



Document title	Candidate indicator 'Phytoplankton community composition as a food web indicator' – proposal to shift to core indicator status
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Background

Development of the candidate indicator 'Phytoplankton community composition as a food web indicator' has been discussed and agreed on intersessionally in the HELCOM PEG group. The indicator proposal was presented and discussed in detail at the HELCOM [PEG 2016 project meeting](#). Also the current data-availability and development of data arrangements from the HELCOM COMBINE database for the purposes of the indicator was discussed.

HELCOM HOD 48-2016 agreed on a Lead Country approach for the further development of the core indicators (paragraph 3.63 of the outcome). The Lead Country for the indicator is Finland, with the Co-Lead countries Lithuania, Sweden, Latvia, Estonia and Poland.

For the purposes of the indicator development, the indicator assessment protocol has been tested using Finnish HELCOM COMBINE phytoplankton monitoring data from the offshore areas of the northern Baltic Sea. The indicator is considered applicable in all HELCOM offshore and coastal assessment units since the methods are applicable with any quality-checked long-term phytoplankton data and the functional characteristics which are specifically considered, i.e. potential suitability or quality as food, harmfulness, trophy, cell size, are common to all phytoplankton communities. The Lead Country is currently supporting ongoing work to test the indicator approach in other assessment units together with respective national representatives in the HELCOM PEG group.

The work to operationalize the candidate indicator has focussed on developing the assessment protocol and describing on how to define if the phytoplankton community composition reflects GES. The interpretation of results is done using expert judgement to define if the phytoplankton community composition reflects GES in terms of its potential effects on the next trophic levels of the food web. When defining the GES boundary, both current phytoplankton composition and the ongoing changes are taken into account. As a clarification it should be noted that the indicator is not based on an index value, and that categorical values used when determining in an assessment unit reflects GES.

This document presents the indicator report for the candidate indicator 'Phytoplankton community composition as a food web indicator' where the indicator approach is demonstrated using Finnish HELCOM COMBINE phytoplankton monitoring data from the offshore areas of the northern Baltic Sea.

Action requested

The Meeting is invited to:

- endorse the approach for defining GES and endorse the assessment protocol.
- endorse the shift of the indicator status from candidate- to core indicator.

Phytoplankton community composition as a food web indicator – candidate core indicator report proposal

Candidate core indicator report:

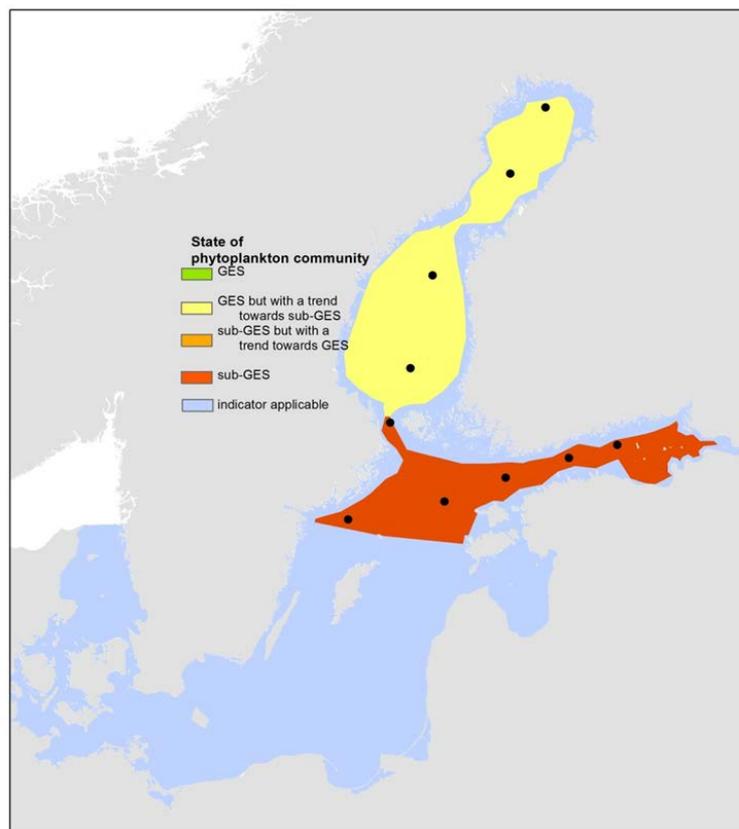
Phytoplankton community composition as a food web indicator

Key Message	3
Policy relevance of the core indicator	5
Cite this indicator	5
Download full indicator report	5
Results and Confidence	6
Step 1: Class-level and total biomass trends	6
Step 2: Genus-level community changes	7
Step 3: Most accurate taxonomic level examination	8
Step 4: Interpretation	8
Confidence of the indicator status evaluation	10
Good Environmental Status.....	11
Assessment Protocol	11
Step 1: Class-level and total biomass trend analyses	12
Step 2: Genus-level community analysis	12
Step 3: The examination of the dominant taxa on the most accurate taxonomic level	12
Step 4: Interpretation of results	13
Relevance of the Indicator.....	13
Food web assessment	13
Policy relevance	13
Role of phytoplankton community composition in the ecosystem	13
Human pressures linked to the indicator	14
Monitoring Requirements	15
Monitoring methodology	15
Current monitoring.....	15
Description of optimal monitoring.....	15
Data and updating	16
Access and use.....	16
Metadata	16
Contributors and references	17
Contributors	17

Archive.....	18
References.....	18
Additional relevant publications	18

Key Message

Within this indicator, both contemporary taxonomic phytoplankton composition and recent trends of changes are used to assess the status of the phytoplankton community in terms of potential effects on the next trophic levels of the food web (Lehtinen et al. *submitted*). The indicator is applicable in all coastal and offshore areas, since the methods are applicable with any long-term phytoplankton monitoring data and the functional characteristics which are specifically considered, i.e. potential suitability or quality as food for grazers, harmfulness, trophic, and cell size, are common to all phytoplankton communities. Sampling should cover the season of tightest coupling between phytoplankton and grazers. The indicator is demonstrated using a 36 years HELCOM COMBINE phytoplankton monitoring data set (288 samples) from the offshore areas of the northern Baltic Sea.



Key message figure 1: Status assessment results based evaluation of the indicator Phytoplankton community composition. The assessment is carried out using Scale3 HELCOM assessment units (defined in the [HELCOM Monitoring and Assessment Strategy Annex 4](#)).

The indicator analysis consists of four steps (Fig. 2). Both contemporary taxonomic phytoplankton composition and recent trends of changes are used to assess the status of the phytoplankton community in terms of potential effects on the next trophic levels of the food web. The statistically significant p-value 0.05 is the reference value for change. Potential suitability and quality of phytoplankton taxa as food for grazers, harmfulness, cell size, and trophic are the characteristics of the dominant or increased or decreased taxa

which are specifically considered when interpreting the results. These characteristics are selected based on existing knowledge on their relevance to the next trophic level (e.g. Koski et al. 1998, Sommer et al. 2000, Berglund et al. 2007, Sopanen et al. 2008).

The interpretation of results is done using expert judgement to define if the phytoplankton community composition reflects good environmental status (GES) in terms of its potential effects on the next trophic levels of the food web: 1= GES is reached; 2 = GES is reached, but trends show ongoing change towards sub-GES; 3 = GES is not reached, but trends show ongoing change towards GES; 4 = GES is not reached, i.e. the indicator reflects sub-GES status.

In this report, the approach is demonstrated with the HELCOM COMBINE phytoplankton monitoring data from the offshore areas of the northern Baltic Sea (Lehtinen et al. *submitted*), but the indicator can be used for all coastal and offshore areas as well, since the methods are applicable with any quality-checked long-term phytoplankton data, and the functional characteristics which are specifically considered (potential suitability or quality as food, harmfulness, trophy, cell size) are common to all phytoplankton communities. A Baltic Sea –wide phytoplankton community composition analysis is to be done every six years along with the other food web indicator analyses as a part of the overall food web assessment.

The confidence of the indicator assessment is dependent on the effort and expertise invested in attaining high-quality phytoplankton monitoring data, performing the statistical analyses, and in interpreting the results. High confidence is assured by maintaining high-quality in phytoplankton monitoring and high expertise in data handling and interpreting the results.

The indicator is applicable in the waters of all countries bordering the Baltic Sea.

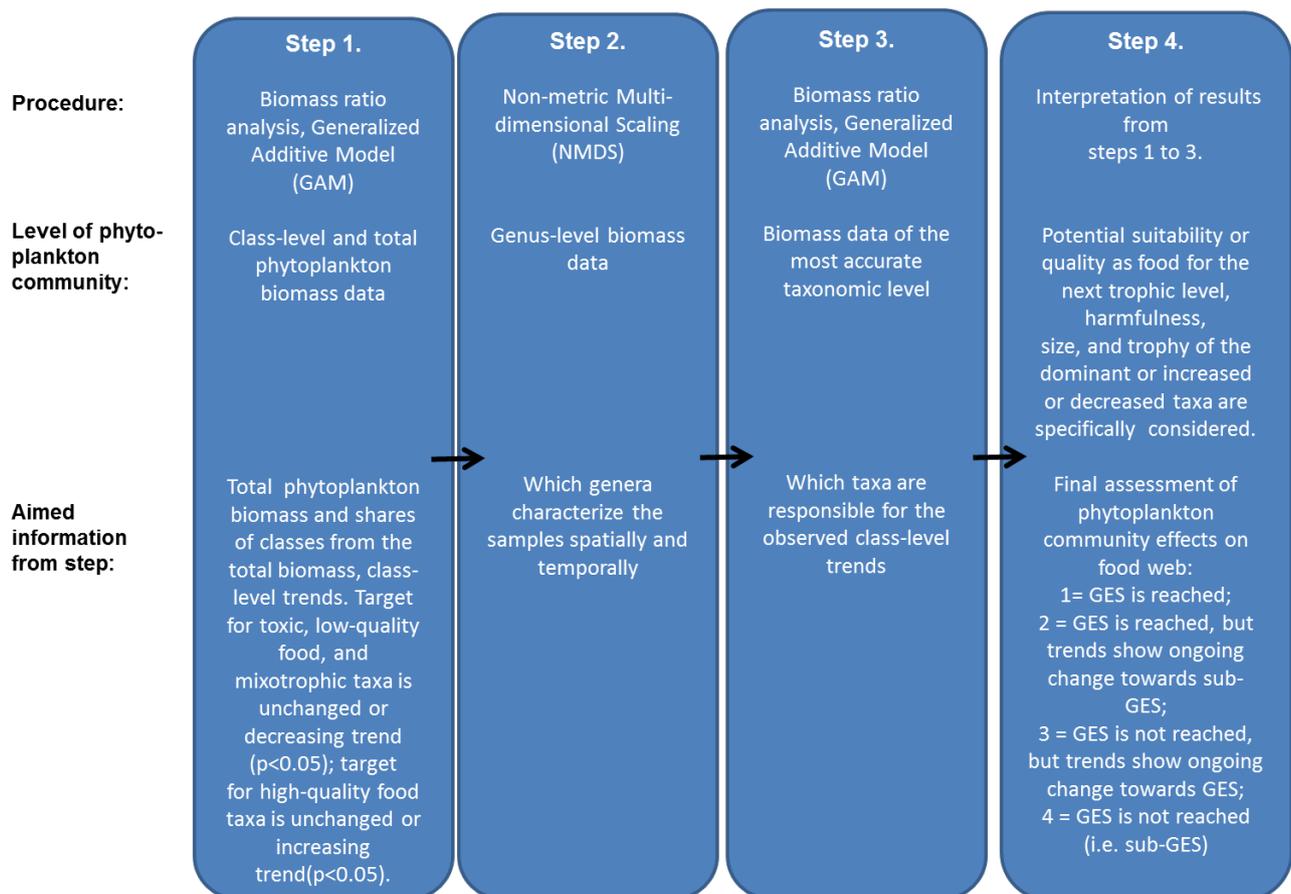


Fig. 2. The approach to assess if the phytoplankton community composition reflects good environmental status (GES) in terms of its potential effects on the next trophic levels of the food web. The figure is modified from Lehtinen et al. (*submitted*).

Relevance of the core indicator

Phytoplankton community composition is a relevant indicator for the Marine Strategy Framework Directive (2008/56/EC) (MSFD) Descriptor 4, food webs (Table 1). Assessments on the structure and functioning of the marine food web are requested also by the HELCOM Baltic Sea Action Plan (BSAP). Within this indicator, a number of phytoplankton properties (potential suitability or quality as food for grazers, harmfulness, trophy, cell size) can be used to assess the potential efficiency of the pelagic food web in a way which cannot be deducted from other monitoring data. This supplements the bottom-up approach, which should then be combined with the results of the other food web indicators for a holistic food web assessment. The analyses of pressures and management options will follow the holistic assessment.

Policy relevance of the core indicator

Table 1. Policy relevance of the phytoplankton community composition indicator for the HELCOM Baltic Sea Action Plan (BSAP) and the Marine Strategy Framework Directive (MSFD).

	BSAP Segment and Objectives	MSFD Descriptors and Criteria
Primary link	<ul style="list-style-type: none"> • Thriving and balanced communities of plants and animals 	D4 - Food webs 4.3. Abundance/distribution of key trophic groups and species
Secondary link	<ul style="list-style-type: none"> • Natural distribution and occurrence of plants and animals • Viable populations of species • Natural level of algal blooms • No introduction of alien species 	1.6. Habitat condition: Condition of the typical species and communities, relative abundance and/or biomass 1.7. Composition and relative proportion of ecosystem components 5.2. Direct effects of nutrients
Other relevant legislation:		

Cite this indicator

Lehtinen., S., Suikkanen, S., Hällfors, H., Kauppila, P., Lehtiniemi, M., Tuimala, J., Uusitalo, L. & Kuosa, H. (2016). Phytoplankton community composition as a food web indicator. HELCOM core indicator report. Online. [Date Viewed], [Web link].

Download full indicator report

Core indicator report – web-based version [month year] (pdf)

Results and Confidence

The indicator is demonstrated with a 36 years Finnish HELCOM COMBINE phytoplankton monitoring data set from the offshore areas of the northern Baltic Sea (Lehtinen et al. *submitted*). The sampling season was late summer, i.e. the period when zooplankton abundance and biomass are the highest in the area (Ojaveer 1998), following the warming of the water and development of thermocline in the surface layer, but before the downwelling period which breaks up the thermocline.

Step 1: Class-level and total biomass trends

The average total phytoplankton biomass during the study period (1979-2014) was the lowest in the Bothnian Bay ($191 \pm 267 \mu\text{g l}^{-1}$, mean \pm S.D.), and the highest in the Gulf of Finland (average $520 \pm 483 \mu\text{g l}^{-1}$). The average total phytoplankton biomass was $427 \pm 355 \mu\text{g l}^{-1}$ in the northern Baltic Proper, $294 \pm 212 \mu\text{g l}^{-1}$ in the Bothnian Sea, and $365 \pm 159 \mu\text{g l}^{-1}$ in the Åland Sea (Lehtinen et al. *submitted*). The Bothnian Bay differed from the other areas also based on its phytoplankton composition. For example, the average share of cyanobacteria was only ca. 2 % of the total phytoplankton biomass in the Bothnian Bay, while the average share of cyanobacteria was ca. 27-37 % in the other sea areas (Table 2, Lehtinen et al. *submitted*).

Statistically significant increasing trends were found for cyanobacteria (class Nostocophyceae) in the Bothnian Sea, Åland Sea and the Gulf of Finland, for prymnesiophytes (class Prymnesiophyceae) in all sea areas but the Bothnian Sea, euglenophytes (class Euglenophyceae) in the Åland Sea, and prasinophytes (class Prasinophyceae) in the Northern Baltic Proper (Table 2). The autotrophic ciliate *Mesodinium rubrum* increased in the Bothnian Sea and Northern Baltic Proper during the study period when it has been included in the phytoplankton counts (i.e. 1986-2014 within this data set). Cryptophytes (class Cryptophyceae) decreased in all sea areas except the Bothnian Sea, and diatoms (class Diatomophyceae) in the Bothnian Bay. The biomass of unidentified taxa decreased in all sea areas, and biomass of total phytoplankton in the Bothnian Bay. Statistically significant, but non-linear variability was shown by diatoms and prasinophytes in the Bothnian Sea.

Table 2. Results of the generalized additive models (GAMs) for detection of long-term trends and the average biomass share (%) of each phytoplankton class from the total phytoplankton biomass. *p*-values; bold = significant trend, $p < 0.05$; direction: blue = decreasing, red = increasing, orange = non-linear. Samples were collected from the Finnish HELCOM COMBINE open sea monitoring stations annually between July 15th and September 15th in 1979-2014. *Mesodinium rubrum* was included into the phytoplankton counts only in 1986, and thus trends in its biomass were calculated for the period 1986-2014, and the species is not included in the biomass of the total phytoplankton community, except for calculation of its biomass share from the total phytoplankton biomass (including *M. rubrum*). The table is modified from Lehtinen et al. (*submitted*).

Offshore sea area	Bothnian Bay		Bothnian Sea		Åland Sea		Gulf of Finland		Northern Baltic Proper	
<i>n</i>	29 (*24)		30 (*25)		28 (*22)		35 (*28)		35 (*28)	
	<i>p</i> -value	% share	<i>p</i> -value	% share						
Nostocophyceae	0.945	2.10	0.023	30.89	0.002	26.69	0.024	39.02	0.144	37.61
Cryptophyceae	0.001	21.95	0.717	9.85	0.007	12.80	0.019	13.64	0.001	12.24
Dinophyceae	0.176	3.59	0.346	13.79	0.711	14.37	0.989	18.56	0.939	18.27
Prymnesiophyceae	0.003	7.83	0.184	11.87	0.017	11.40	0.006	4.16	<0.001	5.35
Chrysophyceae	0.672	10.53	0.591	12.09	0.260	9.71	0.307	4.21	0.561	5.14
Diatomophyceae	<0.001	16.87	<0.001	5.32	0.131	8.15	0.264	3.92	0.909	4.55
Euglenophyceae	0.228	0.50	0.711	2.76	0.022	0.30	0.098	1.17	0.402	1.71
Prasinophyceae	0.743	22.32	0.006	9.83	0.968	8.73	0.536	9.14	0.046	9.22
Chlorophyta	0.237	5.53	0.332	0.66	0.787	0.33	0.227	0.85	0.140	1.21
<i>Mesodinium</i> ¹	0.682	24.15	<0.001	3.57	0.169	2.17	0.107	6.45	<0.001	4.32
Unidentified	<0.001	8.77	<0.001	2.98	<0.001	7.52	<0.001	5.32	<0.001	4.71
Total phytoplankton	<0.001	100.00	0.447	100.00	0.481	100.00	0.980	100.00	0.932	100.00

¹ = Biomass trends for *Mesodinium rubrum* cover the period 1986-2014 only.
 n = number of sampling years (* = number of sampling years for *Mesodinium rubrum*).

Step 2: Genus-level community changes

Community composition was clearly different in the Bothnian Bay compared to the other sea areas (Fig. 3, Lehtinen et al. *submitted*). Chlorophyte (phylum Chlorophyta in the GAM) genera *Desmodesmus*, *Elakatothrix*, *Dictyosphaerium*, and *Botryococcus*, and the diatoms (class Diatomophyceae) *Diatoma* and *keletonema* characterized the samples originating from the Bothnian Bay. Irrespective of this, the NMDS showed that the phytoplankton community composition changed simultaneously in the same direction during the study period 1979-2014 in all sea areas (Fig. 3).

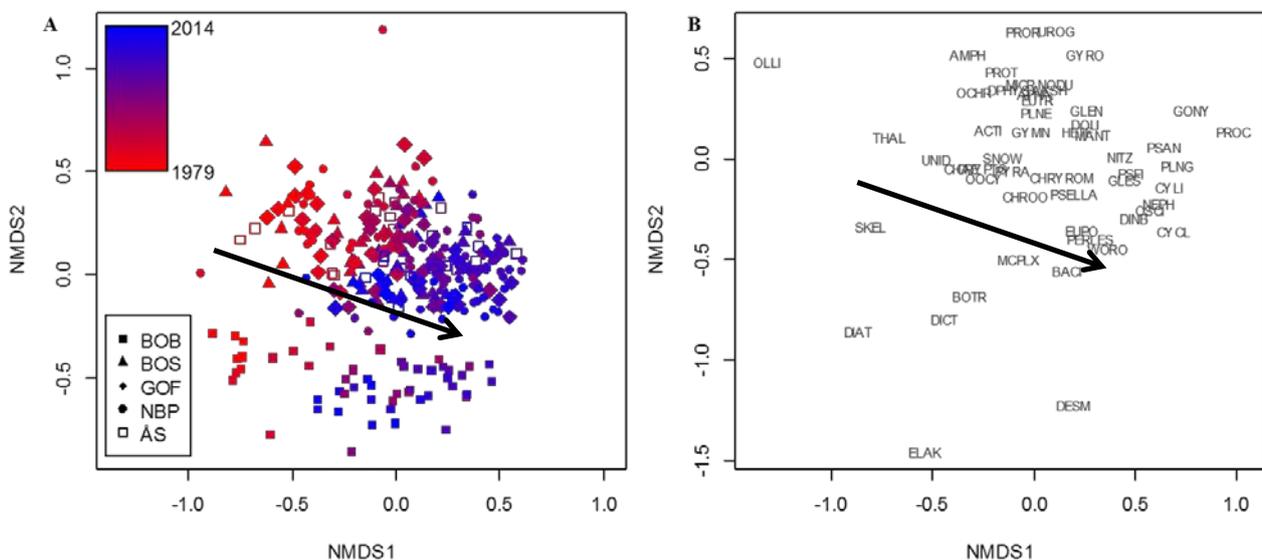


Fig. 3. A demonstration of the non-metric multidimensional scaling (NMDS) results based on northern Baltic Sea phytoplankton monitoring data. NMDS was used to cluster samples (A) based on genus-level biomass composition. The 53 genera (or orders and complexes) that the analysis is based on are plotted separately for clarity (B). The color scale represents sampling years from 1979 (red) to 2014 (blue). The HELCOM sea areas investigated were BOB = Bothnian Bay, BOS = Bothnian Sea, GOF = Gulf of Finland, NBP = Northern Baltic Proper, and ÅS = Åland Sea. Taxa: ACTI = Actinocyclus, AKSH = Akashiwo, AMPH = Amphidinium, APHA = Aphanizomenon, BACI = Bacillariales, BOTR = Botryococcus, CHAE = Chaetoceros, CHROO = Chroococcales, CHRYROM = Chrysochromulina, CRYPTO = Cryptomonadales, CYCL = Cyclotella, CYLI = Cyclodrotheca, DESM = *Desmodesmus*, DIAT = *Diatoma*, DICT = *Dictyosphaerium*, DINB = *Dinobryon*, DPHYS = *Dinophysis*, DOLI = *Dolichospermum*, ELAK = *Elakatothrix*, EUPO = *Eupodiscales*, EUTR = *Eutreptiella*, GLEN = *Glenodinium*, GONY = *Gonyaulax*, GLES = *Gymnodiniales*, GYMN = *Gymnodinium*, GYRO = *Gyrodinium*, HETE = *Heterocapsa*, MANT = *Mantoniella*, MICR = *Micromonas*, MCPLX = *Monoraphidium complex*, NEPH = *Nephroselmis*, NITZ = *Nitzschia*, NODU = *Nodularia*, OCHR = *Ochromonadales*, OLLI = *Ollicola*, OOCY = *Oocystis*, OSCI = *Oscillatoriales*, PERLES = *Peridinales*, PLNE = *Planctonema*, PLNG = *Planktolyngbya*, PROC = *Prochlorothrix*, PROR = *Prorocentrum*, PROT = *Protoceratium*, PSAN = *Pseudanabaena*, PSELLA = *Pseudopedinella*, PSFI = *Pseudoscourfieldia*, PYRA = *Pyramimonas*, SKEL = *Skeletonema*, SNOW = *Snowella*, THAL = *Thalassiosira*, UNID = *Unidentified monads and nanoflagellates*, UROG = *Uroglena*, WORO = *Woronichinia*. The figure is modified from Lehtinen et al. (*submitted*).

Step 3: Most accurate taxonomic level examination

The dominant taxa responsible for the statistically significant class-level trends are listed in Table 3, and the most important characteristics of the taxa are also included (Lehtinen et al. *submitted*).

Table 3. Taxa causing the statistically significant trends shown in Table 2. For each class with significant trends according to GAM (direction: blue = decreasing, red = increasing, orange = non-linear), the share (%) of main taxa of the total class biomass in each sea area is indicated, followed by the *p*-value of the GAM run for that individual taxon. The most important characteristics of the main taxa are also listed. The table is modified from Lehtinen et al. (*submitted*).

Main taxa responsible for class-level biomass and trends, and their characteristics	Bothnian Bay	Bothnian Sea	Åland Sea	Gulf of Finland	Northern Baltic Proper
<i>Aphanizomenon flosaquae</i> (Nostocophyceae) *N ₂ -fixing *filamentous *buoyant *low-quality food for mesozooplankton, but a thiamine source *potentially toxic/harmful for mammals *allelopathic		75% <i>p</i> = 0.001	84% <i>p</i> = 0.009	67% <i>p</i> = 0.002	
<i>Nodularia spumigena</i> (Nostocophyceae) same as <i>A. flosaquae</i>			12% <i>p</i> = 0.048		
<i>Cryptomonas</i> spp. (Cryptophyceae) *autotrophic/mixotrophic *mostly nanoflagellates *favored food for mesozooplankton	47% <i>p</i> < 0.001			60% <i>p</i> = 0.009	35% <i>p</i> < 0.001
<i>Chrysochromulina</i> spp. <i>sensu lato</i> (Prymnesiophyceae) *nanoflagellates *mixotrophic *low-quality food for mesozooplankton *potentially toxic/harmful for fish *allelopathic	100% <i>p</i> = 0.003		100% <i>p</i> = 0.015	97% <i>p</i> = 0.003	99% <i>p</i> < 0.001
<i>Diatoma tenuis</i> (Diatomophyceae) *requires silica	69% <i>p</i> = 0.001				
<i>Eutreptiella</i> spp. (Euglenophyceae) *mostly nanoflagellate-sized in the study area			100% <i>p</i> = 0.022		
<i>Pyramimonas</i> spp. (Prasinophyceae) *nanoflagellates		96% <i>p</i> = 0.007			

Step 4: Interpretation

The Bothnian Bay differed from the other areas based on its lower total phytoplankton biomass and its phytoplankton composition (Table 2). However, the community analysis demonstrated an ongoing change towards the same direction in all five sea areas, including the Bothnian Bay (Fig. 3).

In terms of harmfulness, food quality, and trophy, cyanobacteria, prymnesiophytes, and cryptophytes are the classes which potentially show the most important food web effects in the study area. *Nodularia spumigena* (Table 3) produces hepatotoxin, which accumulates in the pelagic and benthic food web and is toxic for mammals (Sipiä et al. 2001, Karjalainen et al. 2007, Sopanen et al. 2009, Karlson and Mozūraitis 2011). *Chrysochromulina* spp. *sensu lato* (Table 3) includes potentially harmful species which can form fish-killing ichthyotoxins as well as allelopathic substances which are harmful for other phytoplankton species (Granéli and Turner 2008, Reigosa et al. 2006). In addition to the increasing risk of potential harmful algal bloom effects in the ecosystem, the observed phytoplankton community changes can have direct food-web

effects through the changes in the food quality for micro- and mesozooplankton grazers. Cyanobacteria and prymnesiophytes are considered to be low-quality food for herbivorous zooplankton (de Bernardi and Giussani 1990, Sopanen et al. 2008), while cryptophytes, which decreased in most of the study area, are considered high-quality food (Lehman and Sandgren 1985).

Since *Chrysochromulina* spp. *sensu lato* includes mixotrophic species, its clear increase may indicate a shift from an autotrophic, phytoplankton-based food web towards a more mixotrophic, bacteria-based food web. According to Berglund et al. (2007), a shift towards a more bacteria-based food web may reduce pelagic productivity at higher trophic levels in the Baltic Sea, since in a more mixotrophic bacteria-based food web carbon passes additional trophic levels through flagellates and ciliates before reaching mesozooplankton, while in a more autotrophic phytoplankton-based food web there is a direct pathway from phytoplankton to mesozooplankton.

The comparison of results of the trend analyses and the community analysis showed that those taxa which were mainly responsible for the statistically significant GAM trends (i.e. *Aphanizomenon flosaquae*, *Nodularia spumigena*, *Chrysochromulina* spp. *sensu lato*, and *Cryptomonas* spp., Tables 2 and 3) were located quite in the middle of the NMDS ordination plot (*Aphanizomenon*, *Nodularia*, *Chrysochromulina*, *Cryptomonadales*, Fig. 3) suggesting that their importance in the study area as a whole has not changed markedly during the study period despite the distinct significant increase or decrease in their biomass in particular sea areas. Thus, there is obviously an ongoing phytoplankton community change in the northern Baltic Sea offshore areas that cannot be fully explained by changes of biomasses of single taxa in the different sea areas.

In conclusion, the community composition in the Gulf of Finland, the Åland Sea, the northern Baltic Proper, and the Bothnian Sea shows unsatisfactory signs (in terms of high share of cyanobacterial biomass from the total biomass, Table 2), and some trends (Tables 2 and 3), as well as the community analysis (Fig. 3) show an ongoing change towards an unsatisfactory direction in all studied sea areas (Table 4). Phytoplankton community in the Bothnian Sea is very similar to the communities in the Gulf of Finland, the Åland Sea, and the northern Baltic Proper, but based on the existing knowledge (which needs to be considered when interpreting the results), the extensive cyanobacterial blooms are not as common in the offshore areas of the Bothnian Sea as in the more southern studied areas, especially in the Gulf of Finland (Karhu and Elmgren 2014, Wasmund et al. 2015, Öberg 2015). Thus, the offshore areas of the Gulf of Finland, the Åland Sea, and the northern Baltic Proper do not reach GES (Fig. 1). In the Bothnian Bay and the Bothnian Sea, GES is reached, but trends show an ongoing change in an unsatisfactory direction, i.e. towards sub-GES (Fig. 1). To reach GES in the next EU MSFD assessment in 2018, phytoplankton class-level trends with statistically significant p-values (<0.05) in the offshore Gulf of Finland, the Åland Sea, and the northern Baltic Proper should be negative (instead of the current positive) for cyanobacteria and prymnesiophytes, and positive (instead of the current negative) for cryptophytes. In the Bothnian Sea, the trend for cyanobacteria should be negative (instead of the current positive) and new unwanted changes should not appear. In the Bothnian Bay, the trend for prymnesiophytes should be negative (instead of the current positive), and the trend for cryptophytes should be positive (instead of the current negative), and new unwanted changes should not appear. In addition, the results of the community analysis should also support the results of the trend analyses in 2018.

Table 4. A summary of results from steps 1 to 3, based on the northern Baltic Sea phytoplankton monitoring data which was used to demonstrate the indicator.

Observation	Effect	Bothnian Bay	Bothnian Sea	Åland Sea	Northern Baltic Proper	Gulf of Finland
Overall current phytoplankton community composition	Positive: High quality food items for optimal grazer community, non-toxic taxa. Negative: Low quality food items for optimal grazer community and toxic taxa.	Cyanobacteria form only ca. 2 % of the total biomass. No cyanobacterial blooms in the offshore areas.	Cyanobacteria form ca. 31 % of the total biomass. Cyanobacteria form occasionally blooms in the offshore areas.	Cyanobacteria form ca. 27 % of the total biomass. Cyanobacteria form regularly blooms in the offshore areas.	Cyanobacteria form ca. 39 % of the total biomass. Cyanobacteria form regularly extensive blooms in the offshore areas.	Cyanobacteria form ca. 38 % of the total biomass. Cyanobacteria form regularly extensive blooms in the offshore areas.
Increasing risk of potential harmful algal bloom effects in the ecosystem	Increase in algal toxins	—	↑	↑	—	↑
Cyanobacteria and prymnesiophytes increased	Direct food-web effects: more low-quality food for micro- and mesozooplankton grazers	—	↑	↑	—	↑
Cryptophytes decreased	Direct food-web effects: less high-quality food for micro- and mesozooplankton grazers	↓	—	—	↓	↓
A shift from an autotrophic, phytoplankton-based food web towards a more mixotrophic, bacteria-based food web	Reduction of pelagic productivity at higher trophic levels	autotr: ↓ mixotr: ↑	autotr: — mixotr: —	autotr: — mixotr: ↑	autotr: ↓ mixotr: ↑	autotr: ↓ mixotr: ↑
<p>Key: ↑: increase towards GES direction; ↓: decrease towards GES direction; ↑: increase towards sub-GES direction; ↓: decrease towards sub-GES direction; —: no direction. Colors concerning the overall phytoplankton community composition: green: mostly positive features; yellow: some negative features; red: many negative features.</p>						

Confidence of the indicator status evaluation

Confidence of the data used to demonstrate the indicator within this HELCOM core indicator report is high since the procedures have followed the HELCOM COMBINE Monitoring Manual (HELCOM 2015) with clearly described national details, and the microscopists have participated in HELCOM PEG workshops, and proficiency tests (e.g. Vuorio et al. 2015). Data was quality-checked in detail by the phytoplankton experts (e.g. changes in the common taxonomical knowledge during the study period were taken into account by grouping certain taxa into more appropriate taxonomic levels for the trend and community analyses), and harmonized to follow the HELCOM PEG taxon and biovolume list (version of year 2014). The sampling frequency was only once per year but the study period was as long as 36 years. A higher sampling frequency would possibly allow detecting changes already within a shorter monitoring period.

Good Environmental Status

The interpretation of results is done using expert judgement to define if the phytoplankton community composition reflects good environmental status (GES) in terms of its potential effects on the next trophic levels of the food web. Categorical values from the minimum of 1 to the maximum of 4 are used as GES units:

- 1 (green) = GES is reached,
- 2 (yellow) = GES is reached, but trends show ongoing change towards sub-GES,
- 3 (orange) = GES is not reached, but trends show ongoing change towards GES,
- 4 (red) = GES is not reached, i.e. sub-GES.

When defining the GES value, both current phytoplankton composition and the ongoing changes are taken into account (results from steps 1 to 3; Fig. 2). The statistically significant p-value 0.05 is the reference value for change. The target for toxic, low-quality food, and mixotrophic taxa is unchanged or decreasing trend ($p < 0.05$), and the target for high-quality food taxa is unchanged or increasing trend ($p < 0.05$).

Assessment Protocol

The indicator assessment protocol consists of four steps (Fig. 2, Lehtinen et al. *submitted*). Prior to the analyses, comparable data need to be selected from a data base, quality-checked, and harmonized taxonomically. Data should cover the season of tightest coupling between phytoplankton and grazers. At least one sample per year from the intended season should be available, but preferably there should be a more frequent sampling. The number of samples per year should remain the same in the long-term analyses to ensure equal representation of the years. Missing years are assigned as not available (NA) in the analyses. Multi-decadal data should be used whenever possible. A suitable updating frequency of the indicator analyses is six years, in accordance with the reporting period of the MSFD.

Offshore and coastal data should be analysed separately. Data from offshore stations located within the same sea area should be analysed together, and annual biomass averages for the studied season and sea area should be used in the trend analyses. To increase the confidence of the indicator assessment in the coastal areas, coastal phytoplankton communities should be preferably analysed station-wise for the selected stations, since environmental conditions as well as phytoplankton communities may vary significantly within short distances in coastal areas (Griffiths et al. 2015). Indicator analyses from different data sets (i.e. data originating from different data providers, different sea areas, offshore and coastal areas etc.) can be presented in the common Baltic Sea –wide indicator assessment, even though the data sets were analysed separately.

Phytoplankton biomass (wet weight per volume) data should be used in the analyses because it is more relevant from the food web perspective than abundance (counting units per volume), since the size of different phytoplankton species varies considerably. The nomenclature and biovolumes should be harmonized preferably according to the latest HELCOM PEG taxon and biovolume list (the latest version can be downloaded from <http://helcom.fi/helcom-at-work/projects/phytoplankton>). The nomenclature of the HELCOM PEG list follows that of the World Register of Marine Species (WoRMS, <http://www.marinespecies.org/about.php>). Only taxa estimated to be auto- or mixotrophic (based on light microscopy and the HELCOM PEG taxon and biovolume list) are included in the analyses, while

heterotrophic taxa, cysts, and benthic taxa (which sporadically occur in the plankton) are excluded. Unidentified < 10 µm autotrophic monads (unicellular) and flagellates may be grouped into “Unidentified”.

Within the analyses, taxa should be grouped into e.g. phylum-level instead of class-level or into order-level instead of genus-level, whenever this increases the confidence of the results. Both trend analyses (GAM) and community analyses (NMDS) are used, since their results are complementary to each other and may reveal different aspects; trend analyses study each taxon separately while community analyses aim at a more holistic view. The reason for using different taxonomic levels in the analyses is due to differing properties of the analyses. The average total phytoplankton biomass and average biomass share (%) of each phytoplankton class from the total phytoplankton biomass are calculated to have an overview of the phytoplankton composition.

Step 1: Class-level and total biomass trend analyses

The average total phytoplankton biomass and average biomass share (%) of each phytoplankton class from the total phytoplankton biomass is calculated based on the whole long-term data set. Time series for phytoplankton class biomasses and for the total phytoplankton biomass in each area are analysed using Generalized Additive Models (GAM, R package ‘mgcv’, Wood 2014). Curves estimated with GAM are plotted on the data to visualize the direction of the statistically significant long-term changes (i.e. decreasing, increasing or non-linear trends), but the final results of GAMs are presented in a table. The possible autocorrelation between years is modelled with AR1 (autocorrelation structure with lag 1).

If some taxa have been included into phytoplankton counts only after a certain year, their trends need to be analysed only since that year and their biomass should not be included into the trend analyses of total phytoplankton biomass. Some classes may be grouped into e.g. phylum-level, if this increases the reliability of the results.

Step 2: Genus-level community analysis

The Non-metric Multidimensional Scaling (NMDS, function metaMDS, R package ‘vegan’, Oksanen et al. 2015) is used to make a visual ordination of samples based on the similarities and dissimilarities in the genus-level phytoplankton community composition. Results are presented in graphs since the NMDS visualizes the phytoplankton community composition by positioning the samples in the ordination space based on their taxon-specific biomass composition. The names of the taxa characterizing the samples are plotted likewise from the same NMDS analysis. The NMDS ordination graphs thus give an overview of the phytoplankton community composition and its spatio-temporal changes, to support the results of GAMs which reveal changes in the biomasses of different phytoplankton taxa separately. Biomass values need to be square-root transformed, and the Bray-Curtis dissimilarity is used as the distance metric.

Genera which are present in less than 5% of the samples (if they are present with low biomass in all cases) are excluded from the NMDS analyses. Taxa which are excluded from the NMDS are acknowledged within most accurate taxonomic level examination (step 3). If some taxa have been included into phytoplankton counts only after a certain year, they should not be included into the NMDS. Some genera may be grouped into e.g. order-level or into taxon complexes, if this increases the reliability of the results.

Step 3: The examination of the dominant taxa on the most accurate taxonomic level

The most accurate taxonomic level data is analysed by simple biomass ratio analyses showing which taxa dominate the biomass of each phytoplankton class (step 1, GAMs). The role of the dominant taxa in each class is confirmed by running a separate GAM for these taxa to see if the result agrees with that of the total class. The results are presented in a table, in which also the specific characteristics of the dominant taxa are included.

As taxa are analysed separately within this step, it is possible to use a more accurate taxonomic level than in the NMDS (step 2) in which result of one taxon affect the whole outcome of the community analysis. The most accurate usable taxonomic level varies between taxa. When determining which taxonomic level is appropriate to be used as the most accurate taxonomic level for a certain taxon, e.g. the possible changes in the common taxonomical knowledge and changes in the microscopists need to be considered.

Step 4: Interpretation of results

In interpreting the results, all results from steps 1 to 3 are considered i.e. both current phytoplankton composition and trends are considered when assessing if the phytoplankton community composition reflects GES, in terms of its potential effects on the next trophic levels of the food web (GES: 1 = GES is reached, 2 = GES is reached, but trends show ongoing change towards sub-GES, 3 = GES is not reached, but trends show ongoing change towards GES, 4 = the area is in sub-GES i.e. GES is not reached).

The taxon-specific characteristics specifically considered when interpreting the results are the potential quality or suitability as a food source for grazers, harmfulness, cell size, and trophy (Table 4). Since these characteristics may be affected by even the life stage of the cells or vary within strains, only the potential of taxa to possess the characteristics can be considered when interpreting the results. If the class-level GAM results are based primarily on taxa for which there exists scientific knowledge on these functional properties, the statistically significant long-term trends ($p < 0.05$) are used to indicate if the ongoing changes are positive or negative for grazers. For taxa which are considered low-quality food, as well as for taxa which are potentially harmful or toxic to other organisms of the food web, the preferred trend is 'decreasing or no change', while for taxa which are considered high-quality food the preferred trend is 'increasing or no change'.

The assessment units are defined in the [HELCOM Monitoring and Assessment Strategy Annex 4](#).

Relevance of the Indicator

Food web assessment

The status of the food web is assessed using several core indicators. Each indicator focuses on one important aspect of the complex issue. In addition to providing an indicator-based evaluation of the phytoplankton community composition, this indicator will also contribute to the next overall food web assessment to be completed in 2018 along with the other food web core indicators.

Policy relevance

The policy relevance of the phytoplankton community composition indicator for the HELCOM Baltic Sea Action Plan (BSAP) and the Marine Strategy Framework Directive (MSFD) is described in Table 1.

Role of phytoplankton community composition in the ecosystem

Within this indicator, a number of phytoplankton properties (potential suitability or quality as food for grazers, harmfulness, trophy, and cell size) can be used to assess the potential efficiency of the pelagic food web, which cannot be deducted from other monitoring data. A conceptual model presenting linkages between functional characteristics of phytoplankton taxa and high and low trophic transfer efficiency in pelagic food webs is presented in Table 5 (Lehtinen et al. *submitted*).

Table 5. A conceptual model of the linkage of phytoplankton community properties (defined as functional characteristics of taxa) considered within the phytoplankton community composition indicator, to high and low trophic transfer efficiency in pelagic food webs (DOM = dissolved organic matter). The table is modified from Lehtinen et al. (submitted).

Phytoplankton community	Grazer community	Food web structure	Transfer efficiency from primary producers to top trophic levels
High quality food items for optimal grazer community (high nutritional value, optimal size and other properties, non-toxic) or efficient total particulate productivity (efficient autotrophy-based community, low respirational losses, efficient nutrient utilization, low DOM production and mixotrophy)	Optimal food items for key pelagic fish, e.g. large copepods	Direct phytoplankton-based grazing food chain	High
Low quality food items for optimal grazer community (low nutritional value, toxic, successful grazing deterrence) or a community leading to complex or inefficient food webs (very small cell size, high DOM production, complicated mixotrophy-based community, high respirational losses, inefficient nutrient utilization)	High share of low quality food items, e.g. small-sized zooplankton or gelatinous plankton	Complicated food web with high respiratory losses, e.g. due to extra trophic levels in microbial loop-based communities, or grazers with low value to predators.	Low

Human pressures linked to the indicator

Table 6. Human pressures linked to the indicator.

	General	MSFD Annex III, Table 2
Strong link	Chemicals and other pollutants Energy Biological Hydrological	Input of nutrients and organic matter Change in water temperature Introduction or spread of non-indigenous species Changes to hydrological conditions
Weak link		

The analyses of pressures and management options follow a holistic food web analysis including also other compartments (physical, chemical, and biological) in addition to phytoplankton community composition. In the Baltic Sea, changes in the planktonic food web have been linked to interactions between warming,

eutrophication and increased top-down pressure (e.g. Suikkanen et al. 2013, Elmgren et al. 2015, Kuosa et al. *submitted*) (Table 6).

Monitoring Requirements

Monitoring methodology

Monitoring of phytoplankton in the Contracting Parties of HELCOM is described on a general level in the HELCOM Monitoring Manual in the sub-programme Phytoplankton species composition, abundance and biomass [<http://www.helcom.fi/action-areas/monitoring-and-assessment/monitoring-manual/phytoplankton/species-composition-abundance-and-biomass>]

The indicator is applicable with any quality-checked long-term phytoplankton data, as long as detailed information on procedures is available with the data (in order to be able to select data sets into separate analyses based on their comparability with each other). HELCOM monitoring guidelines for phytoplankton monitoring are given in the HELCOM Monitoring Manual in the Annex C-6: Guidelines concerning phytoplankton species composition, abundance and biomass [<http://www.helcom.fi/Documents/Action%20areas/Monitoring%20and%20assessment/Manuals%20and%20Guidelines/Manual%20for%20Marine%20Monitoring%20in%20the%20COMBINE%20Programme%20of%20HELCOM.pdf>].

Current monitoring

Phytoplankton monitoring efforts (spatial and temporal) vary between the different regions of the Baltic Sea. To be able to utilize the Phytoplankton community composition indicator, monitoring needs to cover the season of tightest coupling between phytoplankton and grazers. At least one sample per year from the intended season needs to be taken, but preferably there should be a more frequent sampling.

The monitoring activities relevant to the indicator that are currently carried out by HELCOM Contracting Parties are described in the HELCOM Monitoring Manual

Sub-programme:

<http://www.helcom.fi/action-areas/monitoring-and-assessment/monitoring-manual/phytoplankton/species-composition-abundance-and-biomass#Concepts>

Description of optimal monitoring

Sampling needs to cover the seasonal period when both phytoplankton and zooplankton are abundant and trophic coupling between phytoplankton and zooplankton is potentially the highest. During that seasonal period, sampling should be preferably once per week. Even though data from all seasons is not used for the indicator, sampling during the other seasons (or at least seasons close to the preferred season) improves the confidence of the results since it facilitates detection of e.g. potential changes in the timing of species in their annual seasonal succession cycle.

Sampling should cover both offshore and coastal areas. In the offshore areas, distance between stations which represent the same sea area may be wider than in coastal areas (Jaanus et al. *submitted*). To increase the confidence of the indicator assessment in coastal areas, station-wise analysis of the phytoplankton communities should be considered, since environmental conditions as well as phytoplankton communities may vary significantly within short distances in the coastal areas (Griffiths et al. 2015).

In an optimal situation, all procedures concerning sampling, preservation, storage of samples, microscopic analysis, taxonomical identification, nomenclature, and biomass calculation follow the same detailed guidance. The HELCOM COMBINE Monitoring Manual (HELCOM 2015) is a good frame for phytoplankton monitoring procedures, but in addition to that, additional detailed information on used procedures needs to be made available with the data, in order to be able to select the comparable results (sampling depth, effort used for the microscopic analysis etc.) for an analysis. However, the indicator can be utilized also even though the procedures differ from the HELCOM COMBINE Monitoring Manual. For example, the indicator is applicable also with data sets utilizing the traditional Russian long-term phytoplankton monitoring procedures.

For the Baltic Sea –wide MSFD assessments performed every six years, the long-term phytoplankton monitoring data sets (since the starting year of the monitoring) should be updated according to the latest version of the HELCOM PEG taxa and biovolume list (available at <http://helcom.fi/helcom-at-work/projects/phytoplankton>) and made available for performing the indicator analyses.

Data and updating

Access and use

The data and resulting data products (tables, figures and maps) available on the indicator web page can be used freely given that the source is cited. Since the indicator (and certain tables and figures) is based on a specific submitted scientific article, also that original article, Lehtinen et al. (*submitted*), needs to be cited whenever appropriate. The HELCOM core indicator should be cited as following:

Lehtinen., S., Suikkanen, S., Hällfors, H., Kauppila, P., Lehtiniemi, M., Tuimala, J., Uusitalo, L. & Kuosa, H. (2016). Phytoplankton community composition as a food web indicator. HELCOM core indicator report. Online. [Date Viewed], [Web link].

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Metadata

The indicator was demonstrated with the Finnish national HELCOM COMBINE monitoring data collected during 36 years from the offshore stations located in the northern Baltic Sea (Lehtinen et al. *submitted*). Samples (the total number of samples was 288, Table 7) were taken in late summer (during the period between 15th of July to 15th of September) in years 1979-2014. Quantitative phytoplankton biomass data was used (wet weight in $\mu\text{g l}^{-1}$). The data was taken from the Finnish national database OIVA (www.ymparisto.fi/oiva). Data is stored also in the ICES database (<http://ecosystemdata.ices.dk/inventory/index.aspx>). A suitable updating frequency of the indicator analyses is six years, in accordance with the reporting period of the MSFD.

The methodology followed the HELCOM COMBINE manual (HELCOM 2015) with the following details: integrated water samples were taken with a tube sampler from the surface layer (0 to 10 m) by mixing equal amounts of water from the depths of 1, 2.5, 5, 7.5, and 10 m. Samples were preserved with acidic Lugol's solution (1 ml per 300 ml sample), and kept refrigerated (+4 to +10°C) in the dark prior to microscopic analysis within a year of sampling. Microscopy was done with an inverted light microscope using the Utermöhl method (Utermöhl 1958). A volume of 50 ml (or 25 ml, depending on the density of cells, HELCOM 2015) of sample was settled in a settling chamber. A magnification of 125x was used to count the taxa larger than 30 μm as well as taxa belonging to the order Nostocales; 250x magnification was used to count the 20 to 30 μm sized taxa, colonies belonging to the order Chroococcales with a cell size larger than 2 μm , as well as taxa belonging to the order Oscillatoriales; and 500x magnification was used to count taxa smaller than 20 μm as

well as Chroococcales colonies with cells smaller than 2 µm. With each of the three magnifications, 60 ocular squares were analyzed, aiming to count at least 400 counting units with each magnification.

During microscopic analysis and when converting the counting results to biomass (wet weight µg per liter), the taxon-specific counting units, size classes and biovolume formulae of the HELCOM PEG taxon and biovolume list v. 2014 were used (Olenina et al. 2006; the annually updated biovolume list is available at <http://helcom.fi/helcom-at-work/projects/phytoplankton>). Only taxa estimated to be auto- or mixotrophic (based on light microscopy and the HELCOM PEG taxon and biovolume list) were included in the analyses, while heterotrophic taxa, cysts, and benthic taxa were excluded. Unidentified < 10 µm autotrophic monads (unicellular) and unidentified flagellates were grouped into "Unidentified". Picoplankton (cells < 2 µm) counting is not possible with this technique.

Table 7. A total of 288 samples were used to demonstrate the indicator. Samples were taken annually in late summer (between 15th of July to 15th of September) during years 1979-2014 from the HELCOM COMBINE monitoring stations situated in the northern Baltic Sea offshore areas. Data are stored in the Finnish national database and in the ICES database. Years with missing data are also shown. BOB = Bothnian Bay, BOS = Bothnian Sea, ÅS = Åland Sea, GOF = Gulf of Finland, NBP = northern Baltic Proper.

Area	Station	Lat	Lon	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Tot		
BOB	F2	65.38367	23.46267								1							1	1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	19			
BOB	FK2	65.40033	23.11267								1																														1	
BOB	BO3	64.30500	22.35833						1	1	1	1	1			1	1	1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	26
BOB	BO3N	64.23333	22.35000			1																																			1	
BOB	BO3S	64.23367	22.34617		1	1																																			2	
BOS	US5	62.60017	20.01300		1	1																																			2	
BOS	US5B	62.58617	19.96883				1				1	1	1	1	1	1	1	1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	27
BOS	SR5	61.08333	19.58333		1	1	1		1		1	1	1	1	1	1	1	1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	29
ÅS	F64	60.18900	19.14250		1	1	1		1	1	1	1	1	1		1		1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	28
GOF	LL3	60.15017	26.33033							1																																1
GOF	LL3A	60.06717	26.34667		1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	29
GOF	LL7	59.84650	24.83782		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	34
NBP	LL12	59.48350	22.89683				1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	28
NBP	LL12A	59.60017	22.89683				1				1																															2
NBP	LL17	59.03333	21.07950		1	1		1	1		1	1	1	1	1	1			1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	27
NBP	TEIL1	59.43333	21.49683																			1																			1	
NBP	LL22	58.68333	18.66333		1																																				1	
NBP	LL23	58.58333	18.23333		1	1	1		1	1	1	1	1	1	1	1			1	1	1						1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	26

Contributors and references

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