A2.3
Pelagic habitats
Main report

Baltic Marine Environment Protection Commission

BLUES

Co-funded by the European Union

Activity 2- Biodiversity

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Activity 2.3

Overview of Task A2.3 – pelagic habitats

Introduction

Distinct gaps for the assessment of pelagic habitat under the Marine Strategy Framework Directive (i.e., MSFD Descriptor 1 (D1C6 – pelagic habitats) but also of high relevance to Descriptor 4 – food webs) and the Baltic Sea Action Plan (BSAP) were identified by the last HELCOM holistic assessment (HOLAS II, 2018). Although some assessment components were developed and applied in selected assessment units, neither a complete regional assessment nor an integrated assessment were comprehensively carried out during HOLAS II. In HELCOM BLUES, we further operationalised key indicators, improved the regional integrated assessment methodology, and provided the complete regional evaluations, as far as data allowed, for HOLAS 3 (currently under regional review, indicator reports approved and thematic assessment for biodiversity with approval after March 2023).

In our work, we focused on: (1) Complete operationalisation of the HELCOM indicator *Zooplankton Mean Size and Total Stock* (MSTS) with approved threshold values, (2) Complete operationalisation of the HELCOM indicator *Seasonal succession of dominating phytoplankton groups* with approved threshold values, (3) Indicator integration for the assessment of the pelagic habitats as a whole, and (4) Exploring the applicability of the OSPAR indicator *PH1/FW5 Plankton Lifeforms* for the Baltic plankton to evaluate the possibility of developing improved and comparative assessment approaches.

Summary

All project objectives were accomplished, and improvements made according to collated available data. More specifically:

- *Zooplankton Mean Size and Total Stock* indicator was operationalised in 10 assessment units for HOLAS 3, which is substantially more than six units assessed in HOLAS II (task A2.3.1).
- Operationalisation of the *Seasonal succession of dominating phytoplankton groups* indicator was completed, and the assessment areas with approved target values have doubled from 7 open basins in HOLAS II to 13 areas in HOLAS 3 and from 6 coastal areas in HOLAS II to 13 in HOLAS 3. (task A2.3.2).
- A new approach was proposed and applied for the indicator integration for the pelagic habitat assessment based on three biodiversity components and two eutrophication components. Four coastal areas were assessed as being in good status when only biodiversity components were used. In contrast, none of the assessment units was in good status when the eutrophication and the biodiversity components were combined and appropriate weighing factors were applied (task A2.3.3).

- A pilot test for the OSPAR *PH11/FW5 Life form* indicator was conducted in three areas of the Baltic Sea, using two lifeform pairs for phytoplankton and one for zooplankton. We have demonstrated that this approach can be used to analyse the Baltic plankton; however, more work is needed to establish ecologically meaningful lifeform pairs in the future and further evaluate the statistical validity of the method for relatively low-frequency data collected, as is commonly the case in COMBINE monitoring in the Baltic Sea (task A2.3.4).

Table A2.3. The tasks and its deliverables of the project

Task	Deliverables
Subtask 2.3.1	Complete operationalisation of the HELCOM *Zooplankton Mean Size and Total Stock* (MSTS) indicator
Subtask 2.3.2	Complete operationalisation of the HELCOM *Seasonal succession of dominating phytoplankton groups* indicator
Subtask 2.3.3	Develop an approach to combine the operationalised indicators
Subtask 2.3.4	Evaluation of unified pelagic habitat assessment approaches and development towards a viable assessment in the Baltic Sea

Key messages

Key messages for **science**

1) All plankton indicators suggest profound changes in the pelagic food web, characterised by shifts towards smaller body size of zooplankton, spatial expansion of cyanobacteria, increased biomass of diatoms and/or the autotrophic ciliate *Mesodinium rubrum*. However, the relative importance of anthropogenic pressures vs climate change for these effects is not sufficiently understood.
2) Indicators based on growth and production are needed to understand better the top-down and bottom-up mechanisms behind the observed plankton community changes.
3) Linking pelagic indicators to biochemical flows in the food web can provide a mechanistic understanding of the consequences of pelagic community changes for higher consumers.

- Key message for **policy makers**

1) Good Environmental Status is generally not achieved, with marked impacts on the pelagic habitat recorded, and eutrophication represents one of a number of determining pressures catalysing this status.
2) A better conceptualisation of GES for pelagic habitat and its components in different sub-basins of the Baltic Sea is needed for meaningful targets and policy requirements.
3) Integration of plankton-based indicators into the food web assessment (D4) is needed to have ecologically relevant targets.
4) Harmonisation of assessment scales would facilitate the integration of plankton indicators.

Use of results so far and in future

HELCOM: The results have been used in the HELCOM indicator reports (zooplankton and phytoplankton) and HOLAS 3 thematic assessment report for biodiversity.
BSAP: The results feed into several goals of the plan of "Baltic Sea ecosystem is healthy and resilient" and the ecological objective "viable populations of all native species", as well as the management objective "reduce or prevent human pressures that lead to imbalance in the food web". BSAP action B33 is addressed, by supporting filling of gaps to enable a holistic assessment for all relevant ecosystem components and pressures.
MSFD: The results will be part of the reporting on D1C6 and D4; art. 8 Guidance and they will be available for national reporting of the MSFD.
New developed approaches and results have also been brought to discussions at JRC pelagic habitat.
The achieved progress and results of the work under HELCOM BLUES A2.3 have also supported the following outputs:

- Magliozzi et al. 2021. Pelagic habitats under the MSFD D1: scientific advice of policy relevance, EUR 30671 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-35958-6, doi:10.2760/081368, JRC124882.
- Labuce, A., Gorokhova, E., 2023. A script-based workflow to calculate zooplankton community indicator for environmental status assessment in the Baltic Sea. Ecological Informatics 74, 101965. https://doi.org/10.1016/j.ecoinf.2022.101965
- Magliozzi et al. 2023. Status of pelagic habitats within the EU-Marine Strategy Framework Directive: Proposals for improving consistency and representativeness of the assessment. Marine Policy 148, 105467. https://doi.org/10.1016/j.marpol.2022.105467

Results A2.3.1 Zooplankton Mean Size and Total Stock (MSTS) indicator

The operationalisation of MSTS was expanded from six sub-basins in HOLAS II to ten sub-basins in HOLAS 3 (Fig. 2.3.1), where the indicator-based status evaluation has been completed for the following sub-basins: Bothnian Bay, Bothnian Sea, Åland Sea, Gulf of Finland, Northern Baltic Proper, Western Gotland Basin, Gulf of Riga, Eastern Gotland Basin, Gdansk Basin and Bornholm. The MSTS operationalisation for seven sub-basins was not completed due to the lack of appropriate monitoring (The Quark) and insufficient data availability and collaboration for the southern basins (Arkona, Mecklenburg Bight, Kiel Bight, The Sound, Great Belt and Kattegat). However, the indicator is applicable in these waters, and further development is needed to make it operational in the entire system (including at the level of national monitoring, harmonisation and reporting of data).

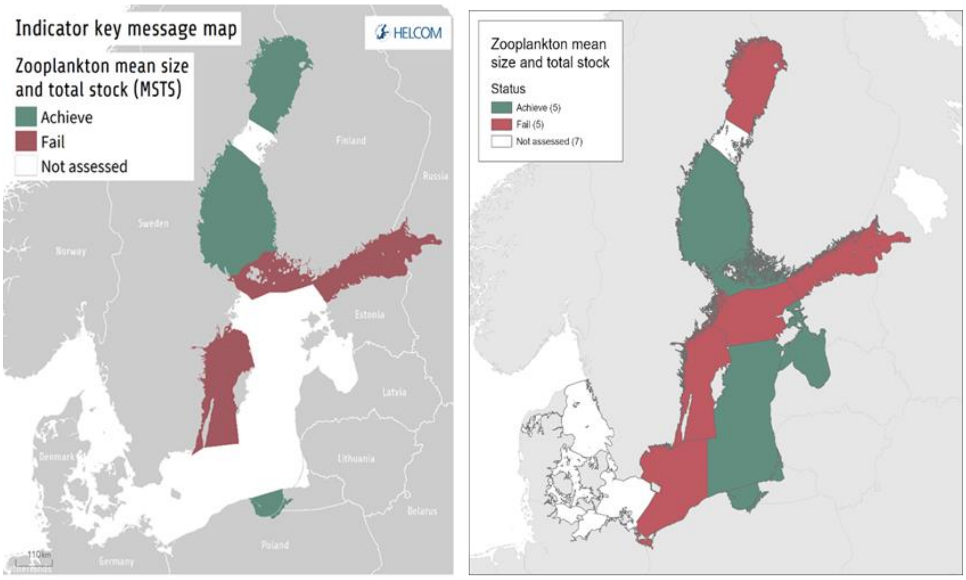

Figure 2.3.1. Evaluation of the status assessment results for zooplankton indicator *'Mean size and total stock*' (MSTS) in Holas II (left panel) and Holas 3 (right panel). The assessment was conducted on scale 2.

We applied MSTS with the pre-existing (six sub-basins) and newly derived (four sub-basins) threshold values in the HOLAS 3 assessment. Good status was found in the Bothnian Sea, Åland Sea, Gulf of Riga, Eastern Gotland Basin and Gdansk Basin. The observed MSTS values did not comply with a good status in all other assessed sub-basins, primarily due to the low mean zooplankton body size values. This negative development results from an increased contribution of rotifers and cladocerans, a possible consequence of eutrophication, and a decreased share of copepods, especially the older life-stages, which is most like due to size-selective predation by zooplanktivorous fish. The detected trends in the mean size and biomass of zooplankton indicate that the pelagic food web structure in many Baltic Sea areas is not optimal for energy transfer from primary producers (phytoplankton) to fish, and thus beyond. It is also possible, albeit not verified, that altered environmental conditions (e.g., decreased salinity, increased temperature and deep-water hypoxia) have contributed to these trends.

Application of MSTS in HOLAS 3 was conducted on the HELCOM Level 2 scale of assessment (i.e., the 17 sub-basins) because most of the zooplankton data available in databases (e.g., ICES DOME, SHARKweb) are for the open sea stations. Therefore, an inventory of the coastal data is needed to explore possibilities of establishing datasets suitable for deriving threshold values and an evaluation for coastal zooplankton communities and elevating the assessment scale to 3 (i.e., sub-basins with national coastal areas divided).
More detailed overview of the zooplankton indicator can be found in HELCOM (2023), and as document A2.3 Annex 1 to this report.

Results A2.3.2 Seasonal Succession of Dominating Phytoplankton Groups (SSDGP) indicator

The indicator evaluates the coincidence of seasonal succession of dominating phytoplankton groups (SSDPG) over an assessment period (commonly 5−6 years) with regionally established reference seasonal growth curves using wet weight biomass data.

Deviations from the normal seasonal cycle will result in failure to meet the threshold values set for acceptable variation, indicating impairment of the environmental status.
The indicator should be applicable in all coastal and open sea waters around the Baltic Sea. As compared to the previous assessment period (HOLAS II), the spatial coverage has increased from seven to 13 sub-basins in the open sea and from six to 13 units in coastal sea. The new areas included are the Bothnian Bay, Bothnian Sea, The Quark (coastal waters), the Gulf of Finland (open sea and Finnish coastal waters), Western Gotland Basin, Kiel Bay and Kattegat (Fig. 2.3.2). According to the updated division of HELCOM assessment units, Northern Baltic Proper Swedish Coastal waters are no more represented in the SSDPG. The assessment units, excluded from the indicator analysis, are not monitored with sufficient frequency and regularity (incl. too short datasets to define reference period) or have no data provided with which to apply the evaluation.
The deviations from the long-term mean reference growth curves have become less frequent during the last decade than in the 1990s and the early 2000s. This may infer an improvement in the current environmental status or at least a stabilisation of it (as the direct linkage between the reference period and GES requires further research). For this reason, compared to the previous assessment, reference periods and threshold values have been changed in the Gulf of Gdansk and in the Gulf of Riga Latvian coastal waters. Minor changes have been made in most assessment units.
Good status for the data period 2015-2020 has been achieved in four coastal water units (The Quark Swedish Coastal waters, Gulf of Riga Latvian coastal waters, Mecklenburg and Kiel bights German coastal waters) and in three open sea units (Arkona and Gdansk basins, Bothnian Bay). Positive trend expressed as a difference in the indicator values equal or more than 15% in comparison to the previous assessment were found only in Arkona Basin, The Quark Swedish Coastal waters and in the Gulf of Riga Latvian Coastal waters.
More detailed overview of the phytoplankton indicator can be found in HELCOM (2023), and as document A2.3 Annex 2 to this report.

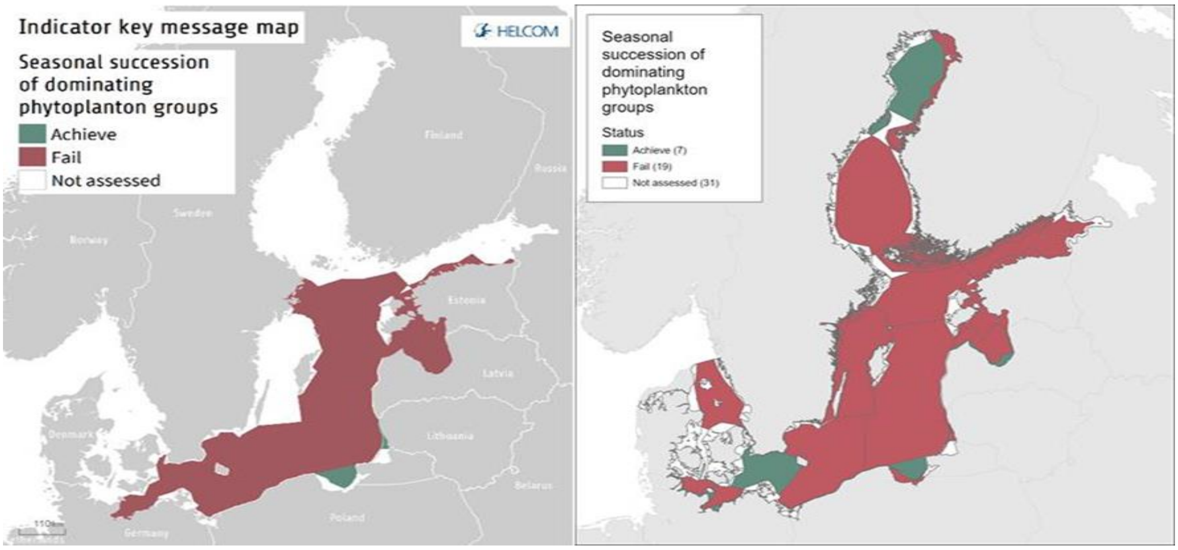

Figure 2.3.2. Assessment results for the indicator '*Seasonal succession of dominating phytoplankton groups*' in HOLAS II (left panel) and HOLAS 3 (right panel). The evaluation is carried out using Scale 3 HELCOM assessment units.

Results A2.3.3 Develop an approach to combine the operationalised indicators

The assessment of pelagic habitats is achieved by integrating the *Zooplankton Mean Size and Total Stock* HELCOM core indicator, via a One-Out-All-Out (OOAO) approach, with phytoplankton related components. The rationale for choosing OOAO was that both, phyto- and zooplankton need to be in a good condition in order to achieve a good condition in a well-balanced and properly functioning pelagic habitat (i.e., the two components are in essence inseparable). Thus, any weighted integration approach or simple averaging would allow for compensating a bad zooplankton status with a good phytoplankton status or vice versa and would not be appropriate assuming that both phyto- and zooplankton constitute essential and non-interchangeable components of a healthy pelagic ecosystem. This assessment approach closely follows the Water Framework Directive, where biological quality components are combined using OOAO. Concerning the phytoplankton related component one core indicator and a pre-core indicator exists for HOLAS 3, the *Seasonal Succession of dominating phytoplankton groups* HELCOM pre-core indicator and the *Cyanobacterial Bloom Index* (CyaBI) HELCOM pre-core indicator, and it is therefore necessary to determine a suitable approach for assessing the phytoplankton component as a whole. A weighted integration was proposed for this assessment. The logic behind the proposal is as follows: although the Cyanobacterial Bloom Index is developed as an indicator predominantly for eutrophication purposes it does directly address a biodiversity aspect (i.e. specific phytoplankton taxa and utilizes biomass/abundance information) and can therefore be more logically integrated with other biodiversity components (unlike Chlorophyll-a or water clarity that are less directly linked to biodiversity). However, since the Cyanobacterial Bloom Index is developed as an indicator predominantly for eutrophication purposes it should receive a lower weighting in the integration process (see figure 2.3.3a).

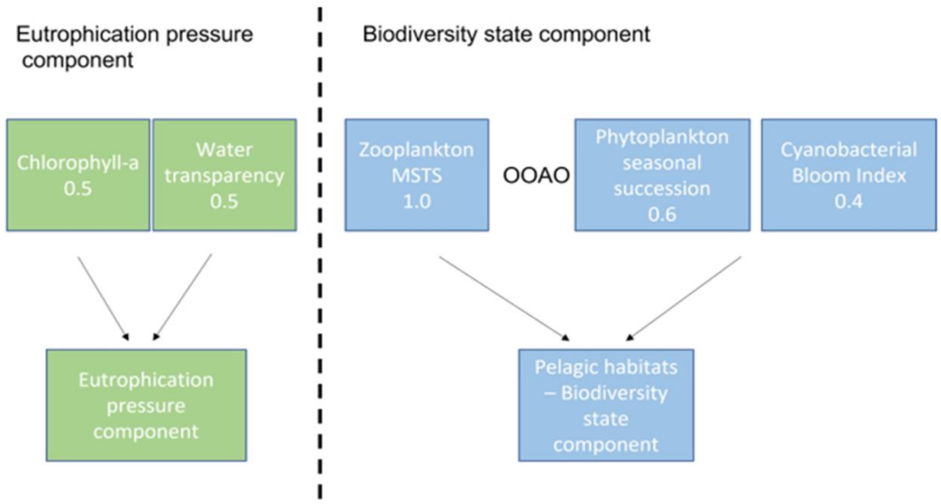

Figure 2.3.3a. Schematic presenting overview of BEAT integration components and weighting for the Assessment of Pelagic habitats for HOLAS 3. Numbers within the boxes (each box representing a separate HELCOM indicator) represent the weighting of that component in the BEAT integration process.

The proposed scheme and approach for the integrated assessment of pelagic habitats also aligns closely to the requirements of the MSFD, where the eutrophication components are ‘to be taken into account’ (though further development is still needed for other pressures to be taken into account suitably in the future). Thus, in the approach developed in BLUES for HOLAS 3, and subsequently approved by HELCOM Contracting Parties, the biodiversity and eutrophication parameters are maintained as separate components and integrated mainly via a description of the interactions between the two (Fig. 2.3.3b). This is also more reflective of the fact that changes in the eutrophication parameters (or pressures) may for example act as drivers of future change that is potentially not seen in status assessment carried out in parallel (e.g., biological impacts or recovery may lag behind changes in a pressure).

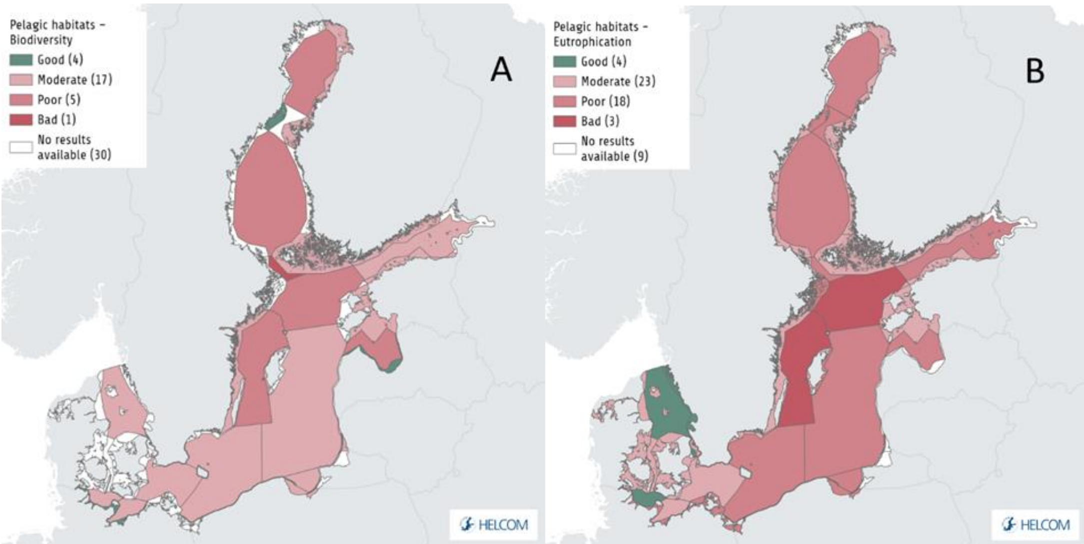

Figure 2.3.3b. Result of Integrated assessment of biodiversity components (A) and eutrophication components (B).

When assessing only the biodiversity components together (fig A) GES is only achieved in Kiel Bight German coastal waters, Mecklenburg Bight German coastal waters, Gulf of Riga Latvian Coastal waters and The Quark Swedish Coastal waters. The eutrophication components (fig B) combined with the biodiversity components (fig A) indicate that the whole Baltic is assessed as below GES from a eutrophication aspect. Confidence values for the integrated assessment range between intermediate-high.
The evaluation information was incorporated into the HOLAS 3 thematic assessment report for biodiversity with a dedicated chapter on pelagic habitats.

Relationships between Zooplankton MSTS and Cyanobacteria bloom index (CyaBI)

In the integrated assessment of the pelagic habitats, CyaBI is being used as both eutrophication and biodiversity indicator because, on the one hand, filamentous nitrogen-fixing cyanobacteria blooms are a sign of eutrophication, but on the other hand, CyaBI values reflect the structural changes of the phytoplankton community and thus, food provisioning for zooplankton grazers. The core biodiversity indicator, Zooplankton MSTS, is related to eutrophication pressure (supporting small-sized grazers that can efficiently use cyanobacteria-dominating food webs) and fishing (changing fish communities and thus predation on large-sized zooplankters). Thus, zooplankton is sandwiched between these two dynamic pressures, and, therefore, it is challenging to delineate their relative contributions and interpret the indicator behaviour.

We explored the relationships between the components of Zooplankton MSTS (mean size and total biomass of zooplankton community) and CyaBI (cyanobacteria surface accumulations and cyanobacterial biomass in the water column) to understand the indicator linkages based on cyanobacteria - zooplankton interactions in the Baltic Sea and provide a rationale for combining CyaBI and MSTS in the integrated assessment of pelagic habitats. The data (2003-2015) for all sub-basins experiencing summer cyanobacteria blooms were used.

There were negative relationships between the mean size of zooplankton and cyanobacteria biomass, and between total zooplankton biomass and CyaBI values, with substantial differences between the sub-basins (Fig. 2.3.3c), whereas no clear relationships with zooplankton variables were found for the surface accumulations of cyanobacteria. Moreover, for several sub-basins, zooplankton communities with GES-compliant mean size and biomass values were observed at moderate CyaBI values close to the target values (0.6-0.8), indicating that cyanobacteria production is important for maintaining a healthy zooplankton community. These findings supported the ecological rationale for eutrophication as a pressure for Zooplankton MSTS in the Baltic Sea. When a suite of indicators is being used for the integrated assessment of the pelagic habitat, CyaBI and Zooplankton MSTS provide independent yet complementary information on the status of the primary producers and primary consumers under varying eutrophication load.

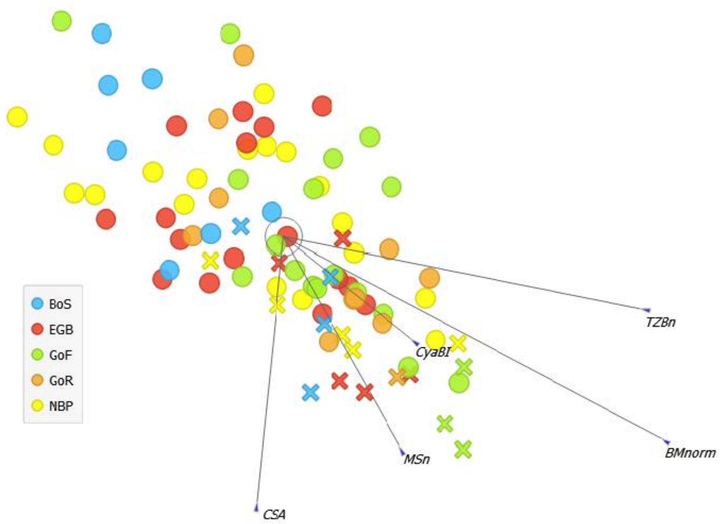

Fig. 2.3.3c. Linear projection (FreeViZ visualisation) of the data on zooplankton mean size (MSn) and total biomass (TZBn), the two components of Zooplankton MSTS indicator (normalized values to account for the differences between the subbasins), and cyanobacteria bloom indicator (CyaBI) based on the surface accumulations (CSA) and

cyanobacteria biomass in the water column (BMnorm; normalised values). The data for 2003-2015 from five sub-basins (Bothnian Sea: BoS, Eastern Gotland Basin: EGB, Northern Baltic Proper: NBP, Gulf of Finland: GoF, and Gulf of Riga: GoR) were used. The vectors pointing in similar directions indicate positive relationships between the attributes. Note that high values for Cyabi, BMnorm and CSA correspond to lower abundances of cyanobacteria.

Results A2.3.4 Evaluation of unified pelagic habitat assessment approaches and development towards a viable assessment in the Baltic Sea

A workshop with participants from NEA PANACEA and HELCOM experts was organised and the conclusions of the PELAGIC WS 1-2021 indicated that the paired approach (lifeform pairs for plankton) being further developed under NEA PANACEA would likely be the most effective approach for the Baltic Sea region for the long-term development, considering aspects like the plankton indicator integration. Thus, test case options were explored and used for a preliminary evaluation in the Baltic Sea for an unified pelagic habitat assessment. Lifeforms are taxa that are not necessarily taxonomically related but play similar functional roles in the food web. When grouped in ecologically complementary pairs, their time trends can be analysed in concert to indicate changes in ecosystem status and function. In the North-East Atlantic (OSPAR) region, an indicator considering plankton lifeform dynamics has been developed (McQuatters-Gollop et al. 2019). Here, a pilot study evaluating the applicability of the mentioned lifeform pair indicator, *PH1/FW5 Plankton lifeforms (PH1/FW5),* was conducted to explore whether its approach could complement pelagic habitat assessment in the Baltic Sea, and, if so, lay the foundations for future development in the region.

*PH1/FW5 i*s a state indicator used in environmental assessment in the OSPAR region by analysing phytoplankton and zooplankton time series obtained with the Continuous Plankton Recorder survey, but also, albeit to a lesser extent, fixed-station sampling. Most of these datasets span more than 55 years, with high-frequency data covering seasonal variability relatively well. The results of the currently available *PH1/FW5* assessment (i.e., OSPAR Intermediate Assessment in 2017; OSPAR, 2017) indicated profound structural changes in the pelagic ecosystem. Moreover, some lifeforms used in *PH1/FW5* have been linked to climate change (Bedford et al., 2020).

Plankton Community Index, also known as *Plankton Index*, is used to quantify changes in PH1/FW5 (see https://www.ospar.org/documents?v=39001 for details on the *PH1/FW5* indicator approach). Selecting ecologically meaningful lifeform pairs is crucial for PH1/FW5 indicator applicability. Eight lifeform pairs are analysed in the OSPAR region assessment (OSPAR, 2017). Unfortunately, these lifeform pairs are not applicable for the Baltic plankton because *PH1/FW5* was developed for plankton communities that are more diverse than in the Baltic Sea and specific to the North Sea environment. Moreover, the OSPAR work includes groups that are not present, not monitored (e.g., jellyfish), or not monitored with a sufficient frequency to allow for the quantitative seasonal development assessment (e.g., meroplankton) in the Baltic Sea. A possible exception is the lifeform pair *Diatoms and dinoflagellates*, which might also be applicable in the Baltic Sea and can provide information

about eutrophication pressure, energy transfer or changes in water column stability (Wasmund et al., 2017).
Current HELCOM indicators for pelagic habitat include the core indicator *Zooplankton Mean Size and Total Stock* (MSTS), the pre-core indicator *Seasonal succession of dominant phytoplankton groups*, and the pre-core indicator *Cyanobacteria Bloom Index*, which is developed foremost as a eutrophication (D5) indicator. These indicators address specific properties of the Baltic plankton communities. The MSTS indicator is conceptually similar to PH1/FW5 lifeform pair *Small and large copepods* because both indicators address variability in zooplankton body size. Similar to the *Small and large copepods* lifeform pair, MSTS reflects the relative contribution of large copepods (e.g., *Limnocalanus macrurus* in the Bay of Bothnia) and large-size classes of cladocerans (e.g., *Evadne nordmanni* and *Bosmina maritima* in the Gulf of Riga and the eastern Gotland basin) to zooplankton community. By contrast, both phytoplankton indicators used in the Baltic Sea represent properties that only slightly overlap with the lifeform approach. Therefore, identifying lifeform pairs for Baltic plankton may provide a complementary approach when assessing the status of the pelagic habitat and requires careful future discussion at the regional (i.e., HELCOM Expert Group) and sub-regional (e.g., sub-basin scale) level.

The pilot study

Testing of *PH1/FW5* approach was carried out to evaluate the potential of the tool in the Baltic Sea region, as well as explore potential harmonisation with OSPAR tools. The current work is preliminary and focusses dominantly on exploring the potential for application (e.g., data suitability and tool functionality) but aims to provide a first step towards improved assessments of pelagic habitats in the Baltic Sea in the future. The pilot study below explores an initial evaluation of the lifeform pair approach utilising data available in selected assessment units of the Baltic Sea.

Calculating PH1/FW5 indicator. An open-source R-script for PH1/FW5 (https://github.com/hollam2/PH1_PLET_tool) was adapted to the data from three sub-basins of the Baltic Sea: Western Gotland Basin, the Bothnian Sea, the Gulf of Riga. Only open-water monitoring stations were considered. The script uses data to calculate PH1/FW5 and outputs the resulting figures providing Plankton Index for each lifeform pair and Kendall's statistic for the entire time series for each lifeform.

Selection of the lifeform pairs. For this test, we provisionally identified the following lifeform pairs based on the functional and taxonomic representation of the Baltic plankton:

1. *Diatoms and dinoflagellates*; this lifeform pair is currently used in OSPAR region, where it primarily reflects vertical mass transport and benthic-pelagic coupling efficiency; moreover, it may indicate eutrophication and food web changes (Wasmund et al., 2017). In the Baltic Sea sub-basins, diatoms and dinoflagellates contribute 60–90% to the total annual biomass. These two groups appear to be functionally surrogates as both are able to effectively exhaust the wintertime accumulation of inorganic nutrients and produce bloom level biomass that contribute to vertical export of organic matter. High diatom biomass, especially during the spring bloom, indicates stronger sedimentation that may support benthic consumers. On the other hand, in deeper areas with permanent hypoxia, sinking quickly out of the euphotic zone, diatoms rather fuel oxygen deficiency.

In contrast, increasing dinoflagellates at the expense of diatoms imply low vertical transport of organic matter and facilitate mineralization in the water column or sink as inert resting cysts. The proliferation of dinoflagellates with high encystment efficiency could increase sediment retention and burial of organic matter, alleviating the eutrophication problem and improve the environmental status of the Baltic Sea (Spilling et al., 2018). This lifeform pair is also known to respond to climate change, especially to milder winters that promote increased dinoflagellates (Wasmund et al., 2017).

2. *Cyanobacteria and mixotrophic ciliate Mesodinium rubrum*; this lifeform pair primarily reflects difference in nutritional status and energy transfer in the system. Cyanobacteria are able to thrive in a phosphorous rich environment as they are able to acquire and use inert nitrogen. The mixotrophic ciliate *M. rubrum,* on the other hand, can use dissolved nutrients but also prey, being part of the microbial food web, when nutrients are low. The rationale is that cyanobacteria dominance implies an excess of phosphorous (eutrophication) and when the system shifts to the prevalence of *M. rubrum* it might be more biased towards the microbial loop and heterotrophic processes.
3. *Microphagous zooplankton grazers* (rotifers, cladocerans and copepod nauplii) and *macrophagous grazers* (copepodites excluding nauplii); the rationale is that microphagous mesozooplankton are favoured by high availability of primary producers with small sizes, such as picoplankton, in eutrophied systems. By contrast, macrophagous copepods feeding preferentially on large and slow-growing phytoplankton and ciliates, would not have that advantage. Therefore, we expected this lifeform pair to respond to an increase in eutrophication manifested as an increase in microphagous zooplankton and a concomitant decrease in the macrophagous copepods.

PH1/FW5 is designed to analyse changes in the seasonal cycle based on monthly observations of plankton abundances, with at least one observation per month. However, the sampling frequency in the Baltic Sea plankton monitoring (COMBINE) is often less frequent, especially during the late fall-early spring. Hence, <u>our first objective</u> was to test whether meaningful PH1/FW5 analysis can be conducted using seasonal instead of monthly averaging. This was done by comparing the PH1/FW5 indicator performance calculated for monthly and seasonal Baltic plankton data obtained from the HELCOM COMBINE database, hosted by ICES. <u>The second objective</u> was to compare the outcome of the PH1/FW5 indicator calculations for *Microphagous zooplankton grazers and macrophagous grazers* between the abundance- and biomass-based data.

Results

<u>PH1/FW5 indicator for the selected lifeform pairs.</u> PH1/FW5 identified significant changes in plankton communities between the assessment period and the comparison period for all the studied sub-basins and lifeform pairs, implying high sensitivity of the approach to the changes in the community. *Cyanobacteria and mixotrophic ciliate Mesodinium rubrum* was the only lifeform pair successfully performed using either monthly or seasonal data in all tested sub-basins. Performance of *Diatoms and dinoflagellates* lifeform pair was acceptable in the Western Gotland Basin and Bothnian Sea but not in the Gulf of Riga,

where the assessment period domain did not result in a well-resolved doughnut shape (see left panel of Figure 2.3.4.B). For the *Microphagous zooplankton grazers and macrophagous grazers* lifeform pair, no reliable assessment domain was established in any sub-basin when the data were aggregated by season.

Applicability of PH1/FW5 for low-frequency data. The conclusions concerning trends were similar for all lifeform pairs regardless of whether the data were aggregated by month or season. For the lifeform pairs that converged to a well-resolved state space, the chi-square test outputs for the calculations based on monthly- and season-aggregated data were also similar. However, consistently lower Plankton Index (PI) values were obtained for the seasonal than for the monthly data ($PI_{monthly}$: 0.43 to 0.73, $PI_{seasonal:}$ 0.32 to 0.52). Therefore, toward our first objective, we can conclude that PH1/FW5 approach can be applied for plankton data analysis with less than monthly temporal coverage by averaging the observations for winter, spring, summer and autumn seasons. However, regular monthly monitoring is crucial for the indicator-based assessment (Magliozzi et al., 2023) if we are to detect changes occurring outside the growth season, which might be particularly indicative of the climate-induced alterations in plankton communities.

Consistency between PH1/FW5 based on abundance and biomass estimates. The comparison between the abundance- and biomass-based evaluation for *Microphagous zooplankton grazers and macrophagous grazers* resulted in similar conclusions regarding the trend direction and significance. Although this is a positive sign, more lifeform pairs comprised of taxa with high seasonal variability in body size/stage should be used for this evaluation. Abundance may be more appropriate for some lifeform pairs and biomass for others.

Conclusions

A systematic search for the ecologically relevant lifeform pairs in the Baltic plankton is needed to introduce the PH1/FW5 indicator for the pelagic habitat assessment in the Baltic Sea. This work should include (1) database compilation for plankton functional traits, (2) identification and validation of relevant lifeform pairs for each sub-basin and the appropriate metrics (abundance or biomass) for the Plankton Index calculations, and (3) implementation of appropriate data aggregation to account for low sampling frequency outside of the growing season. Such work would benefit from future projects and also needs to consider regional and sub-regional aspects. There may also be benefits from reviewing regional monitoring to explore other sources or the need for adjusted monitoring across the Baltic Sea region. The general approach however, does show promise for future improved assessments of pelagic habitats in the Baltic Sea.

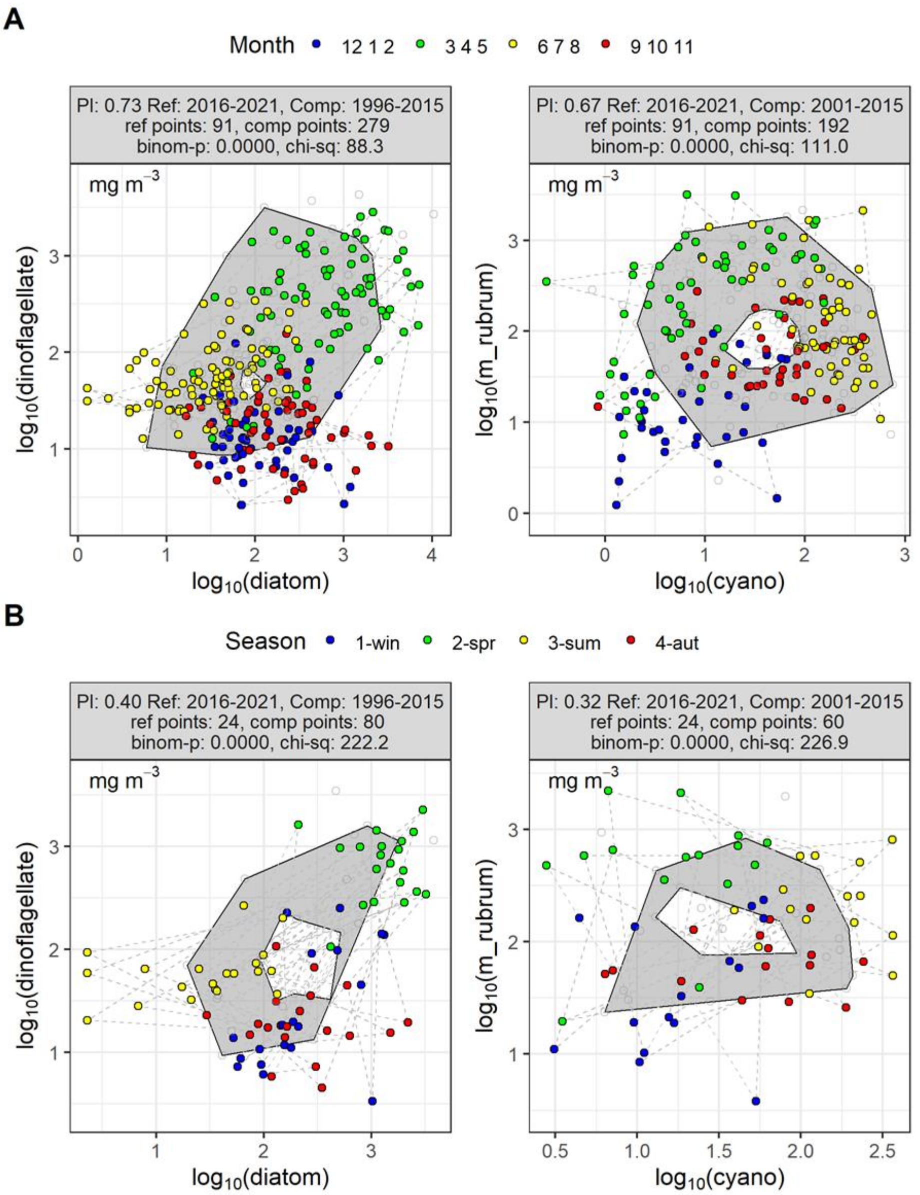

Figure 2.3.4. Application of PH1/FW5 approach for lifeform pairs in the Gulf of Riga: A) monthly and B) seasonal abundances of phytoplankton lifeform pairs plotted in a state-space. Left-side panel: Diatoms and dinoflagellates; right-side panel: Cyanobacteria and mixotrophic ciliate Mesodinium rubrum*. Monthly averages for each lifeform pair are plotted in a two-dimensional space, with the first lifeform plotted on the X-axis and the second on the Y-axis (Tett et al., 2013). Plotting multiple abundances produces a circular 'domain' describing the intra-annual co-variation of the lifeforms during the defined period. The plankton community corresponding to the assessment period is used to establish this domain, whereas the data preceding this period are plotted to overlie the assessment period and represent a comparison period. The change is evaluated by calculating the proportion of the data points for the comparison period falling into the assessment period domain, with 10% of points expected to fall outside in congruent communities. The change is quantified as a standardised PI and used to describe variations in the state of the lifeform pairs.*

Abbreviations: PI – Plankton Community Index; Ref – assessment period; Comp – comparison period; ref points – data points in the assessment period (used in the establishment of the domain (filled in grey)); comp points – data points in the comparison period (coloured according to the legend); binom-p – p value (significance); chi-sq. – chi square coefficient.

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A2.3 Annex 1
Zooplankton indicator report

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HELCOM

Zooplankton

INDICATOR TYPE: State
INDICATOR CATEGORY: Core
BSAP SEGMENT: Biodiversity
MSFD CRITERIA: D4C3

Zooplankton mean size and total stock (MSTS)

Table of contents

1 Key message

This Zooplankton Mean Size and Total Stock HELCOM core indicator evaluates the zooplankton community structure to determine whether it reflects good environmental status (GES). Due to strong environmental gradients and community variations, size distribution and zooplankton total stock corresponding to good status vary between the Baltic Sea sub-basins. As a rule, good status is achieved when large-bodied zooplankters (older stages of calanoid copepods and adult cladocerans) are abundant in the plankton community.

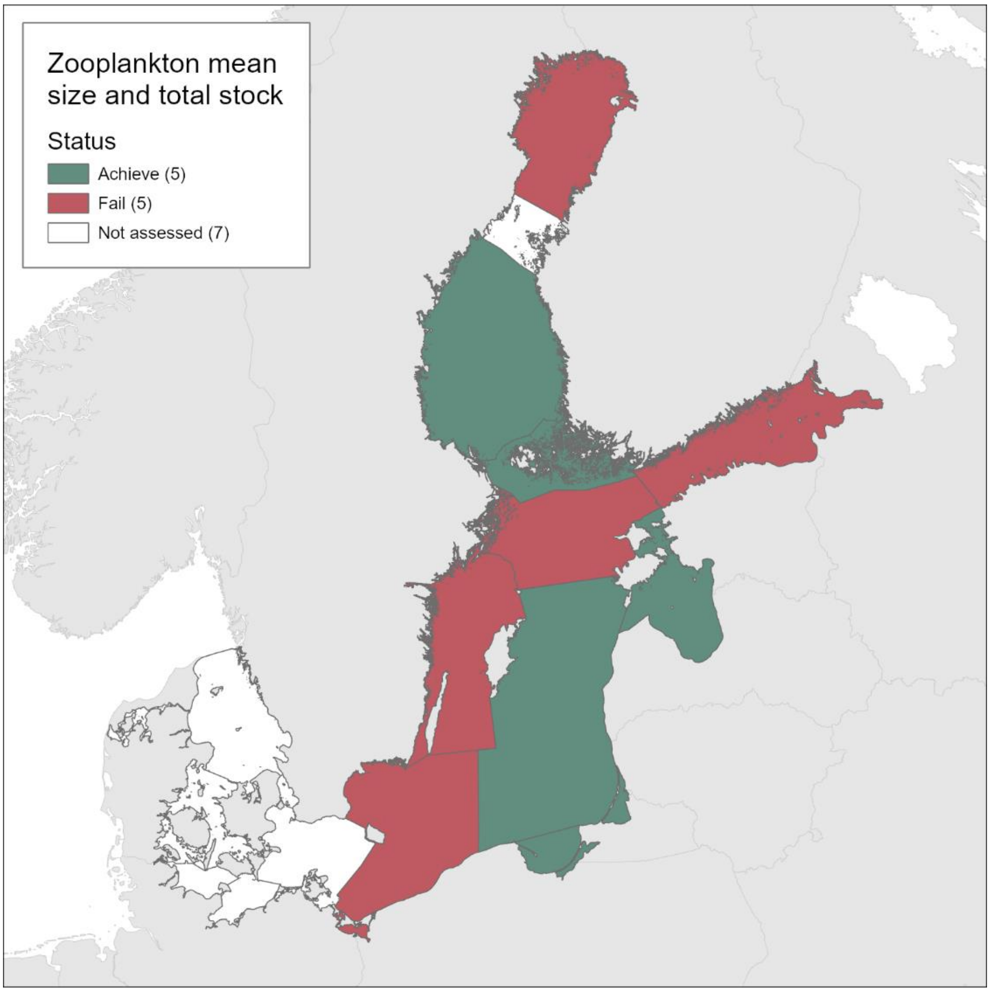

Figure 1. Evaluation of the status evaluation results for zooplankton indicator 'Mean size and total stock' (MSTS) in HOLAS 3. Due to national database issues Danish zooplankton data are not included in this evaluation. The evaluation is carried out using Scale 2 HELCOM assessment units (for more information see the HELCOM Monitoring and Assessment Strategy Annex 4. Click here to access interactive maps at the **See 'data chapter' for interactive maps and data at the HELCOM Map and Data Service.**

The indicator-based status evaluation has been completed for the following subbasins: Gulf of Bothnia, Gulf of Finland, Åland Sea, and Northern Baltic Proper, Gulf of Riga, Western Gotland Basin, Gdansk Basin and Bornholm. The evaluation for the other subbasins is mostly hampered by the availability of the data and lack of national expert engagement.

Good status during the assessment period 2016-2021 was found in the Bothnian Sea, Åland Sea, Gulf of Riga, Eastern Gotland Basin and Gdansk Basin (Figure 1). In the rest of the evaluated subbasins, the MSTS does not comply with a good status during the assessment period, mostly, due to the low mean size values. This negative development results from both an increased contribution of rotifers and cladocerans, a probable consequence of eutrophication, and a decreased share of copepods, especially the older stages, which is a probable consequence of size-selective predation by zooplanktivorous fish. It is also possible, albeit not verified, that altered environmental conditions (e.g., decreased salinity, increased temperature and deep-water hypoxia) have contributed to these trends. The detected trends in the mean size and total stocks of zooplankton communities indicate that in many Baltic Sea areas, today's pelagic food web structure is not optimal for energy transfer from primary consumers (phytoplankton) to fish.

The confidence of the indicator evaluation with regards to spatial and temporal resolution is low to intermediate and varies across the basins since the data used cover fairly long time periods for the sub-basins where the evaluation results are completed, and monthly sampling frequency is usually applied. However, the number of stations is usually low.

The indicator is applicable in the waters of all the countries bordering the Baltic Sea. However, currently the indicator is not operational in some assessment units, and further development work is needed to make it operational in the entire system.

- As a rule, good status is achieved when large-bodied zooplankters are abundant in the plankton community.
- Ten of the 17 HELCOM Scale 2 Assessment Units are evaluated.
- The confidence in the indicator evaluation is deemed to be moderate as the data series are generally reasonably long (i.e. >20 years), sampling frequency is at least monthly, but the number of stations is low.
- Good status is achieved in the Bothnian Sea, Åland Sea, Gulf of Riga, Eastern Gotland Basin, and Gdansk Basin.
- Good status is not achieved in the Bothnian Bay, Gulf of Finland, Northern Baltic Proper, Western Gotland Basin, and Bornholm.
-

1.1 Citation

The data and resulting data products (e.g. tables, figures and maps) available on the indicator web page can be used freely given that it is used appropriately and the source is cited. The indicator should be cited as follows:

HELCOM (2023) Zooplankton mean size and total stock. HELCOM core indicator report. Online. [Date Viewed], [Web link].

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2 Relevance of the indicator

Zooplankton includes an array of macro and microscopic invertebrates. They play a vital role in the marine food web. The herbivorous zooplankton feed on phytoplankton and in turn constitute prey to animals at higher trophic levels, including fish. Therefore, zooplankton are an essential link in aquatic food webs, influencing energy transfer in the pelagic food webs and recruitment to fish stocks as well as ecosystem productivity, nutrient and carbon cycling. Hence, the evaluation of zooplankton communities is a prerequisite for analysis of pelagic food web structure.

The mean size of a zooplankter in the community is indicative of both fish feeding conditions and grazing pressure from zooplankton on phytoplankton. Large stocks of zooplankton composed of large-bodied organisms have a higher capacity for transfer of primary producers (phytoplankton) to fish, i.e. higher energy transfer efficiency. By contrast, dominance of small-bodied zooplankton is usually associated with lower energy transfer efficiency, due to higher losses. Thus, a high community biomass of zooplankton with large individual body size represents both favourable fish feeding conditions and a high potential for efficient utilization of primary production. According to ecological theories, this would represent an efficient food web and correspond to a good environmental status. All other combinations of zooplankton stock and individual size would be suboptimal and imply food web limitations in terms of energy transfer through the food web and productivity.

2.1 Ecological relevance

Zooplankton play an important role transferring primary production to zooplanktivorous fish. However, different zooplankton taxa often have different preferences for trophic state of the ecosystem and are of different value as prey for zooplanktivores, because of the variations in size, escape response, and biochemical composition. In the Baltic Sea, alterations in fish stocks and regime shifts received particular attention as driving forces behind changes in zooplankton (Casini *et al.* 2009). With the position that zooplankton has in the food web – sandwiched between phytoplankton and fish (between eutrophication and overfishing) – data and understanding of zooplankton are a prerequisite for an ecosystem approach to management.

With respect to the eutrophication-driven alterations in food web structure, it has been suggested that with increasing nutrient enrichment of water bodies, total zooplankton abundance or biomass increases (Hanson & Peters 1984), mean size decreases (Pace 1986), and relative abundance of large-bodied zooplankters (e.g. calanoids) generally decrease, while small-bodied forms (e.g., small cladocerans, rotifers, copepod nauplii, and ciliates) increase (Pace & Orcutt 1981).

Total zooplankton abundance and biomass

In lakes and estuaries, herbivorous zooplankton stocks have been reported to correlate with chlorophyll *a* and phytoplankton biomass (Pace 1986; Nowaczyk *et al.* 2011; Hsieh *et* *al.* 2011), but also with total phosphorus (Pace 1986). In general, total zooplankton stocks increase with increasing eutrophication, which in most cases is a result of the increase in small herbivores (Gliwicz 1969; Pace 1986; Hsieh *et al.* 2011). Both parameters have been recommended as primary 'bottom-up' indicators (Jeppesen *et al.* 2011).

In most areas of the Baltic Sea, copepods contribute substantially to the diet of zooplanktivorous fish (e.g. sprat and young herring), and fish body condition and weight-at-age (WAA) have been reported to correlate positively to abundance/biomass of copepods (Cardinale *et al.* 2002; Rönkkönen *et al.* 2004). In coastal areas of the northern and central Baltic Sea, WAA has been suggested to be used as a proxy for zooplankton food availability and related fish feeding conditions to fish recruitment (Ljunggren *et al.* 2010).

Herbivorous zooplankton biomass is indirectly impacted by eutrophication via changes in primary productivity and phytoplankton composition, whereas direct impacts are expected mostly from predation, and to a lesser extent, from introduction of synthetic compounds (at point sources) and invasive species (via predation). The latter can also be indirect if invasive species are changing trophic guilds, which may affect zooplankton species. Finally, zooplankton abundance and biomass are affected – both positively and negatively – by climatic changes and natural fluctuations in thermal regime and salinity.

Mean zooplankter size

Evidence is accumulating that a shift in zooplankton body size can dramatically affect water clarity, rates of nutrient regeneration and fish abundance (Moore & Folt 1993). Although these shifts can be caused by a variety of factors, such as increased temperatures (Moore & Folt 1993; Brucet *et al.* 2010), eutrophication (Yan *et al.* 2008; Jeppesen *et al.* 2000), fish predation (Mills *et al.* 1987; Yan *et al.* 2008, Brucet *et al.* 2010), and pollution (Moore & Folt 1993), the resulting change implies a community that is well adapted to eutrophic conditions and provides a poor food base for fish. It has been recommended to use zooplankton size as an index of predator-prey balance, with mean zooplankton size decreasing as the abundance of zooplanktivorous fish increase and increasing when the abundance of piscivores increase (Mills *et al.* 1987).

2.2 Policy relevance

The indicator on zooplankton mean size and total stock addresses the Baltic Sea Action Plan (BSAP 2021) vision of “a healthy Baltic Sea environment with diverse biological components functioning in balance, resulting in a good ecological status and supporting a wide range of sustainable economic and social activities”, in particular being relevant to the Biodiversity goal of a “Baltic Sea ecosystem is healthy and resilient” and the subsequent ecological objectives of “Natural distribution, occurrence and quality of habitats and associated communities” and “Functional, healthy and resilient food webs”.

The core indicator also contributes to the MSFD in supporting a determination of good environmental status under MSFD Descriptor 4 and Descriptor 1 (Commission Decision (EU) 2017/848). More specifically the indicator addresses size distribution of individuals across the trophic guild and supports an evaluation of condition of the habitat type (pelagic habitats). This core indicator is among the few indicators able to evaluate the structure of the Baltic Sea food web with known links to lower and higher trophic levels (Table 1).

Table 1. Policy relevance of this specific HELCOM indicator.

	Baltic Sea Action Plan (BSAP)	**Marine Strategy Framework Directive (MSFD)**
Fundamental link	Segment: Biodiversity Goal: "Baltic Sea ecosystem is healthy and resilient" • Ecological objective: "Functional, healthy and resilient food webs". • Management objective: "Reduce or prevent human pressures that lead to imbalance in the food web".	Descriptor 4 Ecosystems, including food webs • Criteria 3 The size distribution of individuals across the trophic guild is not adversely affected due to anthropogenic pressures. • Feature – Shelf ecosystems. • Element of the feature assessed – Trophic guilds. • Criterion D4C1 The diversity (species composition and their relative abundance) of the trophic guild is not adversely affected due to anthropogenic pressures. • Feature – Trophic guilds. • Element of the feature assessed – Trophic guilds: Secondary producers • Criterion D4C2 The balance of total abundance between the trophic guilds is not adversely affected due to anthropogenic pressures. • Feature – Trophic guilds. • Element of the feature assessed – Trophic guilds: Secondary producers
Complementary link	Segment: Biodiversity Goal: "Baltic Sea ecosystem is healthy and resilient" • Ecological objective: "Functional, healthy and resilient food webs". • Management objective: "Reduce or prevent human pressures that lead to imbalance in the food web".	Descriptor 1 Species groups of birds, mammals, reptiles, fish and cephalopods • Criteria 6 The condition of the habitat type, including its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), is not adversely affected due to anthropogenic pressures. • Feature – Pelagic broad habitats. • Element of the feature assessed – Trophic guilds.
Other relevant legislation:	UN Sustainable Development Goal 14 (Conserve and sustainably use the oceans, seas and marine resources for sustainable development) is most clearly relevant, though SDG 12 (Ensure sustainable consumption and production patterns) and 13 (Take urgent action to combat climate change and its impacts) also have relevance.	

2.3 Relevance for other assessments

The status of biodiversity 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 mean size and total stock of zooplankton, this indicator, along with the other biodiversity indicators, contributes to the overall biodiversity assessment of pelagic habitat.

3 Threshold values

This core indicator employs zooplankton mean size (MS; µg wet mass/ind.) and total stock (TS as total zooplankton biomass, mg wet mass/m^3) to evaluate pelagic food web structure, with particular focus on lower food webs. MSTS evaluates whether good status is achieved using two threshold values, one for the mean size and one for the total stock of zooplankton (Figure 2 and Table 2).

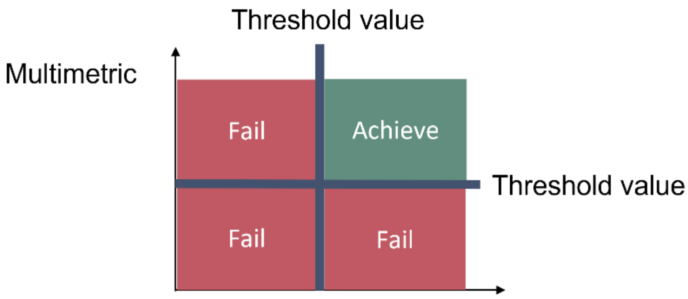

Figure 2. Schematic representation of the threshold value applied in the 'Zooplankton mean size and total stock' core indicator (see Table 2 for the threshold values).

An area is evaluated as having achieved good status using the MSTS indicator when both mean size and total stock are above their specific threshold values (Figure 3), and one-sided lower CuSum values (see section 9.2) confirm no significant deviation from the target.

Table 2. Due to strong environmental gradients affecting structure of plankton communities, including zooplankton, in the sub-basins of the Baltic Sea, the threshold values for each indicator component (mean size and total stock) are specific for each assessment unit. The indicator is evaluated using Scale 2 HELCOM assessment units.

Assessment unit (Scale 2)	**Threshold value** mean size (µg wet weight ind^{-1}) / total stock (mg m^{-3})
Kattegat (SEA-001)	Not currently evaluated
Great Belt (SEA-002)	Not currently evaluated
The Sound (SEA-003)	Not currently evaluated
Kiel Bay (SEA-004)	Not currently evaluated
Bay of Mecklenburg (SEA-005)	Not currently evaluated
Arkona Basin (SEA-006)	Not currently evaluated
Bornholm Basin (SEA-007)	14.9 / 273
Gdansk Basin (SEA-008)	10.2 / 103
Eastern Gotland Basin (SEA-009)	14.1 / 104
Western Gotland Basin (SEA-010)	5.1 / 220
Gulf of Riga (SEA-011)	4.7 / 253
Northern Baltic Proper (SEA-012)	9.8 / 123
Gulf of Finland (SEA-013)	8.6 / 125
Åland Sea (SEA-014)	10.3 / 55
Bothnian Sea (SEA-015)	8.5 / 84
The Quark (SEA-016)	Not currently evaluated
Bothnian Bay (SEA-017)	23.7 / 161

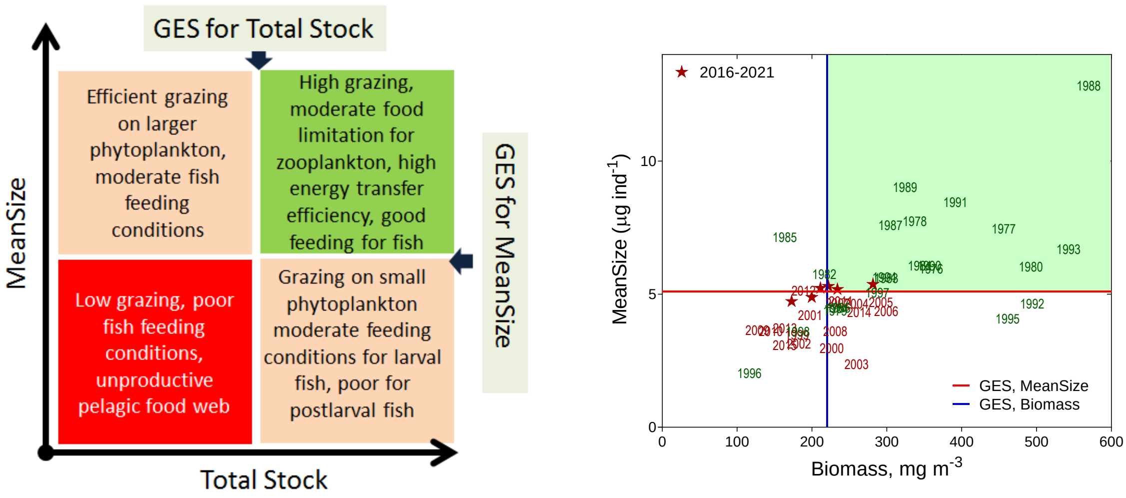

Figure 3. The MSTS concept (left) and a data example (right) to illustrate the use of the indicator. The green area on the left panel represents good status conditions, orange areas represent not good status where only one of the two parameters is adequate and the red area represents not good status where both parameters fail to meet the threshold value. On the right panel, an example of long-term zooplankton data for mean size and total biomass (stations B1 and BY31, Western Gotland Basin) were analysed. The corresponding thresholds are shown as red and blue lines, respectively. The years in green were classified as in good status and those in red as not in good status. Years of the assessment period are shown as stars. Generally, all years located in the right upper quadrant (green area in panel A) reflect good status. However, some years (e.g., 1979, 1985, 1994, etc.) are classified as reflecting good status, although they are placed outside of the green area. For these years, even though the absolute values for the indicator components (MeanSzie and Biomass) are below the threshold value, the deviation is not significant as determined by CuSum analysis. To achieve a significantly sub-GES value, the change must be persistent and cumulative negative change must exceed 5σ difference from the threshold value. Similarly, some years (e.g., 2007) are classified as sub-GES, although they are placed in the green area; during these years the observed values were above the thresholds, however this has not resulted in a significant shift in any of the MSTS component that was sufficiently persistent to return the MSTS values in the GES state. See the Assessment protocol for details.

3.1 Setting the threshold values

The threshold values are set using a reference period which defines a status when the food web structure was not measurably affected by eutrophication and represents good fish feeding conditions within the time series of existing data. Thus, the reference periods for MSTS reflects a time period when effects of eutrophication (defined as 'acceptable' chlorophyll *a* concentration) are low, whereas nutrition of zooplanktivorous fish is adequate for optimal growth. Hence, these are the periods when eutrophication and overfishing related food web changes are negligible. In some cases, reference periods can be adopted from neighbouring areas, for which longer datasets are available.

As the indicator evaluates the structural- and functional integrity of the food web, the threshold values are conceptually achieved when:

- there is a high proportion of large-sized individuals (usually older copepodites and adults of copepods but also adult cladocerans) in the zooplankton community that efficiently graze on phytoplankton and provide good-quality food for zooplanktivorous fish, and

- the biomass (abundance) of zooplankton is at an adequate level to transfer primary production to the higher trophic levels, support fish growth and exert control over phytoplankton production.

Two alternative strategies for setting reference conditions are possible.

1. The first approach should be used when the data series are very short or when chlorophyll *a* concentration or zooplanktivorous fish body condition indices are invariant for the entire length of the zooplankton data series. Conceptually this approach is similar to using a trend as a threshold value. When using this approach, the long-term mean and corresponding variance (95% confidence interval, CI) for both the mean size and the total stock parameter are calculated based on the entire available dataset. The lower bound of 95%-CI is then used as threshold value to evaluate deviations in the current observations. This approach was used in the MSTS-based evaluation of the Gulf of Riga in 2016-2021.
2. The second approach is based on (i) specific reference conditions for chlorophyll *a* concentration ($RefCon_{Chl}$) that have been defined for the different sub-basins of the Baltic Sea (either observed in the past or based on models), and (ii) reference data on clupeid fish (young herring and sprat) that are used to identify the reference time periods ($RefCon_{Fish}$) when both the fish growth (i.e. weight-at-age, WAA, or other body condition indices, such as fat content) and fish stocks were relatively high in the relevant ICES subdivisions. Once the reference time periods have been identified based on chlorophyll *a* and fish time series, the threshold values for both mean size and total stock were defined as the lower bound of the 99%-CI for the respective mean values calculated for zooplankton time series during the reference time period. This approach was used for the 2016-2021 assessment period in most of the assessment units (Figure 3).

4 Results and discussion

The results of the indicator evaluation that underlie the key message map and information are provided below.

4.1 Status evaluation

The evaluation of zooplankton mean size and total stock (MSTS) for the period 2016-2021 indicates that in the Bothnian Sea, Åland Sea, Gulf of Riga, Eastern Gotland Basin, and the Gdansk Basin, the MSTS values were above the threshold values indicating good status. By contrast, in the Bothnian Bay, Northern Baltic Proper, Gulf of Finland, Western Gotland Basin, and Bornholm, the MSTS values were significantly below the threshold values, which implies that good status has not been achieved. The details for each of the evaluated sub-basins are presented below.

In the Bothnian Bay (Figure 4a), MSTS has changed over the assessment period, with biomass value decreasing below its threshold, whereas MS value did not change appreciably. The biomass decline occurred over the entire data series (1979 – 2016); however, up to the 2016-2021 period, the occasional deviations of the annual average values were not sufficient to produce a significant deviation from the threshold value as indicated by CuSum analysis. During 2016-2021, however, the continued biomass decline resulted in sub-GES values, with annual values for 2020 and 2021 falling below the 5σ below the threshold. The populations that were responsible for this decline will be identified in the final evaluation report.

In the Bothnian Sea (Figure 4b), MSTS suggests good food web structure, with no indication of the decline in the overall status over the assessed period. In the Åland Sea (Figure 4c), starting from 1996, zooplankton mean size stayed significantly below the threshold and the total biomass values were often below the threshold values. In 2016-2020, the mean size has increased (most likely, due to the increased population of the large copepod *Limnocalanus macrurus*, following that in the Bothnian Bay (Mann-Kendall test, $P < 0.01$). As a result, the mean size reached the target values, and the threshold crossing was significant. The overall evaluation for MSTS indicator in this basin was, therefore in-GES, although this evaluation was based on the limited number of observations and will be revised when the Finnish data are incorporated.

In the Gulf of Finland (Figure 4d), the values of the mean size indicate that the system was not in good status from 2001 onwards. Also, the biomass failed the threshold during the same years on multiple occasions, albeit not significantly. Thus, MSTS indicates that in 2016-2021, the zooplankton community was not in good status, i.e., the overall status did not change since the previous assessment period. Moreover, the mean size was significantly decreasing for the entire time series, including the last decade (trends table 1).

In the Northern Baltic Proper (Figure 4e), the MSTS indicates that the system was not in good status since 1997, progressively worsening. In 2016-2021, the zooplankton community was not in good status due to the low mean size, whereas the total biomass was not significantly below the threshold.

In the Western Gotland Basin (Figure 4f), the MSTS indicates that the system was not in good status since 1998, although some signs of recovery, such as significantly increasing mean size, appear during the last decade (trends table 1). Nevertheless, during the assessment period 2016-2021, zooplankton community was not in good status.

In the Gulf of Riga (Figure 4g), the MSTS indicates that the system was in good status, although occasionally low values for the mean size were observed in some years. Moreover, the biomass in this sub-basin is progressively increased since the start of the time series (i.e., 1993).

In the Eastern Gotland Basin, (Figure 4h), the MSTS indicates that the system was still in good status, although both mean size biomass values were very close to the threshold for several years. Moreover, the increasing trend for the abundance without a corresponding increase in biomass (Table 3) suggests changes in the community structure, which need to be evaluated in order to understand the population-level responses over time.

In the Gdansk Basin (Figure 4j), the MSTS values indicate that the system was in good status, with no decrease from its reference state for the last 30 years with regard to both mean size and biomass values. On the contrary, zooplankton abundance, biomass and mean size have been increasing during the last 12 years (Table 3).

In the Bornholm Basin (Figure 4k), the MSTS indicates that the system was not in good status, mostly due to the significantly sub-GES mean size values. Notably, the threshold for the mean size was crossed already in the year 2006. When only the last 12 years of the data were considered, no significant trend in either the mean size or the biomass were detected.

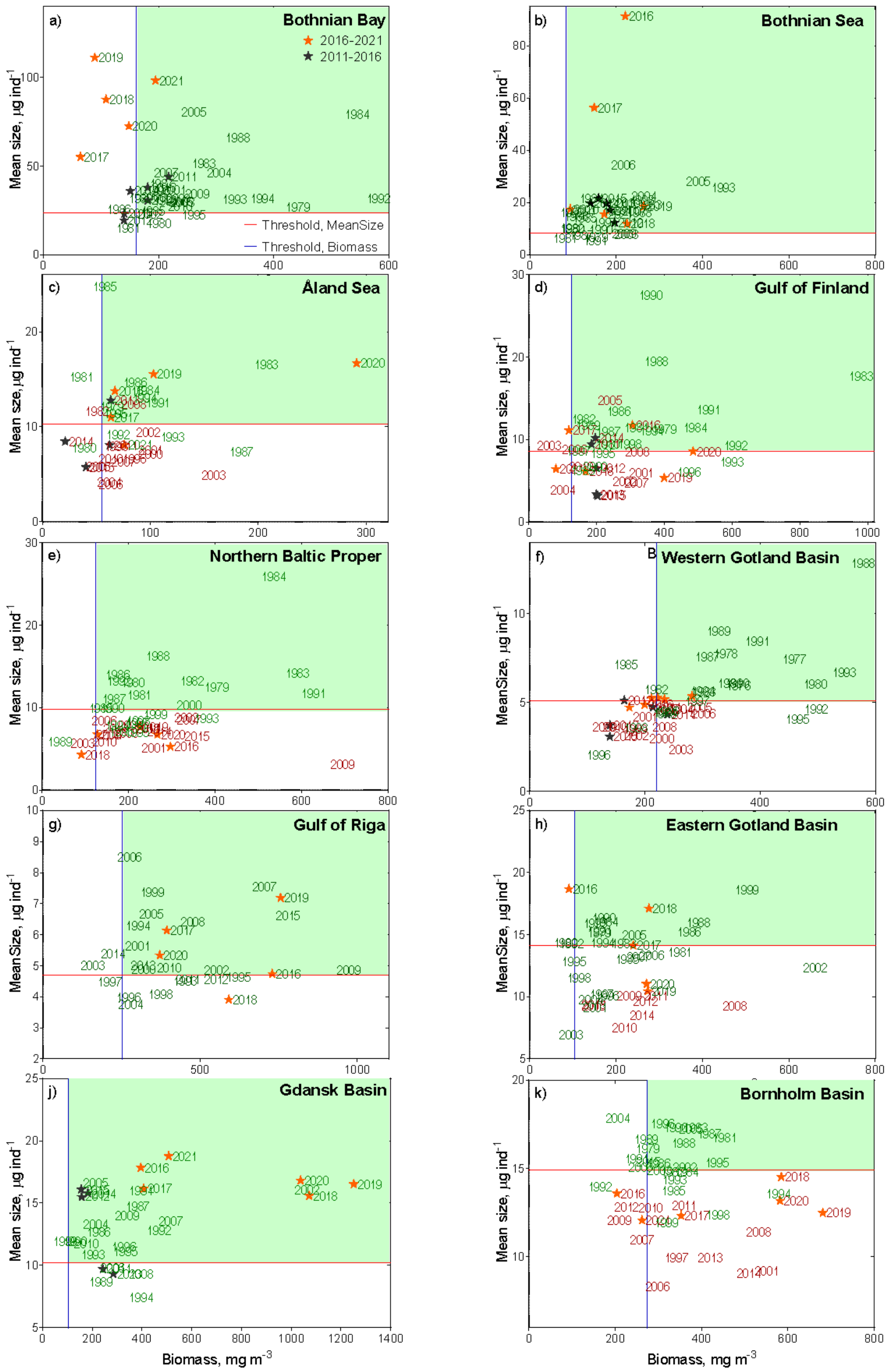

Figure 4. Evaluation results on the performance of MSTS indicator, which integrates mean size (Y axis) and total biomass of zooplankton (X axis). Blue and red lines show threshold values for the total biomass and mean size, respectively. The green-shaded quartile indicates good status. Observations in good and in not good status are shown as green and red years, respectively. Stars indicate the assessment period years (black: 2011 to 2016, orange: 2016-2021). Note that some years falling below the threshold values were assigned as being in good status because these values were not judged as significantly different from the threshold value according to the CuSum analysis, which is based on the cumulative summing of the persistent deviations from the reference mean.

4.2 Trends

Significant long-term trends were observed for zooplankton stock metrics (abundance and biomass) and the mean size in all evaluated sub-basins with the Bothnian Sea as a single exception (Table 3 and Figure 5). When the entire time series were considered, the most prominent change was observed for the mean size that declined in six out of 10 sub-basins. This decline was no longer detectable for the last 12 years in the Åland Sea, Northern Baltic Proper, Eastern Gotland Basin, and Bornholm Basin, partly due to the lower statistical power for the shorter data sets, but also because in some cases (e.g., Western Gotland Basin), the trend became positive.

Table 3. Long-term trends for zooplankton biomass, abundance, and mean size in the sub-basins evaluated in HOLAS 3. The Mann-Kendall test for trend was applied using the entire data series available and then repeated for the last 12 years to understand the most recent changes. The significant ($p < 0.05$) increasing and decreasing trends are indicated as ↑ and ↓, respectively, and → indicates no significant change. Data are taken as provided in ICES DOME database.

Sub-basin	***Entire time series***				***Last 12 years***		
	Biomass	**Abundance**	**Mean size**	**Period (years)**	**Biomass**	**Abundance**	**Mean size**
Bothnian Bay	↓	↓	↑	1979-2021	↓	→	↑
Bothnian Sea	↑	→	↑	1979-2021	→	→	→
Åland Sea	→	→	↓	1982-2021	→	→	→
Northern Baltic Proper	→	↑	↓	1979-2021	→	→	→
Gulf of Finland	→	↑	↓	1980-2021	→	→	→
Gulf of Riga	↑	→	→	1993-2021	→	→	→
Eastern Gotland Basin	→	↑	↓	1979-2021	→	→	→
Western Gotland Basin	↓	→	↓	1976-2021	→	→	↑
Gdansk Bay	→	→	↑	1986-2021	↑	↑	↑
Bornholm	→	→	↓	1979-2021	→	→	→
All subbasins assessed	→	↑	↓	1976-2021	→	↑	→

To understand the reasons behind the observed changes in the indicator components, and, especially, the mean size, population studies are needed, with a particular focus on the demography of the key taxa. For example, when the zooplankton community structure in the Western Gotland Basin is explored.

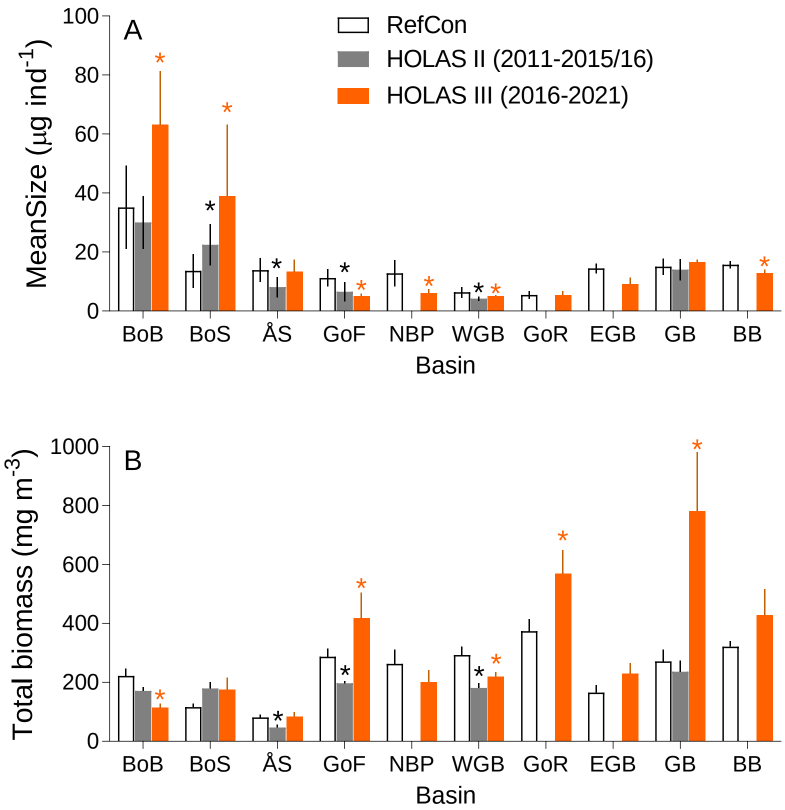

Figure 5. Pair-wise comparisons between the MSTS values observed during the current assessment period (2016-2021) and in HOLAS II (2011-2016) for mean zooplankton size (Mean size; A) and total zooplankton biomass (Total biomass; B) in the Bothnian Bay (BB), Bothnian Sea (BS), Åland Sea (ÅS), Northern Baltic Proper (NBP), Gulf of Finland (GoF), Western Gotland Basin (WGB), Gulf of Riga (GoR), Gdansk Basin (GB), and Bornholm Basin (BB). The basin-specific data were compared using unpaired t-test with Welch correction and statistically significant differences ($p < 0.05$) are indicated with percent change and red asterisk. For the nearly significant difference in ÅS, the *p* value is shown. Percentage values indicate change (positive or negative) in the value observed for the assessment period relative to the reference period. Data are shown as means and standard deviations for the untransformed data; the statistical comparisons were done using Box-Cox transformed values that were normally distributed.

4.3 Discussion

Developments from the last assessment period

The difference in the MSTS components between the reference conditions and the HOLAS 3 assessment period varied from -34% to +75% for the mean zooplankter size (µg ind^{-1}) and from -42% to +42% for the total biomass (mg m^{-3}) among the sub-basins (Figure 5). Prominent decreases in both body size and total biomass of zooplankton were observed in the Åland Sea, Gulf of Finland and Western Gotland Basin, where size and total biomass decreased by 39% and 38%, respectively, from the reference period to the assessment period (2011-2016). Similar changes occurred in the Bornholm Basin (preliminary evaluation) where mean size and biomass decreased by 20% and 39%, respectively.

Contrary to all other sub-basins, both mean size and biomass have increased in the Bothnian Sea from the reference period to the assessment period (Figure 4). The increase observed in the Bothnian Sea is related to an increased population size of the large-bodied copepod *Limnocalanus macrurus*. This species, which is a glacial relict in the Baltic Sea, responded positively to the low salinity conditions during the last decade, which improved herring feeding conditions (Rajasilta *et al.* 2014) as well as MSTS values in this sub-basin. In the other sub-basins, species that contributed to the detected changes in the MSTS components varied. However, regardless of the variability among the species and species groups contributing to general declines in body size and biomass values among the sub-basins, an increase in proportion of small-sized taxa and groups was observed in all assessment units (except the Bothnian Sea). In the Gulf of Finland, the change is largely attributed to a decline in the biomass of large cladocerans. In the Western Gotland Basin and the Bornholm Basin, the decline in mean size and total biomass is mostly due to declining copepod populations and thus shifting size spectra and biomass of the zooplankton communities.

An overview is provided in Table 4.

Table 4. Evaluation summary for each assessment unit and comparison between assessment periods, where relevant.

HELCOM Assessment unit name (and ID)	**Threshold value achieved/failed**	**Distinct trend between current and previous evaluation.**	**Description of outcomes, if pertinent.**
Kattegat (SEA-001)	Not evaluated	NA	Not evaluated
Great Belt (SEA-002)	Not evaluated	NA	Not evaluated
The Sound (SEA-003)	Not evaluated	NA	Not evaluated
Kiel Bay (SEA-004)	Not evaluated	NA	Not evaluated
Bay of Mecklenburg (SEA-005)	Not evaluated	NA	Not evaluated
Arkona Basin (SEA-006)	Not evaluated	NA	Not evaluated
Bornholm Basin (SEA-007)	Failed	First iteration of indicator	Zooplankton abundance (but not the biomass) has been increasing from the mid-1990-ties (significant trend), whereas the mean size of the organisms significantly decreased. In 2016-2020, the mean size of zooplankton was significantly lower than in the reference period.
Gdansk Basin (SEA-008)	Achieved	No change	Zooplankton abundance, biomass, and mean size have been increasing since the mid-1980-ties (significant trend). In 2016-

			2020, the biomass was significantly higher than in the reference period.
Eastern Gotland Basin (SEA-009)	Achieved	First iteration of indicator	Zooplankton abundance has been increasing since the mid-1980-ties (significant trend), whereas biomass and mean size decline. If the trend continues, the status in the next reference period can be sub-GES.
Western Gotland Basin (SEA-010)	Failed	Partially improved	Zooplankton biomass and mean size have been decreasing since the mid-1970-ties (significant trends). However, during the last decade, the mean size significantly increased and now is close to the target value, although both biomass and mean size are still significantly lower than during the reference period.
Gulf of Riga (SEA-011)	Achieved	First iteration of indicator	Zooplankton biomass and mean size are not below their respective target values. Moreover, the biomass has been significantly increasing since the beginning of the time series. The increase is related to both large-sized (copepods) and small-sized (rotifers and small cladocerans) biomasses (data not shown).
Northern Baltic Proper (SEA-012)	Failed	First iteration of indicator	Abundance of small-sized organisms has been significantly increasing since the mid-1980-ties, resulting in a significant decrease of the total biomass and the mean size of zooplankton. In 2016-2021, the mean size was significantly lower than during the reference period and a similar, albeit not significant, tendency was observed for the biomass.
Gulf of Finland (SEA-013)	Failed	Partially improved.	Zooplankton abundance has been increasing since the beginning of the time series, due to increase in small-sized taxa, which resulted in a significant decrease of the mean size. During the previous and the current assessment periods, the mean size was significantly below the target value, with no appreciable change between the periods. However, the biomass significantly increased in relation to both reference period and 2011-2015/16, which can be considered as, at least, a partial improvement of the fish feeding conditions.
Åland Sea (SEA-014)	Achieved	Improved	The mean size has been decreasing since the beginning of the time series. Although the trend has not been significantly reversed during the last decade, both mean size and biomass values for the 2016-2019

			were above the threshold and were considered as in-GES.
Bothnian Sea (SEA-015)	Achieved	No change	Zooplankton biomass and mean size are not below their respective target values. Moreover, the mean size has significantly increasing compared to the previous assessment period due to increase in the large copepod population abundance and biomass.
The Quark (SEA-016)	Not evaluated	NA	
Bothnian Bay (SEA-017)	Failed	Worsened	The abundance and biomass of zooplankton have been significantly decreasing since the end of 1970-ties. During the assessment period, the biomass decline resulting in crossing the threshold and the overall status became sub-GES.

The methodology and previously established threshold values (i.e. as applied in HOLAS II) have not been altered between the current (HOLAS 3) and prior (HOLAS II) assessment periods. Therefore, a direct comparison between the two periods is valid.

5 Confidence

The overall confidence of the evaluation varies from low to high between the assessment units. With regard to the spatial coverage, the confidence varies from low to intermediate because the number of sampling stations used for the evaluation is relatively low (1 to 4). With regard to the temporal coverage, the sampling frequency varies from annual (e.g., Gdansk Basin) to bi-weekly (e.g., WGB); therefore, the confidence is evaluated as low to high. With regard to the methodology, the confidence is high, because in the Baltic Sea, zooplankton sampling, analysis and evaluation are well harmonized between the national laboratories.

The data availability is the main reason for the variation in the confidence across the assessment units. Also, confidence of the evaluation accuracy depends on the time series length and between-year variability during the reference period. It is also important that confidence is comparable for the reference and the evaluation values, which usually holds for our zooplankton data (Figure 5) as the number of stations and sampling frequency are relatively stable between years.

Zooplankton monitoring stations are generally found in every Baltic Sea sub-basin, and suitable monitoring data series are available for relatively long (>18 years) time periods from most of the sub-basins. A similar confidence in the evaluation (moderate to high) is expected for the most evaluated basins with fairly similar length of the data sets and similar number of observations (number of data points per basin and per year). However, in case of low observation frequency (for example, Åland Sea and Gdansk Basin, where only August data were used from a single station each year), the confidence is low.

The accuracy component of the confidence is considered to be high also because of the statistical method evaluating whether the thresholds for the mean size and biomass values defined as acceptable have been significantly crossed when comparing the values observed during the assessment period and the reference period. This confidence classification is due to:

(1) the CuSum technique that is used to determine whether the observed value reflects good status or not is considered to be a very sensitive method for detecting persistent small changes (Lucas 1982),

(2) the lower bound of 99% confidence interval around the baseline (reference condition) was used as threshold, thus minimizing the risk of false negatives (i.e., assigning not good status to an observation that is in fact reflecting good status), and

(3) using a pre-cautionary principle by selecting the higher value after comparing threshold values obtained for $RefCon_{Fish}$ and $RefCon_{Chl}$ for each part of the indicator (i.e. mean size and total biomass).

In aquatic ecosystems, a hierarchical response across trophic levels is commonly observed; that is, higher trophic levels may show a more delayed response or a weaker response to eutrophication than lower ones (Hsieh *et al.* 2011). Therefore, alterations in planktonic primary producers and primary consumers have been considered among the most sensitive ecosystem responses to anthropogenic stress, including eutrophication (Schindler 1987; Stemberger & Lazorchak 1994).

The core indicator responds to fishing and eutrophication but also other drivers causing changes in the food web, such as salinity and temperature, that are particularly relevant in the context of the Baltic Sea. Other pressures that might be involved are environmental contaminants causing adverse effects on the zooplankton (e.g., Vezi *et al.* 2019) and bottom hypoxia (e.g., Keister and Tuttle, 2013). The regression analysis conducted during the evaluation procedure confirmed that all metrics in question (mean size, total zooplankton abundance, and total biomass) can change significantly when chlorophyll *a* and herring weight-at-age (WAA) values are outside of their reference conditions (Gorokhova *et al.* 2016). The effects of fishery activities and eutrophication, although potentially co-occurring, would have different outcomes:

Increased eutrophication and dominance of bacterio- and picoplankton leads to a selective advantage for grazing by small-sized zooplankton taxa. Hence, the declining trend in mean size, but not total stock are likely to occur due to the increased abundance of the microphagous taxa. In moderately eutrophied systems, an increase in the total abundance and/or biomass can be observed (Figure 6).

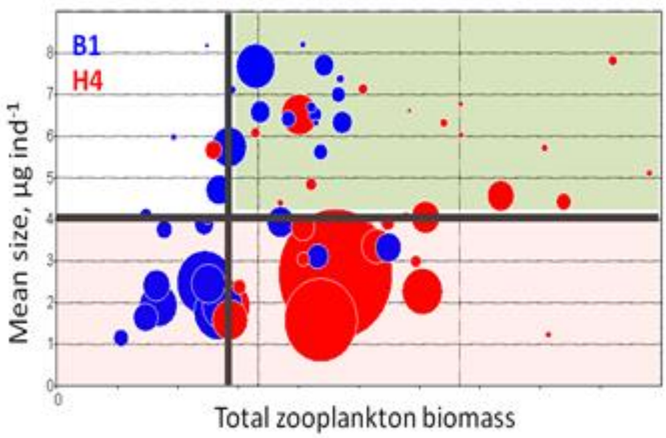

Figure 6. MSTS for two coastal stations (B1 and H4) in the Western Gotland Basin/northern Baltic Proper (years 1976-2010). Data are non-transformed mean values for summer (June-September) and circle size indicates average biovolume of filamentous cyanobacteria during the same period. In the Baltic Sea, the extensive cyanobacteria blooms are commonly considered a sign of eutrophication. Therefore, lower mean size observed during years with particularly strong blooms suggests negative effects of eutrophication primarily on mean size. By contrast, no clear effect on the total stock is apparent. Thick lines show threshold values and the green area corresponds to good status conditions.

Increased fishery leads to an increase in zooplanktivorous fish stocks that would affect both mean size and total zooplankton biomass negatively. Moreover, in addition to the community-level decrease in zooplankton mean size, a decrease in the population-level mean size would be observed due to selective predation on older life stages (Figure 7). Hence, the declining trend in the mean size (most likely) due to the increased relative abundance of the younger stages together with the declining total stock (probably) would be observed.

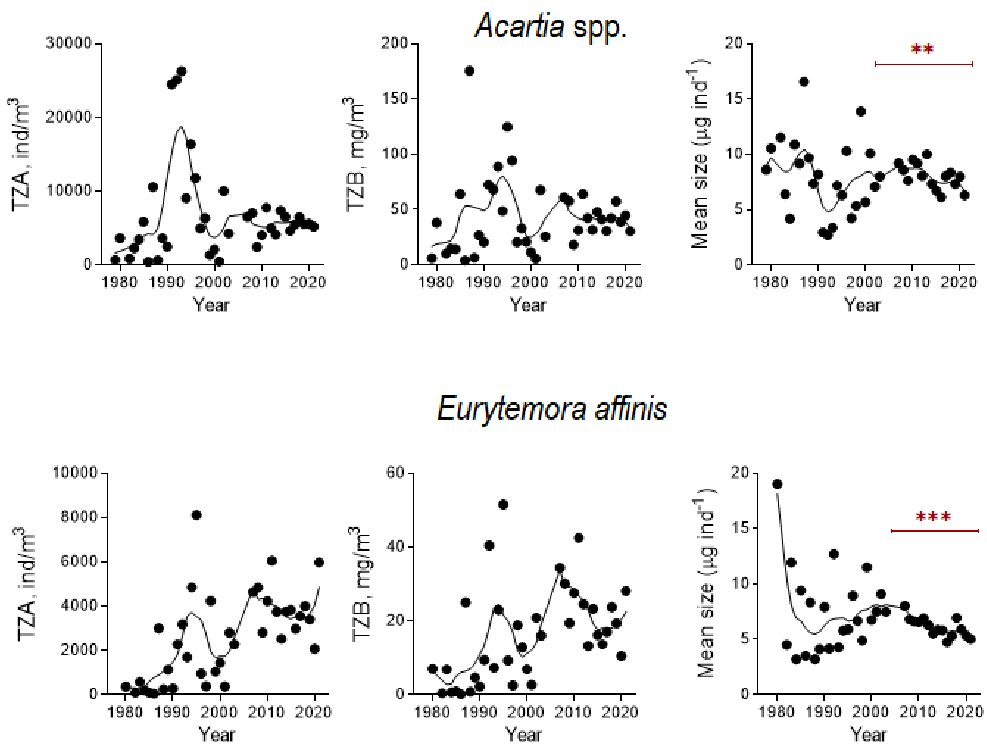

Figure 7. Zooplankton stock metrics (total zooplankton abundance, TZA, and total zooplankton biomass, TZB) and mean size for the dominant copepod populations of *Acartia* spp. and *Eurytemora affinis* in the Western Gotland Basin (years 1979-2021). Data are non-transformed mean values for summer (June-September). The significant decline in the mean size for both copepods was observed during the last two decades (Mann Kendall test, $p < 0.01$ in both cases), whereas the stock metrics were relatively stable (*Acartia*) or decreasing (*Eurytemora*). The decreasing mean size is most likely related to the size-selective predation by zooplanktivorous fish, with preferential removal of the older copepodite stages.

A number of directly policy relevant pressures are listed in Table 5.

Table 5. A brief summary of relevant pressures and activities with relevance to the indicator.

	General	MSFD Annex III, Table 2a
Strong link	Fishery-induced mortality of larger zooplankters (both with populations and communities). Eutrophication leading to dominance of small-sized phytoplankton supporting feeding of microphagous zooplankters.	Biological - Extraction of, or mortality/injury to, wild species (by commercial and recreational fishing and other activities). Substances, litter and energy - Input of nutrients – diffuse sources, point sources, atmospheric deposition.
Weak link	Higher salinity favouring species of marine origin. Higher temperature favouring warm-water taxa and parthenogenic reproduction of rotifers and some cladocerans, which are generally smaller zooplankters. Changes in oxygen concentration decrease habitat space for large-sized copepods. Invasive species (predatory zooplankters) affect native copepods and cladocerans. Environmental contaminants may have adverse effects on the most sensitive species; however, at present they are unknown in the Baltic Sea.	

7 Climate change and other factors

Climate change is expected to add further cumulative pressures to already existing anthropogenic ones in the Baltic Sea (HELCOM/Baltic Earth 2021) and thus to affect zooplankton mean size and total stock via various factors. Although exact details are not yet known in full detail, direct parameters causing changes in zooplankton mean size and total stock are most likely to be caused by changes in water temperature, sea ice, salinity and saltwater inflows, precipitation, river run-off, carbonate chemistry, as well as riverine nutrient loads and atmospheric deposition. Indirect parameters most likely to have direct effects on zooplankton are changes by climate change on the microbial community and processes, pelagic and demersal fish, occurrence of non-indigenous species, and ecosystem functions.

8 Conclusions

For HOLAS 3, the applicability of the MSTS indicator was expanded covering four new assessment units, the Northern Baltic Proper, Gulf of Riga, Eastern Gotland Basin, and Bornholm Basin. In half of the assessment units, zooplankton communities failed to comply with good environmental status. Moreover, the reason for this failure was the mean zooplankter size (in all cases but one), indicating that zooplankton communities show signs of losing large taxa and large (adult) individuals within populations. In several cases (Gulf of Finland, Eastern Gotland Basin, and Bornholm Basin), the decrease in the mean body size coincided with an increase in total zooplankton stocks (biomass and/or abundance), i.e., that change that would be expected at moderate eutrophication. Also, the intrapopulation dynamics of the mean size and increased relative contribution of the young stages at stable population total abundances are indicative of the predation pressure affecting zooplankton community structure. The interpretation of MSTS is facilitated by the integration with the results of the eutrophication status assessment by BEAT and, in the future, would be instrumental for the integrated food web assessment.

8.1 Future work or improvements needed

At present, the MSTS indicator has not been evaluated for all open sea assessment units in the Baltic Sea where zooplankton monitoring is conducted. The applicability of the indicator and the determination of relevant threshold values are still needed in The Quark and much of the southern Baltic Sea, Kattegat and Skagerrak before evaluation for these areas can be conducted.

Temperature- and salinity-induced MSTS responses also need to be further evaluated and, if relevant and significant, they need to be accounted for in the indicator-based evaluation of the pelagic food webs.

In order to evaluate the status of the food webs in the Baltic Sea, further development of the interpretation of the indicator results in relation to other evaluation results is needed. Future development should also include inventory and exploration of the coastal stations and data that can be used for the indicator-based evaluation. This would be critical if we are to establish pelagic habitat evaluation at scale 3.

A full evaluation of pelagic food webs is still to be developed, and the outcome of the MSTS-based evaluation needs to be considered in conjunction with other food web indicators.

Indicator development for HOLAS 3 has been supported by the Baltic Data Flows project, by enabling necessary data flows and indicator calculation improved for a developed R-script. Furthermore the HELCOM BLUES project enabled the development of new threshold values and enabling approval of the proposed threshold values via HELCOM processes. Future developments and improvements might need to secure necessary resources for further work on the indicator.

9 Methodology

The indicator uses mean zooplankton size and total stock (MSTS) for evaluating whether good environmental status is achieved or not. The indicator uses the parameter mean zooplankter size (mean size) which is presented as a ratio between the total zooplankton abundance (TZA) and total biomass (TZB). This metrics is complemented with an absolute measure of total zooplankton stock, TZA or TZB, to provide MSTS. Thus, MSTS is a two-dimensional, or a multimetric, indicator representing a synthetic descriptor of zooplankton community structure. The methodology and basis of the indicator evaluation is provided below. The indicator calculation workflow (Figure 8) is available as an R script from GitHub (Labuce and Gorokhova, in press.).

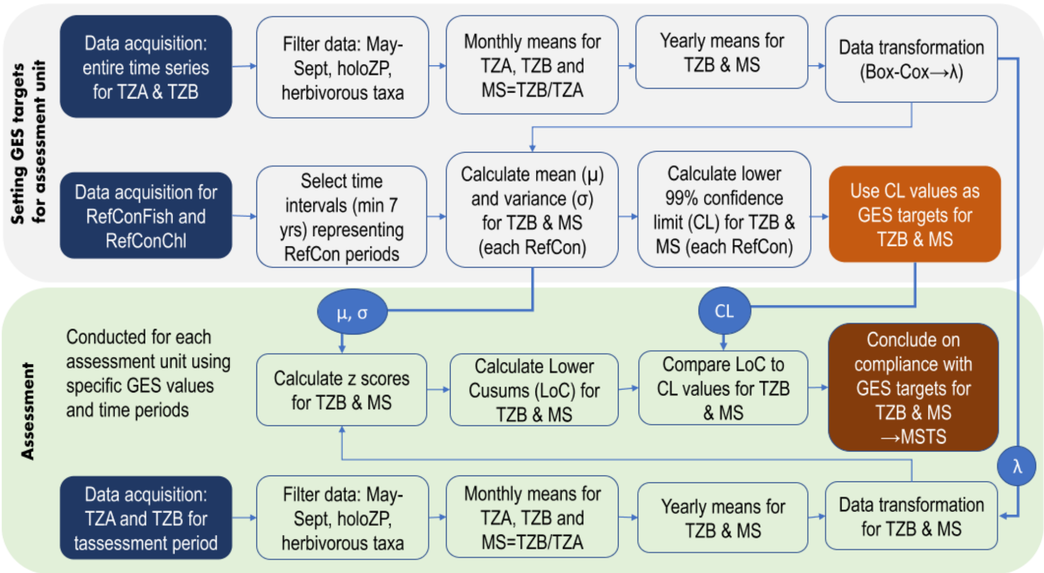

Figure 8. Workflow for MSTS calculations as implemented in the script *runMSTS* consists of Part I (Setting target values for good environmental status (GES) in the assessment unit; upper panel) and Part II (Conducting the evaluation; lower panel). holoZP - holoplanktonic zooplankton; MS - mean zooplankter size; MSTS - zooplankton mean size total stock indicator; RefCon - reference periods, either RefConFish or RefConChl; TZA - total zooplankton abundance; TZB - total zooplankton biomass. See Labuce and Gorokhova, in press., for details, and the script.

9.1 Scale of assessment

The indicator is evaluated using HELCOM assessment scale 2, which is consists of 17 Baltic Sea sub-basins. In the future it should be further discussed whether a higher spatial resolution (i.e. separating coastal and offshore areas) is needed. The assessment units are defined in the HELCOM Monitoring and Assessment Strategy Annex 4.

9.2 Methodology applied

Data period: The MSTS evaluations are currently restricted to the analysis of zooplankton communities observed during June-September. This seasonal time period was chosen because it is covered most extensively by the monitoring sampling programmes supplying the data; moreover, this is also the period of the highest plankton productivity as well as predation pressure on zooplankton (Johansson *et al.* 1993; Adrian *et al.* 1999). The structure of the marine food web is naturally variable; therefore, the indicator is designed to detect changes in the community structure that significantly deviate from the natural variability during the growth season.

Control charts: The time series of the MSTS components (mean size and total stock) for each zooplankton community are analyzed with cumulative sum (CuSum) control charts. The CuSum methods are designed to detect persistent small changes when the long-term mean changes in observed processes or periods. A control chart uses information about the natural variation of the process that is evaluated to examine if the process, i.e. the structure of the zooplankton community, is moving beyond the expected stochastic variability which is defined as desirable tolerance. If the process is *in control*, i.e. the zooplankton community structure is not affected by pressures, then subsequent observations are expected to lie within the tolerance boundaries. The hypothesis that the process is *in control* is rejected if the observations fall outside the desired tolerance boundaries. As a test statistic, control charts employ the controlling mean (μ) and specify control limits of *n* × standard deviations (σ) above and below the mean or the confidence intervals (CI). The upper and lower control limits are defined using a conservative approach of ±5σ for μ estimated for either $RefCon_{Fish}$ (reference conditions for fish) or $RefCon_{Chl}$ (reference conditions for chlorophyll *a* concentrations).

All datasets used for setting the thresholds values for evaluating status are >30 years of observations. The normality of each data series is first tested for normality (D'Agostino & Pearson omnibus normality test, Shapiro-Wilk and Kolmogorov-Smirnov normality tests). As both mean size and total zooplankton biomass often deviate significantly from the normal distribution, the values can be transformed using Box-Cox procedure and all calculations are then carried out on the transformed data. Once a controlling mean (μ_i) and standard deviation (σ_i) have been specified based on the chosen period used to determine the baseline against which status evaluation is made, indicator values ($x_{i,t}$) within the time series are standardized to *z*-scores ($z_{i,t}$) as:

$$z_{i,t} = \frac{x_{i,t} - \mu_i}{\sigma_i}$$

The approach for setting the reference period used a window of the available data corresponding to the selected reference period, i.e. years representing sub-basin specific reference conditions for (*i*) food webs not measurably affected by eutrophication; these are based on environmental quality ratio (EQR) and historical data on chlorophyll *a* (HELCOM 2009) when defining $RefCon_{Chl}$, and (*ii*) high feeding conditions for zooplanktivorous fish when defining $RefCon_{Fish}$ (Figure 9).

The μi and σi are defined based on the conditions during the reference period.

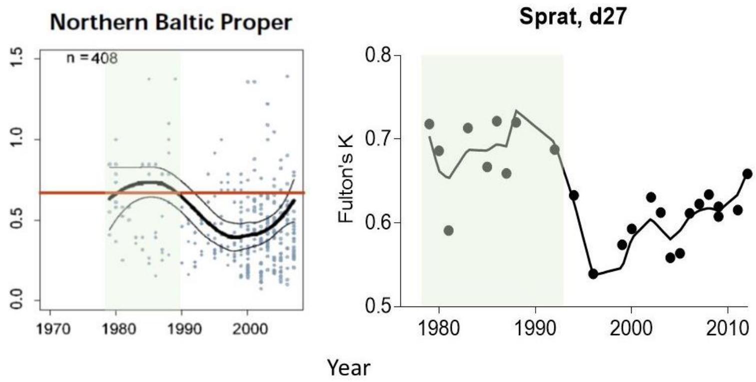

Figure 9. Examples for setting $\text{RefCon}_{\text{Chl}}$ and $\text{RefCon}_{\text{Fish}}$ using long-term variability in chlorophyll *a* expressed as ecological quality ratio (EQR) in the northern Baltic Proper (modified from HELCOM 2009) (left) and body condition index (Fulton's K) of sprat in the ICES subdivision 27 (right) used to identify time period (green area) when zooplankton community was sufficient to efficiently transfer primary production to secondary consumers.

To investigate trends in accumulated small changes for the zooplankton mean size and total stock over long time periods, the CuSum charts (Figure 10) are constructed by first determining a decision-interval CuSum (DI-CuSum) that is calculated by recursively accumulating negative deviations (one-sided lower CuSum) as:

$$S_i^- = \min\left[0, S_{i-1}^- + z_i + k\right]$$

with $S_{i=0} = 0$. The *k* value is the allowance value in the process, expressed in *z* units, reflecting natural variability of the mean shift one wishes to detect. Thus, deviations smaller than *k* are ignored in the recursions. The default choice of $k = 0.5$ is considered appropriate for detecting a 1-σ shift in the process mean (Lucas 1982).

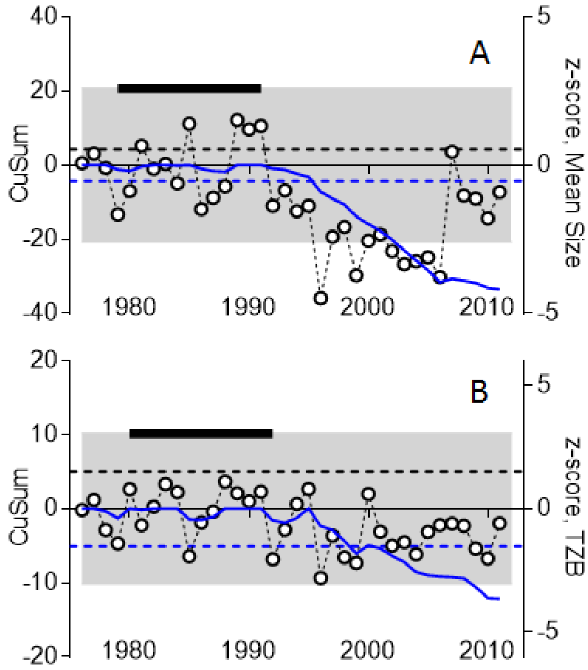

Figure 10. CuSum analysis of mean size (A) and total zooplankton biomass, TZB (B) using data series for station B1 (Askö station, Western Gotland Basin). The data are normalized to z-scores (right Y axis, open symbols). The threshold values are shown as dashed blue lines (-5σ from the mean for the reference period; σ is standard deviation) and the reference period (years) is indicated as a black bar on the top. The lower CuSum (solid blue line) indicates accumulated changes in the mean size and TZB; the CuSum lines are crossing the respective good status threshold values in 1995 (mean size) and 1999 (TZB). According to this chart, from 1995 onwards, MSTS indicates food web structure being in not good status.

A strategy that was used for obtaining an overall status evaluation when several datasets are available for an assessment unit is based on the integrated datasets. Since all zooplankton data are generated by national laboratories following HELCOM-Monitoring Manual guidelines and standardized gears and analysis methods, the data used for MSTS calculations are likely to be comparable. In order to arrive at a meaningful decision scheme, the main properties of the datasets should be considered. This includes issues such as length of the time series, their variability within defined reference periods, length of the time series overlapping with the reference periods, statistical properties of yearly mean values (i.e. number of samples contributing), quality control practices in the analysing laboratories, etc. These issues were carefully considered and discussed before this two-stage evaluation algorithm (first, comparing the datasets, and second, generating integrated data for the assessment unit) was applied.

9.3 Monitoring and reporting requirements

Monitoring methodology

HELCOM common monitoring of relevance to the core indicator is described on a general level in the HELCOM Monitoring Manual in the [Sub-programme: Zooplankton species composition, abundance and biomass](#).

Specific guidelines are under review with the aim to be included in the HELCOM Monitoring Manual at a later stage.

According to HELCOM guidelines for biological monitoring (HELCOM 1988), zooplankton were collected by vertical tows from either ~5 m above the bottom to the surface (shallow stations, ≤ 30 m bottom depth) or by stratified tows (deep stations, ≥ 30 m) as designed and specified by regional monitoring programmes. The standard sampling gear is a 100 µm WP-2 net (diameter 57 cm) equipped with a flow meter.

Samples are preserved upon collection in formalin and analysed by national laboratories within the respective monitoring programmes (see Data table 1). Copepods are classified according to species, developmental stage (copepodites CI-III and CIV-V classified as younger and older copepodites, respectively), and sex (adults); naupliar stages are not separated. Rotifers and cladocerans are identified to the lowest possible taxonomic level; moreover, the latter are classified according to sex, and females as ovigerous or non-ovigerous. Biomass is estimated using individual wet weights recommended by Hernroth (1985); for species not included in this list, either measured or calculated individual weights based on length measurements are used.

Current monitoring

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

Sub-programme: Zooplankton species composition, abundance and biomass Monitoring Concepts table

Zooplankton monitoring stations are located in every Baltic Sea sub-basin. Most of the stations are offshore but there are also some coastal stations.

Time series of zooplankton used for setting thresholds value for mean size and total stock (MSTS) assessment are > 30 years. Due to considerable variations in the sampling frequency between the monitoring programmes and datasets, the data that are currently recommended for use in the MSTS evaluation are restricted to the summer period (June-September) as the most representative in the currently available datasets (due to sampling schedules in the national monitoring programmes).

Description of optimal monitoring

In general, current monitoring is considered sufficient, although effects of the sampling frequency on the indicator performance remain to be evaluated. Evaluating the effect of sampling frequency on the indicator performance would be relevant for evaluating the confidence of the indicator.

Different strategies are employed in the national monitoring programmes with regard to sampling frequency and spatial coverage. In future work, this should be addressed to provide recommendations for zooplankton monitoring in the Baltic Sea.

If more resources are available, they should be used for development and implementation of methods for automated analysis and growth rate evaluation that may complement standard analysis at the existing monitoring sites and provide specific information on zooplankton productivity.

10 Data

The data and resulting data products (e.g. tables, figures and maps) available on the indicator web page can be used freely given that it is used appropriately and the source is cited.

Result: Zooplankton mean size and total stock

Data: Zooplankton mean size and total stock

The data are provided by national monitoring programmes with HELCOM COMBINE parameters and methods. The indicator is based on routine data obtained within current monitoring schemes in the Baltic Sea, and is applicable in all areas where the programme is implemented. All HELCOM Contracting Parties carry out relevant monitoring.

An overview of data utilised for the current evaluation and the establishment of threshold values is provided in Tables 6 and 7, respectively.

Please note that due to national database issues Danish zooplankton data are not included in this assessment.

Table 6. Overview of the datasets used for MSTS evaluation for the period 2016-2021. The sampling stations indicated for the zooplankton data are referred to by their names used in ICES/DOME database.

Sub-basin	Countries providing data	Station names
Bothnian Bay	Sweden, Finland	A5, A13, F3/A5, F9/A13, BO3, F2
Bothnian Sea	Sweden, Finland	C3, C15, SR5, US5B
Åland Sea	Finland	F64
Northern Baltic Proper	Finland, Estonia	LL12, LL17, 25, H1, H2
Gulf of Finland	Finland	GF1, LL3A, LL7, LL7S, LL9, XV1, XIV3, UUS23-Långden
Eastern Gotland Basin	Sweden, Finland, Lithuania, Latvia	32, 34a, BY15, 46, B4, F80
Gulf of Riga	Latvia Estonia,	121, 119, 121A, 142, 114A, G1, 111, 114, 107, 125
Western Gotland Basin	Sweden, Finland	B1, BY31, LL23
Gdansk Basin	Poland	P1
Bornholm Basin	Poland, Germany	PL-P5C (P5)*, BMPK2, (TF-0213, OMBMPK2)*

*In parentheses, the stations names used in national station registers and HELCOM map and data service are provided.

Table 7. Historical data used for setting TVs for MSTS evaluation for the period 2011-2016; deviations in the sampling methods from the HELCOM COMBINE guidelines are indicated.

Data set code	Area	Monitoring station(s)	Geographic coordinates	Max. sampling depth (m)	Time period (gaps)	Sampling frequency [a]	Deviations in sampling methods from HELCOM guidelines
ASKÖ	Western Gotland Basin	B1	N 58° 48' 19, E 17° 37' 52	40 m	1976-2010 (1990, 1993)	8-10	Water bottle[b] (1983-1988), otherwiseWP2, 90-µm mesh size[c]
Landsort	Western Gotland Basin					2-10	WP2, 90-µm mesh size[c]
GoFFI	Gulf of Finland	LL7	N 59.5101, E 24.4981	95 m	1979-2010 (1999, 2009)	1[d]	none
		LL3A	N 60.0403, E 26.8020,	60 m	1979-2010 (1989, 1990, 1999, 2000, 2009)		
ÅlandFI	Åland Sea	F64	N 59.5101, E 24.4981	280 m	1979-2010 (1988-1990,1997, 1999, 2009)		
BoSFI	Bothnian Sea	SR5	N 61.0500, E 19.3478	125 m	1979-2010 (1989, 1997, 1999, 2009)		
		US5B	N 62.3517, E 19.5813	116 m	1980-2010 (1989, 1997, 1999, 2009)		
BoBFI	Bay of Bothnia	BO3[e]	N 64.1812, E 22.2059	100 m	1979-2010 (1989, 1990,1997-1999, 2009)		
		F2[f]	N 65.2302, E 23.2776	90 m	1979-2010 (1983, 1989, 1990,1997-2000, 2009)		
Gdansk Deep	Gdansk Basin	P1	N 54°50.042′ E 19°19.683′	112 m	1986-2016 (1988, 1997-1998, 2000-2001)	1[d]	

[a] if not specified otherwise, this frequency is a number of samples collected during June-September;
[b] 23-L water bottle was used to sample water column every 5 m (bottom to surface) and pooled for counting using a 90-µm sieve;
[c] WP2 nets with mesh size of 90 and 100 µm were compared in 2003 in the Western Gotland Basin/northern Baltic proper and found to provide statistically similar sampling efficiencies for all relevant zooplankton groups (Gorokhova, pers. observations);
[d] August;
[e] or stations BO3N and/or BO3S located in a close proximity;
[f] or station F2A located in a close proximity;
[g] total for all stations

11 Contributors

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HELCOM Zooplankton Expert Network

HELCOM Secretariat: Owen Rowe, Jana Wolf

Contributing projects: ZEN-QAI project, Baltic Data Flows, HELCOM BLUES

12 Archive

This version of the HELCOM core indicator report was published in April 2023:

The current version of this indicator (including as a PDF) can be found on the HELCOM indicator web page.

Earlier versions of the core indicator report are available:

Zooplankton mean size and total stock HELCOM core indicator 2018 (pdf)

HOLAS II component - Core indicator report – web-based version July 2017 (pdf)

Zooplankton mean size and total abundance indicator report 2013 (pdf)

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14 Other relevant resources

No additional information is required for this indicator.

Baltic Marine Environment
Protection Commission

BLUES

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A2.3 Annex 2
Phytoplankton indicator report

For bibliographic purposes this document should be cited as: HELCOM (2023). Seasonal succession of functional phytoplankton groups. HELCOM pre-core indicator report.

2023

HELCOM

Phytoplankton

INDICATOR TYPE: State
INDICATOR CATEGORY: Pre-core
BSAP SEGMENT: Biodiversity
MSFD CRITERIA: D4C1

Seasonal succession of dominating phytoplankton groups

Table of contents

1 Key message

This indicator is a HELCOM pre-core indicator.

This HELCOM pre-core indicator is evaluated for the purposes of the 'State of the Baltic Sea' report (HOLAS 3) and further development towards a core indicator is expected in the future. An overview of indicator development is set out in the HELCOM indicator manual.

The status evaluation has been done for specific assessment units over the period 2015–2020 (Figure 1). The threshold values, based on defined reference periods, assess acceptable deviations from seasonal growth curves of dominating phytoplankton groups. The indictor value is based on the number of data points which fall within the acceptable deviation range, as set for each monthly phase in the reference growth curve and expressed as the percentage to the total number of observations. Strong deviations from the reference growth curves indicate impairment in the environmental status.

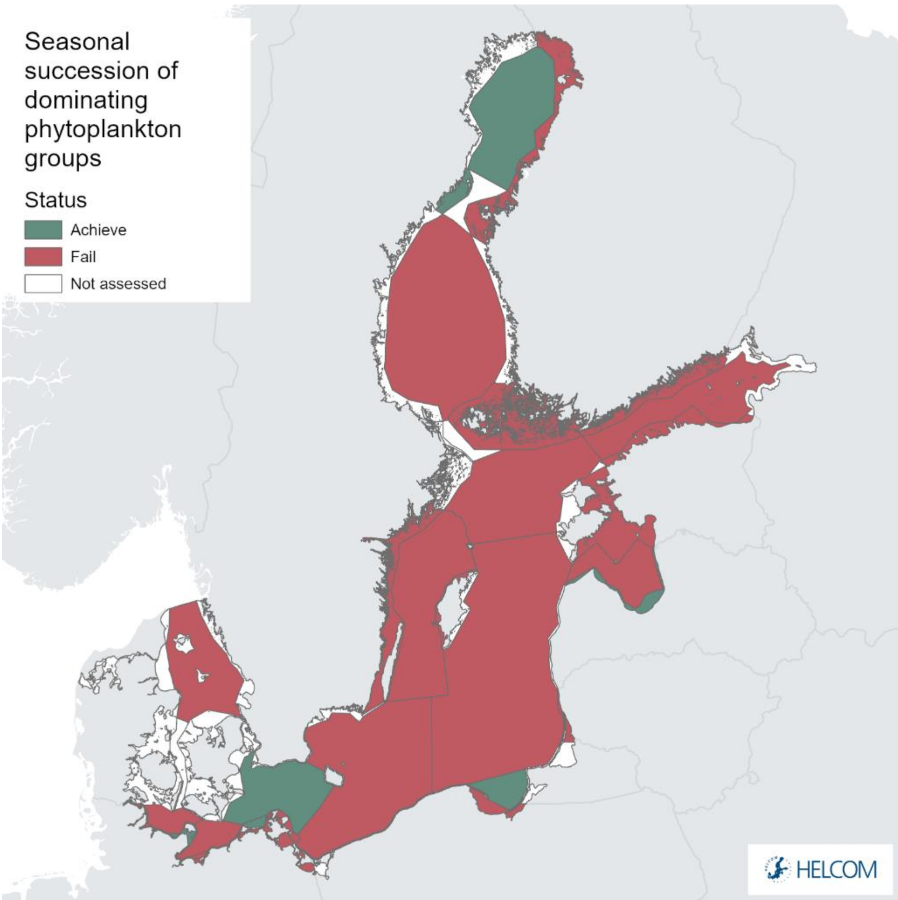

Figure 1. Status evaluation results based evaluation of the indicator 'Seasonal succession of dominating phytoplankton groups'. Due to national database issues Danish phytoplankton data are not included in this evaluation. German costal waters are displayed based on WFD results. The evaluation is carried out using Scale 3 HELCOM assessment units (defined in the HELCOM Monitoring and Assessment Strategy Annex 4). **See 'data chapter' for interactive maps and data at the HELCOM Map and Data Service.**

To develop basin-specific threshold values, all data were analysed to detect periods with lower total biomass and lesser year-to-year fluctuations. This indicator should be applicable also in coastal and open sea waters around the Baltic Sea. The analysis for 27 assessment units resulted in threshold values varying from 0.55 to 0.79 (Table 2). Most of the evaluated areas do not show good environmental status (GES), as seen in the figure 1.

Where applied, the confidence in this indicator evaluation is high or intermediate.

1.1 Citation

The data and resulting data products (e.g. tables, figures and maps) available on the indicator web page can be used freely given that it is used appropriately and the source is cited. The indicator should be cited as follows:

HELCOM (2023). Seasonal succession of functional phytoplankton groups. HELCOM pre-core indicator report. Online. [Date Viewed], [Web link].

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2 Relevance of the indicator

Phytoplankton are the key primary producers in marine ecosystems. The phytoplankton community is comprised of several functionally diverse groups that dominate at different times of the year. Changes in the presence of specific phytoplankton group or the timing of when it dominates and becomes abundant may influence ecosystem function. The consequent altered timing of food and carbon availability for other higher trophic levels (e.g. zooplankton) can have wider food web impacts and the sedimentation of detritus (e.g. dead phytoplankton) can influence the microbial food web and ecosystem balance (e.g. heterotrophy-autotrophy) and the physicochemical state of the ecosystem (e.g. oxygen concentration).

A deviation from the normal seasonal cycle (such as a too high or too low biomass, or absence of some dominating phytoplankton group(s)) is indicative of an impairment of environmental status. Phytoplankton species composition changes if the amount of nutrients or the ratios of important nutrients (e.g. nitrogen and phosphorus) change, and eutrophication has resulted in more intense and frequent phytoplankton blooms during the summer.

2.1 Ecological relevance

Phytoplankton are the main primary producers in the marine pelagic ecosystem. These organisms occur in vast numbers and capture sunlight via photosynthesis to build biomass. These primary producers are commonly autotrophic and photosynthetic (though some can be mixotrophic) and they form a direct link between the environmental conditions (e.g. nutrient status) and the marine food webs. Phytoplankton biomass represents the base of the classical marine food web, forming the carbon and energy (and nutrient) source for grazers and predators such as zooplankton, which in turn are eaten by fish. Furthermore, phytoplankton can also play a role in the regulation of secondary basal producers (i.e. bacteria) that classically rely on exudates, and the degradation of phytoplankton biomass has consequences for biochemical cycles, such as oxygen consumption, and thus the status of the marine environment.

In aquatic ecosystems, a hierarchical response across trophic levels is commonly observed. Because of this, higher trophic levels may show a more delayed response or a weaker response to eutrophication than lower ones. Measurements of biomass (rather than abundance) were used to develop this indicator, since they can readily be translated into understanding biogeochemical cycles, they link to eutrophication, and are considered to give a more accurate depiction of the phytoplankton community. The succession of phytoplankton has a rather regular pattern and the initial event like spring bloom may also influence the formation of summer communities. Firstly, the dominance of either diatoms or dinoflagellates in the spring period determines the rate of sinking organic matter and subsequent oxygen consumption in bottom sediments. The diatoms settle out quickly and may cause oxygen depletion, which may in turn launch the release of phosphorus from sediments. This favours those phytoplankton which benefits from excessive phosphorus, especially bloom-forming diazotrophic (nitrogen fixing) cyanobacteria (e. g. Eilola *et al.*, 2009).

The succession of dominant groups can provide an index that represents a healthy planktonic system, with a natural succession of dominant functional groups throughout the seasonal cycle. Deviations from the normal seasonal cycle, such as a too high or too low biomass, absence or appearance of some dominating groups at unusual time periods of the year, may indicate impairment in environmental status.

2.2 Policy relevance

Most pelagic habitats in the Baltic Sea are currently not in a healthy state and signs of deterioration at the food web and ecosystem levels are becoming more widespread and frequent. The pre-core indicator is among the few indicators able to evaluate the structure of the Baltic Sea food web, since phytoplankton have known links between environmental conditions (e.g. nutrient conditions) and higher trophic levels. Furthermore they have an important influence on other environmental or ecosystem components such as the supplementation of the microbial food web and possible consequences for oxygen conditions. Evaluations on the structure and functioning of the marine food web are requested by the Baltic Sea Action Plan (BSAP 2021) and the EU Marine Strategy Framework Directive (MSFD).

The EU MSFD lists a specific qualitative descriptor for the food webs: 'All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.'

The pre-core indicator is also relevant in supporting a determination of good environmental status under MSFD Descriptor 1 Criteria 6 on pelagic habitats and Descriptor 5 Criteria 3 on harmful algae bloom (Commission Decision (EU) 2017/848).

Table 1. Policy relevance of the pre-core indicator

	Baltic Sea Action Plan (BSAP)	**Marine Strategy Framework Directive (MSFD)**
Fundamental link	Segment: Biodiversity Goal: "*Baltic Sea ecosystem is healthy and resilient*" Ecological objectives: • Functional, healthy and resilient food webs • Viable populations of all native species	Descriptor 4 Ecosystems, including food webs • Criteria 1 The diversity (species composition and their relative abundance) of the trophic guild is not adversely affected due to anthropogenic pressures. • Feature – Shelf ecosystems. • Element of the feature assessed – Trophic guilds.

	• Natural distribution, occurrence and quality of habitats and associated communities Management objective: • Reduce or prevent human pressures that lead to imbalance in the food web	
Complementary link	Segment: Eutrophication Goal: "*Baltic Sea unaffected by eutrophication*" • Ecological objective: Natural distribution, occurrence and quality of habitats and associated communities • Management objective: Reduce or prevent human pressures that lead to imbalance in the food web	Descriptor 1 Species groups of birds, mammals, reptiles, fish and cephalopods • Criteria 6 The condition of the habitat type, including its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), is not adversely affected due to anthropogenic pressures. • Feature – Pelagic broad habitats. • Element of the feature assessed – Trophic guilds. Descriptor 5 Ecosystems, including food webs • Criteria 3 The number, spatial extent and duration of harmful algal bloom events are not at levels that indicate adverse effects of nutrient enrichment. • Feature – Eutrophication. • Element of the feature assessed – Harmful algal blooms species list.
Other relevant legislation:	UN Sustainable Development Goal 14 (Conserve and sustainably use the oceans, seas and marine resources for sustainable development) is most clearly relevant, though SDG 12 (Ensure sustainable consumption and production patterns) and 13 (Take urgent action to combat climate change and its impacts) also have relevance.	

2.3 Relevance for other assessments

The status of biodiversity and food webs can be 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 “Seasonal succession of dominating phytoplankton groups”, this indicator will in the future also contribute to an overall food webs assessment, along with the other biodiversity core indicators. The seasonal succession indicator may also be used as background data for the development of a modified lifeform approach in the monitoring and environmental assessments in the HELCOM area. Lifeform approach has been considered to be taken into use in the MSFD assessments by OSPAR (Gowen *et al.* 2011, McQuatters-Gollop *et al.* 2019).

3 Threshold values

The concept for evaluating good environmental status using the succession of dominant groups in the phytoplankton community is structured around a reference status succession and the acceptable deviation from that pattern. The indicator evaluates the coincidence of seasonal succession of dominating phytoplankton groups over an assessment period (commonly 5–6 years) using regionally established reference seasonal growth curves and wet weight biomass data. The indicator result value is based on the number of data points falling within the acceptable deviation range set for each monthly point of the reference growth curve and expressed as the percentage to the total number of data points. This result value is then compared to regionally relevant threshold values established to represent acceptable levels of variation. Strong deviations from the reference growth curves will result in failure to meet the thresholds set for acceptable variation, indicating impairment of the environmental status and a failure to meet good status (Figure 2).

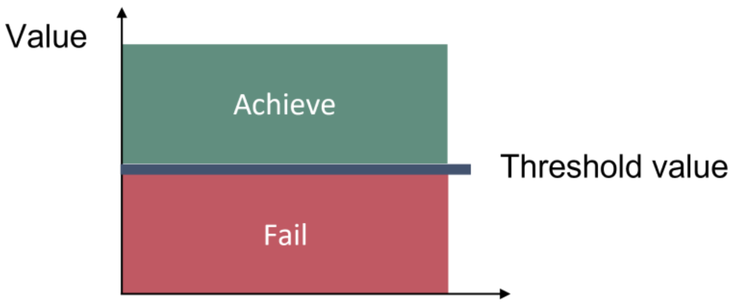


Figure 2. Good status is achieved when the indicator result (number of data points that fall within the established acceptable variation range) is above the regionally defined threshold value.

The specific regional threshold values used in this indicator are presented in Table 2. The final evaluation is based on the average score of single dominant groups. The threshold values are calculated for the periods with lower biomass values and lower interannual variability. If the number of deviations in an assessment unit increases along with the decreasing biomass values reflecting rather improvement in the ecological status, reference period may need to be redefined and threshold value recalculated. Therefore, part of the threshold values may be subjects of possible change for the next assessment period.

Table 2. Threshold values for selected assessment units in the Baltic Sea area, expressed as ratio of data points falling within the acceptable deviation range set for each monthly point.

HELCOM Assessment unit ID and name	Threshold value
SEA-001 Kattegat	0.56
SEA-004 Kiel Bay	0.55
SEA-005 Bay of Mecklenburg	0.61
SEA-006 Arkona Basin	0.55
SEA-007 Bornholm Basin	0.66
SEA-008 Gdansk Basin	0.61
SEA-009 Eastern Gotland Basin	0.68
SEA-010 Western Gotland Basin	0.70
SEA-011 Gulf of Riga	0.68
SEA-012 Northern Baltic Proper	0.70
SEA-013 Gulf of Finland	0.70
SEA-015 Bothnian Sea	0.63
SEA-017 Bothnian Bay	0.61
1 Bothnian Bay Finnish Coastal waters	0.56
3 The Quark Finnish Coastal waters	0.63
4 The Quark Swedish Coastal waters	0.55
7 Åland Sea Finnish Coastal waters	0.74
11 Gulf of Finland Finnish Coastal waters	0.79
12 Gulf of Finland Estonian Coastal waters, western part	0.65
12 Gulf of Finland Estonian Coastal waters, eastern part	0.66
14 Gulf of Riga Estonian Coastal waters	0.68
15 Gulf of Riga Latvian Coastal waters	0.66
16 Western Gotland Basin Swedish Coastal waters	0.71
19 Eastern Gotland Basin Lithuanian Coastal waters	0.66
24 Gdansk Basin Polish Coastal waters	0.60
32 Mecklenburg Bight German Coastal waters	0.62
35 Kiel Bight German Coastal waters	0.63

3.1 Setting the threshold value(s)

Background information on deriving the threshold values

The term ‘Good status’ has, however, to be taken with care as the first eutrophication affected changes in ecosystems emerged already in the mid-1950s in the Baltic Sea (Andersen *et al.*, 2015). Only in a few basins, regular phytoplankton datasets date back to the mid-1980s (Table 3). Mostly the observations begin from the 1990s and in several coastal assessment units, regular sampling started only in 2006-2007 after the implementation of the Water Framework Directive. This means that most areas of the Baltic Sea have been heavily influenced by anthropogenic pressures prior to the initiation of regular monitoring and it may thus be difficult to determine reference conditions for the succession, based on pristine environmental conditions. Due to the lack of confirmed high status waterbodies or historical datasets, the reference seasonal growth curves have been set through observations made after the 1980s and the threshold between GES and sub-GES status is based on expert judgement.

To define unit-specific reference conditions, the periods of stability in long-term biomass data were ascertained. This approach was tested by calculating 5-year moving averages of standard deviations in yearly total biomass values (Figure 2). The recommended minimum time period for setting reference is ten years to include all natural variability. If it is not possible to determine a time period of sufficient length, the reference period can be split. As the data has been updated since the previous evaluation, also the reference periods and threshold values have been subjects of change. Further analysis with data seemed to indicate that in several cases, the deviations from the long-term mean reference growth curves have become less frequent during the last decade than in the 1990s and the early 2000s (Figure 4). This may infer an improvement in the current environmental status. For this reason, compared to the previous evaluation, reference periods and threshold values have changed in the Gulf of Gdansk and in the Gulf of Riga Latvian coastal waters. Minor changes have been made in most assessment units.

The threshold values based on calculations with data points representing reference periods varied from 0.55 (Arkona Basin and the Quark Swedish Coastal waters) to 0.79 (Gulf of Finland Finnish Coastal waters). Most of the threshold values fell within the range 0.6-0.7. This means that during the reference period, approximately 2/3 of observations fit within the acceptable deviation range from the reference growth curves.

Low threshold values should indicate high natural variability in seasonal succession of dominating phytoplankton groups and vice versa. In general, phytoplankton community structure and timely performance of dominant groups are more predictable in the areas with stable hydrological conditions (e.g. no major freshwater discharges and turbulent mixing). Offshore communities might have more coherent responses across the sea than coastal communities that tend to be more isolated and may therefore show little coherence within and among regions (Griffiths *et al.*, 2015). This is also visible in the reference periods, which tend to be more similar in the adjacent open sea basins in comparison with the coastal assessment units belonging to the same sub-basin (Figure 4). Another reason explaining such discrepancy is due to different time periods of regular monitoring between the coastal and open sea areas.

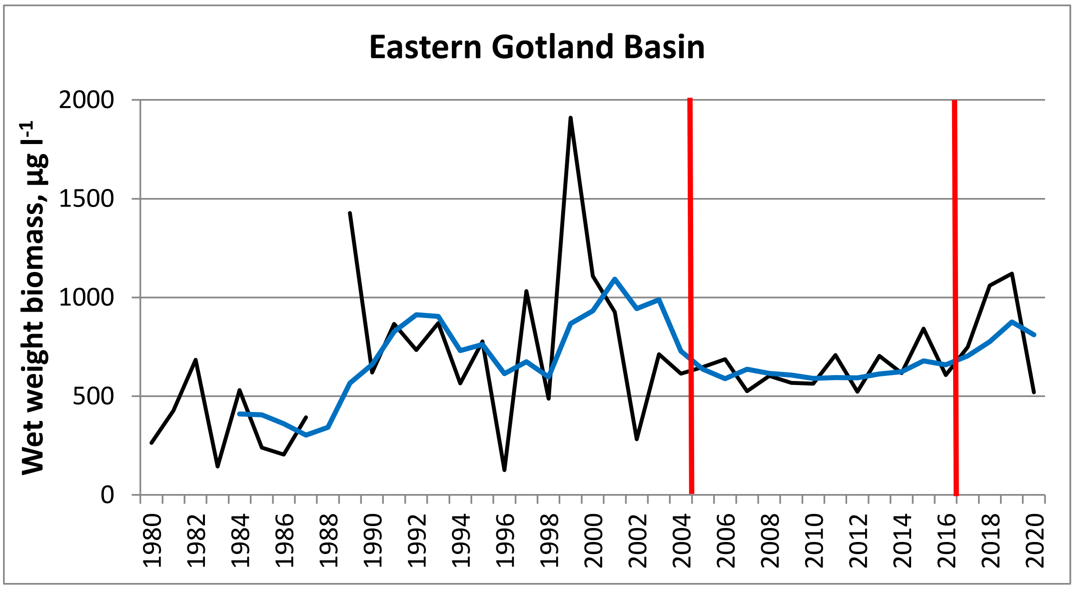
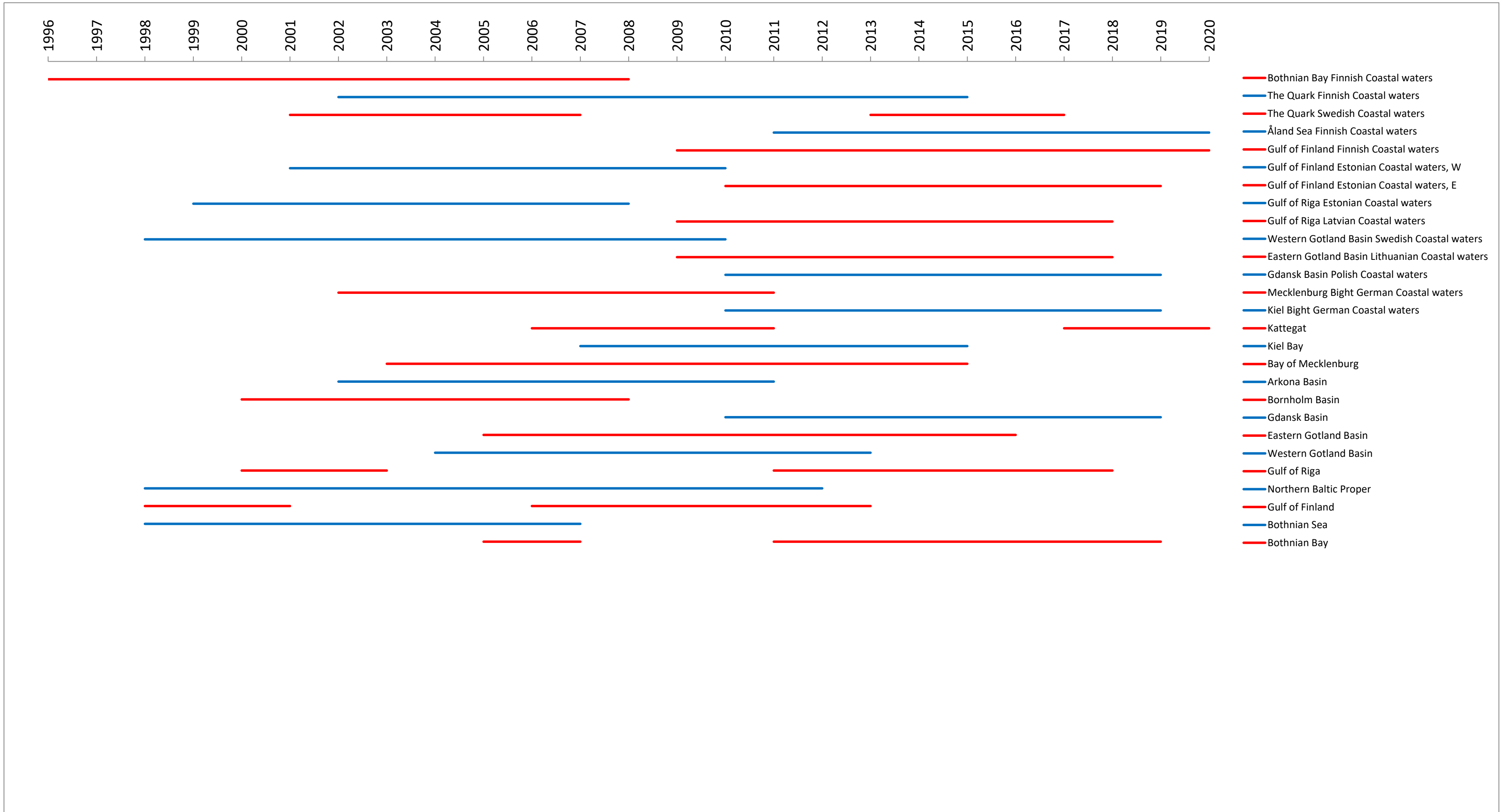


Figure 3. Selection of reference period by calculating 5-years moving averages (blue line) from yearly standard deviations of total phytoplankton biomass values (black line; µg l^{-1}). The period with lowest variability is indicated between red bars.

Figure 4. Scope of reference periods for seasonal succession of dominating phytoplankton groups in different assessment units across the Baltic Sea. Bars represent reference periods in the specific area, with alternating blue and red colours added to enhance readability.



4 Results and discussion

Below, the results of the indicator evaluation underlying the key message map and information are provided.

4.1 Status evaluation

The evaluation results are presented for 13 open sea basins out of 17 and for 13 coastal assessment units out of 40 (Table 3). In the Gulf of Finland Estonian coastal waters, western and eastern parts are evaluated separately due to salinity gradient and differences in phenology resulting in shifts of occurrence of dominant groups. The assessment units, excluded from the indicator analysis, are not monitored with sufficient frequency and regularity (incl. too short datasets to define reference period) or no data provided.

An example of reference growth curves and indicator values within the given assessment period is represented in Figure 5.

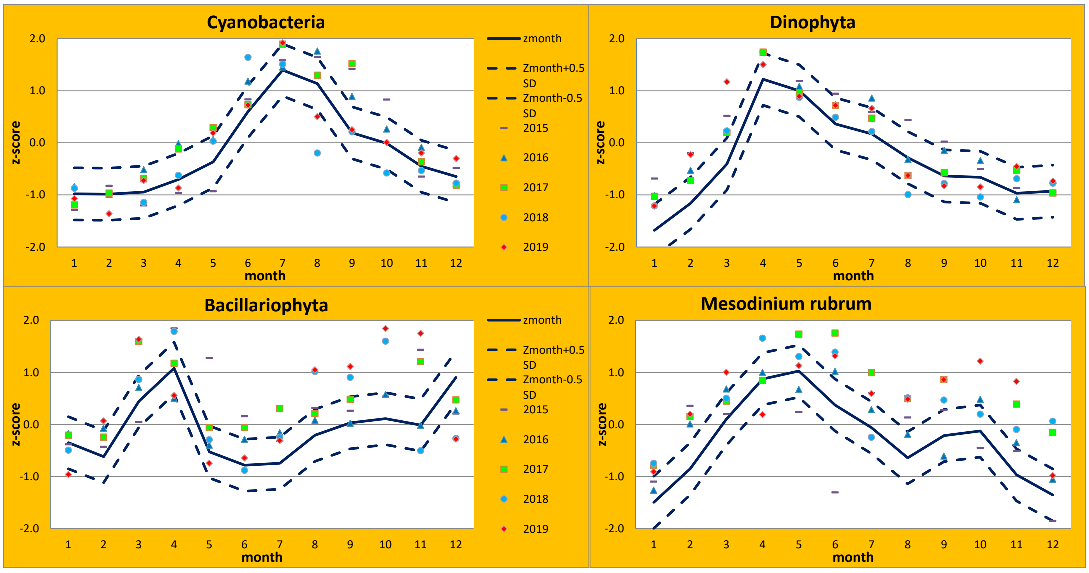


Figure 5. Reference growth curves with monthly averaged normalized biomass values (Z_{month}), acceptable deviations (Z_{month}±0.5) and data points during the period 2015-2019 in the Eastern Gotland Basin.

Table 3. Indicator results for the period 2015-2020 in comparison with threshold values in different assessment units of the Baltic Sea. The indicator value lies between 0 and 1 and is the proportion of data points within the "envelope" of seasonal reference growth curves and acceptable deviations. Data point is the average of all observations in a month of certain year. For overall evaluation, indicator values of individual dominant groups are averaged.

HELCOM assessment unit name and ID	No. of stations	No. of obs./ data points (2015-2020)	Dominant groups	Indicator value	Threshol d value	Reference period	Beginning of regular monitoring
SEA-001 Kattegat	2	174/66	**All groups**	**0.55**	**0.56**	2006–2011; 2017–2020	1994
			Cyanobacteria	0.71	0.75		
			Dinoflagellates	0.62	0.58		
			Diatoms	0.35	0.38		
			M. rubrum	0.50	0.54		
SEA-004 Kiel Bay	2	150/61	**All groups**	**0.50**	**0.55**	2007–2015	2007
			Cyanobacteria	0.75	0.76		
			Dinoflagellates	0.49	0.56		
			Diatoms	0.31	0.44		
			M. rubrum	0.46	0.43		
SEA-005 Bay of Mecklenburg	2	148/59	**All groups**	**0.60**	**0.61**	2003–2015	1980
			Cyanobacteria	0.71	0.80		
			Dinoflagellates	0.56	0.57		
			Diatoms	0.46	0.47		
			M. rubrum	0.68	0.61		
SEA-006 Arkona Basin	6	172/57	**All groups**	**0.61**	**0.55**	2002–2011	1980
			Cyanobacteria	0.63	0.54		
			Dinoflagellates	0.58	0.58		
			Diatoms	0.56	0.53		
			M. rubrum	0.67	0.56		
SEA-007 Bornholm Basin	4	148/54	**All groups**	**0.54**	**0.66**	2000–2008	1980
			Cyanobacteria	0.57	0.68		
			Dinoflagellates	0.61	0.67		
			Diatoms	0.43	0.61		
			M. rubrum	0.56	0.68		
SEA-008 Gdansk Basin	3	28/20	**All groups**	**0.67**	**0.61**	2010–2019	1984
			Cyanobacteria	0.71	0.65		
			Dinoflagellates	0.71	0.65		

			Diatoms	0.75	0.65		
			M. rubrum	0.50	0.47		
SEA-009 Eastern Gotland Basin	5	170/64	**All groups**	**0.64**	**0.68**	2005–2016	1980
			Cyanobacteria	0.71	0.67		
			Dinoflagellates	0.75	0.63		
			Diatoms	0.56	0.53		
			M. rubrum	0.70	0.73		
SEA-010 Western Gotland Basin	2	114/58	**All groups**	**0.56**	**0.70**	2004–2013	1990
			Cyanobacteria	0.71	0.76		
			Dinoflagellates	0.64	0.74		
			Diatoms	0.50	0.62		
			M. rubrum	0.38	0.66		
SEA-011 Gulf of Riga	13	197/44	**All groups**	**0.51**	**0.68**	2000–2003; 2011-2018	1992
			Cyanobacteria	0.36	0.61		
			Dinoflagellates	0.57	0.79		
			Diatoms	0.57	0.65		
			M. rubrum	0.52	0.66		
SEA-012 Northern Baltic Proper	3	184/64	**All groups**	**0.57**	**0.70**	1998–2012	1990
			Cyanobacteria	0.73	0.73		
			Dinoflagellates	0.66	0.74		
			Diatoms	0.52	0.60		
			M. rubrum	0.39	0.72		
SEA-013 Gulf of Finland	4	108/32	**All groups**	**0.62**	**0.70**	1997-2012	1990
			Cyanobacteria	0.66	0.84		
			Dinoflagellates	0.69	0.68		
			Diatoms	0.69	0.78		
			M. rubrum	0.44	0.48		
SEA-015 Bothnian Sea	4	56/46	**All groups**	**0.45**	**0.63**	1993-2004	1995
			Cyanobacteria	0.57	0.61		
			Dinoflagellates	0.49	0.67		
			Diatoms	0.31	0.61		
			M. rubrum	0.43	0.64		
SEA-017 Bothnian Bay	5	55/47	**All groups**	**0.65**	**0.61**	2001-2015	1995
			Cyanobacteria	0.68	0.59		

			Dinoflagellates	0.77	0.75		
			Diatoms	0.55	0.53		
			M. rubrum	0.62	0.59		
1 Bothnian Bay Finnish Coastal waters	28	282/36	**All groups**	**0.47**	**0.56**	1996–2008	1990
			Cyanobacteria	0.42	0.33		
			Dinoflagellates	0.58	0.82		
			Diatoms	0.47	0.60		
			M. rubrum	0.42	0.47		
3 The Quark Finnish Coastal waters	8	142/37	**All groups**	**0.43**	**0.63**	2002–2015	1998
			Cyanobacteria	0.59	0.70		
			Dinoflagellates	0.19	0.55		
			Diatoms	0.51	0.61		
			M. rubrum	0.43	0.68		
4 The Quark Swedish Coastal waters	2	49/45	**All groups**	**0.71**	**0.55**	2001–2007; 2013–2017	1995
			Cyanobacteria	0.64	0.56		
			Dinoflagellates	0.73	0.57		
			Diatoms	0.69	0.52		
			M. rubrum	0.64	0.58		
7 Åland Sea Finnish Coastal waters	51	677/32	**All groups**	**0.71**	**0.74**	2011–2020	1990
			Cyanobacteria	0.75	0.85		
			Dinoflagellates	0.53	0.63		
			Diatoms	0.88	0.74		
			M. rubrum	0.69	0.73		
11 Gulf of Finland Finnish Coastal waters	37	782/48	**All groups**	**0.78**	**0.79**	2009–2020	1990
			Cyanobacteria	0.69	0.67		
			Dinoflagellates	0.71	0.79		
			Diatoms	0.90	0.84		
			M. rubrum	0.83	0.85		
12 Gulf of Finland Estonian Coastal waters, western part	3	195/42	**All groups**	**0.49**	**0.65**	2001–2010	1993
			Cyanobacteria	0.52	0.73		
			Dinoflagellates	0.60	0.64		
			Diatoms	0.43	0.75		
			M. rubrum	0.43	0.47		
12 Gulf of Finland	3	190/42	**All groups**	**0.63**	**0.66**	2010–2019	1990

Estonian Coastal waters, eastern part			Cyanobacteria Dinoflagellates Diatoms M. rubrum	0.55 0.57 0.74 0.64	0.63 0.60 0.75 0.69		
14 Gulf of Riga Estonian Coastal waters	3	213/41	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.61** 0.54 0.68 0.73 0.49	**0.68** 0.63 0.80 0.68 0.62	1999–2008	1993
15 Gulf of Riga Latvian Coastal waters	11	236/41	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.68** 0.66 0.71 0.68 0.66	**0.66** 0.56 0.76 0.73 0.60	2009–2018	1995
16 Western Gotland Basin Swedish Coastal waters	2	201/72	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.64** 0.79 0.76 0.40 0.58	**0.71** 0.79 0.74 0.68 0.63	1998–2010	1983
19 Eastern Gotland Basin Lithuanian Coastal waters	7	165/45	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.62** 0.67 0.69 0.51 0.62	**0.66** 0.72 0.69 0.48 0.62	2009–2018	2001
24 Gdansk Basin Polish Coastal waters	22	65/44	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.56** 0.55 0.59 0.59 0.52	**0.60** 0.57 0.59 0.59 0.64	2010–2019	1987
32 Mecklenburg Bight German Coastal waters	6	238/53	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.64** 0.72 0.68 0.49 0.66	**0.62** 0.57 0.75 0.56 0.59	2002–2011	1997

35 Kiel Bight German Coastal waters	5	169/48	**All groups** Cyanobacteria Dinoflagellates Diatoms M. rubrum	**0.65** 0.76 0.60 0.51 0.57	**0.63** 0.74 0.65 0.52 0.59	2010–2019	2007

Please note that German coastal waters were not part of this evaluation but that WFD results were used instead and are thus displayed accordingly in Figure 1, where a good WFD status is displayed as achieved and anything else as failed. The results in table 4 show the WFD results for German coastal waters.

Table 4. Results for German coastal waters are from the WFD and result based on the biological quality component phytoplankton.

Unit ID	Unit Code	Unit Description	Chl-a [µg/l]	Assessment class Chl-a	Phytoplankton Index [EQR]	Assessment class Phytoplankton Index
1001	GER-001	mesohaline inner coastal waters, Wismarbucht, Suedteil			0.5718	moderate
1002	GER-002	mesohaline inner coastal waters, Wismarbucht, Nordteil			0.5718	moderate
1003	GER-003	mesohaline inner coastal waters, Wismarbucht, Salzhaff			0.5210	moderate
1004	GER-004	mesohaline open coastal waters, Suedliche Mecklenburger Bucht/ Travemuende bis Warnemünde			0.4968	moderate
1005	GER-005	mesohaline inner coastal waters, Unterwarnow			0.5214	moderate
1006	GER-006	mesohaline open coastal waters, Suedliche Mecklenburger Bucht/ Warnemünde bis Darss			0.4261	moderate
1007	GER-007	oligohaline inner coastal waters, Ribnitzer See / Saaler Bodden			0.1760	bad

1008	GER-008	oligohaline inner coastal waters, Koppelstrom / Bodstedter Bodden			0.2597	poor
1009	GER-009	mesohaline inner coastal waters, Barther Bodden, Grabow			0.1837	bad
1010	GER-010	mesohaline open coastal waters, Prerowbucht/ Darsser Ort bis Dornbusch			0.8720	good
1011	GER-011	mesohaline inner coastal waters, Westruegensche Bodden			0.3780	poor
1012	GER-012	mesohaline inner coastal waters, Strelasund			0.3677	poor
1013	GER-013	mesohaline inner coastal waters, Greifswalder Bodden			0.3820	poor
1014	GER-014	mesohaline inner coastal waters, Kleiner Jasmunder Bodden			0.1800	bad
1015	GER-015	mesohaline open coastal waters, Nord- und Ostruegensche Gewaesser			0.5119	moderate
1016	GER-016	oligohaline inner coastal waters, Peenestrom			0.2722	poor
1017	GER-017	oligohaline inner coastal waters, Achterwasser			0.2364	poor
1018	GER-018	mesohaline open coastal waters, Pommersche Bucht, Nordteil			0.3493	poor
1019	GER-019	mesohaline open coastal waters, Pommersche Bucht, Südteil			0.2779	poor
1020	GER-020	oligohaline inner coastal waters, Kleines Haff			0.2821	poor
1021	GER-021	mesohaline inner coastal waters, Flensburg Innenfoerde	5.0850	poor		
1022	GER-022	mesohaline open coastal waters, Geltinger Bucht	2.0418	moderate		

1023	GER-023	meso- to polyhaline open coastal waters, seasonally stratified, Flensburger Aussenfoerde	2.0418	moderate		
1024	GER-024	mesohaline open coastal waters, Aussenschlei	2.0553	moderate		
1025	GER-025	mesohaline inner coastal waters, Schleimuende	21.6758	bad		
1026	GER-026A	A.mesohaline inner coastal waters, Mittlere Schlei	53.3100	bad		
1027	GER-026B	B.mesohaline inner coastal waters, Mittlere Schlei	68.3740	bad		
1028	GER-027	mesohaline inner coastal waters, Innere Schlei	68.3740	bad		
1029	GER-028	mesohaline open coastal waters, Eckerfoerder Bucht, Rand	1.7330	good		
1030	GER-029	meso- to polyhaline open coastal waters, seasonally stratified, Eckerfoerderbucht, Tiefe	1.9657	moderate		
1031	GER-030	mesohaline open coastal waters, Buelk	1.9657	moderate		
1032	GER-031	meso- to polyhaline open coastal waters, seasonally stratified, Kieler Aussenfoerde	2.0357	moderate		
1033	GER-032	mesohaline inner coastal waters, Kieler Innenfoerde	4.5272	poor		
1034	GER-033	mesohaline open coastal waters, Probstei	1.8000	good		
1035	GER-034	mesohaline open coastal waters, Putlos	1.8000	good		
1036	GER-035	meso- to polyhaline open coastal waters, seasonally stratified, Hohwachter Bucht	1.6492	good		

1037	GER-036A	A.mesohaline open coastal waters, Fehmarnsund	1.7680	good		
1038	GER-036B	B.mesohaline open coastal waters, Fehmarnsund	1.7860	good		
1039	GER-037	mesohaline inner coastal waters, Orther Bucht	1.8623	good		
1040	GER-038A	A.mesohaline open coastal waters, Fehmarnbelt	1.4699	good		
1041	GER-038B	B.mesohaline open coastal waters, Fehmarnbelt	1.4699	good		
1042	GER-039	meso- to polyhaline open coastal waters, seasonally stratified, Fehmarn Sund Ost	1.5851	good		
1043	GER-040	mesohaline open coastal waters, Groemitz	2.1330	moderate		
1044	GER-041	mesohaline open coastal waters, Neustaedter Bucht	2.1957	moderate		
1045	GER-042	mesohaline inner coastal waters, Travemuende	19.7657	bad		
1046	GER-043	mesohaline inner coastal waters, Poetenitzer Wiek	19.7657	bad		
1047	GER-044	mesohaline inner coastal waters, Untere Trave	20.1872	bad		
1048	GER-111	mesohaline inner coastal waters, Nordruegensche Bodden			0.2250	poor

4.2 Trends

Distinct trends between the current and previous evaluation are considered if there is a difference in the indicator values equal or more than 15% (HELCOM, 2018). Indicator values for the previous period (2011–2016) have been also calculated in the assessment units not included in HOLAS II. The changes in groups do not follow the same trends in all areas and an increase or a decrease in the same group in different areas can only be evaluated against the specific reference period (threshold value setting period) for the region. The threshold value reflects the balance between the dominating groups from that period and the evaluation is carried out in relation to that. Thus, an increase or decrease in a group may alter the balance from the selected reference period, but changes alone in those groups are not themselves indicative of a specific directional change that can be used to infer status overall and must be considered as a change related to the balance between the groups relative to the threshold value setting period.

An overview is provided in Table 5.

Table 5. Assessment units, threshold values and trends

HELCOM Assessment unit ID and name	Threshold value achieved/failed – HOLAS II	Threshold value achieved/failed – HOLAS 3	Distinct trend between current and previous evaluation	Description of outcomes
SEA-001 Kattegat	Not evaluated	failed	NA	Increasing dinoflagellate and decreasing diatom biomass
SEA-004 Kiel Bay	Not evaluated	failed	NA	Increasing diatom and de-creasing dinoflagellate biomass
SEA-005 Bay of Mecklenburg	Not evaluated	failed	NA	Increasing cyanobacteria and diatom biomass
SEA-006 Arkona Basin	failed	achieved	Deterioration. The status has deteriorated in the current assessment period, possibly the availability of a larger data set compared to the test evaluation in the previous period plays a role in this change.	
SEA-007 Bornholm Basin	failed	failed	No change in status between assessment periods.	Increasing diatom and *Mesodinium rubrum* biomass
SEA-008 Gdansk Basin	achieved	achieved	No change in status between assessment periods.	
SEA-009 Eastern Gotland Basin	failed	failed	No change in status between assessment periods.	Decreasing dinoflagellate biomass
SEA-010 Western Gotland Basin	Not evaluated	failed	NA	Increasing diatom and *Mesodinium rubrum* biomass

SEA-011 Gulf of Riga	failed	failed	No change in status between assessment periods.	
SEA-012 Northern Baltic Proper	failed	failed	No change in status between assessment periods.	Increasing diatom and *Mesodinium rubrum* biomass
SEA-013 Gulf of Finland	Not evaluated	failed	NA	Increasing diatom and *Mesodinium rubrum* biomass
SEA-015 Bothnian Sea	Not evaluated	failed	NA	Increasing biomass in all dominant groups
SEA-017 Bothnian Bay	Not evaluated	achieved	NA	
1 Bothnian Bay Finnish Coastal waters		failed	NA	Increasing biomass in all dominant groups, except dinoflagellates
3 The Quark Finnish Coastal waters	Not evaluated	failed	NA	Decreasing dinoflagellate and increasing *Mesodinium rubrum* biomass
4 The Quark Swedish Coastal waters	Not evaluated	achieved	NA	
7 Åland Sea Finnish Coastal waters	Not evaluated	failed	NA	
11 Gulf of Finland Finnish Coastal waters	Not evaluated	failed	NA	
12 Gulf of Finland Estonian Coastal waters, western part	failed	failed	No change in status between assessment periods.	Increasing diatom and *Mesodinium rubrum* biomass
12 Gulf of Finland Estonian Coastal waters, eastern part	failed	failed	No change in status between assessment periods.	Increasing *Mesodinium rubrum* biomass
14 Gulf of Riga Estonian Coastal waters	failed	failed	No change in status between assessment periods.	
15 Gulf of Riga Latvian Coastal waters	failed	achieved	Positive. The status is approved in the current assessment period.	

16 Western Gotland Basin Swedish Coastal waters	Not evaluated	failed	NA	Increasing diatom and *Mesodinium rubrum* biomass
19 Eastern Gotland Basin Lithuanian Coastal waters	achieved	failed	No	Decreasing cyanobacterial biomass
24 Gdansk Basin Polish Coastal waters	Not evaluated	failed	NA	Increasing *Mesodinium rubrum* biomass
32 Mecklenburg Bight German Coastal waters	Not evaluated	achieved	NA	Increasing diatom biomass
35 Kiel Bight German Coastal waters	Not evaluated	achieved	NA	Increasing cyanobacteria and diatom biomass

4.3 Discussion text

Phytoplankton communities are comprised of several functionally diverse groups that dominate at different times of the year. The consequent altered timing of food and carbon availability for other higher trophic levels (e.g. zooplankton) can have wider food web impacts and the sedimentation of detritus (e.g. dead phytoplankton) can influence the microbial food web and ecosystem balance (e.g. heterotrophy-autotrophy) and the physicochemical state of the ecosystem (e.g. oxygen concentration). Phytoplankton species composition also changes if the amount of nutrients or the ratios of important nutrients (nitrogen and phosphorus) change, and eutrophication has resulted in more intense and frequent phytoplankton blooms.

The selected dominant groups for the seasonal succession indicator – cyanobacteria, dinoflagellates, diatoms and the autotrophic ciliate *Mesodinium rubrum* contribute usually at least 80–90% to the total phytoplankton biomass and make the base of marine food web. The relevance of different dominant groups is, however, highly variable across the Baltic Sea and mainly governed by salinity (e.g. Gasiūnaitė *et al.*, 2005). It is most prominent in cyanobacteria, which make up 10-25% of the annual phytoplankton biomass in the northern (except Bothnian Bay), eastern and central parts of the Baltic Sea, but only 0.3-2% in the southern basins and the Kattegat. 21 assessment units out of 27 analysed for this indicator are more or less diatom dominated. However, a distinction must be made here, as in the northern and central parts of the Baltic Sea diatoms make the bulk biomass in spring period, while in the south and the Kattegat, the peak biomasses are observed rather in autumn. The contribution of diatoms to the annual biomass is the largest in the coastal waters of Bothnian Bay and in the Kattegat (85-86% among the four dominant groups) and only the three basins (northern Baltic Proper, eastern and western Gotland basins) are dinoflagellate dominated during the spring bloom. The autotrophic ciliate

Mesodinium rubrum plays an important role in Bothnian Bay, Bothnian Sea, the Gulf of Riga and eastern and western Gotland basins (20–30% of annual biomass on average).

The results presented in 4.2 indicate that in comparison of previous and current assessment periods, most of the increasing trends in the biomasses of dominant groups are due to diatoms – in Bothnian Bay, Bothnian Sea, the Gulf of Finland, Northern Baltic Proper, Western Gotland Basin, Bay of Mecklenburg and Kiel Bay. The share of dinoflagellates has been increasing only in the Kattegat and Bothnian Sea between these two periods. Except the Bay of Mecklenburg and Kiel Bay, these changes concern the spring bloom, the period with the highest annual primary production and sinking of organic matter to the sediment. The fate of this organic matter is a key driver for material fluxes, affecting ecosystem functioning and eutrophication feedback loops. The dominant diatoms and dinoflagellates appear to be functionally surrogates as both groups are able to effectively exhaust the wintertime accumulation of inorganic nutrients and produce bloom level biomass that contribute to vertical export of organic matter (Kremp *et al.*, 2008; Spilling *et al.*, 2018). However, the groups have very different sedimentation patterns, and the seafloor has variable potential to mineralize the settled biomass in the different sub-basins. While diatoms sink quickly out of the euphotic zone, dinoflagellates sink as inert resting cysts, or decompose in the water column contributing to slowly settling phyto-detritus. The dominance by both phytoplankton group thus directly affects both the summertime nutrient pools of the water column and the input of organic matter to the sediment but to contrasting directions. The proliferation of dinoflagellates with high encystment efficiency could increase sediment retention and burial of organic matter, alleviating the eutrophication problem and improve the environmental status of the Baltic Sea. Thus, the increasing dominance of diatoms impacts sedimentation of phytoplankton biomass quantitatively, with higher vertical export of fixed carbon from the atmosphere to great depths (Smetacek, 1998). The conclusions must be drawn, however, with caution as we compare rather short time periods. Over a wider period before the 2010s, the proportion of dinoflagellates has been on the rise at least in the northernmost basins, in the gulfs of Bothnia and Finland (Klais *et al.*, 2011).

In the northern and central basins, also the autotrophic ciliate *M. rubrum* indicates an upward trend between the two assessment periods. Intensive studies in the Gulf of Finland have revealed that the blooms of this species are more prominent in years of earlier warming in spring (Lips & Lips, 2017). An increase in cyanobacterial biomass was observed in the areas where blooms have not been common – Bothnian Bay, Bothnian Sea, Bay of Mecklenburg and Kiel Bight. Statistically significant increasing trend in the Bay of Mecklenburg and Western Gotland Basin has been also detected by Kownacka *et al.* (2021). The same authors have revealed decreasing trend in cyanobacterial biomass in the central parts of the Baltic Sea – Arkona, Bornholm and Eastern Gotland basins during 1990-2020.

At the same time, the overall evaluation results of the seasonal succession indicator show opposite trends in different sub-basins of the Baltic Sea. In the open sea assessment units, phytoplankton communities seem to be heading for greater stability in the southern parts (Arkona, Bornholm and Gdansk basins, the Bay of Mecklenburg), while in the northern assessment units and in the Western Gotland Basin the status is moving further from good.

It has been noted for this indicator, that it is challenging to define a threshold value for good or not good environmental status, and since defining status is a complex process when addressing complex systems such as food webs, an expression or understanding of change (or no change) may be a more appropriate way to evaluate food webs. This applies in particular where data may simply not be available from a non-disturbed period, e.g. without eutrophication effects. The indicator seasonal succession of dominating phytoplankton groups is therefore primarily not a status indicator, but rather reflects trends by comparison of reference and assessment periods. There is also a danger that increasing deviations judged as bad are in fact positive because they are caused by declining eutrophication. Furthermore, using a recent reference period means that we also include the impact of climate change which might be more influential than eutrophication.

5 Confidence

Confidence is assessed based on expert evaluation of the information that underlies the confidence scoring. Specifically, this requires a categorical scoring of four different criteria: accuracy of estimate (where if present standard error or statistical outputs are used), temporal coverage, spatial representability of data, and methodological confidence. Confidence can be scored as high, intermediate or low for these criteria. Temporal coverage is scored based on monitoring data cover during the assessment period (year range for assessment and variation such as temporal frequency). For spatial representability, spatial cover (e.g. patchiness) is evaluated. For methodological confidence, scoring of conducted monitoring and data quality are scored. The result for confidence in this phytoplankton pre-core indicator evaluation reflects all of these criteria. The approach is applied in all biodiversity indicators following harmonised guidance provide for the integrated biodiversity assessment tool (BEAT) so that these values can be utilised in downstream assessments. Spatio-temporal coverage differs between the assessment units. For most of the assessed areas, the confidence of indicator status is intermediate to high according to temporal and intermediate according to spatial resolution. Confidence level depends on the length of the time-series and regularity of phytoplankton sampling during the growth period. On the other hand, once the reference growth curves have been established, some compromises in the frequency of sampling and total number of samples used in the evaluation are possible. The indicator value is the proportion of biomass values fitting into the reference growth envelope (region of acceptable deviation) and the values for individual months are independent. It means that if some data points for some months are missing during the assessment period, the evaluation is still feasible.

Methodological confidence of monitoring data used for this indicator is rather high since all laboratories providing data follow the same guidelines. The quality of data is substantially improved after implementing a standardised species list with fixed size-classes and biovolumes (Olenina *et al.*, 2006).

6 Drivers, Activities, and Pressures

The shift in the plankton community is most probably due to complex interactions between climate change impacts, eutrophication and increased top-down pressures due to overexploitation of resources, and the resulting trophic cascades. Eutrophication is commonly noted as being the major driver behind current impacts on the phytoplankton community. A shift in functional groups may affect ecosystem function in terms of the carbon available to higher trophic levels or settling to the sediments. The examination of seasonality shows the broad temporal variability of phytoplankton populations. Succession of dominant groups can potentially provide an index that represents a healthy planktonic system, with a natural progression of dominant functional groups throughout the seasonal cycle. Alterations in the seasonal cycle may be related to nutrient enrichment. Expert judgement must be used when alterations in the seasonal cycle, and their causes, are interpreted.

It has been pointed out that phytoplankton indicators show complex pressure-response relationships, and their use is therefore demanding. However, phytoplankton indicators have additional value for the implementation of the MSFD. Ecosystem components often respond non-linearly to environmental drivers and human stressors, where small changes in a driver cause a disproportionately large ecological response. In pelagic ecosystems, non-linearities comprise more than half of all driver-response relationships (Hunsicker *et al.*, 2016). The effects of eutrophication on phytoplankton may be expressed by shifts in species composition and increases in the frequency and intensity of nuisance blooms.

Table 6. Brief summary of relevant pressures and activities with relevance to the indicator.

	General	**MSFD Annex III, Table 2a**
Strong link	the most important anthropogenic threat to phytoplankton is eutrophication	Input of nutrients — diffuse sources, point sources, atmospheric deposition. Input of organic matter — diffuse sources and point sources.
Weak link	Biological disturbance (introduction of non-native species)	

7 Climate change and other factors

Seasonal succession indicator also reflects climate-induced changes in phenology with the consequences on productivity and food webs. Phytoplankton phenology has even been proposed as an indicator to monitor systematically the state of the pelagic ecosystem and to detect changes triggered by perturbation of the environmental conditions (Racault *et al.*, 2012). The duration of sunshine and sea surface temperature (SST) are the main factors governing the onset and the length of vegetation. At high-latitudes, higher SST is associated with prolongation of the growing period – both the earlier onset of spring bloom and the extension of phytoplankton peak biomasses during summer and autumn (Kahru *et al.*, 2016; Racault *et al.*, 2012; Sommer & Lewandowska, 2011; Wasmund *et al.*, 2019). On the example of cyanobacteria, dominating mainly in the summer period, significantly higher growth rates and peak abundances have been measured in the average and warm spring scenarios than in the cold spring scenario (De Senerpont Domis *et al.*, 2007).

Temperature has both a nutrient-independent effect and a nutrient-shared effect on phytoplankton community size structure (Askov Mousing *et al.*, 2014). Although the correlation between the duration of the growing season and the concentrations of nutrients may not be causative, the macroecological patterns show an increase in the fraction of large phytoplankton with increasing nutrient availability and a decrease with increasing temperature. Response of phytoplankton to precipitation depends upon the season and region. Using long-term time-series data worldwide, Thompson *et al.* (2015) concluded that in general phytoplankton responded more positively to increased precipitation during summer rather than winter. Analyses in Chesapeake Bay revealed increased abundance of diatoms in wet years compared to long-term average or dry years (Harding *et al.*, 2015).

It is predictable that the community structure becomes increasingly unstable in response to climate change (Henson *et al.*, 2021). Here is also a direct reference to the seasonal succession indicator, where the deviations from the reference growth patterns reflect impairment in the environmental status.

8 Conclusions

The indicator evaluates the coincidence of seasonal succession of dominating phytoplankton groups over an assessment period (commonly 5−6 years) with regionally established reference seasonal growth curves using wet weight biomass data. Deviations from the normal seasonal cycle may indicate impairment in the environmental status.

Phytoplankton data are not available from a non-disturbed period, e.g. without eutrophication effects. Status may be highly complex to define and an expression or understanding of change (or no change) may be a more appropriate way for the evaluation. The seasonal succession of dominating phytoplankton groups is therefore primarily not a status indicator, but rather reflects trends by comparison of reference and assessment periods.

The status evaluation has been done for specific assessment units over the period 2015–2020. The assessment results are presented for 13 open sea basins out of 17 and for 13 coastal assessment units out of 40. GES has been achieved in two open sea basins and in four coastal water assessment units.

Most of the increasing trends in the biomasses of dominant groups are due to diatoms. The dominance of either diatoms or dinoflagellates in the spring period determines the rate of sinking organic matter and subsequent oxygen consumption in bottom sediments. The diatoms settle out quickly and may cause oxygen depletion, which may in turn launch the release of phosphorus from sediments. This can favour blooms of diazotrophic (nitrogen fixing) cyanobacteria, which benefits from excessive phosphorus.

An upward biomass trend of the autotrophic ciliate *Mesodinium rubrum* in the northern and central basins of the Baltic Sea may be related to earlier warming in spring.

In the southern Baltic Sea (Arkona, Bornholm and Gdansk basins, the Bay of Mecklenburg), phytoplankton communities seem to be heading for greater stability, while in the northern assessment units and in the Western Gotland Basin the deviations from the normal succession growth curves have become more frequent.

The confidence of indicator status is intermediate to high according to temporal and intermediate according to spatial resolution. Methodological confidence of monitoring data used for this indicator is rather high since all laboratories providing data follow the same guidelines.

8.1 Future work or improvements needed

In some areas, especially offshore, phytoplankton monitoring can be supported by FerryBox sampling. For time being, microscopic analysis is a part of Ferrybox sampling only in the Estonian and Swedish monitoring programs.

Additional work could be explored in relation to linking the threshold values (and periods applied) to a harmonised period known to reflect an environmental condition that is classified as good (e.g. a pre-eutrophication impacted state). Another issue that could be further explored is the handling of imbalances and gaps in data sets. Future work on this indicator could further aim on strengthening the rationale for the indicator, including demonstrating the link to anthropogenic pressures. Future work could also continue to develop the methodology of threshold setting.

All of these aspects may be challenging due to the availability of historic and sufficient data to achieve improvements.Indicator development for HOLAS 3 has been supported by the Baltic Data Flows project, by enabling necessary data flows and indicator calculation via a developed R-script. Furthermore the HELCOM BLUES project enabled the development of new threshold values and enabling approval of the proposed threshold values via HELCOM processes. Future developments and improvements might need to secure necessary resources for further work on the indicator.

Further work on the indicators and approaches for the evaluation broader and more complex interactions in pelagic habitats (e.g. life-form pairs analyses) should also be progressed and the general concepts of this indicator may be relevant for such work. An initial pilot study on the potential of such approaches is expected to be available in the HOLAS 3 thematic assessment of biodiversity.

Calculations and data requirements

The input data required is wet weight biomasses of major functional or dominating phytoplankton groups over a sampling year. Sampling frequency should be at least once per month. The selection of groups may differ between sub-basins or assessment units of the Baltic Sea, and expert judgement based on long-term monitoring data is required to identify the correct and most suitable candidate groups. In all test areas cyanobacteria, auto- and mixo-trophic dinoflagellates, diatoms and the autotrophic ciliate *Mesodinium rubrum* were selected. In the Eastern Gotland Basin Lithuanian Coastal waters and in the Quark Swedish Coastal waters, green algae were included in the analysis as an extra component.

The process of establishing phytoplankton group reference growth curves for marine water bodies was originally described by Devlin *et al.* (2007). Type- or site-specific seasonal growth curves can be designed for each dominating phytoplankton group:

1) Skewed data is accounted for by the transformation of phytoplankton biomass (x) on a natural log scale (ln x+1);

2) Overall and monthly means and standard deviations are calculated for each functional group over a reference period;

3) Monthly Z scores are calculated as follows:

$$\mathbf{Z}_{\boldsymbol{month}} = \frac{(\mathbf{Monthly\ mean} - \mathbf{Overall\ mean})_{\boldsymbol{reference\ period}}}{\mathbf{Overall\ standard\ deviation}_{\boldsymbol{reference\ period}}}$$

A positive z-score implies that the observed type and site-specific growth curve for a certain month is greater than the mean. And this in turn indicates that the phytoplankton group has grown more in that month than average. A negative score indicates that the observation is less than the mean and the phytoplankton group is missing or constitutes only minor part of biomass in the whole community.

4) Acceptable deviations for monthly means (reference envelopes) are calculated (z_{month}±0.5).

The indicator value is calculated:

$$\mathbf{Z}_{\boldsymbol{score}} = \frac{\mathbf{Monthly\ mean}_{\boldsymbol{year}} - \mathbf{Overall\ mean}_{\boldsymbol{reference\ period}}}{\mathbf{Overall\ standard\ deviation}_{\boldsymbol{reference\ period}}}$$

The indicator value is based on the number of data points from the test area which fall within the acceptable deviation range that has been set for each monthly point of the reference growth curve. Percentage-based thresholds are established for each dominating group to determine index values for the evaluation of the ecological status:

$$\mathbf{Index\ value}_{\boldsymbol{assessment\ period}} = \frac{\textbf{No. of data points within the reference envelope}}{\mathbf{Overall\ no.\ of\ data\ points}}$$

9.1 Scale of assessment

Currently this indicator has been tested in a selection of assessment units. The indicator has the potential to be applied for the entire Baltic Sea. The set of dominating phytoplankton groups can vary between different sub-basins, for example cyanobacteria do not generally occur among the dominant groups in high salinity areas.

The underlying characteristics vital to the function of this indicator differ between areas of the Baltic Sea due to seasonal and environmental factors, thus derivation of assessment unit specific reference conditions and threshold values is critical. The indicator values may also differ between the coastal and open sea zone within the same sub-basin. The aim is to use known characteristics of individual waterbodies to assess status on the largest possible scale. Currently, level 3 is used for the coastal assessment units.

Data for the open sea units are aggregated from 3-13 stations with most regular monitoring covering the whole vegetation period. The number of stations in coastal water units ranges from two to 51 (Table 3). Due to different hydrological conditions, mainly salinity (5–7 *vs*. 3–5 PSU), Estonian coastal waters in the Gulf of Finland are divided into two separate assessment units (western and eastern part). High number of stations in the Finnish coastal waters is due to different strategy, where nearshore areas are sampled more extensively in July-August. Most of selected stations belong to the current monitoring programs.

The assessment units are defined in the HELCOM Monitoring and Assessment Strategy Annex 4.

9.2 Methodology applied

The data required for this indicator are attained by quantitative phytoplankton analysis (cf. HELCOM, 2021).

9.3 Monitoring and reporting requirements

Monitoring methodology

HELCOM common monitoring of the phytoplankton community, the methods for sampling, sample analysis and calculation of carbon biomass are described in general terms in the HELCOM Monitoring Manual.

For time-series calculations, it is important to have as regular datasets as possible. At least monthly sampling during the growth period is needed to design reference growth curves. If sampling dates or numbers of samples are very irregularly distributed, monthly means have to be calculated before further analysis. The time-scale for data sets should be at least 10 years to include natural variability and to create type- or site-specific reference growth curves. Some recommendations for spatial resolution have been given recently (Jaanus *et al.*, 2017) and this will be an important consideration when defining the appropriate scale of assessment units monitored.

If historical datasets are not available, time-series data should be collected over a period of at least 10-15 years. The data must represent the upper mixed layer. FerryBox data can be additionally used assuming that that the sampling depth (usually 4–5 m) represents the upper surface layer as the ship creates turbulence when moving.

Current monitoring

Current monitoring is not formalised for this indicator. Sufficiently frequent sampling is seldom available through monitoring programs (see also Heiskanen *et al.*, 2016). Moreover, the open sea monitoring activities of many countries have been reduced during the last years. This is in some areas (Gulf of Finland, Northern Baltic Proper) compensated by increasing activities of sampling by FerryBox systems. A more detailed scheme of stations and sampling times of recent monitoring activities can be provided.

The seasonal succession indicator is operational as:

- National monitoring programs for getting the samples are established.
- Samples are taken and processed according to the guidelines (HELCOM monitoring manual).
- Data are delivered by experts belonging to the HELCOM Expert Group on Phytoplankton (EG PHYTO) and are therefore of high quality.
- The data are regularly reported and stored in national and international databases (e.g. ICES).

10 Data

The data and resulting data products (e.g. tables, figures and maps) available on the indicator web page can be used freely given that it is used appropriately and the source is cited.

Result: Seasonal succession of dominating phytoplankton groups

Data: Seasonal succession of dominating phytoplankton groups

The methods of collection, counting and identification should be unified between all laboratories sharing the same assessment area. For this report data has been collected directly from the persons responsible for phytoplankton monitoring. ICES Data Centre has made a script available that reads phytoplankton data extract from the ICES Data Portal, groups the data based on the taxonomic information and aggregates biomasses for the groups needed for indicator calculations. In addition, there is also R script (M1-eng-R) that can be used for indicator calculation.

The indicator will be updated once in 6-year assessment period to detect reliable trends in seasonal dynamics of dominant phytoplankton groups.

Please note that due to national database issues Danish phytoplankton data are not included in this assessment.

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Projects: Baltic Data Flows, HELCOM BLUES

12 Archive

This version of the HELCOM core indicator report was published in April 2023:

The current version of this indicator (including as a PDF) can be found on the HELCOM indicator web page.

Earlier version of the HELCOM indicator report was published in July 2018:

Seasonal succession of dominating phytoplankton groups HELCOM pre-core indicator 2018 (pdf)

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