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Detection of Autumnal Concentration of *Coscinodiscus granii* in the Southern Baltic—A Method for In Situ Measurement of Marine Particles

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Abstract: Efficient means for measuring the abundance and species composition of phytoplankton in situ continue to pose a big challenge to scientists. Hitherto, analyses and interpretations have been based mainly on small numbers of data acquired from microscopic examinations of water samples. Hence, information on devices facilitating such measurements is highly desirable. This paper examines the opportunities offered by the LISST-100X instrument for measuring in situ the concentrations and spatially variable biovolumes of a species dominant in the southern Baltic during the autumnal bloom. Microscopic analysis of phytoplankton in water sampled from different locations in the southern Baltic confirmed earlier results, indicating that this bloom was due to the overriding prevalence of one microplankton diatom species—*Coscinodiscus granii*. Combining the microscopic measurements of *C. granii* cell sizes with the size distribution ranges employed by the LISST-100X yielded equivalent spherical diameters (EDSs) ranging from 47.4 to 188.0 µm, with maxima in the 78.4–92.6 and 92.6–109 ranges. Comparative analysis of the particle size distribution (PSD) spectra was used to separate the abundance of *C. granii* from the total suspended particulate matter (SPM). Spatial in situ measurements in 2012 and 2014 of *C. granii* concentrations in surface waters showed that both its abundance and its percentage contribution to the total SPM were highly variable. Over a distance of several km, these concentrations varied from values close to zero to 0.2 µL L^{-1} in 2012 and from 0.3 to 0.9 µL L^{-1} in 2014, whereas the percentage in the total SPM was found to vary locally from a few to c. 50%. The proposed method and results demonstrate the success of the LISST-100X instrument in detecting size and volume concentrations of phytoplankton in size classes ranging from 1.25 to 250 µm. However, the correct interpretation of LISST data requires that the dominant phytoplankton species concentration in the suspensions be large enough for the signal (peak) to be readily visible against the background PSD of other SPM.

Keywords: *Coscinodiscus*; coastal zone; LISST-100X; biovolume; the Baltic Sea; phytoplankton

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1. Introduction

Phytoplankton is a key component of the marine food web and plays an important role in shaping the ecosystem of the Baltic Sea. The function of the pelagic habitat is not only dependent on productivity but also on the species composition and size structure of the system. The major groups of phytoplankton in the Baltic Sea are diatoms, dinoflagellates and cyanobacteria, together with the common ciliate species *Mesodinium rubrum*, all with different functions in the system. The seasonal succession mechanisms of phytoplankton have been quite well identified in both fresh and sea waters [1–6]. Every year, regular cyclic changes in the biomass and taxonomic composition of phytoplankton assemblages are recorded in Baltic Sea waters. However, the spatial and temporal variability of individual phytoplankton groups is not the same in different regions of the Baltic Sea. Depending on the season and the availability of nutrients, blooms of cyanobacteria, dinoflagellates, chlorophytes and diatoms (Bacillariophyceae) can occur [7–16].

Advances in quantitative studies of phytoplankton have revealed ecological patterns in its distribution in the marine environment. In this context, several methods are currently in use. The usual and most precise technique is to count, under a microscope, all the individuals present in a given volume of water and then to estimate the biomass of each taxon. However, phytoplankton concentrations are highly variable in time and space, and so are the dynamics of their changes. Therefore, routine microscopic analyses to evaluate their dimensions, abundance and species composition by examining water samples taken from specific locations by no means guarantee results adequately reflecting the real distributions of these particles in the water. This method, moreover, is extremely labour-intensive, time-consuming and costly, so usually only a small number of samples is collected for analysis. A possible solution to this problem is to use state-of-the-art optical instruments permitting in situ measurements to be made with a high frequency. One such instrument for measuring the concentration and particle size distribution (PSD) of suspended particulate matter (SPM) is the LISST-100X PSD analyser. However, its application requires a certain expertise if the results are to be correctly interpreted. The main difficulty as regards interpretation emerges from the impossibility of separating phytoplankton from other SPM, such as terrestrial detritus and mineral particles, very large quantities of which are carried into the Baltic by rivers and other watercourses, e.g., [17–19]. Another limitation of this device relates to the highly irregular shapes of phytoplankton suspensions: these can be extremely variable, so one cannot talk about their diameters in the literal sense of the word. In an attempt to address this problem, the equivalent spherical diameter (ESD) has been introduced. However, different instruments can variously interpret ESDs, particularly when the particles are not spherical [20–22]. These limitations notwithstanding, this instrument is perfectly suitable for determining the PSDs of phytoplankton [22,23], bacteria [24,25] and other SPM [26–28]. To date, no in situ measurements of the PSDs and concentrations of phytoplankton using the LISSTA-100X have been carried out in the Baltic Sea. However, since the succession of species in the Baltic is both predictable and repeatable, the LISSTA-100X is eminently suitable for monitoring this marine ecosystem.

The main aim of this research was to investigate the possibilities of using the LISST-100X for detecting the autumnal concentrations of phytoplankton in Baltic waters due to the evident preponderance during this season of one particular species, namely, *Coscinodiscus granii*. Further aims were to compare the PSDs obtained with the LISSTA-100X with those acquired from microscopic measurements and to make an assessment of the spatial and temporal variability of the dominant autumn species, *C. granii*.

2. Materials and Methods

2.1. Study Area

The experimental material was collected during an autumn cruise of the research vessel s/y Oceania in different regions of the southern Baltic Sea in 2012 and during research cruises on the fishing vessel Sea Angel in southern Baltic coastal waters in 2014. The study area covered the open waters of the southern Baltic as well as the coastal regions of the Gulf of Gdańsk. The exact positions of the sampling stations are shown in Figure 1.

In 2012 and 2014, the water for continuous measurements (along transects) was sampled from a surface layer and then pumped through a hose to the LISST instrument installed on the deck. At the same time, surface water was sampled at selected stations and immediately preserved in Lugol's solution for microscopic analysis.

In addition, data gathered during a cruise in 2011 were used to verify the species composition and the ESDs of *Coscinodiscus granii* (Table 1). Material for the quantitative and qualitative analysis of phytoplankton was obtained from a total of 49 sampling stations.

At each station, we measured: water temperature and salinity. These have been performed with the Multiparameter Water Quality Sonde (YSI 6600 V2, YSI Incorporated, Yellow Springs, OH, USA).

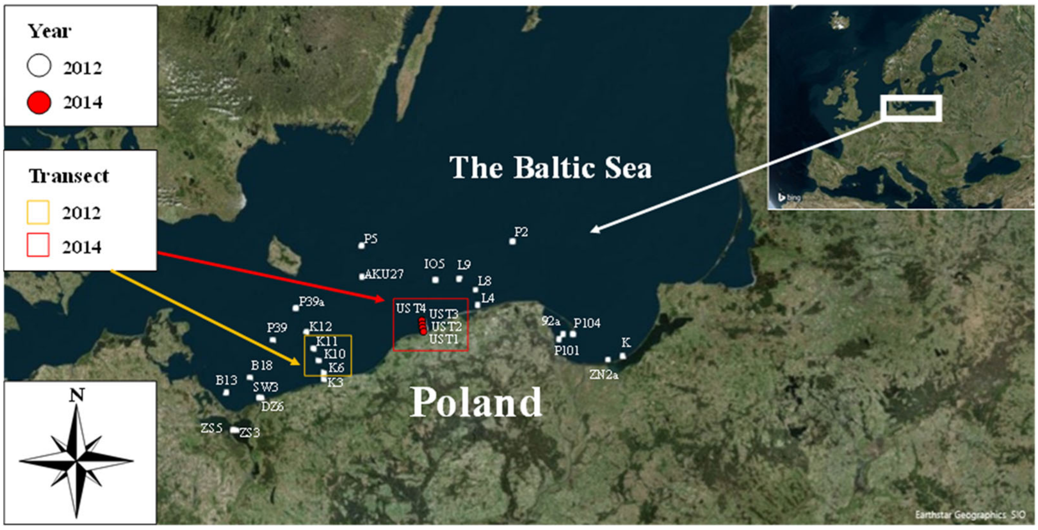


Figure 1. Location of stations and transects where concentration of *Coscinodiscus granii* in situ measurements were conducted within the periods 2012 and 2014 in the waters of the southern Baltic Sea.

Table 1. Specification of empirical material collected in autumn in the southern Baltic in 2011, 2012 and 2014 for species identification.

Ship	Sampling Month		Number of Samples Collected for Species Indentification	Total
s/y Oceania	October	2011	9	49
	October	2012	25	
Fishing boat "Sea Angel"	August	2014	3	
	September	2014	4	
	October	2014	4	
	November	2014	4	

2.2. Methods

During the cruises, the volume concentration of marine SPM was measured continuously using a LISST-100X type B laser instrument (Sequoia Scientific, Inc., Bellevue, WA, USA). This device measures volume concentrations in 32 size classes, divided logarithmically from 1.25 to 250 µm, using small-angle forward-scattering laser diffraction. The scattered light is measured in 32 size bins using a red laser diode at 670 nm and a 32-ring silicon detector. At these small angles, laser diffraction is unaffected by the composition of particles, because light scattering is determined almost entirely by light diffracted by the particle. With the software provided by the manufacturers, the scattering intensities measured by the detector are mathematically inverted to obtain the particle volume concentration, assuming that the particles are spheres. Detailed information on the operation and specifications of the LISST-100X is given in Agrawal and Pottsmith [29].

Sea water at all the sampling stations was also taken for the microscopic analysis of phytoplankton. The samples were fixed with Lugol's solution immediately after collection [29] and stored in the dark at a temperature of 4 °C until analysis. The cells were measured and the qualitative and quantitative analyses of the phytoplankton were carried out in accordance with Utermöhl's method [30] using an OLYMPUS CKX41 (Olympus Soft Imaging Solutions GmbH, Münster, Germany) inverted microscope. The cells were counted on the basis of the procedure given in the Manual for Marine Monitoring in the COMBINE Programme of HELCOM [31]. During the microscopic analysis, individual phytoplankton organisms were identified to the species or a higher systematic level [32]. The volume of each cell was calculated by comparing cells, chains and colonies to stereometric figures [33]. In addition, the length and width of each phytoplankton cell were measured under a microscope, and these dimensions were then used to calculate its surface area and ESD [22]. Jonasz [20], Jennings and Parslow [21] and Karp-Boss et al. [22] showed that the LISST-100X measurements were better correlated with ESDs. Different physical measurements are sensitive to different attributes of the particle (for example, whereas near-forward light scattering is sensitive to the particle's cross-sectional area, electrical impedance is sensitive to the particle's volume). In line with the results of those authors, we used ESDs for the LISST-100X. In this case, we calculate ESDs, the surface-equivalent spherical diameter of a sphere [22] from the formula:

$$\mathrm{ESDs} = \left(\frac{6}{\pi}\mathrm{S}_{\mathrm{particle}}\right)^{-2}$$

The calculated ESDs were used for interpreting the empirical data obtained with the LISST-100X.

3. Results and Discussion

While the biomass of phytoplankton varies distinctly during the annual cycle, the year-on-year variation is not very great. Characteristically, these cycles exhibit two peaks when the biomass is higher: in spring, when blooms are very intense, and again in autumn [7,12]. This cyclic increase in phytoplankton in temperate climate zones is mediated by the seasonal variability in the physicochemical parameters of the water, e.g., nutrient content and light availability. Moreover, there is a distinct succession of species in the annual phytoplankton growth cycle. The dominance of any one species is usually restricted to a short, strictly defined period in the annual cycle, while the maximum biomass of a species can occur earlier or later, as dictated by differences in the weather conditions in particular years. Furthermore, the dominant species succession model outlined above may also be subject to modification due to increasing fertility, pollution of the sea and, finally, climate change. Warming is reflected in an increase in surface water temperature in summer and a decrease in the extent of ice cover in winter. Thus, the temperature of Baltic waters, which fluctuates both seasonally and over many years, is an important factor in determining the dominance of particular species. In addition, it is a fundamental ecological factor, conditioning the development of a given species through its specific thermal optimum. The second important factor for Baltic phytoplankton growth is salinity. In 2012, the average water temperature during the present study was 12.7 °C, while in 2014 it was 11.4 °C, while the average salinity value in 2012 was 7.4 PSU, and in 2014 it was 7.7 PSU. No statistically significant relationships were found between the *Coscinodiscus granii* concentration and water temperature or salinity. Low coefficients of determination (R^2 = 0.0978 and 0.0408) indicate a lack of correlation between these quantities (Figure 2).

Long-term studies have shown that the autumn peaks of phytoplankton biomass in Baltic waters are regularly dominated by centric *Coscinodiscus* species (Table 2).

Our microscopic examinations of phytoplankton in different regions of the southern Baltic in 2011, 2012 and 2014 showed that in autumn (Figure 3b and Table 2), the highest percentage of biomass was due to the large microplankton diatom *Coscinodiscus granii*, in association with *Mesodinium rubrum*.

In contrast, nanoplankton organisms growing in vast numbers (Figure 3a), such as cryptophytes (*Plagioselmis prolonga*, *Teleaulax* spp.) and chlorophytes (*Pyramimonas* spp.), exhibited the highest percentage abundance. However, their biomass was too small for them to be dominant in this respect.

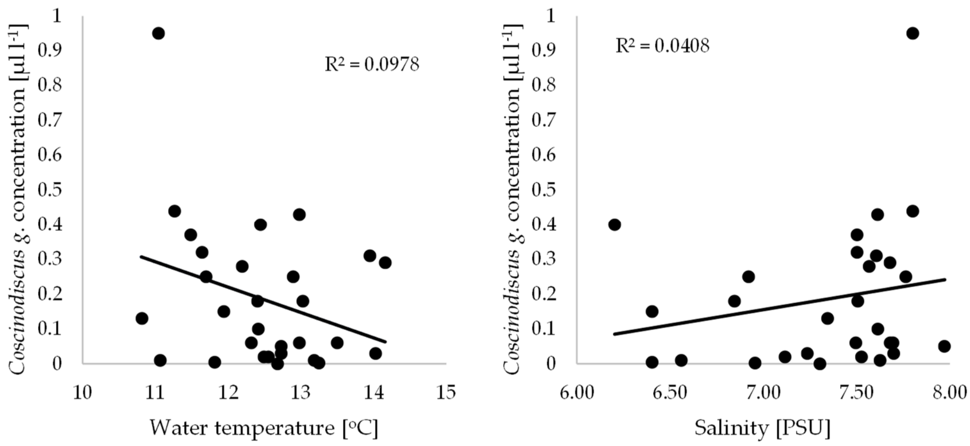


Figure 2. Relationships between *Coscinodiscus granii* concentration measured by LISST-100X and (**a**) water temperature; (**b**) salinity.

Table 2. Percentage of *C. granii* in total phytoplankton biomass in the different sea areas.

Month	Year	Central Arkona Basin	Eastern Arkona Basin	Bornholm Basin	Southern Gotland Basin	Eastern Gotland Basin	References
XI	2010	78.4	84.9	96.5	93.5	91.7	[8]
XI	2015	93.5	95.5	67.9	no date	50.6	[13]
XI	2016	8.4	7.4	55.8	79.8	81.2	[14]
XI	2017	5.7	9.7	50.5	12.3	no date	[15]
XI	2018	No date	64.0	77.1	94.8	79.7	[16]
XI	2020	No date	No date	19.5	The Gotland basin > 70.0		[34]
				Gulf of Gdańsk and Pomeranian Bay			
X/XI	1994 1997			73.2			[35]
				Southern Baltic Sea			
X	2011			58.0			Own research
X	2012			48.0			Own research
VIII/IX	2014			49.0			Own research
X/XI	2014			86.5			Own research

Biovolume is of prime importance in studies of phytoplankton ecology [36–38]. The measurement of this parameter begins with cell size, which plays a crucial role in the dynamics of phytoplankton communities. In accordance with the size classification of Sieburth et al. [39], phytoplankton can be divided into micro-, nano- and picoplankton fractions, although they are also less abundant in the meso- and macroplankton.

Microscopic measurements of the diameters (D) of *C. granii* cells were used to calculate their ESDs. Table 3 lists the maximum and minimum values of D and ESDs for *Coscinodiscul granii* calles, along with their mean values, medians and standard deviations. D ranged from 48.0 to 188.0 µm with a mean of 97.3 µm and ESDs from 47.4 to 188.0 µm with a mean of 89.7 µm.

The dimensions of 962 *C. granii* cells, grouped into the size classes specified by the LISST-100X instrument, are given in Figure 4. This shows that the cell diameters (D) ranged from 47.7 to 212 µm, the most numerous group being cells with D ≈ 101 µm. The corresponding ESDs ranged from 40.5 to 212 µm, with the most numerous group containing cells with ESDs ≈ 85.2 µm.

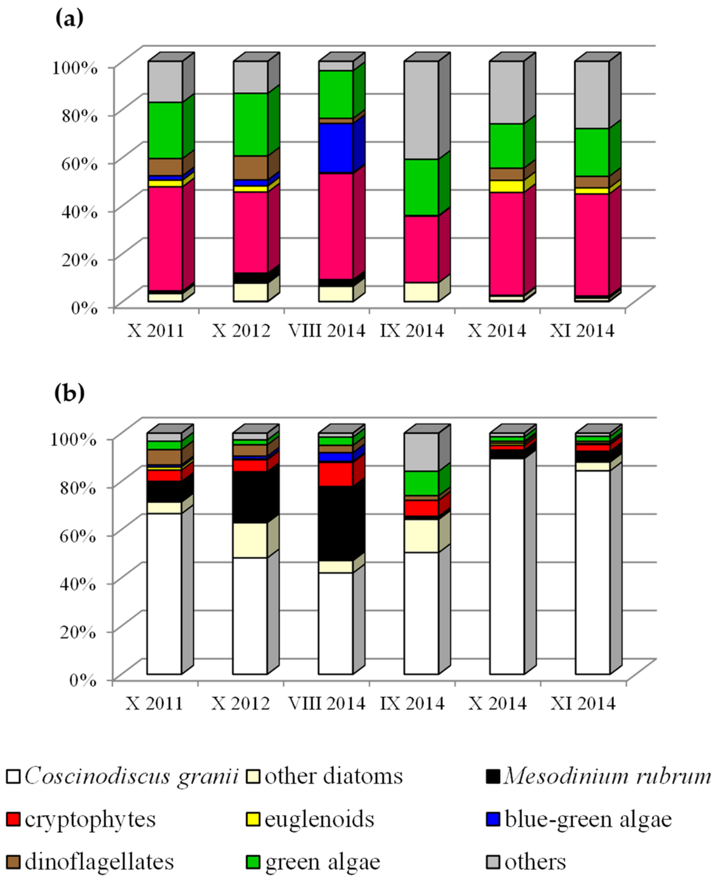


Figure 3. (**a**,**b**) The phytoplankton community structure identified in autumn 2011, 2012 and 2014 at the Baltic Sea with highlighted *Coscinodiscus granii*.

Table 3. Basic statistical size characteristics of *Coscinodiscus granii* (n = 962).

	Diametr [μm]	**Equivalent Spherical Diameter [μm]**
Minimum value	48.0	47.4
Maximum value	188.0	188.0
Average value	97.3	89.7
Median	94.4	89.4
Standard deviation	17.2	14.9

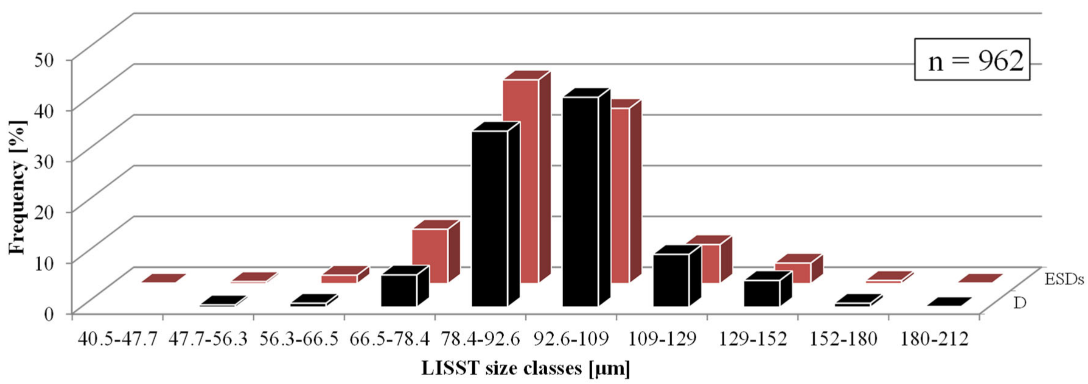


Figure 4. Diameter (D, microscopic measurements) and equivalent spherical (ESD) diameter of *C. granii* sampled from different Baltic Sea areas in particular size classes of LISST-100X. Black bars represent D diameters and red bars ESD diameters.

As mentioned earlier, the labour-intensive, time-consuming and costly qualitative and quantitative analyses of phytoplankton using a microscope are rather ineffective. Because they are based on a small number of data, they do not guarantee results reflecting the actual distributions of these particles in natural waters. For this reason, other instruments are used nowadays, which measure the particles indirectly. The LISST-100X that we used is one such device measuring the concentrations and size distributions of SPM. This information was used to interpret the PSD spectra obtained from the LISST-100X meter. However, phytoplankton concentrations in Baltic waters have never yet been measured in situ with this instrument. To make a valid interpretation of the LISST data, the phytoplankton (bloom) signal must be clearly distinct from background noise. Such a signal was recorded in autumn, specifically in early October 2011, and also towards the end of October 2012 and 2014 along the transects and at particular measurement stations (Figure 1).

In those years, the water at the stations was dominated by large aggregations of SPM with ESDs ranging from 47.4 to 188.0 µm with a distinct peak from 92.6 to 109 µm in 2011, and from 78.4 to 92.6 µm in 2012 and 2014. Figure 5 gives examples of the particle size distributions; these were measured in 2014 at stations UST1, UST2, UST3 and UST4 in the southern Baltic coastal zone (see Figure 1).

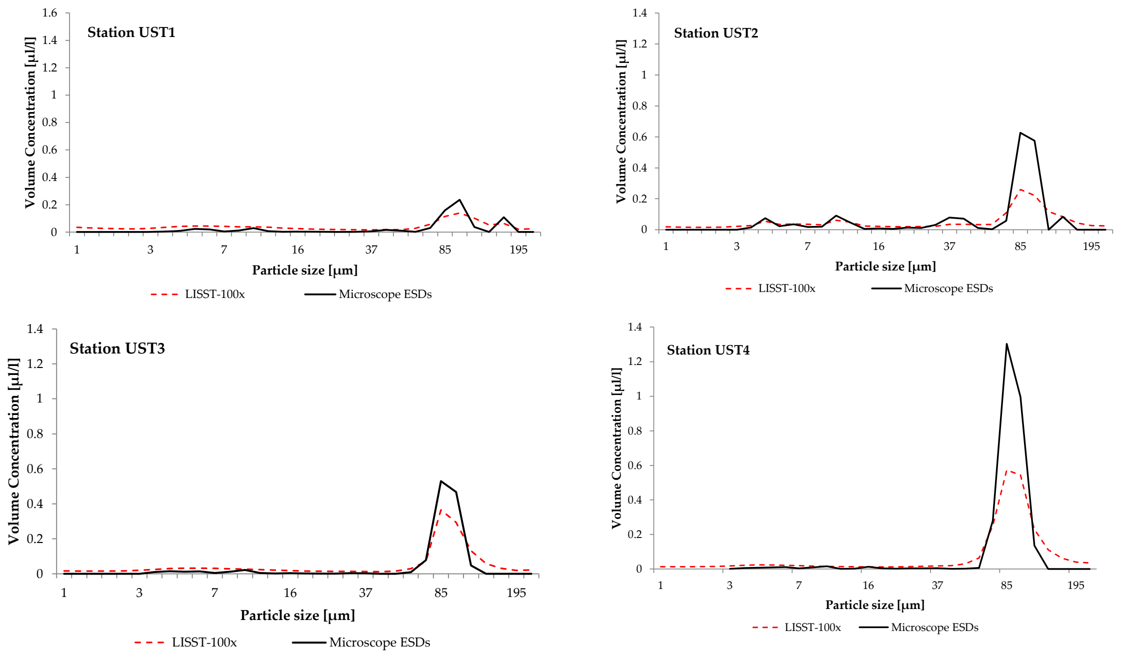


Figure 5. Volume concentration of suspended particulate matter measured in surface water at stations UST 1, UST2, UST3 and UST4. LISST-100 data are represented by the dashed red solid line. Microscopy data are represented by the black line (ESDs, equivalent spherical diameter of a sphere of an equal cross-sectional area).

It is clear from the figures that a feature common to these distributions is the appearance of irregularities in the form of distinct maxima from 92.6 to 109 µm at station UST1 and from 78.4 to 92.6 µm at UST2, UST3 and UST4. These maxima provide evidence for the presence in the water of distinctive groups of SPM constituents, the ESDs of which lie between 47.4 and 188.0 µm.

Microscopic analysis of the water samples showed that within the range of diameters of the dominant maxima, the principal and only constituent of the SPM were microplankton diatoms *Coscinodiscus granii*. The concentrations of *C. granii* were the largest at UST4 (the station farthest from the river mouth), but the lowest at UST1, near the mouth of the River Słupia. During this period, pico- and nanoplankton cyanobacteria were no longer dominant with respect to both abundance and biomass. Microscopic measurements also showed that besides *C. granii*, the samples did not contain any other concentrations of zooplankton SPM with dimensions within the range of the dominant maxima.

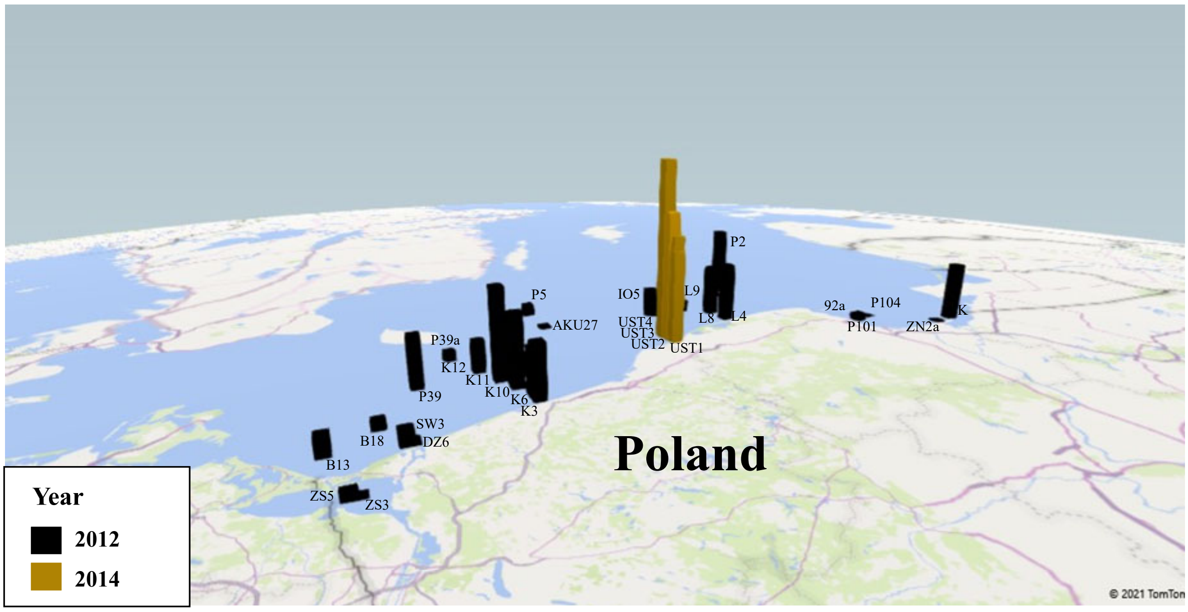
Based on the obtained results, the concentration of centric *Coscinodiscus* diatom species was estimated for each of the measurement stations and along the transect surveyed on 22 October 2012 and 31 October 2014 (Figure 1).

The results for the stations are listed in Table 4 and on the spatial map (Figure 6a); those for the transects are shown in Figure 7a,c. They show that the volume concentrations of *C. granii* are spatially highly variable. In 2012, the highest volume concentrations were recorded at stations P39a (0.43 μL L^{-1}), SW3 (0.40 μL L^{-1}) and K10 (0.31 μL L^{-1}), the lowest ones at P104 (0.0002 μL L^{-1}) and 92a (0.002 μL L^{-1}). In 2014, the highest volume concentration was at UST4 (0.95 μL L^{-1}). In 2012, the highest concentration (>0.20 μL L^{-1}) on the transects was recorded around 22 km from station K6, whereas in 2014, it was 0.9 μL L^{-1} between UST3 and UST4. At stations UST3 and UST4 themselves, the respective concentrations were 0.37 and 0.32 μL L^{-1}. Along both transects, there were also a series of larger and smaller local maxima due to the non-homogeneous distribution of *Coscinodiscus granii* concentrations.

Table 4. The geographical characteristics of measuring stations and absolute and relative concentrations of *Coscinodiscus* spp. during two cruises in the Southern Baltic.

Station Symbol	Latitiude	Longitude	Date of Sampling	Absolute Concentration of *Coscinodiscus* spp. [μL L^{-1}]	Relative Concentration of *Coscinodiscus* spp. [%]
AKU27	54°99.995 N	15°99.975 E	21 October 2012	0.01	0.85
P5	55°14.395 N	15°59.107 E	21 October 2012	0.05	3.49
K3	54°12.466 N	15°31.972 E	21 October 2012	0.25	14.76
K6	54°15.380 N	15°31.910 E	22 October 2012	0.18	12.28
K10	54°34.028 N	15°17.027 E	22 October 2012	0.31	19.43
K11	54°26.499 N	15°22.969 E	22 October 2012	0.29	24.77
K12	54°34.026 N	15°17.019 E	22 October 2012	0.06	8.73
P39	54°74.255 N	15°13.175 E	22 October 2012	0.03	2.71
P39a	54°29.485 N	14°50.460 E	22 October 2012	0.43	14.01
B13	54°03.985 N	14°14.983 E	24 October 2012	0.18	5.01
B18	54°11.976 N	14°33.277 E	24 October 2012	0.06	2.30
DZ6	54°02.512 N	14°43.050 E	24 October 2012	0.03	0.89
SW3	53°57.073 N	14°15.770 E	24 October 2012	0.40	3.90
ZS3	53°46.822 N	14°21.685 E	25 October 2012	0.15	1.66
ZS5	53°46.798 N	14°24.538 E	25 October 2012	0.005	0.07
IO5	54°59.470 N	16°58.588 E	27 October 2012	0.06	8.80
L4	54°48.150 N	17°32.489 E	27 October 2012	0.28	17.38
L9	55°00.343 N	17°29.085 E	27 October 2012	0.02	3.68
L8	54°55.209 N	17°30.620 E	27 October 2012	0.10	15.11
P101	54°32.543 N	18°36.553 E	28 October 2012	0.02	2.30
92a	54°35.057 N	18°40.001 E	28 October 2012	0.002	0.35
P104	54°34.895 N	18°47.440 E	28 October 2012	0.0002	0.03
P2	55°17.501 N	18°00.200 E	29 October 2012	0.13	21.51
ZN2a	54°23.029 N	19°15.036 E	30 October 2012	0.01	0.54
K	54°24.522 N	19°26.573 E	30 October 2012	0.25	17.68
UST4	54°40.422 N	16°49.433 E	31 October 2014	0.95	51.32
UST3	54°38.085 N	16°50.014 E	31 October 2014	0.44	36.93
UST2	54°36.701 N	16°51.430 E	31 October 2014	0.37	30.50
UST1	54°35.453 N	16°50.685 E	31 October 2014	0.32	26.36

(a)



(b)

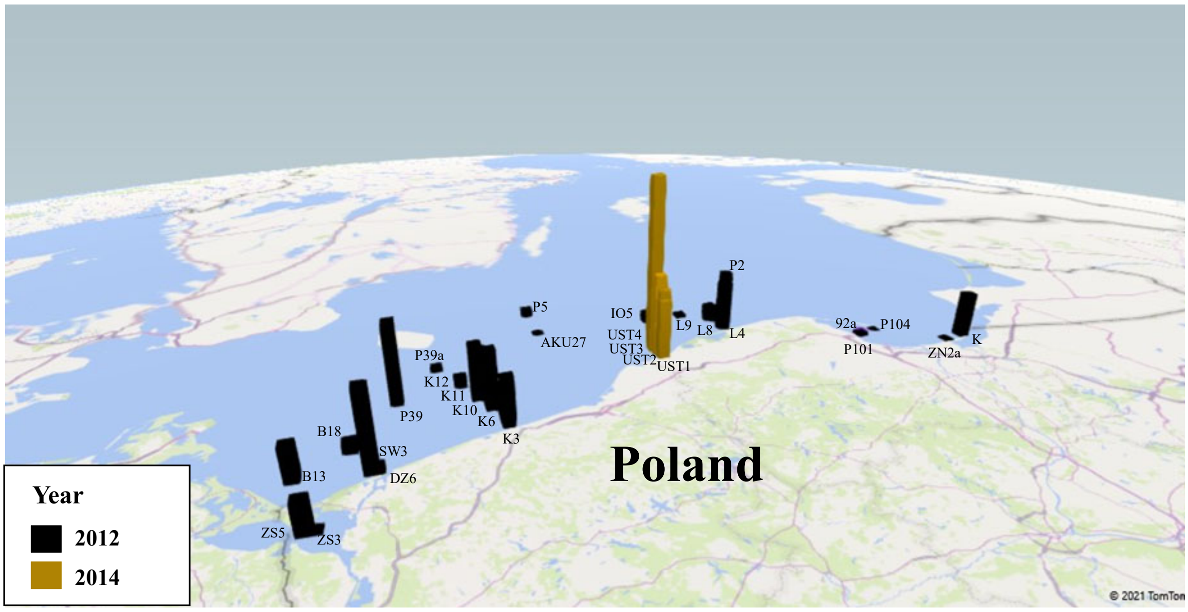


Figure 6. The spatial distribution of concentration of *Coscinodiscus granii* in surface water in 2012 and 2014: (**a**) absolute concentration [µL L^{-1}], (**b**) relative concentration [%], i.e., the ratio of the number of *Coscinodiscus granii* to the total number of suspended particles ranged from 1.25 to 250.0 µm.

The relative percentage concentration of *C. granii* in relation to the total SPM concentration in the 1.25–250 µm range is shown on the spatial map (Figures 6b and 7b,d, and in Table 4).

We see that the relative percentage of *C. granii* in the SPM in 2012 was at a low level, in contrast to 2014. In 2012 at a distance of some 25 km from station K6, the concentration of *C. granii* fell gradually to near-zero values. But in 2014, the lowest *C. granii* concentrations were recorded up to 1 km from the mouth of the River Słupia, when *C. granii* diatoms made up > 20% of all SPM. As a result of a drop in the total SPM concentration between stations

UST3 and UST 4, the relative percentage of *C. granii* was greater even than that recorded at UST4, locally exceeding 47%.

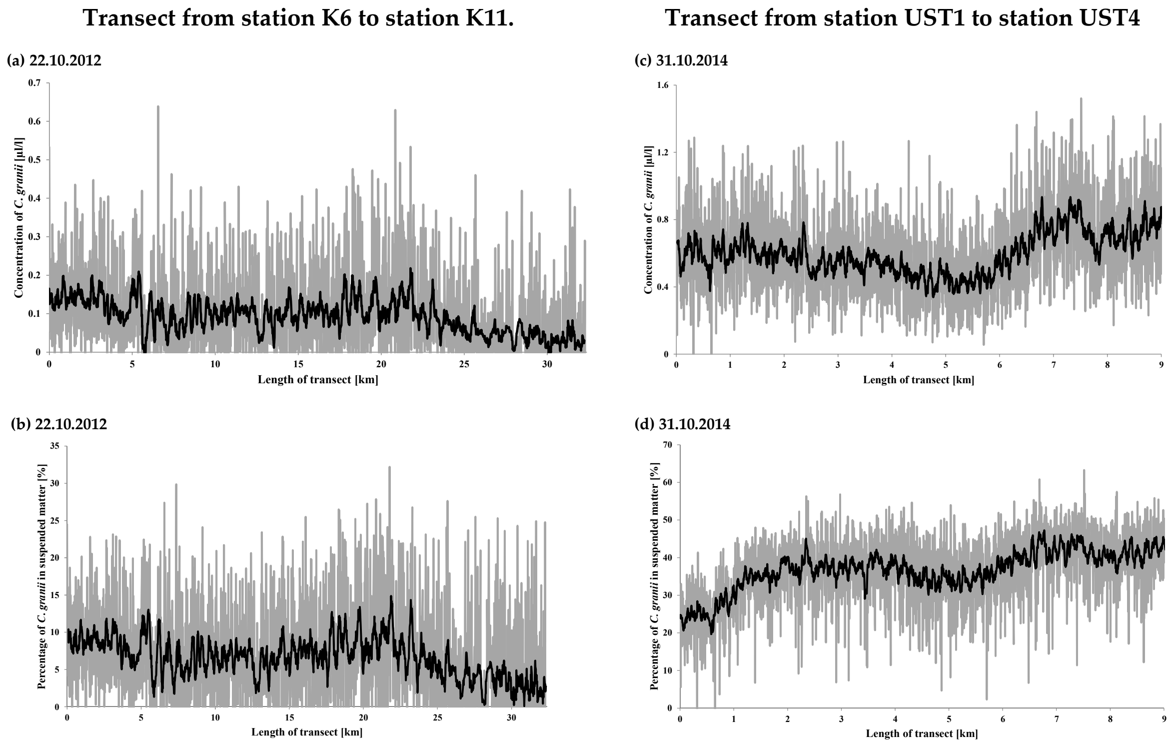


Figure 7. Concentrations of *Coscinodiscus granii* measured on 22 October 2012 and 31 October 2014 along the transects shown in Figure 1: (**a**,**c**) absolute concentration, (**b**,**d**) relative concentration: volume ratio of *C. granii* to the total SPM volume. The mean values on the figures are shown in black.

4. Conclusions

Our measurements have shown that in autumn, Baltic Sea waters are dominated by large phytoplankton suspensions with ESDs from 47.4 to 188.0 µm. Microscopic examinations confirmed that the maxima were due to the presence of microplankton diatoms of one species, namely, *Coscinodiscus granii*, the biovolume of which increases intensively in autumn.

Moreover, the measurements made in 2014 showed that the local relative concentration of *C. granii* in surface waters can exceed 47% of the total SPM.

If supported by microscopic analysis, the LISST-100X device is reliable, because size distributions and volume concentrations of marine SPM can be measured in situ fairly quickly and easily. These results highlight the potential of the LISST-100X to track phytoplankton fluctuations and changes in phytoplankton structure when target species are known and their LISST-100 size distribution signatures have been characterized. What is more, our results highlight the potential applicability of the LISST-100X in Baltic Sea monitoring programmes. This is particularly relevant in the case of the Baltic, given that the greatest threat to this sea is considered to be its eutrophication. Phytoplankton blooms are a natural phenomenon in the Baltic Sea ecosystem. However, due to eutrophication, the phytoplankton blooms are becoming more frequent and extensive [40]. In addition, the effects of global warming, among them, changing water temperatures, may alter the species structure of the Baltic's phytoplankton populations. Such changes have already been documented elsewhere in the world [41–44].

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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