

ORIGINAL RESEARCH ARTICLE

# Canthaxanthin in recent sediments as an indicator of heterocystous cyanobacteria in coastal waters

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Received 15 May 2018; accepted 10 July 2018 Available online 31 July 2018

# **KEYWORDS**

Cyanobacteria; Carotenoids; Canthaxanthin; Gulf of Gdańsk (southern Baltic); Oslofjord Calting and their abundance has increased in the last thirty years, in comparison with previous tim In that period they made up ca 4.6% of the total chlorophyll- <i>a</i> production in the Gulf of Gdańsk The estimate for Oslofjord, at the same assumptions, suggests that heterocystous cyanobacter occurred there also (up to 5.8% of the total chlorophyll- <i>a</i> production), were of marine origin, b their abundance has decreased during the last thirty years. Such an estimate may be used environmental modelling and can be applied to other coastal areas, once the marker pigments the main cyanobacteria species have been identified, and the percentage of total chlorophyll produced in a basin, preserved in sediments, has been determined for such area. © 2018 Institute of Oceanology of Polish Academy of Sciences. Production and hosting by Elsevi	KLIWORD3	Summary The mean share of heterocystods cyanobacteria in total entorophytic a production in
org/licenses/by-nc-nd/4.0/).	Cyanobacteria; Carotenoids; Canthaxanthin; Gulf of Gdańsk (southern Baltic); Oslofjord	coastal waters, based on cyanobacterial marker carotenoid and chloropigments preserved in recent sediments (0–5 cm, ca 30 years), has been studied in the Gulf of Gdańsk (southern Baltic) and for comparison in the Oslofjord/Drammensfjord (southern Norway). First of all, Baltic cyanobacteria, both from laboratory cultures and field samples, were analysed to select marker heterocysteous cyanobacteria carotenoids for sediments. The pigment relation to diatom percentages of different salinity preferences has been tested, to confirm origin of cyanobacteria in the southern Baltic Sea. These filamentous cyanobacteria inflow to the Gulf of Gdańsk from the open sea and their abundance has increased in the last thirty years, in comparison with previous time. In that period they made up ca 4.6% of the total chlorophyll- <i>a</i> production in the Gulf of Gdańsk. The estimate for Oslofjord, at the same assumptions, suggests that heterocystous cyanobacteria occurred there also (up to 5.8% of the total chlorophyll- <i>a</i> production), were of marine origin, but their abundance has decreased during the last thirty years. Such an estimate may be used in environmental modelling and can be applied to other coastal areas, once the marker pigments of the main cyanobacteria species have been identified, and the percentage of total chlorophyll- <i>a</i> produced in a basin, preserved in sediments, has been determined for such area. © 2018 Institute of Oceanology of Polish Academy of Sciences. Production and hosting by Elsevier Sp. z o.o. This is an open access article under the CC BY-NC-ND license (http://creativecommons.

The mean share of heterocystous cyanobacteria in total chlorophylla production in

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Peer review under the responsibility of Institute of Oceanology of the Polish Academy of Sciences.



Production and hosting by Elsevier

#### https://doi.org/10.1016/j.oceano.2018.07.002

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## 1. Introduction

Cyanobacteria (blue-green algae) blooms are presently a serious problem in the Baltic, a brackish, semi-enclosed sea (Kahru and Elmgren, 2014), as in many other coastal zones and lakes (Bianchi et al., 1993; Carstensen et al., 2015; Cook et al., 2016; Paerl et al., 2003). Some cyanobacteria species can produce toxins, so the blooms are often not only a nuisance but also pose a danger to marine life and humans (Karlson et al., 2005; Mazur-Marzec and Pliński, 2009; Sivonen et al., 1989). The cyanobacteria massive blooms which develop in the Baltic each summer may be transferred by waves and water currents to far distances. They are composed of atmospheric nitrogen-fixing (diazotrophic) cyanobacteria, of three planktic taxa: Nodularia spumigena, Aphanizomenon flos-aquae and Dolichospermum sp., classified as heterocystous cyanobacteria (Mazur-Marzec et al., 2013). All three taxa can produce toxins, though there are no reports on toxic Aphanizomenon flos-aquae from the Baltic - only on its freshwater strains (Lehtimäki et al., 1997). The most abundant of those three taxa in the southern Baltic - N. spumigena - produces the toxin nodularin (NOD) (Laamanen et al., 2001; Mazur-Marzec and Pliński, 2009; Stal et al., 2003). It forms blooms also in estuaries and brackish lakes of coastal zones in Australia, New Zealand, inland waters of the USA, South Africa and a saline lake in Turkey (Cook et al., 2016; Henriksen, 2005; Sahindokuyucu Kocasari et al., 2015), as well as off the North Sea coast (Nehring, 1993). N. spumigena needs higher salinities than A. flos-aquae for optimal growth, so the latter is more abundant than the former in freshwater lakes and the northern Baltic (Laamanen et al., 2002; Lehtimäki et al., 1997; Mazur-Marzec et al., 2005).

In order to determine the intensity of cyanobacteria blooms, microscopic analysis of phytoplankton samples is mostly used. Such an analysis provides only temporary information, not always relevant, since the cyanobacteria distribution in water can change very quickly in time and space (Kowalewska et al., 2014; Stal et al., 2003). Satellite detection methods (Kahru and Elmgren, 2014) give a much larger picture than the above-mentioned discrete, point monitoring, although it is still a two-dimensional, fleeting image, encumbered with such impediments like clouds, high costs, etc.

To supplement the above techniques it is worth analysing pigments in sediments. Concentrations and relative compositions of pigments in sediments depend on such factors as primary production, phytoplankton composition, sedimentation/accumulation rate, hydro-meteorological and postdepositional conditions (Jeffrey et al., 1997). Pigments differ in stability and can be degraded by both abiotic and biotic factors e.g. oxygen, light, herbivore grazing or microorganism activity (Leavitt, 1993). In consequence, studies on parent pigments and their derivatives in sediments provide information about time-averaged primary production, phytoplankton taxa and the environmental conditions (Jeffrey et al., 1997). Zeaxanthin is known as a major carotenoid, characteristic of cyanobacteria occurring in an aquatic environment (Jeffrey et al., 1997; Roy et al., 2012); echinenone and canthaxanthin are also mentioned (Henriksen, 2005; Roy et al., 2012). Zeaxanthin has been considered a universal cyanobacteria marker in the Baltic water (Stoń et al., 2002) and sediments (Bianchi et al., 2000; Łotocka, 1998). A 4keto-myxoxanthophyll-like pigment was proposed as a marker of *N. spumigena* in seawater — the major toxic cyanobacteria species forming blooms in the Baltic Sea (Schlüter et al., 2004).

The aim of this paper was to estimate the mean contribution of heterocystous cyanobacteria to total chlorophyll-a production in coastal waters, based on biomarker carotenoids and chloropigments preserved in recent sediments. Such estimate may be used in environmental modelling. Sediments originated from the Gulf of Gdańsk and for comparison from the Oslofjord/Drammensfjord (southern Norway). It is a part of Skagerrak strait, connecting the North Sea and the Kattegat sea area, which leads to the Baltic Sea. Both the Gulf of Gdańsk and Oslofjord are under human impact and of restricted seawater exchange, but differ in natural conditions (salinity, water depth, geomorphology, hydrology, etc.). The sediment results were related to the Baltic cyanobacteria in laboratory cultures and seawater samples collected during summer cyanobacteria blooms to select marker carotenoids characteristic for heterocysteous cyanobacteria in this area. Correlation of cyanobacterial marker carotenoids in sediments with diatom percentage of different salinity preferences has been tested, to confirm marine/ brackish origin of cyanobacteria.

## 2. Material and methods

#### 2.1. Study areas

#### 2.1.1. Gulf of Gdańsk

The Gulf of Gdańsk (area - 4940 km², water volume -291 km<sup>3</sup> (Majewski, 1994)) is situated in the south-eastern part of the Baltic Sea and is part of the Gdańsk Basin. It has an average depth of 59 m and a maximum of 110 m (Gdańsk Deep). The input of nutrients from the waters of the Wisła (Vistula), the largest Polish river ( $\sim$ 39 km<sup>3</sup> yr<sup>-1</sup>), leads to a high level of primary production (up to 225 g  $C^{-1}$  yr<sup>-1</sup>) (Pastuszak and Witek et al., 2012). High sedimentation/accumulation rates and limited water exchange favour intensive blooms of algae and cyanobacteria (including species producing toxins) (Conley et al., 2011). The Gdańsk Deep is a sink for particulate matter both autochthonous (originating from marine primary production) (Maksymowska et al., 2000) and allochthonous (carried by the Wisła). The salinity varies between 7 and 8 in the surface water, except for an area close to the Wisła mouth. The salinity of deeper water is higher, ca 10-15. At depth 60-80 m a halocline is formed resulting in hypoxic/anoxic conditions in the bottom waters (IMGW, 2013). The sediments in the Gulf of Gdańsk are diverse: from sand near the coast to silty clay in the Gdańsk Deep. Stations P110, P116, M1 and P1 were situated along the pathway of Wisła water inflow into the Gulf. Stations BMPK10 and P104 were located in the shallow, western part of the basin (Puck Bay), the latter near the shore but also close to the open sea.

# 2.1.2. Oslofjord/Drammensfjord

Oslofjord/Drammensfjord, the northward extension of the Skagerrak, lies at the entrance to the Baltic. The two main, inner and



Fig. 1 Location of the sediment sampling sites.

outer fjord basins are separated by a sill at 19.5 m depth (Drobak Sound). Stations A and B were located in Drammensfjord, separated from the Greater Oslofjord by a sill at Svelvik. The other four stations were located in the inner Oslofjord: station C in the deepest, southernmost part of the Vestfjord, and stations D, E, F in the Bunnefjord. The water depths were from 113 to 152 m at stations A–D, and 77–78 m at stations E and F. The surface salinity ranged from 0 (stations A, B) to nearly 20 (Oslofjord). The halocline lay at depth 10–20 m at all stations.

#### 2.2. Sample collection

Sediment samples were collected at six stations in the Gulf of Gdańsk and at six in the Oslofjord/Drammensfjord (Fig. 1). Sediments were collected with a Niemistö core sampler ( $\Phi = 10 \text{ cm}$ ) during a cruise of r/v 'Oceania' in April 2014 (Gulf of Gdańsk) and in June 2014 (Oslofjord/Drammensfjord). Immediately after collection, the cores were divided into layers (0–1, 1–5, 5–10, 10–15, 15–20 cm) and frozen at -20°C on board ship and next preserved frozen until pigment and diatom analyses.

Phytoplankton samples were collected from the surface seawater at the Sopot pier during the exceptionally intensive cyanobacteria bloom in August 2015 and a much less intensive one in July 2016. The seawater samples were passed through Whatman GF/F filters, which were then stored at  $-20^{\circ}$ C until HPLC analysis. The water samples were analysed under the microscope in order to identify cyanobacteria taxa.

#### 2.3. Cyanobacteria cultures

The strains originated from the Culture Collection of Northern Poland (CCNP) at the Institute of Oceanography University of Gdańsk. The cyanobacteria had been isolated from the littoral and pelagic zones of the Baltic Sea (Table 1). Additionally, one strain of Aphanizomenon flos-aquae (CCAP 1401/2), isolated from a freshwater lake in the U.K., was analysed. The cyanobacteria cultures were passed through a Whatman GF/F glassfibre filter and next analysed for pigment content.

#### 2.4. Pigment analysis

Pigments (carotenoids and chloropigments) were analysed in sediment samples, cyanobacteria cultures and seston samples. Extraction and analysis of pigments were carried out by HPLC, according to the procedures described in detail elsewhere (Krajewska et al., 2017a; Szymczak-Żyła and Kowa-lewska, 2007, Szymczak-Żyła et al., 2017).

# 2.5. Diatom analysis

Samples for diatom analyses (ca 0.1-0.2 g dry sediment) were prepared following the standard procedure for diatom observation under a light microscope (Battarbee, 1986). The diatom samples were treated with 10% HCl to remove calcium carbonate. Next, the organic matter was digested using 30%  $H_2O_2$ , after which mineral matter was removed by decantation. To estimate the concentration of siliceous microfossils per unit weight of dry sediment (absolute abundance), a random settling technique was used (Bodén, 1991). Permanent diatom preparations were mounted in Naphrax® (refractive index  $n_D = 1.73$ ). The analysis was performed with a NIKON microscope under a  $100 \times$  oil immersion objective; 500 to 800 valves were counted in each sample to estimate the percentage abundance of particular taxa. The raw counts were transformed into relative abundance of the total frustules counted. The taxonomy and ecological information with respect to habitat and salinity preferences was based primarily on Krammer and Lange-Bertalot (1986), Denys (1991), Hasle and Syvertsen (1996).

Table 1 Marker cyanobacteria carotenoids in laboratory cultures and field samples.

Cyanobacteria	Мухо	Zea	Cantha	Echin
Nostocales — planktic				
Nodularia spumigena CCNP1401 (a)	myxo-like (16.4 min)	_	+	+
Nodularia spumigena CCNP 1401 (b)	_	_	+	+
Nodularia spumigena CCNP 1403	myxo-like (16.7 min)	_	+	+
Nodularia spumigena CCNP 1430	_	_	+	+
Nodularia spumigena CCNP 1440	_	_	+	+
Aphanizomenon flos-aqua CCAP1401/2ª	+	_	+	+
Nostocales – benthic				
Anabaena sp. CCNP1417 (a)	myxo-like (17.1 min)	_	+	+
Anabaena sp. CCNP 1417 (b)	myxo-like (17.3 min)	_	+	+
Trichormus variabilis CCNP 1404	myxo-like (17.4 min)	_	+	+
Chroococcales				
Synechocystis sp. CCNP1108 (a)	myxo-DHI standard (20.1 min)	+	_	+
Synechocystis sp. CCNP 1108 (b)	myxo-DHI standard (20.1 min)	+	_	+
Synechocystis salina CCNP1104 (a)	myxo-DHI standard (20.2 min)	+	_	+
Synechocystis salina CCNP 1104 (b)	myxo-DHI standard (20.1 min)	+	_	+
Cyanobium sp. CCNP 1109	_	+	_	+
Synechococcus sp. CCNP 1110	_	+	_	+
Microcystis aeruginosa CCNP1101 (a)	myxo-like (17.5 min)	+	_	+
Microcystis aeruginosa CCNP 1101 (b)	myxo-like (17.1 min)	+	_	+
Pseudoanabaenales				
Pseudoanabaena galeata CCNP1313 (a)	myxo-DHI standard (20.1 min)	+	_	+
Pseudoanabaena galeata CCNP 1313 (b)	_	+	_	+
Oscillatoriales				
Lyngbya aestuari CCNP 1315	myxo-like (18.6 min)	+	_	+
Blooms – Gulf of Gdańsk				
Cyanobacteria bloom — August 2015 (Nostocales)	myxo-like (17.4 min)	+	+	+
Cyanobacteria bloom — July 2016 (Nostocales)	myxo-like (17.3 min)	+	+	+

#### 2.6. Statistical analysis

The results were statistically processed using STATISTICA 12.5 software (StatSoft, Poland); correlation analysis was used. A non-parametric method (R-Spearman correlation analysis) was applied as the basic conditions necessary for using parametric methods were not fulfilled. A correlation of p < 0.05 was regarded as significant.

# 3. Results

#### 3.1. Carotenoid distribution in sediments

The highest concentration of sum of carotenoids ( $\Sigma$ 11Cars) in the Gulf of Gdańsk was in the first (0-1 cm) sediment layer at each station, and varied from minimum concentration 15 nmol/g at station BMPK10 (5-10 cm) to ~860 nmol/g dw of sediment, at station P1 (0-1 cm) and were higher at the Deep of Gdańsk than at the coastal stations; the corresponding results from Oslofjord were from ~11 nmol/g at station F (15-20 cm) to 500 nmol/g at station D (5-10 cm) (Fig. 2a). The carotenoid content was the highest at stations D and E. Moreover, the carotenoid content in the 0-1 cm layer of the cores from all the fjord stations was not the highest: in fact it was sometimes even lower than in the deeper core layers.

Besides  $\beta$ -carotenes in sediments, there were markers of the main phytoplankton groups occurring in the southern Baltic: zeaxanthin (Zea), canthaxanthin (Cantha) and echinenone (Echin) – cyanobacteria; lutein (Lut) – green algae; alloxanthin (Allo) and  $\alpha$ -carotene ( $\alpha$ -Car) – cryptomonads; fucoxanthin (Fuco) and diatoxanthin (Diato) - diatoms and dinoflagellates. The percentage of three cyanobacteria pigments (zeaxanthin, echinenone and canthaxanthin) in the sum of 11 carotenoids was the lowest in the 0-1 cm layer owing to the high percentage of fucoxanthin, an unstable pigment (Appendix 1). In the deeper layers it was around 20% of the sum at all stations, although both the amounts and proportions of particular cyanobacteria carotenoids differed between stations and with depth in sediments (Fig. 2b, Appendix 2). Generally, all three carotenoids were present in distinctly higher concentrations in the sediments of the Gdańsk Deep stations than in the coastal ones. In particular, the zeaxanthin concentration rose towards the Deep and was highest in the sediments at P1. Canthaxanthin was very similarly distributed in the sediments of the four stations P1-P110, although there were differences in particular layers, while echinenone occurred in lower concentrations than zeaxanthin and canthaxanthin (Appendix 2). The three carotenoids were present in the following percentages of their sum: zeaxanthin (57-85%), canthaxanthin (10-30%) and echinenone (2-18%) (Fig. 2b). The average percentage of zeaxanthin in the sum ranged from 70 to 80% in the Gdańsk



**Fig. 2** (a) Sum of 11 carotenoids in recent sediments from the Gulf of Gdańsk and Oslofjord/Drammensfjord (in nmol/g). (b) Percentage of three cyanobacteria carotenoids in their sum.

Deep to 60–75% in the Oslofjord. Station C had the smallest zeaxanthin percentage and an exceptionally high percentage of canthaxanthin, ca 60%. The percentage of canthaxanthin was higher in the Oslofjord than in the Gulf of Gdańsk, while that of echinenone was lower in the Oslofjord compared to the Gulf of Gdańsk.

## 3.2. Diatoms

The results of the diatom analysis indicate that the sum of valves was about ten times higher in the Gulf of Gdańsk than in the Oslofjord and rarely highest in the 0-1 cm layer. At stations C and F the valves occurred only in the surface layers (0-1 and 0-5, respectively). The percentage of marine diatoms in the sum of all diatom valves in the Gulf of Gdańsk was the highest at station P110 and the lowest at station P116, generally lower in the Deep of Gdańsk sediments than in the coastal ones (Fig. 3). In the fjord sediments the content of marine planktic diatoms was much higher in the inner Oslofjord than in Drammensfjord.

# 3.3. Baltic cyanobacteria laboratory cultures and field samples

In order to elucidate the results for sediments, nineteen cyanobacteria cultures were analysed to determine their carotenoid composition (Table 1). Eight were of the order *Nostocales*: five of planktic *Nodularia spumigena*, three of the benthic *Anabaena* sp. and *Trichormus variabilis*, isolated from the Baltic Sea, and one of *Aphanizomenon flos-aquae* — isolated from a lake. The cyanobacteria cultures of the order

Nostocales contained canthaxanthin and echinenone (Table 1). These cultures contained also different myxol glucoside carotenoids. The next cultures analysed were non-colonial cyanobacteria of the order Chroococcales: Synechocystis, Synechococcus and Cyanobium sp. (all non-toxic). The Synechocystis, Synechococcus and Cyanobium are included in the planktic picocyanobacteria which, according to literature data, may constitute as much as 80% of the cyanobacteria biomass in the Baltic Sea (Stal et al., 2003). The next one culture was also included in Chroococcales a freshwater toxic strain of the nanocyanobacteria Microcystis aeruginosa CCNP1101, isolated from the Wisła Lagoon, although M. aeruginosa occurs in the Gulf of Gdańsk and other parts of the Baltic coastal zone where the salinity is low (Belykh et al., 2013; Łotocka, 1998; Stoń et al., 2002). The last cultures were Pseudanabaena, a benthic cyanobacteria of the order Pseudoanabaenales and a representative of Oscillatoriales - Lyngbya aestuari. The analyses indicated that Chroococcales, Pseudanabaena sp. and Oscillatoriales contained echinenone and zeaxanthin but did not contain canthaxanthin. Different myxoxanthophyll-type carotenoids occurred in majority of cultures (Table 1).

The seawater samples collected from the Sopot pier during cyanobacteria blooms contained mainly canthaxanthin and echinenone of the three cyanobacteria carotenoids, characteristic of *Nostocales* (Fig. 4, Appendix 3). Microscopic analysis showed that during the blooms one cyanobacteria taxa, of the order *Nostocales*, *Nodularia* was predominant (ca 90%), *Aphanizomenon* and small amounts of *Dolichospermum* were also present. These samples contained small quantities of zeaxanthin and myxox-



Fig. 3 Percentage of diatoms of different salinity groups.

anthin-type pigments; the letter were under limit of detection in sediments (Fig. 4, Appendix 3).

# 4. Discussion

#### 4.1. Cyanobacteria marker carotenoids

Based on the laboratory culture and field sample analyses, one may infer that canthaxanthin is a marker of heterocystous cyanobacteria in the Gulf of Gdańsk. Of course, there are different cvanobacteria species in the Baltic and its coastal zone and even more numerous strains, some of which may have other sets of marker carotenoids. Nevertheless, when looking for markers of the most abundant species of filamentous diazotrophic cyanobacteria forming massive blooms in Baltic seawater, canthaxanthin seems to be the best. This carotenoid has already been considered as a marker of filamentous heterocystous cyanobacteria for Baltic seawater (Henriksen, 2005; Schlüter et al., 2004; Wojtasiewicz and Stoń-Egiert, 2016), Baltic coastal lakes (Freiberg et al., 2011), and also other aquatic basins in the world, such as an urban lake in Canada (Desphande et al., 2014). The present results disagree with those of Bianchi et al. (2000) for the Baltic, who showed that zeaxanthin is a marker of atmospheric nitrogen-fixing cyanobacteria. However, those authors stated also that zeaxanthin occurred in minor amounts in Aphanizomenon sp. and was absent in Nodularia spumigena (Bianchi et al., 2000, 2002), and they did not determine canthaxanthin. In those papers zeaxanthin and echinenone were presented as markers of filamentous atmospheric nitrogen-fixing cyanobacteria; like other authors (Desphande et al., 2014), we did not observe this. Wojtasiewicz and Stoń-Egiert (2016) also confirmed that zeaxanthin was absent in the toxic *N.spumigena* and in *Anabaena* sp.

Zeaxanthin, in turn, appears to be the best marker of picocyanobacteria which contain higher ratio Zea/

 $\Sigma$ Chlns-*a* than other cyanobacteria (Appendix 3) and are most abundant among cyanobacteria (Stal et al., 2003). In fact, zeaxanthin has already been used as a marker of noncolonial cyanobacteria for sediments from a Canadian lake (Desphande et al., 2014). Although some authors have described the occurrence of zeaxanthin in Nostocales samples and from the Baltic Sea, e.g. in small amounts, in some laboratory cultures of Nodularia spumigena (Schlüter et al., 2008) or the sole available Baltic strain culture of Apanizomenon flos-aquae - KAC15 (Schlüter et al., 2004; Wojtasiewicz and Stoń-Egiert, 2016), one should treat these reports with caution. Such samples are not axenic cultures and may contain an admixture of other species, e.g. picocyanobacteria, which contain zeaxanthin. The seawater samples collected during filamentous cyanobacteria blooms on the Sopot coast also contained small amounts of zeaxanthin (Fig. 4, Appendix 3), besides small amounts of diatom and green algae markers. Moreover, zeaxanthin has a very similar structure to lutein (it differs in the location of just one double bond); because of this, it is often coeluted with lutein in HPLC (Tse et al., 2015). Finally, not only genetic traits but also environmental conditions can considerably influence the carotenoid pattern. Intensive irradiation, light spectrum change, can enhance production of canthaxanthin and zeaxanthin as photo-protective carotenoids or convert other carotenoids to zeaxanthin (Grant and Louda, 2010; Jeffrey et al., 1997; Schlüter et al., 2000). This is very probably why Nostocales strains in field samples and laboratory cultures differ in their zeaxanthin content. In addition, zeaxanthin may originate from green phytoplankton algae, macroalgae or higher plants (Hall et al., 1997), but in the southern Baltic their input in total primary production is rather small and concentrating at the seashore, due to turbidity caused by eutrophication (HELCOM, 2009); in coastal Baltic waters it may also be derived from Microcystis sp.



**Fig. 4** HPLC chromatograms at 450 nm of (a) *Nodularia spumigena* culture, (b) Cyanobacteria bloom – 2015, at the Sopot coast, (c) sediment sample from the Gulf of Gdańsk.

Finally, it can be inferred from the culture analysis that echinenone in sediments could be a marker of all cyanobacteria, as was proposed for Baltic (Henriksen, 2005), providing that there is hypoxia, as it is evidently the least stable of the three cyanobacteria marker carotenoids even though it has been included, along with canthaxanthin and zeaxanthin, in the first pigment stability class (Leavitt and Hodgson, 2001). This is clear in the surface sediments at stations P116 and P1, where the level of hypoxia was the highest and echinenone occurred in the largest concentration. It may decompose already in water column as its ratio to chloropigment-a in laboratory cultures was evidently higher than in sediments. When deposited in sediments, canthaxanthin and zeaxanthin can remain stable for millennia, preserved in spite of very harsh environmental conditions (Watts and Maxwell, 1977).

As a 4-keto-myxoxanthophyll-like carotenoid was suggested as a specific marker of toxic Baltic cyanobacteria (Schlüter et al., 2004), it should be highlighted that myxol glucoside carotenoids, generally referred to as myxoxanthophylls (Roy et al., 2012), make up a whole group of compounds, some of which are even strain-specific (Schlüter et al., 2008). Analysis of these compounds in a culture extract or even an extract from monoclonal bloom in lakes is a much simpler task than their identification in coastal marine sediments. Above all, they are less stable than the other cyanobacteria marker carotenoids (zeaxanthin, canthaxanthin and echinenone) (Leavitt and Hodgson, 2001). Myxoxanthophylls were determined mainly in lake water samples in the past, often by thin-layer chromatography (TLC) (Hickman and Schweger, 1991). This implies that they might have been mistaken for other carotenoids or their derivatives. In HPLC analysis, separation of the particular glucoside derivatives requires chromatographic mobile and stationary phases different from those used for non-glycoside carotenoids. We extract these pigments from the sediments using method (Krajewska et al., 2017a), which gives a very high yield (98%), as can be judged from

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the last 3	0 years.
tous cyano	obacteria in the total chlorophyll- $a$ production (Chl- $a_{CYAN}$ ) in the Gulf of Gdańsk (P1-P110) and Oslofjord (E), averaged over
Table 2	Concentration of canthaxanthin (Cantha), sum of chloropigments- $a$ ( $\Sigma$ Chlns- $a$ ) and estimated percentage of heterocys-

Station	Cantha [nmol/g]	$\sum$ Chlns- <i>a</i> [nmol/g]	Chl-a <sub>CYAN</sub> [%]	Averaged Chl-a <sub>CYAN</sub> [%]
P1	12.09	794.60	4.01	4.64
0–1 cm				
P1	16.03	345.70		
1—5 cm				
M1	15.87	604.86	4.98	
0–1 cm				
M1	14.85	266.87		
1–5 cm				
P116	16.07	616.36	3.75	
0–1 cm				
P116	10.96	271.48		
1–5 cm				
P110	12.31	353.98	5.82	
0–1 cm				
P110	16.23	253.27		
1–5 cm				
E	11.80	324.71	5.75	5.75
0–1 cm				
E	15.63	248.65		
1–5 cm				
-				

tests with the myxoxanthin DHI standard. However, natural myxoxanthin-type pigments in marine samples are difficult to quantify because they have a variety of structures (analogues) and there are no standards for all of them. The HPLC retention time of this group of compounds was between 16 and 20 min (Fig. 4, Table 1); Rt of the available myxox-anthin standard (DHI) was 20.1 min.

# 4.2. Cyanobacteria origin

Correlation of cyanobacteria carotenoids in sediments with diatoms (Appendix 4) let us conclude on cyanobacteria origin. A positive correlation with planktic marine and brackish diatom percentage suggests that they are of marine not of freshwater origin. This is in agreement with the statements that *N. spumigena* can grow at salinities between 5 and 20 (Lehtimäki et al., 1997), and the fact that the surface salinity is around 7–8 in the Gulf of Gdańsk and <20 in the Oslofjord (Szymczak-Żyła et al., 2017) and they inflow to the Gulf of Gdańsk from the open sea, where very intensive blooms are observed (Stal et al., 2003).

The concentrations of particular carotenoids in sediments depend not only on their sources but also on their stability (Leavitt and Hodgson, 2001). However, the very high percentage of zeaxanthin in sum of the three cyanobacteria carotenoids (Fig. 2b), much higher than in algae cultures of both colonial and non-colonial species (Fig. 2b, Appendix 3), suggests that zeaxanthin in sediments originates not only from cyanobacteria. Zeaxanthin correlated well with echinenone and canthaxanthin (r = 0.90 and 0.83, respectively, p < 0.05), but the best correlation was with lutein (r = 0.96, p < 0.05), a marker of green algae (zeaxanthin also occurs in green algae including macroalgae) (Hall et al., 1997; Kra-

jewska et al., 2017b) or may be formed as a photo-protective pigment at intensive irradiation (Chen et al., 2015). The comparatively low percentage of echinenone supports the hypothesis presented above regarding the lower stability of this carotenoid than canthaxanthin and zeaxanthin.

# 4.3. Estimation of heterocystous cyanobacteria share in total chlorophyll-*a* production

Based on marker cyanobacterial carotenoid (canthaxanthin) and chloropigment-*a* results the percentage of heterocystous cyanobacteria in the total chlorophyll-*a* production in the Gulf of Gdańsk (Chl- $a_{CYAN}$ ), averaged over the last 30 years, was calculated from the following formula (Table 2):

$$Chla_{CYAN} = \frac{Car_{0-5}/R_{CULT}}{\sum Chlnsa_{0-5}/F_d} \times 100\%,$$

where  $Chl-a_{CYAN}$  — percentage of heterocystous cyanobacteria in the total chlorophyll-*a* production;  $Car_{0-5}$  — concentration of canthaxanthin (in nmol) in the 0-5 cm sediment layer;  $\sum Chlns-a_{0-5}$  — concentration of the sum of chloropigments-*a* (in nmol) in the 0–5 cm sediment layer;  $R_{CULT}$  = 0.10 — canthaxanthin/sum of chloropigments-*a* mean ratio in *N*. *spumigena* cultures;  $F_d$  = 0.1 — coefficient of chlorophyll-*a* preservation in sediments of the Gulf of Gdańsk.

The assumptions underlying the above are: 1) the sum of chloropigments-a (in nmol) is equal to the initial amount of chlorophyll-a, both in sediments and in laboratory cultures; 2) canthaxanthin does not decompose during organic matter sedimentation and burial in sediments; 3) 90% of the initial amount of chlorophyll-a produced in the Gulf of Gdańsk decomposes to colourless products and only 10% is preserved in sediments (Szymczak-Żyła and Kowalewska, 2007); 4) 0-5 cm layer of sediments in the Deep of Gdańsk, was accumulated during the last ca 30 years, as the sediment accumulation rate for the four stations P1-P110 was about 0.16 cm/a (Szymczak-Żyła et al., 2017).

The calculated value, averaged for the four stations where laminated sediments occurred (P1-P110) was 4.6%. This means that on average 4.6% of chlorophyll-a production in the Gulf of Gdańsk during the last 30 years originated from heterocystous cyanobacteria. An estimate based on the same assumptions, done for station E in Oslofjord, where there were also laminated sediments and a similar accumulation rate as that in the Deep of Gdańsk (0.17 cm/year by Szymczak-Żyła et al., 2017) yielded 5.8% for heterocystous cyanobacteria. We realize, of course, that this is only a very rough estimate. However, it is of a similar order as the cyanobacteria species composition of blooms determined for estuarine and coastal sites in Europe and North America by Carstensen et al. (2015). The occurrence of diazothrophic cyanobacteria Nodularia spumigena in Oslofiord is quite probable, as this species was discovered at the North Sea coast (Nehring, 1993), in the Lindaspollene fjord near Bergen, western Norway, in the Kattegat (Henriksen, 2005; Lehtimäki et al., 1997) and in the Baltic entrance area (Henriksen, 2005). One can imagine, therefore, that as a result of the periodic great abundance of Nodularia, this species could be transferred from the Baltic by water currents via the Danish Sound and Skagerrak to Oslofjord, where the surface salinity is between 10 and 20 (they grow best at salinities from 7 to 18) (Lehtimäki et al., 1997). In Oslofjord, picocyanobacteria (Synechococcus sp.) were also found to be one of the most common groups (Ypma and Throndsen, 1996).

# 5. Conclusions

Summing up, zeaxanthin in sediments cannot be used as a universal marker of cyanobacteria in the Baltic Sea. Instead, echinenone may serve as a universal marker of cyanobacteria, though not quantitative as is less stable than zeaxanthin and canthaxanthin. Myxoxanthophylls are even less stable than these three carotenoids and are strain-specific, occur also in Chroococcales and other cyanobacteria, so are not good markers for sediments of cyanobacteria groups. It is canthaxanthin, a very stable compound, that is a best marker of heterocystous cyanobacteria. These filamentous cyanobacteria flow into the Gulf of Gdańsk from the open sea and their abundance has increased in the last thirty years comparing to the previous time. In the Gulf of Gdańsk, they made up to ca 4.6% of phytoplankton chlorophyll-a production. Similar estimate done for Oslofjord, on the same assumptions, suggests that heterocystous cyanobacteria also occurred there (ca 5.8% of total chlorophyll-a production in the inner Oslofjord), and were also of marine origin, but their abundance, in contrast to the Gulf of Gdańsk, has decreased during the last thirty years. Such an estimate can be applied to other coastal areas of enhanced sedimentation and laminated sediments, once the marker pigments of the major cyanobacteria species occurring there have been identified and the percentage of total chlorophyll-a produced in a basin, which is preserved in sediments, has been determined for such area.

#### Acknowledgements

This work was carried out within the framework of the Polish-Norwegian Research programme operated by the National Centre for Research and Development under the Norwegian Financial Mechanism 2009-2014, grant CLISED no 196128, M. Krajewska received a fellowship from the Leading National Research Centre (KNOW), Centre for Polar Studies 2014-2018. The authors would like to thank to Dr Anna Filipkowska and Dr Ludwik Lubecki of the Institute of Oceanology, PAN, Sopot, Poland, and Dr Tomasz Ciesielski from NTNU, Trondheim, Norway, for their help in organizing cruises and for their assistance in sample collection, Dr Gijs D. Breedveld and Amy M.P. Oen of NGI, Oslo, Norway, for their help in organizing the cruise to Oslofjord/Drammensfjord. Finally, we thank the crew of 'Oceania' for their assistance during cruises to the Gulf of Gdańsk and Oslofjord/Drammensfjord. We are grateful to Prof. Hanna Mazur-Marzec for her comments to the manuscript.

# Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10. 1016/j.oceano.2018.07.002.

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