff, as well as conducting the extraction of pigments by traditional laboratory methods. However, as the sensors' producers indicate, there are some constraints that affect the data reliability obtained by *in situ* fluorometric measurements such as water temperature and turbidity or chlorophyll interference (YSI Incorporated 2009).

In our research we analyzed whether the *in situ* quantification of CHL-*a* and PC concentrations by fluorometry is useful for determining the abundance of total phytoplankton and cyanobacteria in the aspect of their rapid monitoring and detection of bloom risk and the influence of cyanobacterial taxonomic structure on the measurement results.

Study area and methods

Phytoplankton samples and samples used to determine the content of chlorophyll *a* and nutrients were taken once a month (from April to October) in 2009 from ten lakes with a surface area exceeding 50 ha located in the Wel River catchment (Fig. 1). Morphometric characteristics of the studied

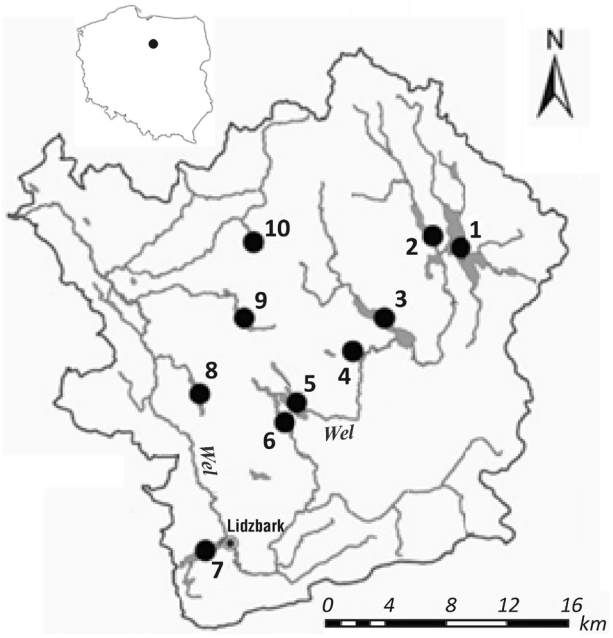

Fig. 1. Location of studied lakes: 1 – Dąbrowa Wielka, 2 – Dąbrowa Mała, 3 – Rumian, 4 – Zarybinek, 5 – Tarczyńskie, 6 – Grądy, 7 – Lidzbarskie, 8 – Kiełpińskie, 9 – Hartowieckie, 10 – Zwiniarz

lakes and water quality parameters are summarized in Table 1.

For each of the surveyed reservoirs with a smaller surface area and a barely complicated shape, one survey site located in the deepest place of the lake was selected. In larger reservoirs, the survey was carried out at two (Dąbrowa Mała and Rumian) or three (Dąbrowa Wielka and Grądy) sites in very distinct pools of these lakes. Altogether, samples were taken from 16 sites, including 112 samples for quantitative analysis, 112 for qualitative analysis and 112 for CHL-*a* and nutrient analysis.

In stratified lakes, water for quantitative phytoplankton tests was taken at 1m depth intervals with the Bernatowicz sampler (Bernatowicz 1953) from the euphotic layer in the spring and autumn circulation periods and from the epilimnion in the summer stagnation period. In polymictic lakes, water for analyses was taken from the surface to the bottom. The samples were fixed using the buffered Lugol's solution directly in the field (Utermöhl 1958) and next preserved by adding 1 mL of formalin per 100 ml of the sample. Qualitative phytoplankton samples were taken using a plankton net No. 25 with 55-60 µm mesh, from the same water layers as those for quantitative samples. The samples for the determination of the concentration of chlorophyll *a* and nutrients were taken on the same dates and at the same stations as the phytoplankton samples.

Simultaneously, Secchi disc visibility (SD) was measured and field measurements such as water temperature, pH, conductivity and oxygen concentration were made using a multi-parametric probe YSI 6131 (Yellow Springs Instruments, Ohio, USA). The chemical analyses of total phosphorus (TP) and nitrogen (TN) content were carried out in the laboratory using the standard methods (TP – spectrophotometric method after mineralization before determination – PN-EN ISO 6878:2006 standard; TN – $N_{Kjeldahl}$ + $N\text{-}NO_3$ – PN-EN25663:1, PN-82/C-04576.08). A water quality sonde 6131 YSI, fitted with probes for the detection of CHL-*a* (expressed in µg L^{-1}) and PC by in-situ fluorometry recorded as RFU (Relative Fluorescence Units) was

Table 1. Morphological characteristics, physicochemical and biological parameters of the studied lakes (mean values of 2009)

Parameter	Lake									
	Dąbrowa Wielka	Dąbrowa Mała	Rumian	Zarybi-nek	Tarczyńskie	Grądy	Lidzbar-skie	Kiełpiń-skie	Hartowieckie	Zwiniarz
Area [ha]	615.1	173.4	305.8	73.8	163.8	112.7	121.8	60.8	69.6	50.0
Max. depth [m]	34.7	34.5	14.4	7.0	9.2	9.1	25.5	11.0	5.2	5.8
Mean depth [m]	8.2	10.0	6.5	2.4	3.8	4.7	10.1	6.1	2.9	3.0
pH[1]	8.43	8.43	8.28	8.46	8.47	8.46	8.29	8.41	8.36	8.85
Conductivity[1] [µS cm^{-1}]	260	277	310	297	300	294	316	292	284	219
TP[1] [µg L^{-1}]	48.48	53.64	80.21	77.57	87.57	84.14	72.43	58.50	68.86	112.20
TN[1] [mg L^{-1}]	1.03	1.01	1.09	1.12	1.28	1.29	1.47	0.88	1.14	1.35
Secchi Disc[1] [m]	2.7	1.7	1.7	1.1	0.8	0.8	1.4	3.4	1.2	0.9
Chlorophyll *a*[2] [µg L^{-1}]	11.37	19.91	25.51	35.54	55.33	56.73	27.93	6.76	26.74	42.72
Chlorophyll *a** [µg L^{-1}]	9.30	11.06	10.74	16.31	29.86	28.92	17.67	3.70	14.78	23.04
Total biomass[2] [mg L^{-1}]	6.4	9.9	12.9	14.6	23.8	21.2	15.5	2.3	12.8	27.5
Biomass of cyanobacteria[2] [mg L^{-1}]	1.5	2.9	5.2	6.3	7.3	7.3	3.5	0.3	5.2	16.0
Relative Fluorescence Units (RFU)*	1.24	2.14	4.18	4.85	5.12	5.54	3.43	0.49	3.65	15.30

[1]results of laboratory analysis according to Soszka and Ochocka (2011), [2] according to Hutorowicz et al. (2011), *results of YSI probe measurements

used for measures of algae pigments at the same time as the sampling was done and in the integrated water sample taken for laboratory analysis. All fluorometric measures were made in overshadow to avoid the influence of light on optical sensors and the final measurement results.

The microscopic analysis of phytoplankton density and biomass was done following the Utermöhl method (1958). For counting, the samples were transferred to a settling chamber (2.5 or 5 ml capacities were used, depending on algal density), and at least 100 individuals of the most numerous algae were counted per sample. Biovolumes were determined according to Kawecka and Eloranta (1994). The results of phytoplankton analysis as well as physicochemical parameters were partly published in Napiórkowska-Krzebietke et al. (2009, 2012), Hutorowicz et al. (2011, 2016); Soszka and Ochocka (2011).

In this study the applicability of PC-sensor measurements as an early warning system was assessed using as reference values the limits for biomass of cyanobacteria in recreational waters derived from the recommendations of the WHO (2003) by Carvalho et al. (2013). These border values are defined for three health alert categories: low – with a threshold of 2 μg L^{-1} (short-time adverse health outcomes), moderate – 10 μg L^{-1} (potential for long-term illness) and high – when scum formation appeared (potential for acute poisoning) (Carvalho et al. 2013).

Statistical analyses were performed with the StatSoft Statistica 10.0 PL for Windows. The Spearman rank correlation coefficient was used in order to determine the relationships between chlorophyll *a* concentration determined by traditional laboratory procedure and obtained as a result of the CHL-*a* sensor (YSI probe) measurements as well as between phycocyanin RFU values (YSI probe) and the total biomass of cyanobacteria. For statistical purposes, the values of chlorophyll *a* and biomass were log-transformed to reduce the skewed distribution. To receive positive values, the following formula was used: x = Ln (x+1). The linear multiple regression analysis was used for the comparison of the relationship between RFU taken as a dependent variable and the total biomass of cyanobacteria regarding the main cyanobacterial orders domination (*Chroococcales*, *Oscillatoriales* and *Nostocales*) as independent variables. The non-parametric Mann-Whitney U test was applied to show the statistical differences between the RFU value at different levels of cyanobacterial biomass categories.

Results

The Wel River basin lakes are characterized by varying degrees of eutrophication expressed by nutrients and phytoplankton parameters (Table 1). Nevertheless, cyanobacterial taxa appeared in all water samples, and in about half of them, there was at least a 30% share of cyanobacteria. The lowest biomass values (below 0.05 mg L^{-1}) were noted in spring samples of lakes: Kiełpińskie, Dąbrowa Wielka and Rumian and extremely high summer biomass occurred in Lake Zwiniarz (31.6 and 43.6 mg L^{-1} in July and August, respectively). The mean biomass of cyanobacteria ranged from 0.3 mg L^{-1} in Lake Kiełpińskie to 16.0 mg L^{-1} in Lake Zwiniarz (Table 1).

Considering the taxonomic structure of cyanobacteria communites in the studied lakes, the most abundant were taxa: *Microcystis aeruginosa* (Kützing) Kützing, *M. wesenbergii* (Komárek) Komárek ex Komárek, *M. smithii* Komárek & Anagnostidis, *Aphanocapsa incerta* (Lemmermann) G.Cronberg & Komárek and *Aphanothece* spp. (from Chroococcles order); *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek, *Limnothrix redekei* (Goor) Meffert, *Pseudanabaena* spp. and *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg (from Oscillatoriales); *Aphanizomenon gracile* Lemmermann, *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, *Dolichospermum flosaquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek and *Gloeotrichia echinulata* P.G.Richter (from Nostocales).

In this study we observed a strong correlation between the CHL-*a* concentration determined by traditional laboratory procedure and obtained as a result of chlorophyll sensor (YSI CHL-*a*) measurements (Fig. 2). The Spearman rank correlation coefficient was 0.78 ($N=112$, $p<0.001$). The comparison of the trend of changes in chlorophyll *a* results measured with the YSI probe along with the increasing concentration gradient of chlorophyll *a* measured in the laboratory indicates similar values of both measurements in the low range of concentration, not exceeding approx. 20 μg L^{-1} (log-transformed value

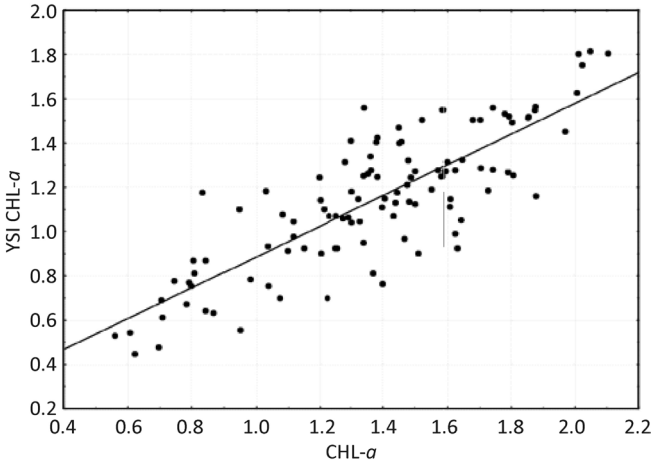

Fig. 2. Relationship between chlorophyll *a* concentration determined by traditional laboratory procedure (CHL-*a*) and obtained as a result of chlorophyll sensor (YSI CHL-*a*) measurements (all data log-transformed)

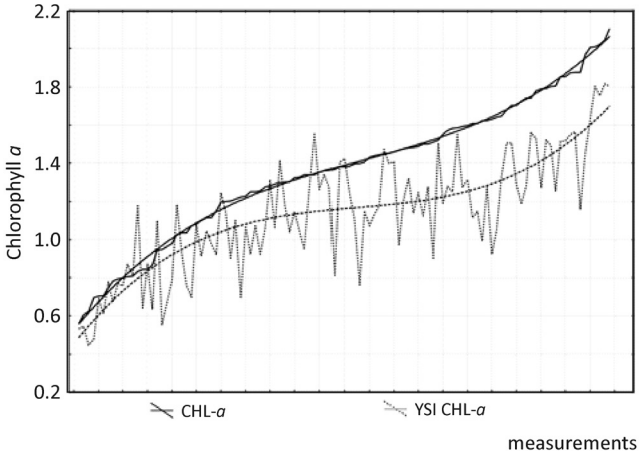

Fig. 3. The third degree polynomial course of variability of chlorophyll *a* measurements carried out with the YSI probe (YSI CHL-*a*) in relation to the values determined by the traditional method (CHL-*a*). Data-set with increasing concentration (all data log-transformed)

= 1.3) (Fig. 3). Generally, chlorophyll-*a* concentrations registered by the YSI probe were usually lower than those estimated in the laboratory. In some cases, however, higher chlorophyll *a* results were noted by YSI probe than determined in the same samples by traditional techniques, and the scatter of YSI CHL-*a* results at a similar range of chlorophyll *a* concentration was considerable. A good relationship of the YSI CHL-*a* and the total phytoplankton biomass was observed ($R=0.73$, $p<0.001$, $N=112$). However, the YSI CHL-*a* was not a good predictor of cyanobacterial biomass and the Spearman rank correlation coefficient of these parameters was significant but weak, 0.24 ($N=112$, $p<0.009$).

The PC concentration recorded as RFU in the analyzed lakes ranged from 0.00 to 43.00. No signal at the lowest values of cyanobacterial biomass (0.01-0.41) appeared in nine spring samples. The extreme values (37.4 and 43.00 RFU, in Zwiniarz Lake in September and August, respectively) were noted when the oscillatorian taxon – *Planktothrix agardhii* – constituted the majority of cyanobacterial biomass. The amount of phycocyanin recorded by the YSI probe was proportional to the total biomass of cyanobacteria and had a relatively strong relationship with the Spearman rank correlation coefficient equal to 0.86 ($N=112$, $p<0.001$). A factor likely to influence measurement was the taxonomical structure of a cyanobacteria group. The regression analysis of samples divided them into three groups based on the main cyanobacterial order domination (*Chroococcales*, *Oscillatoriales* and *Nostocales*) with an assumed domination level of at least 50% of the total cyanobacterial biomass or – in some cases – near it shows that the course of the relationship between the biomass of cyanobacteria (estimated by microscopic analysis) and PC-RFU is always significant ($p<0.001$) but differs in terms of slopes. The slope of regression lines between PC-RFU and biomass of cyanobacteria was stronger in samples dominated by *Oscillatoriales* than in those dominated by *Chroococcales* and *Nostocales* species. As a result, in the same biomass of cyanobacteria, the measurements of phycocyanin can differ considerably depending on the quality structure of the cyanobacterial community (Fig. 4). The predicted RFU value at the boundary of cyanobacteria biomass indicating an alert condition, i.e., 10 mg L^{-1}, is 3.75 RFU in situations when Nostocales species predominate in water, and 7.93 and 9.41 in *Chroococcales* and *Oscillatoriales* domination, respectively (Fig. 4).

The non-parametric Mann-Whitney U test was effective in distinguishing between cyanobacterial biomass categories determined on the basis of adverse health risk thresholds (Fig. 5). The distribution of phycocyanin measurement results and their means at different levels of cyanobacterial abundance (below 2 mg L^{-1}, between 2 and 10 mg L^{-1}, and above 10 mg L^{-1}) differed significantly. The analysis of data shows that the recorded RFU was generally very low (median value = 0.9) when cyanobacterial biomass did not pass 2 mg L^{-1} with only

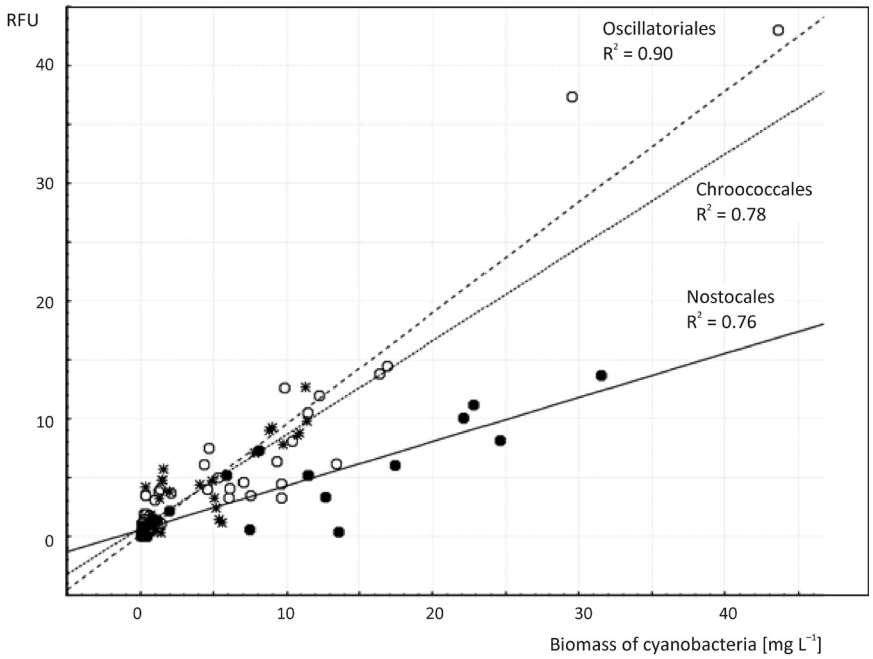

Fig. 4. The relationship between the biomass of cyanobacteria (mg L^{-1}) and phycocyanin measurement results (expressed as RFU – Relative Fluorescence Units) in three groups of samples dominated by taxa from different orders: *Oscillatoriales* (N=55), *Chroococcales* (N=40) or *Nostocales* (N=17)

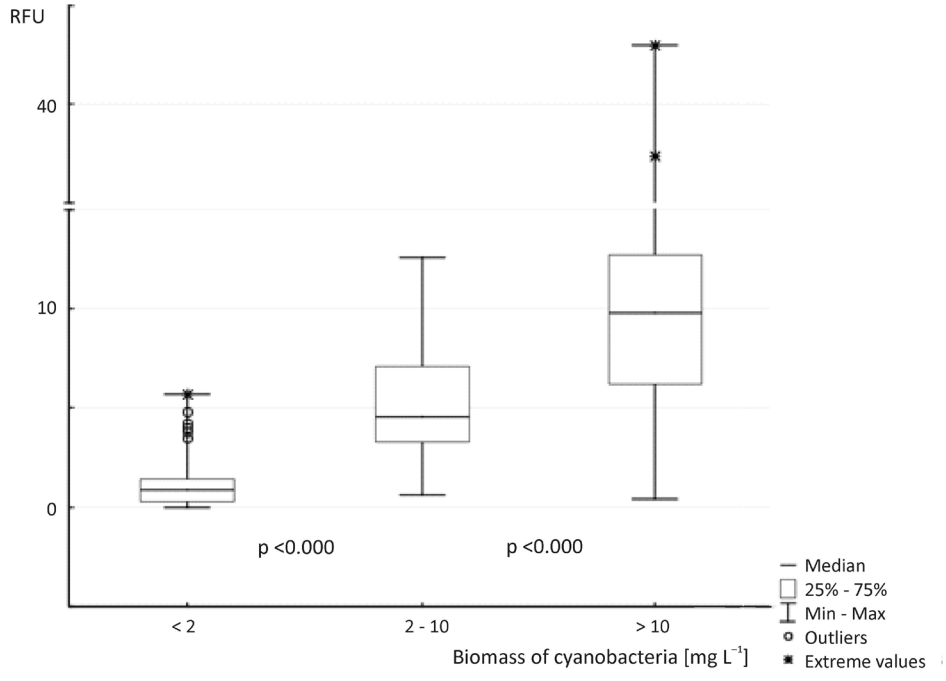

Fig. 5. Distribution of phycocyanin measurement results (expressed as RFU – Relative Fluorescence Units) in samples characterized by three different levels of cyanobacterial biomass: <2 mg/L (N=65, below low health risk threshold) between 2 and 10 mg L^{-1} (N=26, between low and moderate health risk thresholds) and >10 mg L^{-1} (N=21, above moderate health risk threshold). The level of confidence in comparison with the distribution of phycocyanin between subsequent groups was obtained in the Mann-Whitney U test

several outliers reaching more than 3 RFU. PC-RFU median values together with lower and upper percentiles drastically increased with increasing biomass of cyanobacteria. The phycocyanin concentration value near 5 RFU corresponds to the median of cyanobacterial abundance between 2 and 10 mg L^{-1}.

Discussion

The results of trials of the YSI probe fitted with sensors for the detection of chlorophyll *a* and phycocyanin concentrations by *in situ* fluorometry in the lakes located in the Wel River catchment show that this technique has considerable usefulness for rapid monitoring of cyanobacteria in lakes. A strong correlation was noted between chlorophyll *a* values measured with the YSI probe and the concentration of this pigment estimated in the laboratory as well as between phycocyanin expressed as RFU values and biomass of cyanobacteria. These results are consistent with reports by other authors: a strong relationship between the YSI CHL-*a* and chlorophyll *a* concentration estimated with the traditional method as well as between the YSI CHL-*a* and total phytoplankton biomass has been described elsewhere (e.g., Bowling et al. 2016). These authors pointed to the lack of a relationship of the YSI CHL-*a* and cyanobacterial biomass, which was also observed in our study, and results from additional contributions from eukaryotic algae are also present during the *in situ* survey. Nevertheless, the fluorometric measurement of chlorophyll *a* remains a good indicator of the density of cyanobacteria in the case of their domination in phytoplankton.

A significant correlation between phycocyanin concentration and cyanobacteria biomass has been found, among others by Izydorczyk et al. (2005), Brient et al. (2008), Bastien et al. (2011), Bowling et al. (2016) and Hodges et. al. (2018).

It should be noted, however, that the fluorometric signal may be disturbed by several environmental conditions, such as light, water turbidity and distance from the probe to the recipient boundary (e.g. sediment surface or bottom of bottles) (Brient et al. 2008). The detection of cyanobacterial biomass by fluorometry is not effective in very turbid water above 50 NTU (NSW Office of Water, 2012), and during a vertical profile in a reservoir, the reduction in the signal may be due to the absorption of particles rather than induced by the decreased number of cyanobacterial cells (Brient et al. 2008). The content of phycocyanin in a cell depends on the cell morphology and growth phase. It also depends on previous lighting conditions (before the measurement). Phycocyanin content in cyanobacteria cells is higher if the light conditions are worse. There are reports that during periods of intense exposure, cyanobacteria floating on the surface produce less photosynthetic pigments (Izydorczyk 2005). However, such a phenomenon was not found during experimental studies done by Brient and coauthors (2008). The content of phycocyanin in water may also be affected by the concentration of nutrients, primarily nitrogen as a component of proteins. Hence, low nitrogen concentrations may contribute to the degradation of phycocyanin in cyanobacterial cells (Izydorczyk 2005).

Despite the significant positive correlation between phycocyanin measured with the YSI 6131 probe and total cyanobacterial biomass found in our study, there was great variability in the PC-RFU results obtained at the same level of cyanobacterial density estimated by microscopic analysis. It can be assumed that the taxonomic composition of the cyanobacteria community influences the fluorometric signal. This could be associated with different factors arising from the taxa's morphometry, growth stage and/or physiology of the cells (Gregor et al. 2007). As the producer of the sensor YSI 6131 reports, if cyanobacteria forms colonies that are visible to the naked eye, the sensitivity of the sensor that reacts to the fluorescence of individual cells is reduced because it was designed to detect free-floating microscopic cells rather than floating macromolecules. For this reason, underestimation of cyanobacteria in clump-forming colonies is possible (YSI Incorporated 2009). Other factors suggested in literature come from the methodology of phytoplankton counting and biovolume estimations (e.g., Izydorczyk et al. 2009).

In spite of some restrictions, the use of a probe with fluorometric phycocyanin and chlorophyll sensors allows for the rapid location of increased phytoplankton and cyanobacteria biomass. This can be exploited in monitoring in situations when advanced microscopic laboratory analyses are not crucial, too expensive and/or prolonged. The presented analysis indicates the usefulness of PC-YSI measurements in the detection of increased risk of

exceeding health alert thresholds recommended by the WHO (2003). It seems that based on phycocyanin concentration distribution at different levels of cyanobacterial density, a PC value of about 5 RFU is a signal of increased risk of adverse health effects. This value pointed to cyanobacterial presence in the density range from 2 to 10 mg L^{-1} (above the low alert level), and thus, the risk of negative health effects (e.g., skin irritations or gastrointestinal diseases) (WHO 2003). At a similar level of PC-RFU the probability of greater biomass of cyanobacteria, exceeding 10 mg L^{-1} (the moderate alert level), occurs, which can be associated with serious long-term health problems.

The current bathing regulation only provides for the visual assessment of cyanobacterial blooms (observations of streaks, scums of foam on the water) (MZ RP 2019). This seems to be insufficient since a negative health outcome is possible even if the above-mentioned phenomena are not yet noted. The use of fluorometry devices with phycocyanin sensors could offer earlier detection of forming water cyanobacterial blooms and thus better protection of public health in bathing and recreational waters.

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