Cyanobacteria in Baltic waters – preliminary analysis of the possibilities of using different methods to determine their biomass

Introduction

Cyanobacteria (cyanobacteria) found in the marine environment are a taxonomically diverse group of algae. They occur throughout the year as single cells, cenobia, colonies, and filaments. They are considered prokaryotic organisms involved in photosynthesis, and the organization of their intracellular structures exhibits characteristics of both plants and bacteria. The antenna system in the photosynthetic apparatus of cyanobacteria, in addition to carotenoids, also contains complexes of various biliproteins, so-called phycobilisomes, located in the thylakoid membrane (e.g., MacColl 1998). The most common phycobilins are phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (AP), and their properties allow them to absorb light in the band from 450 to 660 nm (e.g., Grossman et al. 1993). Among the carotenoids, zeaxanthin is widely recognized as a biomarker for this group of algae (Jeffrey and Vesk 1997, Roy et al. 2011), while other carotenoids, such as echinenone, myxoxanthophyll, aphanizophyll, and canthaxanthin, have been reported in cyanobacterial cells in amounts not exceeding 10% of the total content of other cellular components. The presence of various types of pigments, the concentration of which varies and depends on environmental conditions, is the main factor influencing the visible light absorption spectra of phytoplankton communities.

In recent decades, an increase in the productivity and occurrence of potentially toxic cyanobacterial blooms has been observed in the coastal and open waters of the Baltic Sea and some Polish Pomeranian lakes (e.g., Pliński et al. 1998). Massive cyanobacterial blooms appear almost every summer, covering an area up to 100,000 km^2 (e.g., Kahru et al. 1994). *Nodularia spumigena*, *Aphanizomenon flos-aquae*, and some species of the genus *Anabaena* are the dominant cyanobacteria found in the Gulf of Gdańsk (Pliński et al. 2007), while *Microcystis* spp. are by far the most common cyanobacteria in freshwater bodies (Kobos et al. 2005, 2013).

Picocyanobacteria such as *Synechococcus* spp. or *Synechocystis* spp. also play an important role during blooms (Mazur-Marzec et al. 2013). Their biomass can constitute up to 80% of the total algal biomass and they can be responsible for up to 50% of the primary production in a water body (e.g., Stal et al. 2003). In addition to these dominant cyanobacterial species, other organisms such as *Pseudanabaena* spp. are also present in the Baltic Sea and freshwater cyanobacterial communities (Stal et al. 2003, Mazur-Marzec et al. 2013).

Due to their ability to produce biologically active compounds, both toxins and potential pharmaceuticals, Baltic cyanobacteria have become an interesting subject of research in recent years (Mazur-Marzec et al. 2015). Determining the biomass of these organisms is an extremely important issue, not only because of the productivity of a given water body or the amount of solar energy absorbed by these organisms, but also because of their toxic properties.

The classic method for determining the biomass of individual algal groups, including cyanobacteria, is microscopic analysis of the phytoplankton species composition occurring in a given region and season. This method is extremely time-consuming and requires highly specialized qualifications for taxonomic assessment of the organisms.

Another method for estimating the biomass of a given phytoplankton group is to use an identified set of indicator pigments characteristic for a given class of algae. This approximation method, which utilizes the optical properties of active cell components, has been used in oceanographic studies (Roy et al. 2011; Sobiechowska et al. 2010; Ston-Egiert et al. 2010). Pigments, naturally occurring in cellular structures in the form of pigment-protein complexes, are isolated from algal cells using organic solvents (acetone, ethanol, methanol) or other media and mechanical disruption of the cell walls. The concentration of a given indicator pigment is determined by chromatographic separation of a mixture of colored compounds or by measuring their fluorescence intensity, followed by appropriate quantitative analysis based on previous calibrations of the systems used. The use of this method gives good results in determining the total phytoplankton biomass depending on the concentration of chlorophyll a, or the biomass of the appropriate phytoplankton group forming the bloom, i.e. constituting over 90% of the total phytoplankton biomass, depending on the concentration of the appropriate indicator carotenoid. It can then be assumed that the concentration of a given indicator pigment is related solely to the presence of a specific class of algae (Latelier et al. 1993, Andersson et al. 1996, Peeken 1997, Mackey et al. 1996, Stoń-Egiert et al. 2010). In the case of phytocoenoses composed of mixed groups of algae, the situation is more complicated, as the concentration of the pigment-marker characteristic of a given class does not always correspond to the class. Pigments, as is well known, occur in varying qualitative and quantitative proportions in the cells of organisms belonging to a given class, but they also occur in the cells of algae belonging to different classes. Furthermore, the pigment content in cells can be modified by various stress factors. Therefore, estimating the biomass of different phytoplankton groups based on the concentrations of the respective pigments is subject to certain inaccuracies.

Another method used to determine the biomass of algal groups is based on the ability of phytoplankton to fluoresce, i.e., to emit radiation by excited particles of appropriate pigments contained in cells (Ostrowska et al. 2015, Houliez et al. 2017). This property is used in measuring devices that allow the identification of appropriately defined algal groups directly in the marine environment based on characteristic fluorescence excitation spectra. In situ, in vivo fluorometry (IVF) is considered a valuable tool for obtaining very rapid information and a large amount of data on spatial and temporal changes in phytoplankton, enabling the detection of cyanobacteria in various aquatic ecosystems, especially in coastal zones (Seppälä 2009). Typically, these probes utilize the fluorescence wavelengths of characteristic extracted pigments, although in many instruments of this type the wavelength of light used to excite the fluorescence may not be optimal for phytoplankton communities in which cyanobacteria are an important component (Kromkamp and Forster, 2003, Raateoja et al., 2004b, Simis et al., 2012).

Accurately determining the biomass of cyanobacteria is crucial for monitoring purposes, especially during the summer. We will present a comparison of various (including remote) methods for determining the biomass of this group of algae. For this purpose, we will utilize the empirical database of the Institute of Oceanology of the Polish Academy of Sciences, which contains long-term measurements of the physicochemical properties of cyanobacteria, the concentrations of colored compounds contained in their cells, and the results of microscopic determinations.

Empirical material and marking methodology

The empirical material used to achieve this objective covered the years 2010–2015. During this period, during cruises in various regions of the Baltic Sea in different months (see Fig. 1 and Table 1), seawater samples were collected from the surface and additional depths for appropriate laboratory analysis, and in situ measurements of various characteristics of the marine environment were performed. The cruises were carried out on the r/v 'Oceania' and 'Oceanograf' (in the summers of 2011, 2012, and 2013 in the Gulf of Gdańsk).

The following empirical data were collected: - results of phytoplankton pigment concentrations determined using RP-HPLC chromatography and spectrofluorimetry in water samples, - results of phytoplankton taxonomic composition and biomass determinations based on microscopic measurements, - results of concentrations of relevant algal groups determined based on in situ fluorescence measurements performed with a FluoroProbe bbe fluorometer (Moldaenke).

Table 1. List of months in the individual measurement years 2010–2015, in which empirical material was collected in various regions of the Baltic Sea. Designations: ob. – open Baltic waters, zg – Gulf of Gdańsk, zp – Pomeranian Bay, zs – Szczecin Lagoon.

	2010				2011				2012				2013				2014				2015			
	ob.	zg	zp	zs	ob.	zg	zp	zs	ob.	zg	zp	zs	ob.	zg	zp	zs	ob.	zg	zp	zs	ob.	zg	zp	zs
styczeń																								
luty										x							x	x	x	x				
marzec	x	x			x	x	x		x	x			x	x			x				x	x	x	x
kwiecień					x	x			x	x							x	x			x	x		
maj	x	x			x	x			x	x			x	x			x	x			x	x		
czerwiec						x				x				x				x						
lipiec						x				x				x				x						
sierpień						x				x				x				x						
wrzesień					x	x			x	x			x	x	x	x	x	x			x	x		
październik					x	x			x	x				x										
listopad													x	x			x	x				x		
grudzień																								

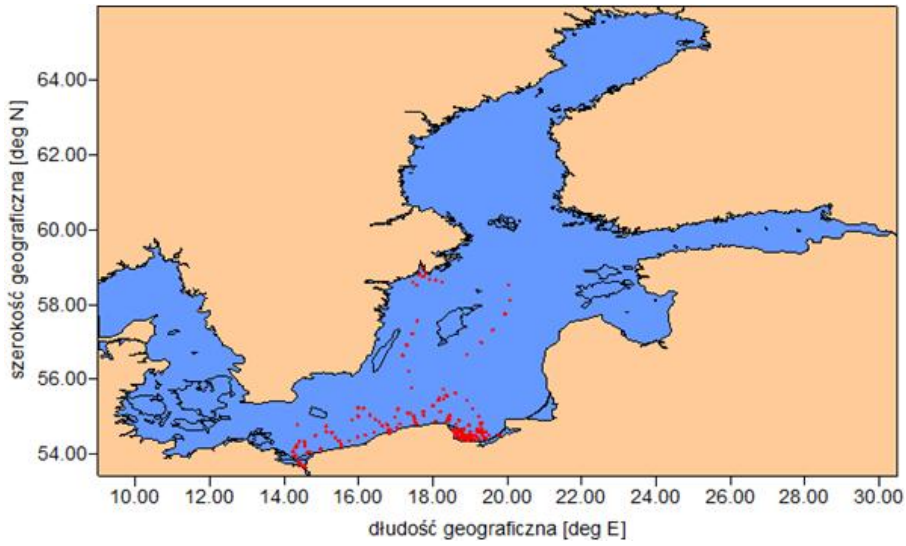

Fig. 1. Location of measurement stations in 2010-2015.

Description of the measurement methodology

- methodology for marking chlorophyll and carotenoid concentrations using the chromatographic method

Pigments were isolated from phytoplankton cells using 90% acetone (Strickland and Parsons 1972, Parsons et al. 1984) and then separated from the mixture using high-performance liquid chromatography (HPLC) using appropriate procedures and system configurations described in (Stoń and Kosakowska 2002; Stoń-Egiert and Kosakowska 2005). Calibration of the chromatographic system was performed based on commercially available chlorophyll and carotenoid standards (The International Agency for 14C Determination, DHI Institute for Water and Environment, Denmark). Qualitative and quantitative identification of the corresponding chlorophylls and carotenoids was performed based on the calibration curves obtained for each pigment.

- methodology for marking phycobiliprotein concentrations using the spectrofluorimetric method

Phycobilin pigments, i.e., phycocyanin and phycoerythrin, were extracted using an appropriate extraction medium consisting of 0.25 M Trizma Base, hydrated with 10 mM disodium EDTA (2H2O), and 2 mg cm−3 lysozyme, at pH 5.5 (Steward and Farmer 1984). The qualitative and quantitative characterization of phycocyanin and phycoerythrin in the analyzed samples was based on the results of spectrofluorimetric measurements (Cary Eclipse, Varian, Agilent Technologies) of previously prepared extracts of these compounds. Instrument calibration and determination of calibration coefficients were performed using commercially available phycobilin standards (ProZyme Inc., USA). A detailed description of the methodological procedures is provided in Sobiechowska-Sasim et al. (2014).

- methodology for microscopic marking of the species composition of phytoplankton and determining the biomass of individual classes of algae

Microscopic determination of phytoplankton species composition was performed on appropriately preserved (aqueous potassium iodide solution) and stored seawater samples. Qualitative and quantitative analysis of phytoplankton in the samples was performed using the Utermohl sedimentation chamber technique (Willen 1962) and an inverted microscope (Axiovert 35, Carl Zeiss, Germany). Phytoplankton cell counts were performed based on the procedure outlined in the Manual for Marine Monitoring in the COMBINE Programme of HELCOM (1997).

During microscopic analysis, individual individuals were identified to species or higher taxonomic units according to the current taxonomic classification (Hällfors 2004). The volume of each cell was determined based on measurements of its relevant morphometric characteristics, assuming similarity of cell shapes to stereometric figures (Olenina et al. 2006). The volume of identified cells was converted to biomass, assuming that 1 μm3 equals 1 pg (Edler 1979). Carbon content in phytoplankton samples was calculated using the equations presented in Menden-Deuer and Lessard (2000):

• for diatoms (due to lower carbon content):
Carbon [pgC cell-1] = 0.288 * cell volume $^{0.811}$

• for other phytoplankton groups (except diatoms):
Carbon [pgC cell-1] = 0.216 * cell volume $^{0.939}$
where cell volume is expressed in $\mu m^{3.}$

- methodology for measuring the concentrations of appropriate algae groups determined based on in situ fluorescence measurements using a FluoroProbe bbe fluorimeter (Moldaenke).

Fluorescence measurements of individual algal groups were performed using a FluoroProbe fluorometer (Moldaenke). This highly sensitive instrument is used to measure chlorophyll a and can distinguish the following algal groups: green algae (Chlorophyceae), cyanobacteria (also known as blue-green algae), diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae), and cryptophytes (Cryptophyceae). These groups can be distinguished by similarities in the composition and relative proportions of photosynthetic pigments in the cells of algae belonging to a given class; hence, the fluorescence excitation spectra for each group are characteristic of that class. Cyanobacterial algae are characterized by a maximum excitation at 610 nm, associated with the properties of the photosynthetic antenna pigment – phycocyanin. The fluorescence of chlorophyll a present in cyanobacterial cells exhibits a low intensity at 450 nm, resulting from the masking effect of phycocyanin on the fluorescence emission spectrum of these algae.

Comparison of Various Methods for Determining the Concentrations of Optically Active Components in Algal Cells and the Biomass of Phytoplankton, Including Cyanobacteria

The collected empirical data, including the results of pigment concentrations, algal biomass, and fluorimetrically determined algal content presented in the previous chapter, formed the basis for exploring the interrelationships between various methods for determining the concentrations of optically active components present in algal cells and the biomass of phytoplankton, including cyanobacteria identified in water.

As a result of statistical analyses, the following relationships were established:

- concentrations of individual carotenoids found in cyanobacterial cells: echinenone, zeaxanthin, β-carotene, canthaxanthin, aphanizomenon, and myxoxanthophyll identified using the HPLC method; and the concentrations of phycobiliproteins (phycocyanin and phycoerythrin) identified using the spectrofluorimetric method and the concentration of chlorophyll a determined by the HPLC method (Fig. 2),
- total concentrations of carotenoids characteristic of cyanobacteria identified by the HPLC method and the concentration of chlorophyll a, phycobiliproteins and the concentration of chlorophyll a, and the sum of pigments characteristic of cyanobacteria and the concentration of chlorophyll a (Fig. 3),
- total biomass of phytoplankton species identified based on microscopic determinations and the concentration of chlorophyll a determined by the HPLC method as an indicator of phytoplankton biomass (Fig. 4a),
- total chlorophyll a content determined based on the fluorescence of the groups. algae in in situ measurements performed using the FluoroProbe bbe Moldaenke fluorometer and the chlorophyll a concentration determined by HPLC as an indicator of phytoplankton biomass (Fig. 4b),
- the total biomass of phytoplankton species identified by microscopic determinations and the total chlorophyll a content determined by fluorescence of algal groups in in situ measurements performed using the FluoroProbe bbe Moldaenke fluorometer (Fig. 4c),
- the total biomass of cyanobacteria identified by microscopic determinations and the total concentration of carotenoids and phycobiliproteins characteristic of cyanobacteria determined by HPLC and spectrofluorimetry (Fig. 5a),

• the total biomass of cyanobacteria identified by microscopic determinations and the cyanobacterial content determined by fluorescence of this algal group in in situ measurements performed using the FluoroProbe fluorometer bbe Moldaenke (Fig. 5b),

• the total concentration of carotenoids and phycobiliproteins characteristic of cyanobacteria, determined using HPLC and spectrofluorimetry, and the cyanobacterial content determined based on the fluorescence of this group of algae in in situ measurements performed using a FluoroProbe bbe Moldaenke fluorometer (Fig. 5c).

The resulting relationships are presented in Tables 2-4, including the data number N and the coefficient of determination r^2.

First, the relationships were determined in the following form (Figure 2):

$$C\ \ pigment = C_{chl\ a}^{A.} + B$$

between the concentrations of pigments ($C_{pigment}$) determined in phytoplankton samples using HPLC and spectrofluorimetric methods in relation to the concentration of chlorophyll a $C_{chl\ a}$, while the obtained relationships are presented in Table 3. It should be emphasized that the analyzed pigment concentrations were determined by carrying out extraction processes appropriate for the given compounds from cells of naturally occurring phytoplankton.

As mentioned in the introduction, pigments are the main cellular components determining the presence of specific taxonomic groups in the studied phytocoenosis. Compounds characteristic of cyanobacterial cells include carotenoids, such as echinenone, zeaxanthin, b-carotene, canthaxanthin, aphanizophyll, myxoxanthophyll, and phycobilins, such as phycocyanin and phycoerythrin. However, it should be remembered that these pigments can occur in algal cells belonging to other taxonomic classes (Jeffey and Vesk 1997, Roy et al. 2011). Zeaxanthin, considered a carotenoid marker for cyanobacteria, also occurs as the main pigment, accounting for 10% of the total chlorophyll and carotenoid content in the cells of prochlorophytes (*Prochlorophyceae*) and red algae (*Rhodophyceae*).

Studies of the pigment composition of Baltic cyanobacteria have shown that zeaxanthin is not the dominant pigment in all cultivated cyanobacterial strains (Wojtasiewicz and Stoń-Egiert 2016). Furthermore, zeaxanthin is a pigment involved in the so-called 'xanthophyll cycle,' associated with the processes of non-photochemical suppression of particle excitation energy. This cycle involves a reversible sequence of two independent enzymatic reactions: epoxidation and de-oxidation, triggered by changes in light intensity (Goss et al. 1998). Under conditions of excessive light intensity, violaxanthin is converted to zeaxanthin (via an intermediate link – antheraxanthin) via a de-epoxidation reaction (i.e., removal of two or one oxygen groups from the molecule), and conversely, under low light conditions, zeaxanthin is epoxidized. De-epoxidation is a rapid reaction (seconds/minutes to hours), while epoxidation can last even days when exposed to additional stress factors. This cycle is widely described in the literature as crucial in the dissipation of excess excitation energy and the regulation of energy flow in photosynthetic antennae (Demmig-Adams 1990, Demmig-Adams and Adams 1996, Young et al. 1997, Larkum 2003). Therefore, the presence of zeaxanthin in the samples may result from cell adaptation to lighting conditions. Echinenone, in turn, has been additionally isolated from cells of organisms belonging to the green algae prasinophytes and euglenas, and β-carotene from cells of algae belonging to most taxonomic classes. Similarly, phycobiliproteins, in addition to cyanobacteria, are also noted as the main pigments in cryptophytes and red algae (*Rhodophyceae*).

The best correlation was obtained for the dependence of β-carotene (r^2=0.74) on the concentration of chlorophyll a, relatively the most numerous in the resulting data set N=1456.

The coefficient of determination of zeaxanthin was r^2=0.50 (at N=1100), while that of myxoxanthophyll was 0.56 (N=171).

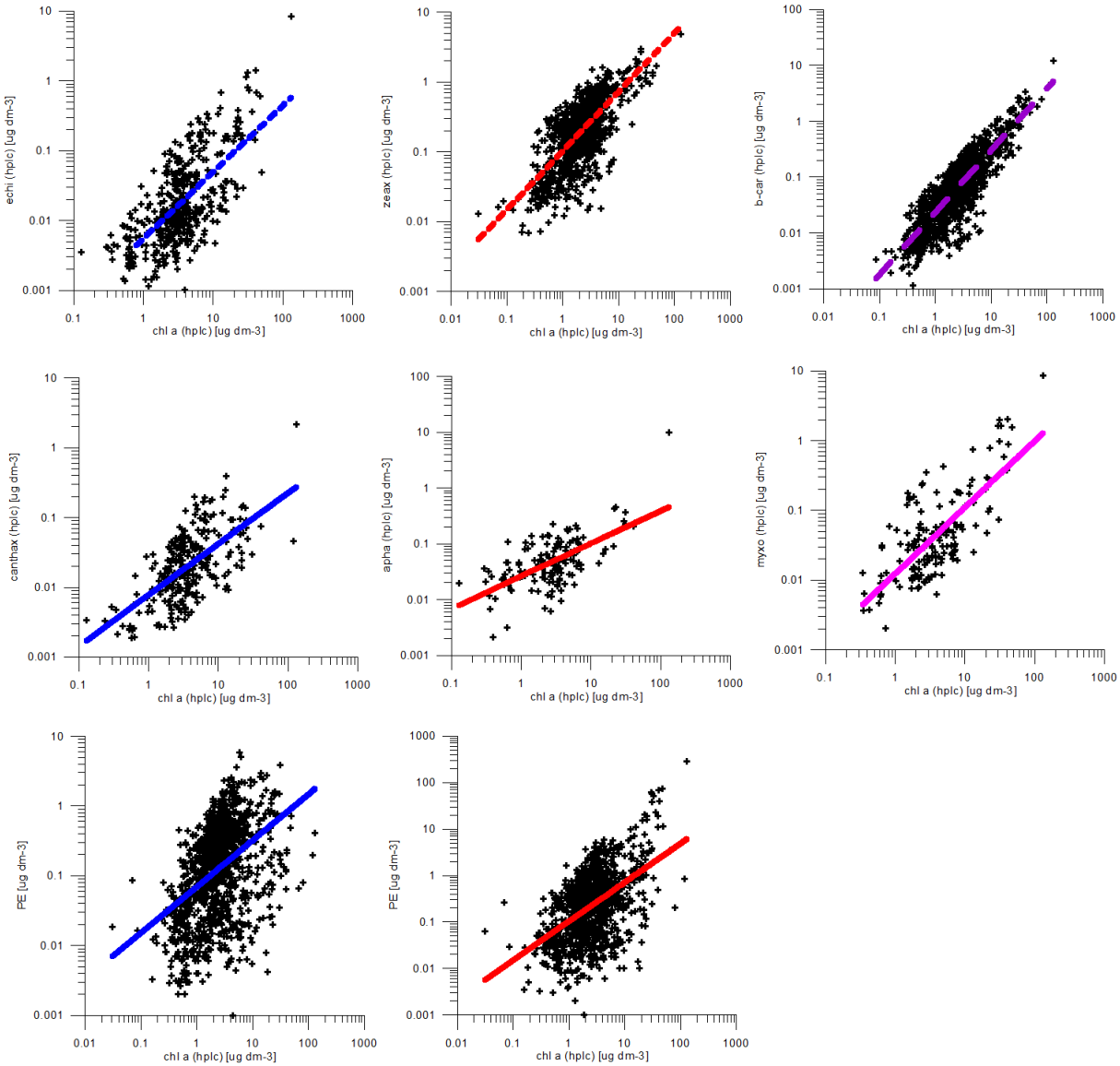

Fig. 2. Relationships between the concentrations of identified carotenoids found in cyanobacterial cells: echinenone (echi), zeaxanthin (zeax), b-carotene (b-kar), canthaxanthin (canthax), aphanizomenone (apha), myxoxanthophyll (myxo), and the concentrations of phycobiliproteins: phycocyanin (PC) and phycoerythrin (PE) identified by spectrofluorimetry and the concentration of chlorophyll a determined by HPLC.

In turn, the relationship between the total content of carotenoids characteristic for cyanobacteria and the concentration of chlorophyll a (Fig. 3) was characterized by the coefficient of determination r^2=0.37 (N=1361), between the total content of phycobiliproteins and chlorophyll a r^2=0.28 (N=1298), while the relationship taking into account carotenoids and phycobiliproteins characteristic for cyanobacteria in relation to the concentration of chlorophyll a r^2=0.33 (N=1461).

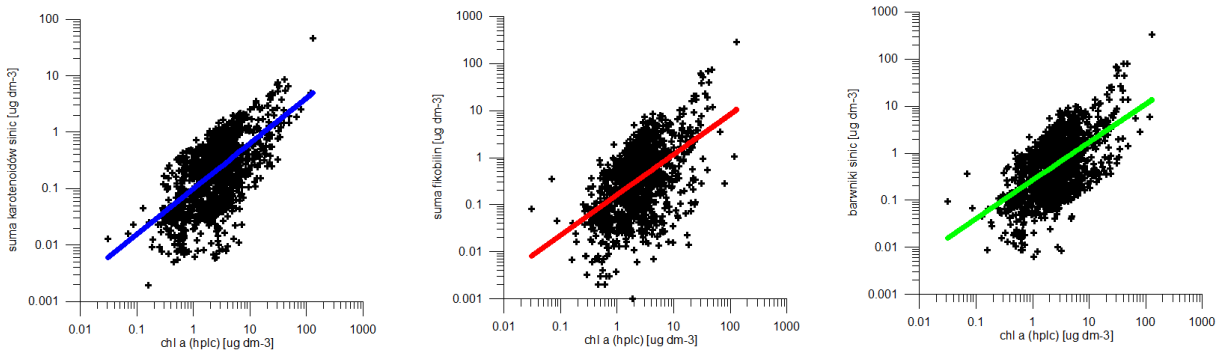

Fig. 3. Relationships between the values of total concentrations of carotenoids identified by HPLC and the concentration of chlorophyll a, phycobiliproteins and the concentration of chlorophyll a, and the sum of pigments characteristic for cyanobacteria and the concentration of chlorophyll *a*

Table 2. Summary of dependency coefficients in the form C_pigment =C_(chl a)^(A.)*B of the obtained dependencies, the number of data N and the coefficient of determination.

	Dependence			Number of data N	Factor of determination R^2
	Pigment	Factor A	Factor B		
1	echinenon	0.9533	0.0055	431	0.45
2	zeaxanthin	0.8442	0.1029	1100	0.50
3	b-carotene	1.1098	0.02349	1456	0.74
4	canthaxanthin	0.7312	0.00779	270	0.40
5	aphanizomenon	0.5846	0.0263	148	0.41
6	myxoxanthophyll	0.9513	0.0123	171	0.56
7	phycoerythrin	0.6631	0.0693	1278	0.19
8	phycocyanin	0.8385	0.1025	1244	0.27
9	total carotenoids of cyanobacteria	0.8151	0.0976	1361	0.37
10	Total phycobiliproteins	0.8577	0.1604	1298	0.28
11	sum of cyanobacterial pigments (carotenoids + phycobilins)	0.8107	0.2631	1461	0.33

In the next stage of analysis, relationships were established between the total biomass of identified phytoplankton expressed in µgC dm^{-3}, determined based on microscopic species identification in natural samples, and the chlorophyll concentration determined by HPLC from the pigment mixture extract (Fig. 4a). The 4 points marked in red represent data sets that may contain erroneous measurement data or non-standard biooptical situations and should be reanalyzed and clarified in further work.

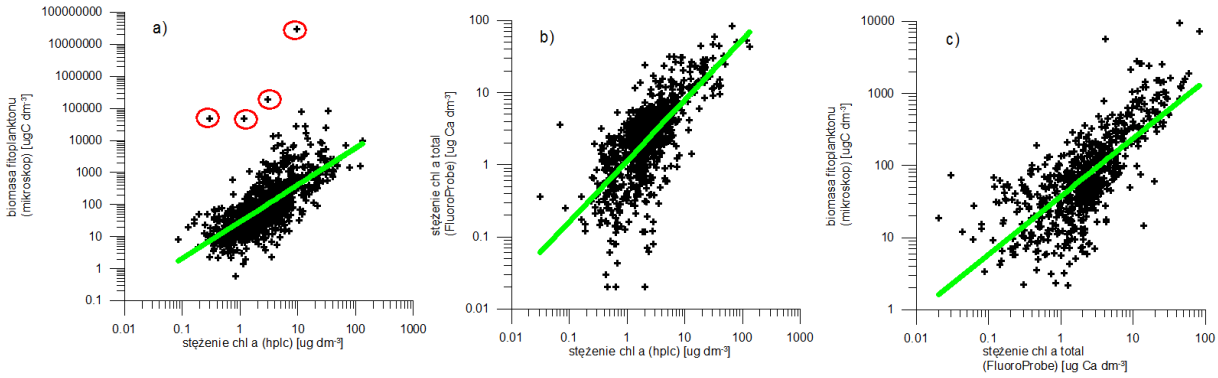

Fig. 4. Relationships between a) the total biomass of phytoplankton species identified based on microscopic determinations and the concentration of chlorophyll a determined by the HPLC method, b) the total chlorophyll a content determined based on the fluorescence of algae groups in in situ measurements performed using the FluoroProbe bbe Moldaenke fluorimeter and the concentration of chlorophyll a determined by the HPLC method, c) the total biomass of phytoplankton species identified based on microscopic determinations and the total chlorophyll a content determined based on the fluorescence of algae groups in in situ measurements performed using the FluoroProbe bbe Moldaenke fluorimeter.

As it is well known, chlorophyll a is widely considered an indicator of phytoplankton biomass. For the analyzed dataset, a relationship was obtained based on 1020 sets of phytoplankton biomass data (determined by microscopic analysis) and chlorophyll a concentration determined by HPLC, and the coefficient of determination of this relationship was $r^2 = 0.48$.

Next, a relationship was established between chlorophyll concentration determined based on HPLC measurements and total chlorophyll content determined based on the fluorescence of algal groups in in situ measurements performed using a FluoroProbe bbe Moldaenke fluorometer (Figure 12.b). In this case, the analyzed dataset contained 987 sets of such measurements, and the obtained coefficient of determination was 0.51. Two alternative methods for determining algal biomass were also compared: a time-consuming and specialized method for microscopic assessment of taxonomic composition, and a method based on fluorescence spectra of phytoplankton groups measured in situ using a FluoroProbe bbe Moldaenke fluorometer (Figure 4c). The resulting relationship was characterized by $r^2 = 0.53$ for the 688 data sets.

Table 3. Summary of the obtained dependencies, the number of data N and the coefficient of determination presented in Fig. 4.

	Dependence	Number of data N	Factor of determination r^2
1	$Biomasa_{fitoplanktonu}(mikroskop) = C_{chl\ a\ (hplc)}^{1.150966648.} * 29.05288895$	1020	0.48
2	$C_{chl\ a\ tot}(FluoroProbe) = C_{chl\ a\ (hplc)}^{0.8406380765} * 1.135410227$	987	0.51
3	$Biomasa_{fitoplanktonu}(mikroskop) = C_{chl\ a\ tot\ (FluoroProbe)}^{0.8007627788} * 37.58922075$	688	0.53

The possibility of using the results obtained using various measurement techniques and methods to determine the biomass of cyanobacteria was also analyzed.

A relationship was established between the total biomass of cyanobacteria identified by microscopic analysis and the total concentration of carotenoids and phycobiliproteins characteristic of cyanobacteria determined by HPLC and spectrofluorimetry (Figure 5a). This relationship was characterized by $r^2 = 0.4$ (for N = 826).

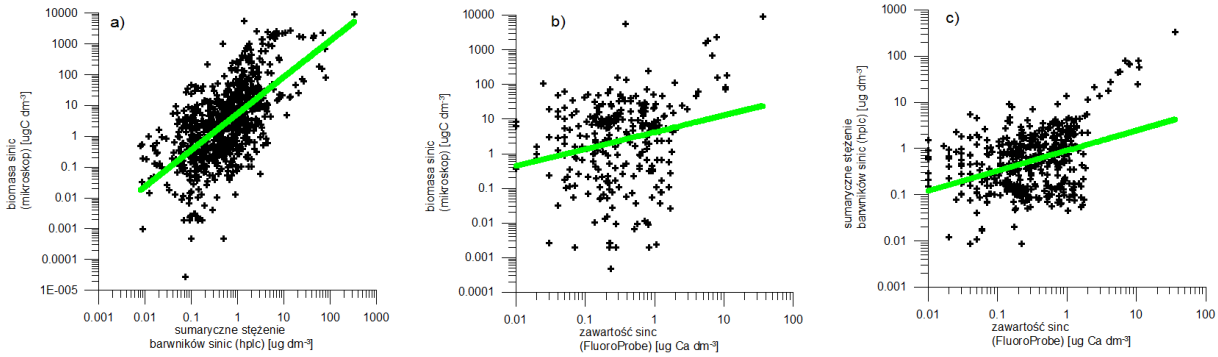

Fig. 5. Relationships between: a) the total biomass of cyanobacteria identified based on microscopic determinations and the total concentration of pigments characteristic for cyanobacteria determined by HPLC and spectrofluorimetry; b) the total biomass of cyanobacteria identified based on microscopic determinations and the content of cyanobacteria determined based on fluorescence of this group of algae (FluoroProbe bbe fluorimeter); c) the total concentration of pigments characteristic for cyanobacteria (HPLC and spectrofluorimeter) and the content of cyanobacteria determined based on fluorescence of this group of algae (FluoroProbe bbe fluorimeter).

In turn, the relationship between the total concentration of carotenoids and phycobiliproteins characteristic of cyanobacteria, determined by HPLC and spectrofluorimetry, and the cyanobacterial content determined based on the fluorescence of this algae group in in situ measurements performed using a FluoroProbe bbe Moldaenke fluorometer (Figure 5c) had a coefficient of determination of 0.13 (for 457 data sets).

The relationship between the total biomass of cyanobacteria identified by microscopic determinations and the cyanobacterial content determined based on the fluorescence of this algae group in in situ measurements (Figure 5b) had the lowest correlation (r2 = 0.05, N = 270).

Table 4. Summary of the obtained dependencies, the number of data N and the coefficient of determination presented in Fig. 5.

Lp	Dependency – pic. 5	Number of data N	Factor of determi-nation r^2
1	$Biomasa_{sinic}(mikroskop) = C_{barwniki\ sinic\ (hplc)}^{1.182064922} * 5.505113756$	826	0.40
2	$Biomasa_{sinic}(mikroskop) = biomasa_{sinic\ (FluoroProbe)}^{0.4886483732} * 4.13688582$	270	0.05
3	$C_{barwiniki\ sinic}(hplc) = biomasa_{sinic\ (FluoroProbe)}^{0.433813358} * 0.895817151$	457	0.13

Summary

As part of the project, a database was collected from 2010 to 2015, containing empirical measurements of the physicochemical properties of cyanobacteria and the color compounds contained in their cells. While the completed set of parameters contains a vast amount of data from various areas of the Baltic Sea, unfortunately, the summer period is represented by the smallest number of data, limited to the Gulf of Gdańsk.

The collected data were initially analyzed for variation resulting from seasonal and regional variability and for comparison of various (including remote) methods for determining the biomass of these algae, which utilize the diverse biooptical properties of cyanobacterial cells and their optically active components.
Methods based on the classical assessment of phytoplankton species composition in samples and the determination of cyanobacterial biomass through microscopic determinations were compared with methods utilizing characteristic cell characteristics and properties, such as pigment composition determined by chromatographic and spectrofluorometric determination of appropriately isolated compounds and fluorescence of appropriate pigments. Generally, the obtained values of the coefficients of determination for some approximations may result from the specific characteristics of the naturally occurring algal population, which is subject to various environmental factors, often posing stress to the cells, which may ultimately modify the values of the biooptical parameters used.

This is the first stage of research planned to be continued in the coming years.

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