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53/2021

Final report

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List of abbreviations

MSFD	Marine Strategy Framework Directive
MSTS	Mean Size Total Stock
TZA	Total Zooplankton Abundance
TZB	Total Zooplankton Biomass
GES	Good Environmental Status
RefCon	Reference Conditions for the Evaluation of GES
EQR	Environmental quality ratio
HELCOM	Helsinki Convention on the Protection of the Marine Environment of the Baltic Sea Area
ICES	International Commission for the Exploration of the Sea
EU	European Union
BSH	Bundesamt für Seeschifffahrt und Hydrographie
LLUR	Landesamt für Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein
IOW	Leibniz Institute for Baltic Sea Research Warnemünde
SMHI	Swedish Meteorological and Hydrological Institute
WW	Wet weight

Kurze Zusammenfassung

Die vorliegende Studie evaluiert die Anwendbarkeit des von HELCOM im Rahmen der Meeresstrategie-Rahmenrichtlinie (MSRL) verwendeten Kern-Indikators ‘Zooplankton mean size and total stock (MSTS)‘ zur Beschreibung des guten ökologischen Zustands (GES) des Nahrungsnetzes in der westlichen Ostsee. Anhand einer monatlichen Datenerhebung in der Kieler Bucht, der Mecklenburger Bucht, der Arkona See und im Bornholmbecken in den Jahren 2015 - 2016 wurde untersucht inwieweit indikatorrelevante Zooplankton-Taxa im Hinblick auf ihr saisonales Vorkommen, die interannuelle Variabilität im zeitlichen Auftreten oder die Variabilität der Probenahme ausreichend durch die derzeitig bestehende Probenahme-Strategie im Monitoring erfasst werden.

Die Ergebnisse zeigen eine große zeitliche und interannuelle Variabilität im Untersuchungsgebiet insbesondere bei denjenigen Gruppen, die durch parthenogenetische Fortpflanzung schnell auf sich ändernde Umweltbedingungen reagieren können, wie z.B. die Rotatorien und die Cladoceren. Zur quantitativen Erfassung dieser Gruppen und des Zooplanktons im Allgemeinen ist daher eine höhere Frequenz in der Probenahme notwendig, da sich ihr zeitliches Auftreten über eine lange produktive Phase von März bis September erstreckt, die nur unzulänglich mit dem bestehenden Monitoring beprobt wird. Bedeutende Bestände eines kleinen cyclopoiden Copepoden in der Kieler und Mecklenburger Bucht schränken die Anwendbarkeit des Indikators im Hinblick auf seine Aussagekraft bezüglich eutrophierungsbedingter Verschiebungen in der Größenstruktur des Zooplanktons ein. Hier ist bisher nicht ausreichend geklärt, ob das Auftreten dieser Gruppe durch die Erhöhung von Nährstoffeinträgen gefördert wird.

Auf Basis der Langzeitdaten der mittleren Größe und Gesamtbiomasse wurden Referenzperioden für einen guten Umweltzustand bezüglich Eutrophierung und Ernährungszustand des Fischbestandes für die Arkonasee und das Bornholmbecken definiert. Dies erfolgte auf Basis von Langzeitdaten für Chlorophyll und altersspezifisches Gewicht der Sprotten. Die jeweils für die Sommerperiode und das Jahresmittel berechneten Schwellenwerte zeigten für das Zooplankton in der Arkonasee einen guten Zustand, da die mittlere Größe und die Gesamtbiomasse über den Zeitraum von 1983 bis 2018 zugenommen haben. In der Bornholmsee zeigt sich hingegen für die mittlere Größe seit den frühen 90er Jahren ein schlechter Zustand, während die Biomasse kritische Schwellenwerte nicht unterschritten hat.

Short summary

The present study assessed the applicability of the HELCOM Core indicator ‘Zooplankton mean size and total stock (MSTS)’ to describe the good environmental status of the zooplankton in the western Baltic Sea in the framework of the Marine Strategy Framework Directive. Based on monthly data from the Kiel Bight, the Bay of Mecklenburg, the Arkona Sea and the Bornholm Basin in 2015 - 2016, the present monitoring scheme was evaluated with regard to the quantitative record of the seasonal variation of major zooplankton taxa, the interannual variation in their temporal occurrence and their stock sizes.

The results showed a large interannual variability in the seasonal timing and along a salinity gradient, particularly in the cladocera and rotifers that are characterised by parthenogenetic reproduction enabling them to react fast to environmental changes. The quantitative record of these groups, but also of the zooplankton in general, therefore, requires a higher sampling frequency to capture sufficiently the seasonal maxima over the period from March to September. The present frequency does not fulfil this demand. High stocks of a small cyclopoid copepod in the Kiel Bight and the Bay of Mecklenburg restrict the power of the indicator to reflect eutrophication-induced changes in the size structure of the zooplankton until the stimulation in abundance of the group by nutrient input has unequivocally been demonstrated.

Reference periods for the good environmental status with regard to eutrophication and fish condition were defined for the Arkona Sea and the Bornholm Basin based on the long-term variation of chlorophyll a and wet-at-age of sprat. Based on these, the threshold values for the GES of mean size and total stock of the zooplankton for the summer period or as annual mean were established. In the Arkona Sea, the threshold was not passed in long-term data reflecting increasing mean size and zooplankton stocks since the reference period in the early 1980s. In the Bornholm Sea, however, the lower threshold for mean size was exceeded since the 1990s indicating sub-optimal conditions. The stock size, in contrast, displayed a constant increase similar to the Arkona Sea.

1 Introduction

1.1 The Core indicator 'Zooplankton mean size and total stock (MSTS)'

The European Union Marine Strategy Framework Directive (MSFD) aims at the sustainable use and the conservation of marine ecosystems. The implementation of the directive requires the development of a set of indicators aiding the achievement/maintenance of a good environmental status (GES) for the several descriptors of ecosystem health (EU Directive 2008/56/EC). Marine zooplankton is an important structural element of the pelagic ecosystem and is for the first time included in the assessment of biodiversity (MSFD descriptor D1) and of the food web (MSFD descriptor D4) response to anthropogenic stressors. Its relevance arises from the central role that zooplankton possess in food webs due to its position sandwiched in between primary production and higher trophic levels such as planktivorous fish and invertebrates. Zooplankton, thus, accounts for a large proportion of transfer efficiency between trophic levels in pelagic ecosystems (e.g., Banse 1995, Adrian et al. 1998).

A number of indicators have been evaluated for their ability to sufficiently track the community state in relation to GES criteria regarding eutrophication and fish feeding conditions in the Baltic Sea (Gorokhova et al. 2016). Single group indicators as well as aggregated zooplankton metrics were assessed in their ability to predict zooplankton being in GES. The mean body mass in the zooplankton community and the zooplankton stock size in terms of abundance (TZA) or biomass (TZB) performed generally best across the monitoring datasets that are available from the diverse national Baltic Sea monitoring programs. These parameters were integrated in a single two-dimensional indicator 'Zooplankton mean size and total stock (MSTS)' and adopted by HELCOM as a core indicator to assess the food web response to anthropogenic impact (HELCOM 2018a).

The core indicator addresses both the structural and numerical properties of the zooplankton community. Its rationale is based on the dependence of ecological processes such as prey preferences of zooplankton or preferences of their vertebrate and invertebrate predators on body size. The mean size of a zooplankter in the community is, thus, indicative of both fish feeding conditions and grazing pressure from zooplankton on phytoplankton and changes under anthropogenic pressures such as eutrophication (Gorokhova et al. 2016). A large stock of zooplankton composed of large-bodied organisms has a high capacity for transfer of primary production to fish, while the dominance of small-bodied zooplankton is associated with a lower energy transfer efficiency. 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, and vice versa (Rönkkönen et al. 2004, Casini et al. 2009).

1.2 Applicability of the indicator in the western Baltic Sea

The indicator-based evaluation of the status of different areas has been completed for the Gulf of Bothnia, the Gulf of Finland, the Åland Sea, the Western Gotland Basin and the Gdansk Basin for the assessment period 2011-2016 (Gorokhova et al. 2016, HELCOM 2018a). In contrast, the assessment of the ecological status in the western Baltic Sea, namely the Kiel Bight, the Bay of Mecklenburg, the Arkona Sea and the Bornholm Basin is still pending. Some fundamental gaps in knowledge exists, however, that presently might hinder the applicability of the core indicator and the assessment. These relate primarily to the low sampling frequency of monitoring relative to the long productive phase in the western Baltic Sea and to the gradient in the biodiversity and seasonal development related to the prevailing geomorphological and hydrographical conditions in the assessed areas.

1.2.1 Timing of the seasonal development and indicator-relevant groups

The assessment of the zooplankton state by the MSTS indicator is related to the determination of the relevant zooplankton metrics during the major growth period of zooplankton. In the eastern and north-eastern Baltic Sea, the period from June to September is considered most relevant since the seasonal maxima of important zooplankton groups such as copepods, cladocera and rotifers lie within this time window (Simm et al. 2014, Gorokhova et al. 2016, Ojaveer et al. 2018). The productive season in the western Baltic Sea, in contrast, appears to begin considerably earlier. Increased phytoplankton stocks can be found as early as February in the Kiel Bight, and are progressively delayed along the depth gradient to the Bornholm Basin (Wasmund et al. 1998, Wasmund & Uhlig 2003, Wasmund & Siegel 2008). The present monitoring data indicates that the seasonal development of the zooplankton in the western Baltic Sea follows this spatial trend with increasing stocks during February-March in Kiel Bight and the Bay of Mecklenburg, but a progressive delay to early - late May in zooplankton stocks in the Arkona Sea and Bornholm Basin (Wasmund et al. 2015, 2016). A quantitative assessment of the stock size and long-term trends in abundance of these groups, however, is hampered by the presently static frequency of the monitoring, which is restricted to five sampling periods (February, March, May, August, and November). This does not account for biogeographical gradients and potential variation in the seasonal timing. Therefore, it remains largely open whether the relevant zooplankton groups are quantitatively sampled and sufficiently recorded.

1.2.2 Gradient in biodiversity

In addition to the gradient in seasonal timing, strong changes in the composition of the zooplankton occur that related to the salinity gradient in the western Baltic Sea. Kiel Bight and the Bay of Mecklenburg separate from Arkona Sea and Bornholm Basin due to a stronger marine influence with higher concentrations of marine pelagic organisms – in particular the cyclopoid copepod *Oithona* spp. and the calanoid copepod *Pseudocalanus* spp., while the indicator-relevant groups of rotifers and small cladocera play only a minor role, if at all. At present, it is unclear whether this general absence reflects the insufficient detection due the low sampling frequency or whether it is a ‘real’ feature of the salinity influence on biodiversity. In addition, the role of *Oithona* spp. on the performance of the indicator remains to be assessed. The individual mass of copepodites of this small cyclopoid copepod is in the range of the mass of *Synchaeta*. This genus is an important group in the pelagic ecosystem of the Baltic Sea and its abundance has been related to eutrophication conditions (Heerkloss et al. 1991, Gorokhova et al. 2016). Due to the low individual mass, the presence of *Oithona* spp. in the western Baltic Sea might have a strong influence on the mean size of the community without necessarily indicating condition of eutrophication.

1.2.3 Sample variance

Considering the fundamental gradients in biodiversity and seasonal timing across the monitored areas, a common assessment of the zooplankton indicator in the western Baltic Sea is unlikely. Thus, the assessment of GES status in the separate areas will critically depend on restricted data, because sampling during the seasons is restricted to more or less one cruise only and 1 - 2 samples per area. At present, it is unknown whether this low sample number and the lack of replicated sampling is critical for the indicator assessment with regard to small-scale patchiness in the distribution of zooplankton.

1.2.4 Reference conditions

In the assessment of the GES, the status of the zooplankton community with regard to mean size and total biomass in a particular region will be evaluated against a reference condition (HELCOM 2018a). In time series less than 12 years, the indicator values are generally assessed against the long-term mean and the corresponding variance without defining specific reference periods. In longer time series, however, a controlling mean is defined from periods during which eutrophication had no measureable effect on zooplankton stocks ($RefCon_{Chl\,a}$) or during which the food web structure support adequate feeding conditions for planktivorous fish ($RefCon_{Fish}$). $RefCon_{Chl\,a}$ have been defined as the period in which the environmental quality ratio (EQR) assessed from historical Chl *a* data is larger than 0.67. Correspondingly, $RefCon_{Fish}$ are set using periods of successful foraging in the relevant ICES subdivisions when both fish growth assessed as weight at age (WAA) and stocks were relatively high.

Considering the limited temporal coverage of the available time series that started around the beginning of the 1980s, pristine conditions especially with the regard to eutrophication are difficult to define.

1.3 Objectives

At present, the monitoring data is insufficient for a comprehensive evaluation of the applicability of the MSTS indicator and its performance in the Kiel Bight, the Bay of Mecklenburg, the Arkona Sea and the Bornholm Basin. The present study aimed at closing the identified knowledge and data gaps resulting from the infrequent sampling of the zooplankton. Based on the analysis of a regular, monthly sampling in each of the major areas along the salinity gradient, the applicability of the indicator is evaluated and recommendations are provided how the MSTS indicator can be applied in the western Baltic Sea. In detail, the study aimed at addressing the following objectives:

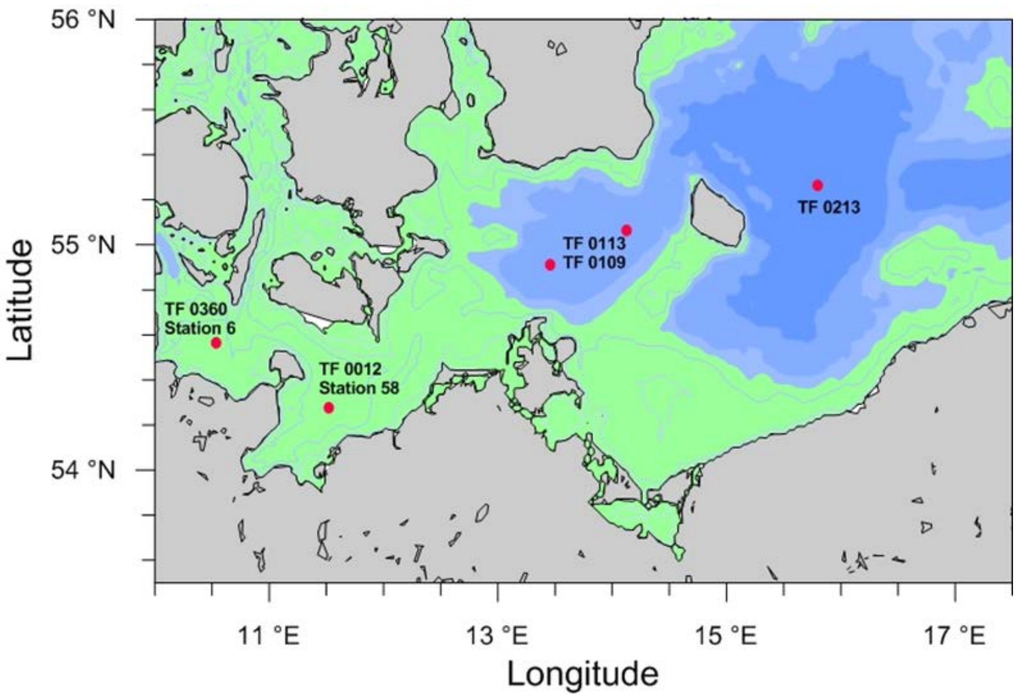
- Analyse whether the indicator relevant groups are sufficiently recorded with regard to timing and interannual variability in their occurrence in the different monitoring areas.
- Analyse the effect of the changing zooplankton community composition and the presence of the small cyclopoid copepod *Oithona* spp. on the applicability of the MSTS indicator in the western Baltic Sea
- Determine the confidence limits for the present sampling design within the BSH monitoring
- Provide recommendations for the monitoring of the zooplankton and the assessment of the indicator in the western Baltic Sea

In addition, literature and the available datasets were surveyed and analysed in order to

- Describe the long-term variation of the indicator values for mean body mass of zooplankton (MS) and total zooplankton biomass (TZB) in August for the period 1984 - 2017 in the Arkona Sea and Bornholm Basin
- Provide suggestions for reference conditions for eutrophication ($RefCon_{Chl\,a}$) and fish nutrition ($RefCon_{Fish}$) according to HELCOM (2018a)
- Assessment of zooplankton mean size and total stock based on combined Shewart- and CuSum-evaluation protocols (HELCOM 2018a)
- Identify historical data suitable to determine indicator values for a better definition of a GES for mean size and total biomass before the 1980s

This analysis is restricted to the Arkona Sea and Bornholm Basin because, according to current knowledge, the biodiversity in the Kiel Bight and the Bay of Mecklenburg diverges strongly from the Baltic Proper.

Figure 1 Investigation area and location of sampling stations. Stations 6 and 58 denote the LLUR monitoring stations, TF-360, -0012, -0109, -0113 and -0213 denote the BSH/IOW sampling stations.



2 Methods

2.1 Field study

2.1.1 Data sources

The analysis of the seasonal composition of the zooplankton, the occurrence of indicator relevant groups and their effect on the indicator performance was investigated in the open areas of the German exclusive economic zone for the years 2015 - 2016. The sampling sites were generally defined by the long-term monitoring programme of the BSH which is run by the Leibniz Institute for Baltic Sea Research Warnemünde (Fig. 1). A delay in the project start prevented the sampling for a full seasonal cycle in 2015. For this year, the analysis is, therefore, based on the combination of data obtained from different sources and the analyses of collected samples (Tab. 1).

Data for the Kiel Bight and the Bay of Mecklenburg was obtained from the ‘Landesamt für Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein’ (LLUR, Flintbeck) using their station 6 and 58, which are concurring with the BSH monitoring stations TF-0360 and TF-0012, respectively (Fig. 1). This data was supplemented with the data from the IOW monitoring; no data was available for station 58/TF-0012 in April 2015. Regarding data from February to June for the Arkona Sea, data from the IOW monitoring was combined with data from the Swedish monitoring programme hosted at the Swedish Meteorological and Hydrological Institute (SMHI, Stockholm); samples for data from July to November were analysed at IOW, except for October, for which no samples could be obtained. All data for the Bornholm Basin in 2015 was taken and analysed by the IOW. All data from 2016 originated from samples taken originally by LLUR/IOW and analysed at the IOW, except September, for which data from the Swedish monitoring programme was used. These samples were analysed in triplicate except for a few occasions when bad weather conditions prohibited this.

Table 1: Samples analysed for the seasonal composition of the zooplankton, the occurrence of indicator relevant groups and mean size during 2015 - 2016.

Month	Station	2015: date /number	2016: date/number	Data origin: 2015/2016
January	TF-360 TF-0012 TF-113/109 TF-213	07.01./1 - 13.01./1 -	26.01./1 27.01./1 27.01./3 30.01./3	LLUR/IOW -/IOW SMHI/IOW -/IOW
February	TF-360 TF-0012 TF-113/109 TF-213	02.02./1 03.02./1 04.02./2 05.02./3	18.02./3 23.02./3 27.02./1 06.02./1	IOW/IOW IOW/IOW IOW, SMHI/IOW IOW/IOW
March	TF-360 TF-0012 TF-113/109 TF-213	15.03./1 18.03./1 18.03./2 19.03./1	28.03./3 28.03./3 27.03./1 18.03./3	LLUR/IOW LLUR/IOW IOW, SMHI/IOW IOW/IOW
April	TF-360 TF-0012 TF-113/109 TF-213	- 01.04./1 22.04./2 19.04./1	20.04./3 18.04./3 15.04./3 16.04./3	-/LLUR IOW/LLUR SMHI/IOW IOW/IOW
May	TF-360 TF-0012 TF-113/109 TF-213t	05.05./1 14.05./1 06.05./2 13.05./1	10.05./3 10.05./3 11.05./3 26.05./3	IOW/IOW IOW/IOW IOW/IOW IOW/IOW
June	TF-360 TF-0012 TF-113/109 TF-213	09.06./1 15.06./1 13.06./2 28.06./1	15.06./2 15.06./3 09.06./3 26.02./3	LLUR/LLUR LLUR/LLUR SMHI/IOW IOW/IOW
July	TF-360 TF-0012 TF-113/109 TF-213	06.07./1 24.07./1 14.07./1 12.07./1	13.07./3 12.07./3 13.07./3 12.07./3	LLUR/LLUR IOW/LLUR IOW/IOW IOW/IOW
August	TF-360 TF-0012 TF-113/109 TF-213	01.08./1 01.08./1 24.08./1 23.08./1	02.08./3 03.08./3 03.08./3 05.08./3	IOW/IOW IOW/IOW IOW/IOW IOW/IOW
September	TF-360 TF-0012 TF-113/109 TF-213	14.09./1 29.09./1 17.09./1 23.09./1	05.09./3 13.09./3 14.09./1 08.09./3	LLUR/LLUR LLUR/LLUR IOW/SMHI IOW/IOW
October	TF-360 TF-0012 TF-113/109 TF-213	05.10./1 30.10./1 - 12.10./1	19.10./3 18.10./3 31.10./3 31.10./3	IOW/LLUR IOW/LLUR -/IOW SMHI/IOW
November	TF-360 TF-0012 TF-113/109 TF-213	07.11./1 16.11./1 08.11./1 15.11./3	03.11./1 13.11./2 04.11./1 06.11./3	IOW/LLUR IOW/IOW IOW/IOW IOW/IOW

For the location of the stations see Fig.1.

All data used in the study originates from samples that were analysed according to the HELCOM guidelines (HELCOM 2015), except a lacking taxonomic differentiation of nauplii of diverse copepod species in the LLUR dataset. This has only minor implications for the data analysis and calculations of the mean individual mass of the zooplankton.

2.1.2 Sampling and sample analysis

All samples were taken with a WP-2 ring net of 57 cm diameter and equipped with 100 µm mesh size and flowmeter. They were taken either as single hauls integrating the water column from the bottom to the surface or as combined hauls of stratified samples. All samples were stored in buffered formalin (4 %) until analysis. In the laboratory, they were analysed to standard procedures (HELCOM). Individuals were identified at least to the taxonomic level whenever possible. Copepods were separated into copepodite stages CI–CIII, copepodite stages CIV–CV, females, and males. The developmental stages of the cladocerans and the rotifers, however, were not distinguished. At least 100 individuals from at least three major taxonomic groups were counted. Abundance (number of individuals m^{-3}) was calculated from the single or stacked vertical hauls as mean concentration over the whole sampling depth. Biomass concentrations (µg wet weight m^{-3}) for single taxa and developmental stages were calculated from abundance and individual, taxon-specific body wet weight values (µg wet weight individual^{-3}) available from the literature (Hernroth 1985). Mean size of zooplankton in each station was calculated from the total biomass divided by the total abundance as described below.

2.2 Long-term data analysis

2.2.1 Data sources

For the establishment of long-term variation of the mean weight of zooplankton and the total biomass, monitoring data for the years 1984 - 2017 at station TF-0113, TF-109 and TF213 hosted at IOW was used. Earlier data lack information for the abundance of copepod nauplii stages. For the assessment, data obtained in the period from June to September is recommended for the calculation of the yearly indicator values (HELCOM 2018a). Data from spring and summer was, however, used to establish separate indicator values for each season because of the earlier start of the productive season in both the Arkona Sea and Bornholm Basin. The monitoring programme has fixed sampling months (February, March, May, August, and November). Therefore, May was chosen as the representative month for the spring period, and August for the summer period. Station TF-0109 was only irregularly sampled in the past and, therefore, does not provide a continuous time series. Data from this series was, however, used to fill data gaps occurring in the time series of station TF-0113. Missing values occurred in August 1990 and May/August 1995/1996 for in the Arkona Sea and for May 1996 and August 1994/1996/2009 in the Bornholm Basin, respectively. They were replaced by the long-term mean.

The abundance was obtained from the single or stacked vertical hauls as the mean zooplankton concentration integrated over the whole water column (number of individuals m^{-3}). Biomass values for the total zooplankton (µg wet weight m^{-3}) were calculated as the sum of the products of the individual abundances and the individual, taxon-specific body wet weight values (Hernroth 1985). Only indicator relevant groups were used in the calculations; protists, cnidarians, ctenophores and diverse meroplankton as well as predatory cladocerans/lophogastrids/chaetognaths were excluded. The indicator value for mean size (µg wet weight individual^{-3}) was calculated as the ratio of total biomass (µg wet weight m^{-3}) and total abundance (number of individuals m^{-3}) for each sampling event. The number of sample events in May and August varied within the time series. The analysis of the interannual variation in the mean size and total stock is based on the monthly means for each year.

2.2.2 Data analysis

The indicator values were analysed for the good environmental status following the protocol in Gorokhova et al (2016) and HELCOM (2018a). Reference conditions were defined for eutrophication ($RefCon_{Chl\,a}$) and fish nutrition ($RefCon_{Fish}$) for each indicator value (mean size, total biomass). The long-term environmental quality ratio for Chl *a* (EQR) during summer (HELCOM 2009), the summer Chl *a* values provided by HELCOM (2018b), the eutrophication ratio (ER) in Andersen et al. (2017) and long-term variation in spring and summer by Wasmund & Siegel (2008) were consulted for the definition $RefCon_{Chl\,a}$. The definition of the $RefCon_{Fish}$ are based on the stock size and weight-at-age (WAA) of planktivorous fish (HELCOM 2018a). Seasonal data for the condition of planktivorous fish was not available. The definition of the good feeding conditions for fish are, therefore, based on the interannual variation of the weight-at-age (WAA) of sprat provided by HELCOM ZEN-ZIIM for the Bornholm Basin. Data on WAA of sprat in the Arkona Sea was, however, not available. Due to the similarity of the Bornholm time series of WAA of sprat to the general assessment of WAA in the ICES subdivisions 28 - 32, the Bornholm dataset was used to define the good feeding conditions in the Arkona Sea as well.

The time series of the mean size and total biomass of the zooplankton community were analysed with combined Shewart and cumulative sum (CuSum) control charts. The indicator values were tested for significant deviations from normality by the Kolmorov-Smirnov normality test and Box-Cox transformed if necessary. Z-scores were calculated as the difference of indicator values and the controlling mean standardized to the standard deviation. Upper and lower control limits for the Shewart and CuSum analysis were defined using ±5 standard deviations for $RefCon_{Chl\,a}$ and $RefCon_{Fish}$ (HELCOM 2018a). Trends in accumulated changes for each indicator in question were investigated by calculating a decision-interval CuSum (DI-CuSum) by recursively accumulating positive and negative deviations separately with two statistics (Gorokhova et al. 2016, HELCOM 2018a). The trend analysis was performed for the summer using the long-term data for July/August as originally outline by HELCOM (2018a) and, in addition, for the annual mean of spring/summer using the May and July/August monitoring data in order to integrate the occurrence of indicator relevant zooplankton over the longer seasonal cycle in the western Baltic Sea.

2.3 Historical data

The data available from the monitoring data is restricted to the period from 1986 onwards. This period is unlikely to provide indicator values for condition that are unaffected by nutrient input to the Baltic Sea and human fishery activities (Gorokhova et al. 2016). Historical data before 1986 was searched by two approaches. First, a search on Google Scholar was conducted using the key words ‘zooplankton’ and ‘Baltic Sea’. Once literature has been identified, the available data was checked whether it contained data including all indicator-relevant zooplankton groups. In the second approach, the references of all publications referring to zooplankton work in the Baltic Sea were scanned for grey literature such as project or institutional reports that might contain additional data. In case data was identified, it was extracted and digitized in order to provide historical values for the mean size and total stock for comparison with the present-day time series. The calculations were done as described above; the individual, taxon-specific body wet weight values (µg wet weight individual^{-3}) based on Hernroth (1985) were used to calculate the biomass, similar to analysis of the long-term data series.

3 Results

3.1 Synopsis of seasonal dynamics and interannual variation of zooplankton

The seasonal variation in the abundance and biomass of zooplankton in 2015 and 2016 indicate an early start of zooplankton production in the western Baltic Sea (Figs. 2 and 3). The timing of the increase in zooplankton stocks, the relative composition of the zooplankton and the occurrence of indicator-relevant groups showed area-specific differences and inter-annual variations.

Kiel Bight

Considerable differences in the zooplankton stock size occurred between the consecutive years. The abundance and biomass values in 2016 were about twice those observed during 2015. The start of the productive phase, defined as the major increase in stock size, occurred in April - May in 2015. Stocks remained high until August. In 2016, the stocks increased already in March and the productive phase lasted until October. Nauplii and copepodites stages of the calanoid copepods were the major groups in both years. Largest stocks occurred during May - July. Rotifers and cladocerans were only minor contributors to the zooplankton stock. Rotifers were not observed in2015, which could be related to missing observations in April. They occurred, however, in April during 2016. Cladocerans were rare in both years. While they were confined to the period May to July in 2015, they occurred year-round in 2016. The cyclopoid copepod *Oithona* spp. was observed in high abundance. In 2015, high concentrations occurred during April - August; in 2016 maximum numbers were recorded in August - September. Calanoid copepods dominated generally in terms of biomass.

Bay of Mecklenburg

The seasonal variation of the zooplankton resembled that in the Kiel Bight with regard to timing, composition, difference in stock size and interannual variability in both years. The abundance increased continuously during winter - spring 2015 with a maximum recorded in April and remained high until July (Fig. 2). In 2016, the major increase in abundance occurred in March and the abundance was high until September (Fig. 3). Nauplii and copepodites stages of the calanoid copepods were dominating in terms of numbers over the entire period from March to August. Compared to 2015, a pronounced seasonality with maximum concentrations in May was observed in 2016. Rotifers and cladocera were minor contributors to the stock in both years. The timing of rotifers was variable. They occurred in April in 2015 and in March in 2016. Cladocerans peaked in July 2015, but displayed continuously low concentrations during the period April to September in 2016. *Oithona* spp. was present in April - May 2015, but was restricted to autumn in 2016. The maximum total biomass was recorded during April - August and March - September in 2015 and 2016, respectively. Calanoid copepods dominated on an annual basis, cladocera contributed primarily during June - July to the stocks.

Arkona Sea

The seasonal increase in the zooplankton stocks occurred during April - May in both years and, thus, later compared to the shallower Kiel Bight and Bay of Mecklenburg (Figs. 2 and 3). The stocks remained high until September. In 2016, the total stock was about one third larger than 2015. Nauplii and calanoid copepods were the most important groups on an annual basis in both years. However, rotifers and cladocerans contributed considerably to the zooplankton. Calanoid copepods dominated in terms of number and biomass during April to August in both years. Rotifers displayed a large inter-annual variation in abundance and timing. They occurred in low numbers during April - May 2015, but were found in peak concentrations in April 2016. The cladocera were confined to the summer and occurred in the period June to September in 2015 - 2016. During this period, they contributed considerably to the biomass of zooplankton. In contrast to the Kiel Bight and the Bay of Mecklenburg, *Oithona* spp. was only rarely observed in the Arkona Sea. The species showed high concentrations in November 2016 only.

Figure 2 Seasonal variation in the community composition in terms of abundance (Ind. m^{-3}, left panel) and biomass (mg wet weight m^{-3}, right panel) of the main zooplankton groups in the Kiel Bight, Bay of Mecklenburg, Arkona Sea and Bornholm Basin during 2015.

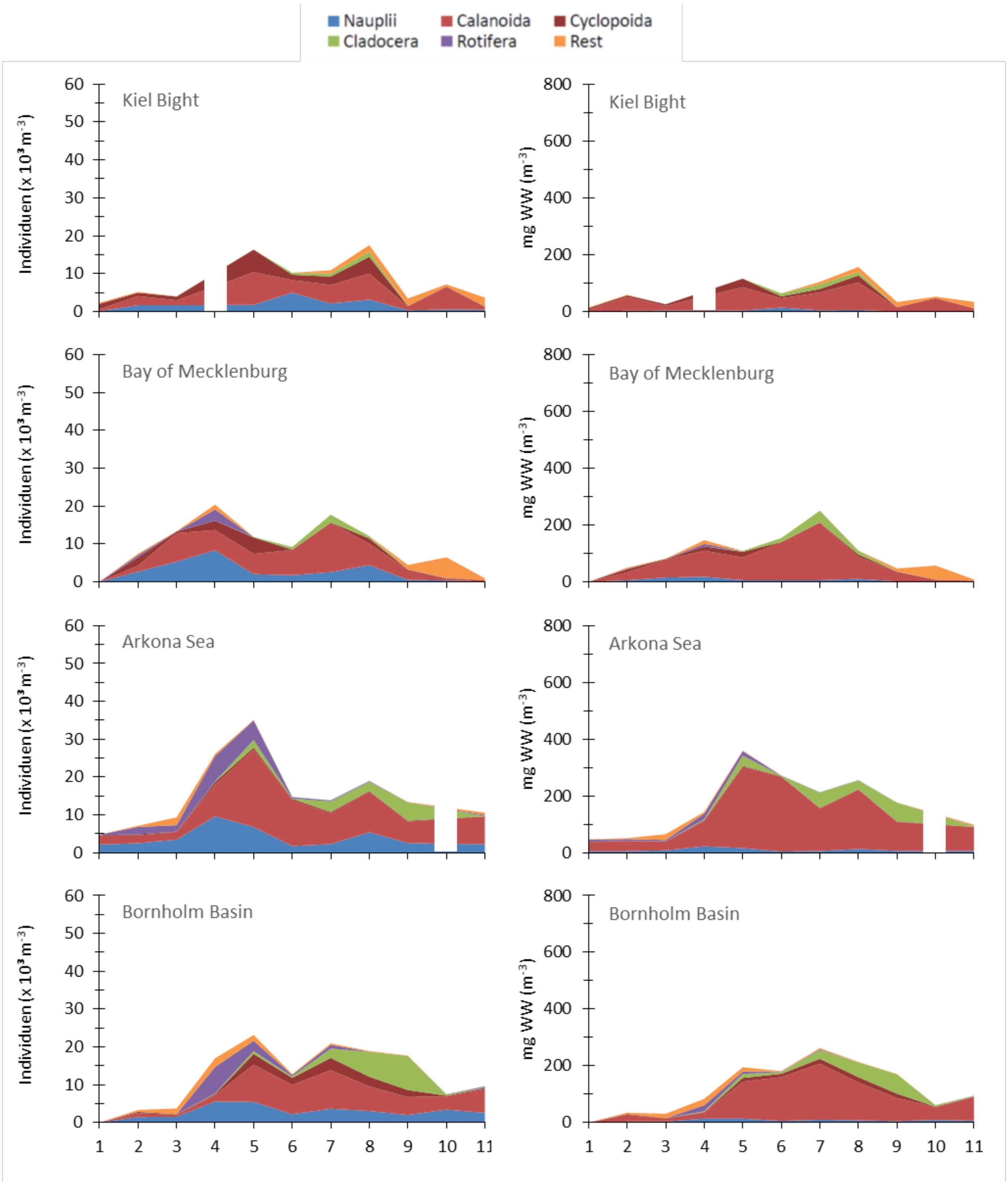


Figure 3 Seasonal variation in the community composition in terms of abundance (Ind. m^{-3}, left panel) and biomass (mg wet weight m^{-3}, right panel) of the main zooplankton groups in the Kiel Bight, Bay of Mecklenburg, Arkona Sea and Bornholm Basin during 2016.

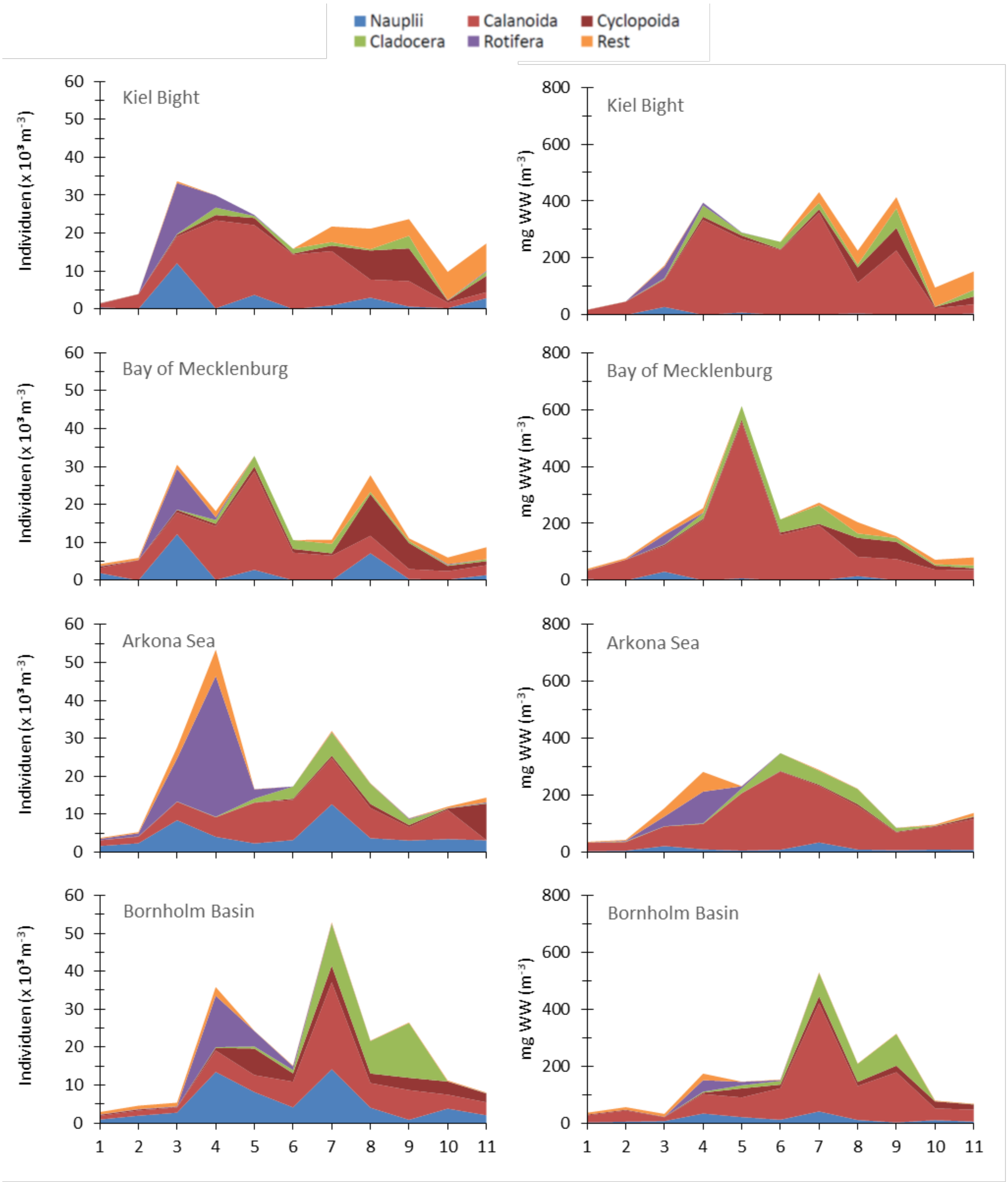
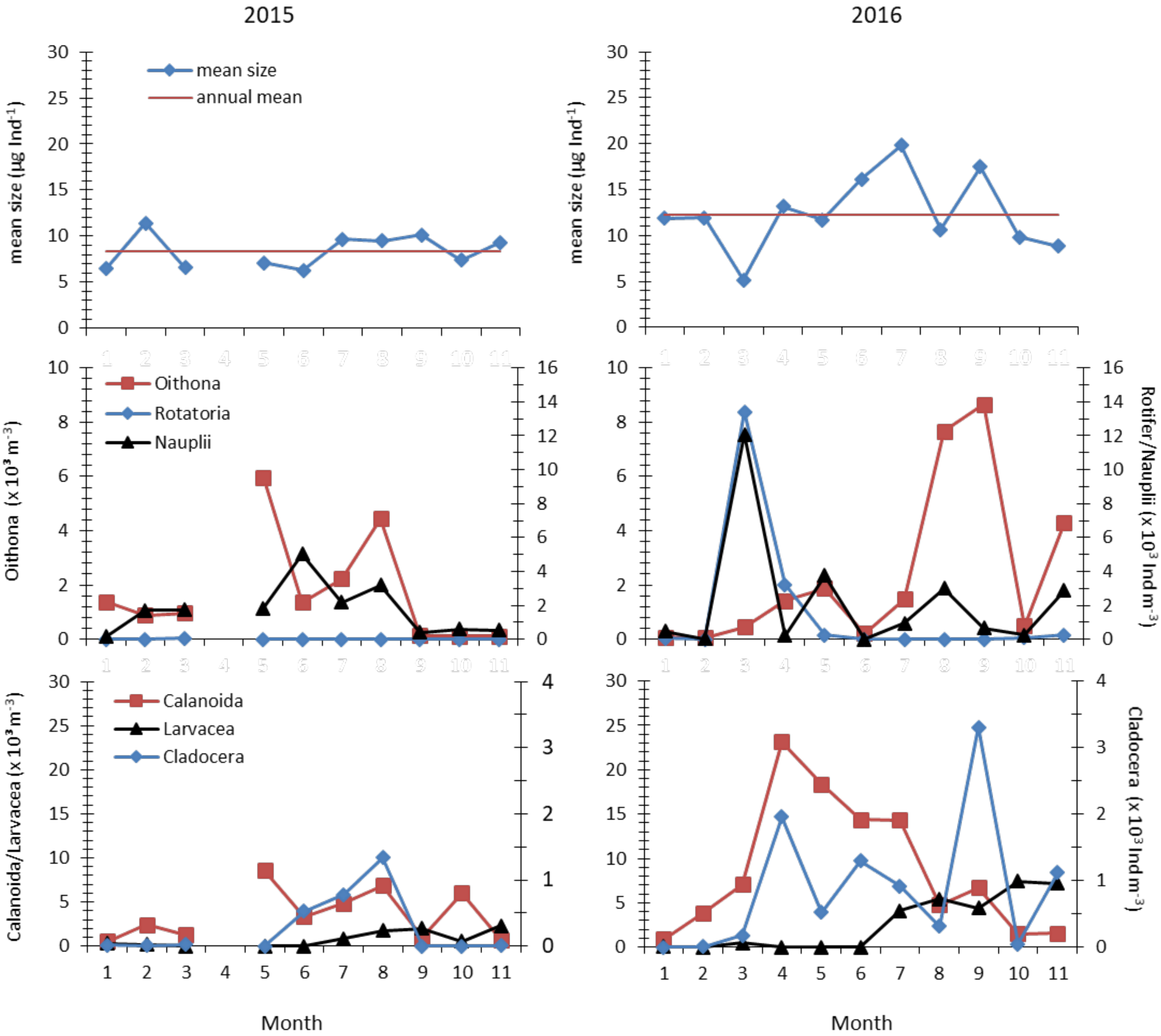


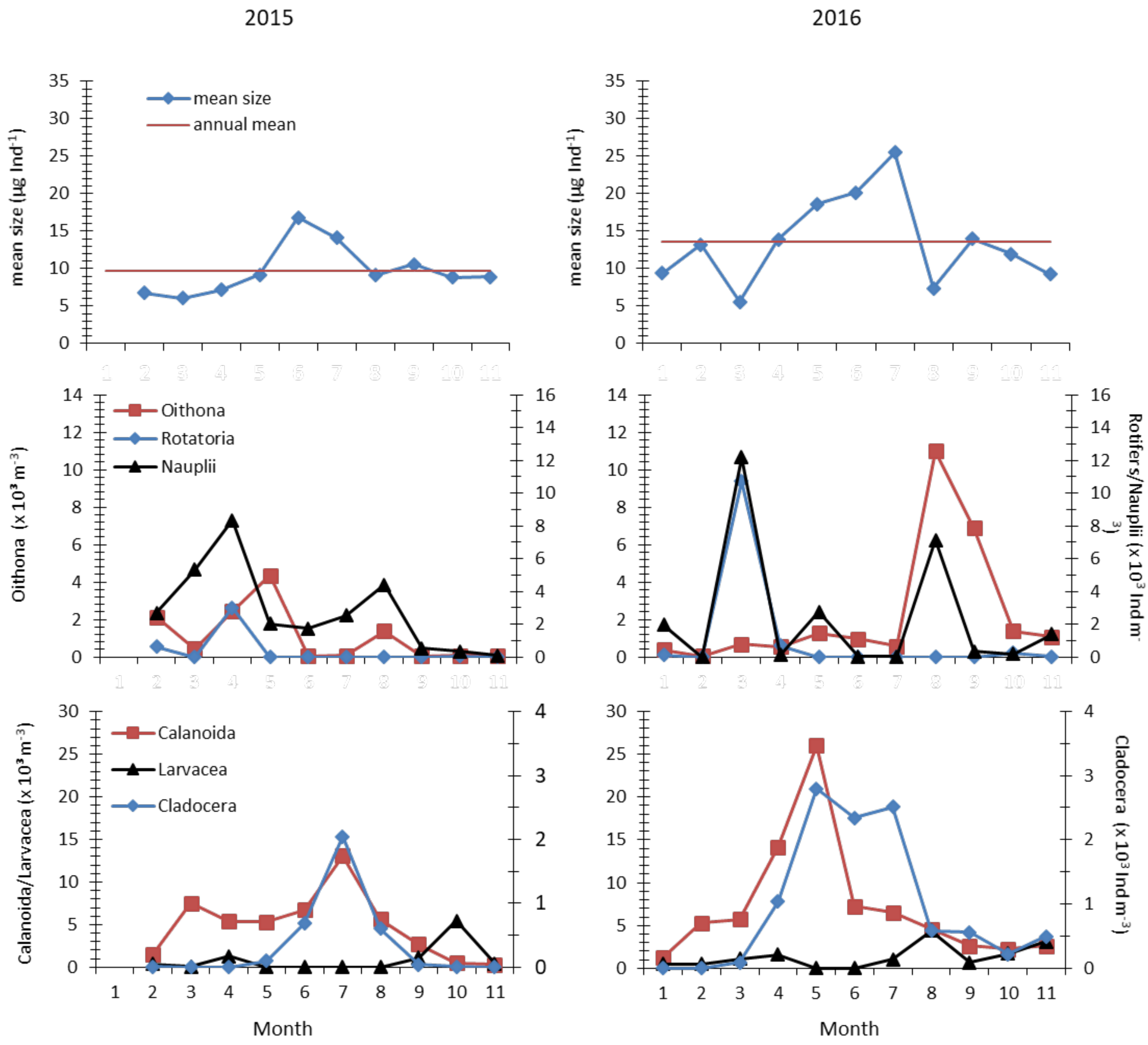
Figure 4 Seasonal variation of ’mean size‘ and the annual mean (upper panel), the abundance of *Oithona* spp., rotifers, copepod nauplii (middle panel) and calanoid copepods, larvacea, cladocerans (lower panel) during 2015 (left) and 2016 (right) in the Kiel Bight.



Bornholm Basin

The seasonal development of the zooplankton community resembled closely the dynamics in the Arkona Sea. The major increase in zooplankton abundance occurred in April in both 2015 and 2016. The abundance and biomass remained high until September. Nauplii and calanoid copepods were the major groups on an annual basis. Compared to the other areas, however, their abundance and biomass maxima were shifted to the second half of the year and occurred during May - September, particularly in 2016. Rotifers were observed during spring (April - May) in both years. Their maximum occurred in April, but the concentrations were generally lower compared to the Arkona Sea. In contrast, cladocerans were more abundant than in the Arkona Sea. They occurred mainly during summer with maximal concentrations in September in both years. *Oithona* spp. was present in low abundance during the entire season except in May 2016. Calanoid copepods and cladocera dominated the seasonal variation of total zooplankton biomass. Due to their increasing abundance during the summer, the biomass maximum was shifted to June-September compared to the shallower western areas.

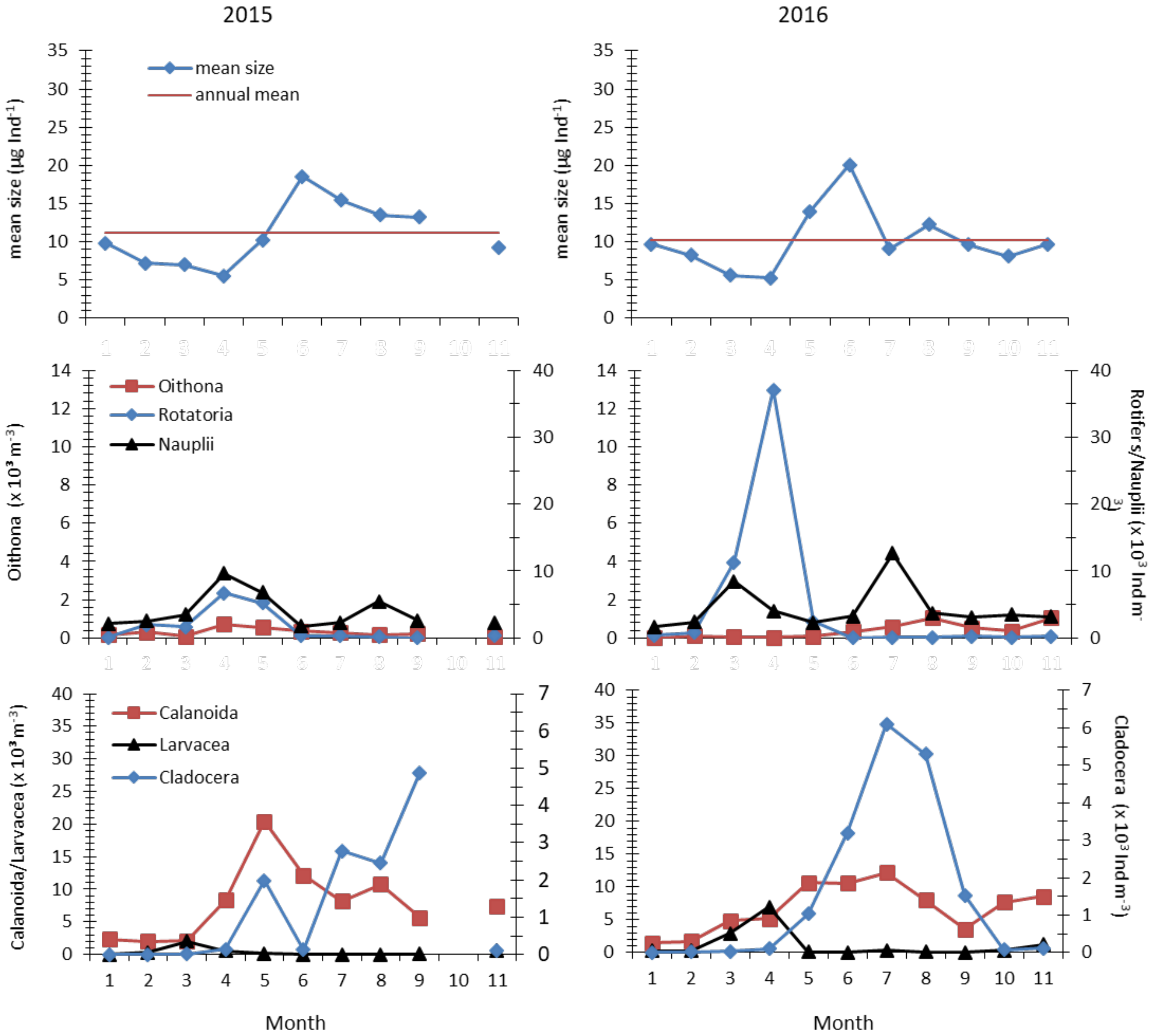
Figure 5 Seasonal variation of 'mean size' and the annual mean (upper panel), the abundance of *Oithona* spp., rotifers, copepod nauplii (middle panel) and calanoid copepods, larvacea, cladocerans (lower panel) during 2015 (left) and 2016 (right) in the Bay of Mecklenburg.



3.2 Community composition and seasonal variation of 'mean size'

The community composition has a strong influence on the indicator value of mean size. Major groups differ considerably in their average individual mass. Rotifers and copepod nauplii have a low individual mass ranging from 1.5 - 3.0 and 1.5 - 6.0 µg WW ind^{-1}, respectively. Calanoid copepodite stages (4.0 - 20.0 µg WW ind^{-1}), adult copepods (15.0 - 70.0 µg WW ind^{-1}) and cladocera (7.0 - 40.0 µg WW ind^{-1}) are generally heavier, while the cyclopoid copepod *Oithona* spp. (4.0 - 10.0 µg WW ind^{-1}) and larvacea (9.0 - 10.0 µg WW ind^{-1}) have an intermediate weight. The seasonal variation and the annual mean differ in the Kiel Bight, the Bay of Mecklenburg, Arkona Sea and Bornholm Basin due to the seasonal and regional differences in the community composition of the zooplankton (Figs. 4 - 7).

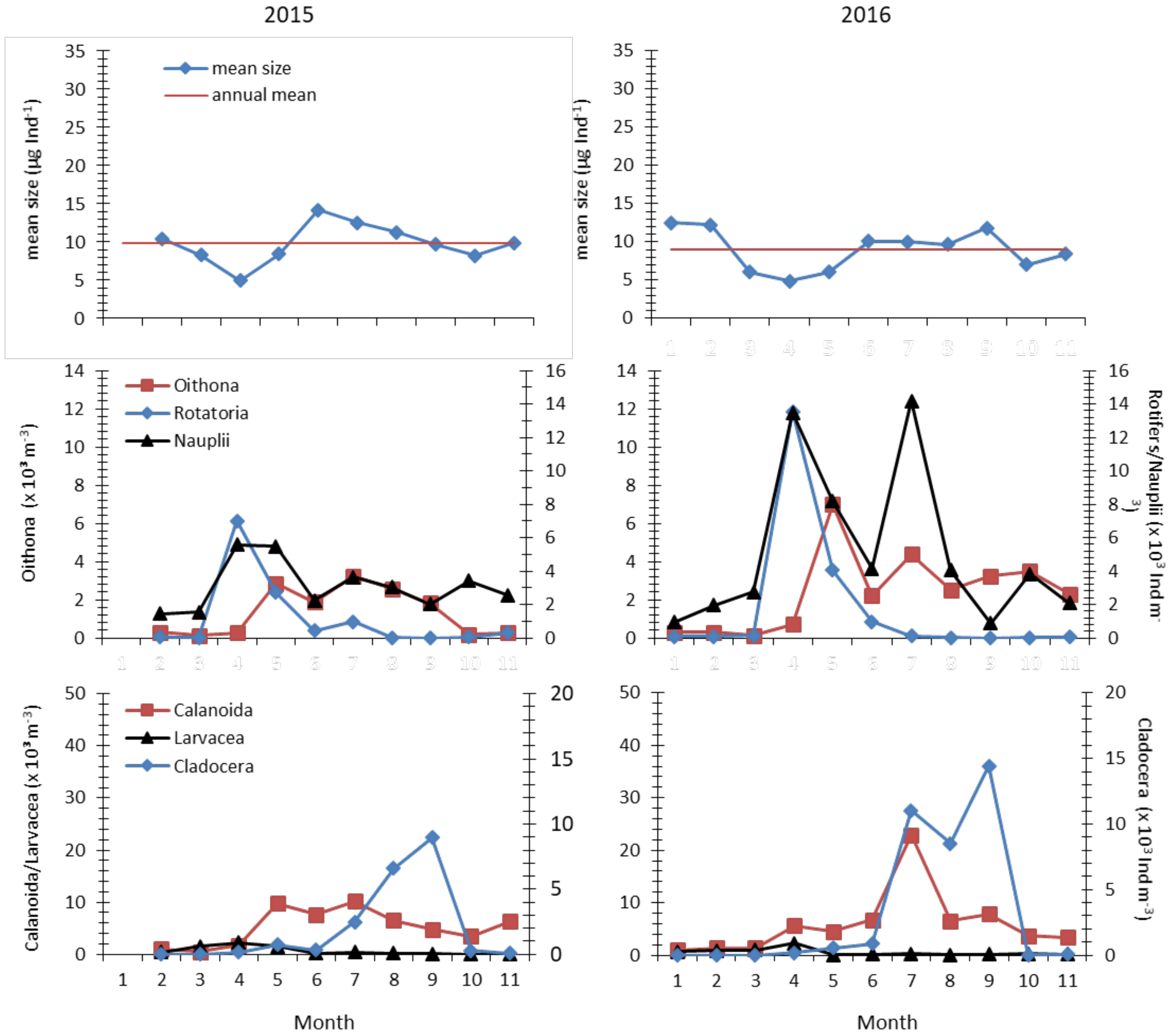
Figure 6 Seasonal variation of 'mean size' and the annual mean (upper panel), the abundance of *Oithona* spp., rotifers, copepod nauplii (middle panel) and calanoid copepods, larvacea, cladocerans (lower panel) during 2015 (left) and 2016 (right) in the Arkona Sea.



Kiel Bight

Zooplankton mean size displayed irregular patterns and a pronounced interannual variation during the study (Fig. 4). The annual mean was 8.3 and 12.3 µg WW ind^{-1} in 2015 and 2016, respectively. The range in mean size was considerably smaller in 2015 (6.3 - 10.1 µg WW ind^{-1}) than in 2016 (5.2 - 19.8 µg WW ind^{-1}). In 2015, when no rotifers were recorded, values below the annual mean resulted from increased concentrations of *Oithona* spp. and nauplii during March - June (6.6 - 7.1 µg WW ind^{-1}). The increasing abundance of calanoid copepods during July - August counteracted the continuously high abundance of small zooplankton. In 2016, a minimum in spring (5.2 µg WW ind^{-1}) was caused by peak concentrations in rotifers in March. Mean size increased during spring due to increasing stocks in calanoid copepods and cladocera, but shows some fluctuations. A minimum in August was caused by peak concentrations of *Oithona*, and a summer minimum of calanoid copepods and cladocera.

Figure 7 Seasonal variation of 'mean size' and the annual mean (upper panel), the abundance of *Oithona* spp., rotifers, copepod nauplii (middle panel) and calanoid copepods, larvacea, cladocerans (lower panel) during 2015 (left) and 2016 (right) in the Bornholm Basin.



Bay of Mecklenburg

Although the zooplankton community in the Kiel Bight and the Bay of Mecklenburg displayed a close resemblance in their composition, the seasonal variation in mean size differed. This is owed to the larger stocks of rotifers and *Oithona* spp. in the Bay of Mecklenburg. The mean size showed a large seasonal variation in both years with a minimum mean size in spring (5.6 - 6.1 µg WW ind^{-1}) and late summer-autumn decline (7.4 - 9.1 µg WW ind^{-1}) and a summer maximum (16.8 – 25.5.1 µg WW ind^{-1}). The seasonal pattern was caused by high concentrations of nauplii and *Oithona* spp. in spring 2015/2016 and autumn 2016, while rotifers and nauplii contributed to low spring values in 2016. Seasonal maxima in the abundance of calanoid copepods and cladocera during May - July are responsible for the early summer maximum in mean size. Due to considerable differences in stock size of these groups, the annual mean was higher in 2016 (13.6 µg WW ind^{-1}) than in 2015 (9.8 µg WW ind^{-1}).

Arkona Sea

A large range in mean size was also observed in 2015 (5.6 - 18.6 µg WW ind^{-1}) and 2016 (5.3 - 20.1 µg WW ind^{-1}). The annual mean of 11.1 and 10.2 µg WW ind^{-1}, however, was similar in both years. The low winter-spring minimum lasted until April and was caused by a high abundance of nauplii and rotifers. The negative influence of the considerable higher abundance of rotifers in 2016 was buffered by larger stocks of calanoid copepods and larvacea. The interannual differences in mean size were therefore small. The shift from nauplii to copepodites in the stocks of calanoid copepods caused the increase of the mean size to maximal values during early summer. The autumn decline was variable in both years. This was mainly related to the interplay of abundant nauplii and the timing in the occurrence of cladocera. In 2015, cladocera were abundant during July - September, postponing the autumn decline in mean size. In 2016, the abundance of cladocera declined already during August - September causing an earlier decline in mean size. Due to low concentrations, *Oithona* spp. had no influence on the variation of the mean size.

Bornholm Basin

The seasonal variation of mean size was rather regular in both years and followed a similar pattern as described for the Arkona Sea with a spring and late autumn minimum and summer maxima in both years. The seasonal variation in mean size was, however, small. Due to the delayed increase in the stocks of calanoid copepods and cladocera, the spring minimum lasted until May (4.9 - 6.8 µg WW ind^{-1}) and the seasonal maximum occurred from June to September (9.7 - 14.2 µg WW ind^{-1}). Rotifers and nauplii were mainly responsible for the low mean size during February - April, while *Oithona* spp. contributed in May and October - November. The seasonal variation in mean size was, therefore, steered by the abundance of nauplii and rotifers on the one hand and calanoid copepods and cladocera on the other hand. Due to the higher concentrations of the smaller groups, in particular nauplii during summer, in comparison to the Arkona Sea, the range in mean size was accordingly smaller. Ranges of 4.9 - 14.1 and 4.9 - 12.4 µg WW ind^{-1} and the annual mean of 9.8 and 9.0 µg WW ind^{-1}, were similar in 2015 and 2016, respectively.

3.3 Variance of sampling

During 2016, triplicate samples were analysed for variation due to sampling error and overdispersion. These triplicates could not always be collected during all months due to bad weather conditions or loss of samples (Tables 2 - 5). Nevertheless, the most relevant periods during April to September were well covered.

The coefficient of variation was analysed for single groups of zooplankton and for the bulk parameter total zooplankton abundance, total zooplankton biomass and mean size. Although the range of the variation coefficients of < 1 to 100 % was considerably large, the error associated with sampling was generally low as most values were lower than 20 %. Differences between the taxonomic groups were also small indicating that patchiness was not related to a specific taxonomic group. In most cases, variation coefficients larger than 20 % were observed when the abundance of the analysed taxonomic unit was low due to a strong seasonally in the life cycle, and, accordingly, counts were low. Examples were the increasing variation of the rotifers and the cladocera when their abundance decrease to a few individuals outside their population maximum. In contrast, during peak concentrations variation was considerably small. Some larger variation (22 – 50 %), however, occurred among nauplii and calanoid copepods during spring and summer when the abundance was generally high. However, these were rather exceptional.

The coefficient of variation of total zooplankton abundance (TZA), total zooplankton biomass (TZB) and mean size (MS) was, in contrast to that of single taxonomic groups, generally very small and did

Table 2: The statistical mean, standard deviation and coefficient of variation of the abundance of major zooplankton groups (ind. m^{-3}), total zooplankton abundance (TZA, ind. m^{-3}), total zooplankton biomass (TZB, mg wet weight m^{-3}) and mean size (MS, µg ind^{-1}) of samples from the Kiel Bight in 2016.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Group	Samples	1	3	3	3	2	3	3	3	3	3	1
Nauplii	Mean	465	22	12088	208	3748	9	958	3014	658	244	2875
	STD		22	1534	338		5	467	475	10	83	
	Var%		55	7	94		32	28	9	1	20	
Calanoida	Mean	916	3884	7072	23178	18387	14371	14315	4762	6695	1479	1592
	STD		776	177	8776		666	2021	1006	387	132	
	Var%		12	1	22		3	8	12	3	5	
Cylopoida	Mean	73	52	461	1416	1870	219	1472	7672	8652	495	4292
	STD		19	34	106		160	456	2553	1349	91	
	Var%		22	4	4		42	18	19	9	11	
Cladocera	Mean	0	5	173	1962	521	1301	915	317	3305	45	1117
	STD		4	40	520		190	282	24	95	16	
	Var%		43	13	15		8	18	4	2	20	
Rotifers	Mean	63	0	13417	3225	255	2	0	0	0	105	250
	STD			2499	1183		4				70	
	Var%			11	21		100				38	
Larvacea	Mean	56	11	480	4	0	0	4096	5429	4394	7447	7188
	STD		5	115	7			629	1660	534	62	
	Var%		28	14	100			9	18	7	< 0	
TZA	Mean	1572	3975	33691	29993	24780	15901	21756	21195	23704	9815	17313
	STD		810	4217	9164		576	3016	4534	2279	61	
	Var%		12	7	18		2	8	12	6	< 0	
TZB	Mean	19	48	195	469	310	304	462	235	532	98	194
	STD		7	10	161		11	43	41	37	2	
	Var%		9	3	21		2	7	11	5	1	
MS	Mean	11,9	12,2	5,8	15,5	12,5	19,1	21,3	11,2	22,5	10,0	11,2
	STD		2,2	0,5	1,2		0,8	1,3	0,9	0,6	0,1	
	Var%		10	5	4		2	4	5	1	1	

not exceed 21 %. A gradient in the variation was found with coefficients for TZA, TZB and MS ranging from 0 – 19 %, 1 – 20 % and 1 – 10 % in the Kiel Bight and Bay of Mecklenburg to 2 – 12 %, 2 – 12 % and 0.4 - 7.3 % in the Arkona Sea and Bornholm Basin, respectively. The variation in the months May and August, in which the indicator values were assessed, was lower than 10 %. The upper and lower 99 % confidence limits of MS and TZB for the areas were, therefore, generally narrow (Fig. 8). A larger variation was observed in the estimates of the zooplankton biomass, while the variation in mean size was generally minor. This indicates that primarily variation in the abundance of the taxonomic group occurred between replicated samples, but that the composition of the zooplankton community, in contrast, remained rather stable.

Table 3: The statistical mean, standard deviation and coefficient of variation of the abundance of major zooplankton groups (ind. m^{-3}), total zooplankton abundance (TZA, ind. m^{-3}), total zooplankton biomass (TZB, mg wet weight m^{-3}) and mean size (MS, µg ind^{-1}) of samples from the Bay of Mecklenburg in 2016.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Group	Samples	1	3	3	3	3	3	3	3	3	3	2
Nauplii	Mean	1955	2	12170	126	2772	39	12	7153	353	188	1396
	STD		3	811	114	2387	35	11	1475	189	162	
	Var%		100	4	52	50	53	52	12	31	50	
Calanoida	Mean	1310	5317	5710	14187	25971	7279	6544	4594	2624	2252	2614
	STD		371	1061	4742	2986	1120	2906	712	655	355	
	Var%		4	11	19	7	9	26	9	14	9	
Cylopoida	Mean	392	57	684	563	1275	973	578	10990	6878	1383	1067
	STD		31	110	66	88	232	205	2590	1900	202	
	Var%		31	9	7	4	14	20	14	16	8	
Cladocera	Mean	0	1	91	1041	2795	2345	2513	588	561	219	499
	STD		2	16	342	546	522	640	154	117	106	
	Var%		100	10	19	11	13	15	15	12	28	
Rotifers	Mean	109	1	10724	702	0	0	0	0	0	246	15
	STD		2	293	485						43	
	Var%		100	2	40						10	
Larvacea	Mean	514	538	1096	1614	0	0	1048	4375	666	1744	3140
	STD		31	265	642			63	341	372	703	
	Var%		3	14	23			3	4	32	23	
TZA	Mean	4280	5916	30474	18234	32813	10636	10694	27700	11081	6032	8730
	STD		364	1685	5779	1799	1253	3463	4750	2932	1344	
	Var%		4	3	18	3	7	19	10	15	13	
TZB	Mean	40	78	186	294	725	299	354	223	173	81	100
	STD		9	47	94	89	41	122	26	40	18	
	Var%		7	16	20	7	9	22	8	14	12	
MS	Mean	9,4	13,2	6,1	16,1	22,1	28,1	33,1	8,1	15,7	13,4	11,4
	STD		0,8	1,2	0,9	1,8	0,7	3,2	0,7	1,0	1,2	
	Var%		4	11	3	5	1	6	5	4	5	

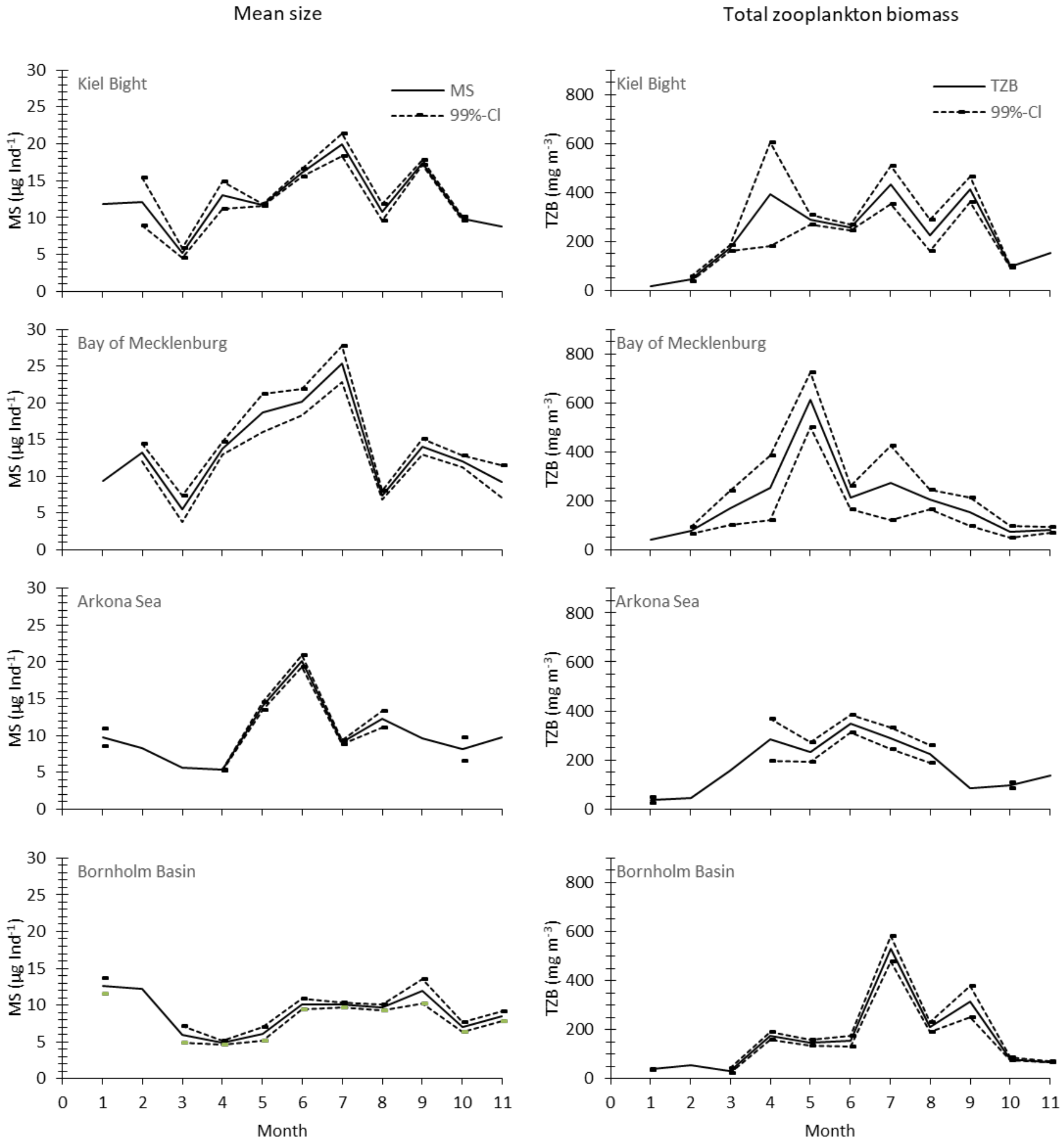
Table 4: The statistical mean, standard deviation and coefficient of variation of the abundance of major zooplankton groups (ind. m^{-3}), total zooplankton abundance (TZA, ind. m^{-3}), total zooplankton biomass (TZB, mg wet weight m^{-3}) and mean size (MS, µg ind^{-1}) of samples from the Arkona Sea.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Group	Samples	3	1	1	3	3	3	3	3	1	3	1
Nauplii	Mean	1643	2359	8432	4066	2325	3208	12653	3708	3093	3471	3153
	STD	542			807	586	202	154	277		450	
	Var%	19			11	15	4	1	4		7	
Calanoida	Mean	1509	1724	4856	5156	10697	10579	12208	7974	3547	7693	8604
	STD	450			1165	1553	314	2205	780		143	
	Var%	17			13	8	2	10	6		1	
Cylopoida	Mean	10	104	50	30	90	343	576	1056	533	368	1062
	STD	4			18	25	8	81	324		49	
	Var%	16			32	9	19	8	15		11	
Cladocera	Mean	3	21	39	105	1028	3192	6102	5294	1529	72	124
	STD	2			17	242	830	953	873		23	
	Var%	51			9	14	15	9	10		19	
Rotifers	Mean	370	800	11339	37089	2429	17	91	64	213	55	233
	STD	95			7269	214	15	85	71		24	
	Var%	15			11	5	52	54	64		25	
Larvacea	Mean	234	296	2922	6943	74	0	331	115	44	359	1236
	STD	232			1586	22	0	12	58		35	
	Var%	57			13	18		2	29		6	
TZA	Mean	3768	5304	27624	53383	16641	17320	31925	18176	8898	11976	14215
	STD	898			10663	2116	1263	2588	826		562	
	Var%	14			12	7	4	5	3		3	
TZB	Mean	37	43	168	331	289	478	421	337	115	93	127
	STD	8			66	36	53	47	34		7	
	Var%	12			12	7	4	6	6		5	
MS	Mean	9,8	8,0	6,1	6,2	17,4	27,6	13,2	18,5	13,0	7,8	9,0
	STD	0,9			0,1	0,12	1,1	0,6	1,1		0,8	
	Var%	5			1	1	2	3	3		6	

Table 5: The statistical mean, standard deviation and coefficient of variation of the abundance of major zooplankton groups (ind. m^{-3}), total zooplankton abundance (TZA, ind. m^{-3}), total zooplankton biomass (TZB, mg wet weight m^{-3}) and mean size (MS, µg ind^{-1}) of samples from the Bornholm Basin.

Group	Samples	Jan 3	Feb 1	Mar 3	Apr 3	May 3	Jun 3	Jul 3	Aug 3	Sept 3	Oct 3	Nov 3
Nauplii	Mean	947	1966	2752	13483	8175	4149	14185	4062	869	3821	2077
	STD	104		106	1590	1904	351	720	128	66	418	228
	Var%	6		2	7	13	5	3	2	4	6	6
Calanoida	Mean	948	1357	1372	5638	4477	6754	22930	6487	7841	3669	3418
	STD	39		114	484	151	728	1091	354	1395	212	104
	Var%	2		5	5	2	6	3	3	10	3	2
Cylopoida	Mean	310	300	143	700	6995	2200	4379	2531	3242	3486	2299
	STD	94		46	65	509	598	479	164	59	105	61
	Var%	18		18	5	4	16	6	4	1	2	2
Cladocera	Mean	< 0		1	174	548	888	11031	8530	14429	32	72
	STD	1		1	31	56	70	1973	768	3276	5	58
	Var%	100		51	10	6	5	10	5	13	9	47
Rotifers	Mean	74	86	150	13550	4064	973	125	27	3	12	43
	STD	19		132	1894	970	320	93	19	5	10	2
	Var%	15		51	8	14	19	43	40	100	49	3
Larvacea	Mean	628	881	964	2304	63	165	245	100	136	231	130
	STD	129		835	468	12	46	50	31	26	85	77
	Var%	12		50	12	11	16	12	18	11	21	34
TZA	Mean	2907	4589	5378	35493	24053	15119	52895	21656	26519	11244	8038
	STD	149		519	3489	3681	801	2510	841	3428	708	344
	Var%	3		6	6	9	3	3	2	7	4	2
TZB	Mean	37	56	32	196	172	183	767	395	630	77	68
	STD	1	0	7	14	9	17	80	31	98	4	2
	Var%	2		12	4	3	6	4	4	8	3	1
MS	Mean	12,6	12,2	6,0	5,5	7,2	12,1	14,5	18,2	23,7	6,9	8,4
	STD	0,7		0,8	0,2	0,8	0,5	0,8	0,7	0,7	0,5	0,6
	Var%	3		7	2	6	3	3	2	2	4	4

Figure 8 Seasonal variation of the indicator values for mean size (MS) and total zooplankton biomass (TZB) and their upper and lower 99 % confidence limits in the Kiel Bight, Bay of Mecklenburg, Arkona Basin and Bornholm Basin during 2016.

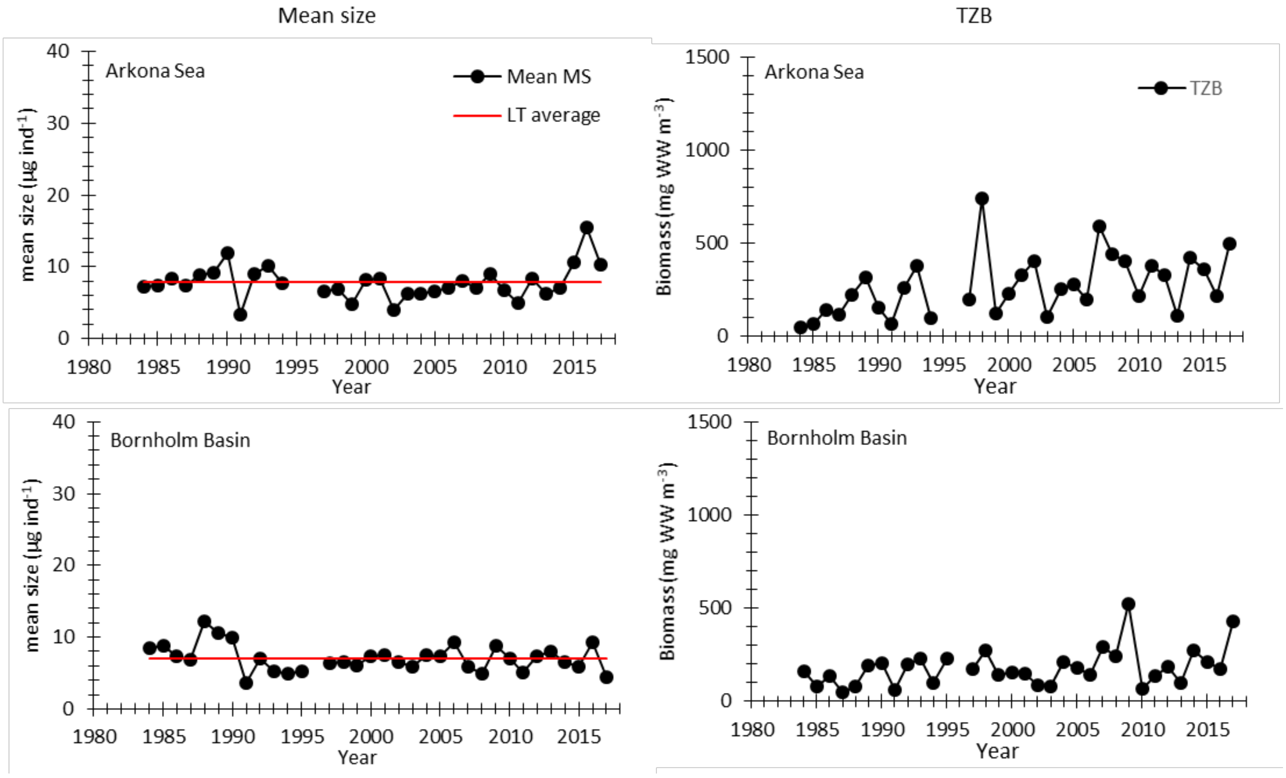


3.4 Reference conditions

3.4.1 Long-term variation of mean size and biomass in the Arkona Sea and Bornholm Basin

The indicator 'Mean size and total stock' is assessed on samples that are collected during the summer period from June to September (HELCOM 2018a). The early seasonal rise in the stocks of zooplankton and relevant zooplankton groups during spring, however, suggest to extend assessment period and cover the entire spring-summer period. The long-term variation of the indicator values for mean size (MS) and total zooplankton biomass (TZB) for the Arkona Sea and Bornholm Basin during the years 1984 - 2017 shows large interannual variability and long-term changes in both seasons, particularly in summer (Fig. 9, 10).

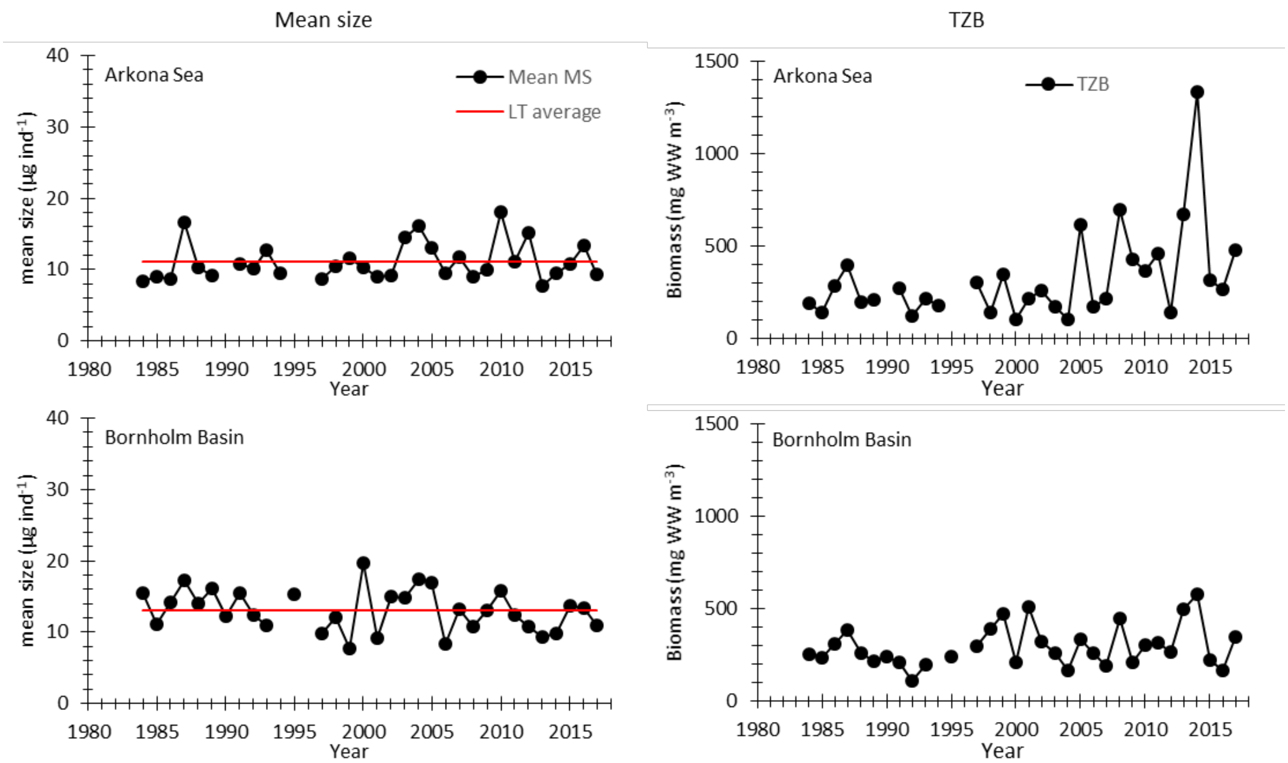
Figure 9 Long-term variation of the indicator values for mean size (MS, left panel) and total zooplankton biomass (TZB, right panel) in the Arkona Sea (upper panel) and the Bornholm Sea (lower panel) during May in the years 1984-2017.



Spring

In the Arkona Sea, the long-term mean for the mean size during spring was 7.8 ± 2.3 µg wet weight ind.$^{-1}$ (Fig. 9). The interannual variation showed alternations of periods of rather stable mean size with periods of larger shifts. Small variations occurred during 1984 - 1989 and 2002 - 2009 during which mean size ranged from 7.2 - 9.1 and 6.2 - 9.0 µg wet weight ind.$^{-1}$, respectively. The range increased considerably during 1990 - 2001 (3.4 - 12.0 µg wet weight ind.$^{-1}$) and 2010 - 2017 (4.9 - 15.5 µg wet weight ind.$^{-1}$). Total zooplankton biomass tended to increase over the observation period, although fluctuations were large (47 - 744 mg wet weight m^{-3}). This was primarily caused by increasing maximal biomass values. While a biomass smaller than 200 mg wet weight m^{-3} was regularly recorded in the beginning of time series, the biomass was rarely lower than that since the year 2000.

Figure 10 Long-term variation of the indicator values for mean size (MS, left panel) and total zooplankton biomass (TZB, right panel) in the Arkona Sea (upper panel) and the Bornholm Sea (lower panel) during August in the years 1984 - 2017.



In the Bornholm Basin, a large interannual variation in mean size was mainly apparent during 1988 - 1991. Three years of an extraordinary high mean size of 10.0 - 12.2 wet weight ind.$^{-1}$ µg was followed by a rapid decline to 7.9 µg wet weight ind.$^{-1}$. During the remainder of the time series mean size fluctuated with low variability around the long-term mean of 7.1 ± 1.9 µg wet weight ind.$^{-1}$ although the year to year variation appears to increase following the year 2005. The total zooplankton biomass did not reveal a clear trend and fluctuated between 51 and 524 mg wet weight m^{-3}. The high biomass recorded in 2009 and 2017, respectively, appears to be rather exceptional.

Summer

In the Arkona Sea, the long-term mean for the mean size was 11.1 ± 2.6 µg wet weight ind.$^{-1}$. In the beginning of the time series, mean size showed large fluctuations from 8.4 to 16.6 µg wet weight ind.$^{-1}$ until the late 1980s. The variability decreased from 1989 - 2002, but the mean size was generally below the long-term mean (9.2 - 12.7 µg wet weight ind.$^{-1}$). The fluctuations increased again with alternating periods of high and low mean size until the present (7.8 - 18.2 µg wet weight ind.$^{-1}$). The total zooplankton biomass fluctuated from 102 to 398 mg wet weight m^{-3} during the beginning of the time series until 2004. Following this period, the biomass showed an increasing trend together with a considerable interannual variability (141 – 1,331 mg wet weight m^{-3}).

In the Bornholm Basin, smaller interannual fluctuations in mean size (12.3 - 17.2 µg wet weight ind.$^{-1}$) mostly above the long-term mean of 13.1 ± 2.9 µg wet weight ind.$^{-1}$ occurred during 1984 - 1990. This was followed by a period of increased variability (7.7 - 19.6 µg wet weight ind.$^{-1}$) from 1990 - 2010. The variation in total zooplankton biomass resembled the pattern observed in the Arkona Sea. Small interannual fluctuations during the initial years of the time series (109 - 383 mg wet weight m^{-3}) were followed by an increased variability (165 - 581 mg wet weight m^{-3}). While the maximal biomass appeared to increase over time-series, an overall trend was less pronounced as in the Arkona Sea.

Table 6: Spring and summer periods in good environmental condition according to the EQR (environmental quality ratio), the Chl *a*-concentration or the ER (eutrophication ratio). Areas are: AS= Arkona Sea, BB=Bornholm Basin.

Indicator	Threshold	Area	Years in GES (spring)	Years in GES (summer)	Source
EQR	0.65	AS BB	-	1975-1985, 2005-2006 1985-2005	HELCOM (2009)
Chl *a*	1.8	AS BB	-	1987-1989, 2003-2005 1985-1995	HELCOM (2018b)
Chl *a*	-	AS BB	1984-1990 1984-1990	no trend no trend	Wasmund & Siegel (2008)
ER	1	AS BB	-	until 1970** until 1965**	Jespersen et al. (2017)

*5-year period of lowest Chl *a* values, for which zooplankton data is available
** years in GES not covered by the zooplankton time series (1984 - 2017)

3.4.2 Reference conditions for eutrophication and fish feeding

The evaluation of indicator performance and the evaluation of the GES of the zooplankton community is based on the definition of reference conditions with regard to eutrophication (RefCon$_{Chla}$) and fish nutrition (RefCon$_{\text{Fish}}$).

RefCon$_{Chla}$

The definition of the RefCon$_{Chla}$ for the zooplankton MSTS indicator in the Arkona Sea and Bornholm Sea is constraint by the shortness of the zooplankton time series. Both time series started in 1984 and are, thus, generally too short to represent the zooplankton status during oligotrophic conditions. The choice of reference period therefore will characterize meso- to eutrophic conditions, a problem already realized during indicator testing (Gorokhova et al. 2016). Concerning eutrophication, the definition of reference conditions has been related to the assessment of the environmental quality ratio (EQR) in the Baltic regions during summer. The period of acceptable nutrient concentrations is defined as those years with an EQR larger than 0.67 (HELCOM 2009, 2018a).

The EQR established by HELCOM (2009) contradicts, however, other assessments of eutrophication that are based on the interannual variation of Chl *a* or the eutrophication ratio (Table 6). In the assessment of the EQR, the periods 1975 - 1985/2005 - 2006 and 1985 - 2005 were identified to pass the threshold values for the Arkona Sea and the Bornholm Basin, respectively (HELCOM 2009). This strongly contrasts with the other estimates that identified sub-optimal conditions during the 1990s - 2000s, particularly in the Bornholm Basin, and deteriorating conditions during the following years (Jespersen et al. 2017, HELCOM 2018b). Moreover, Wasmund & Siegel (2008) could identify clear trends for the spring period only. The Chl *a* values in Wasmund & Siegel (2008) originate from the same station and timing than the zooplankton samples. Therefore, the period reference conditions regarding eutrophication were set to the beginning of the time series for the years 1984 - 1990 for spring and 1984 - 1989 (due to lacking values in 1990) for summer and the annual mean. The threshold of RefCon$_{Chla}$ for mean size for spring, summer and the annual mean were 7.0, 8.2 and 7.6 µg wet weight ind.$^{-1}$ and 7.3, 12.3 and 10.6 µg wet weight ind.$^{-1}$ for the Arkona Sea and the Bornholm Basin, respectively (Tab. 7). Accordingly, RefCon$_{Chla}$ for the total zooplankton biomass were 67.5, 157.2 and 119.9 mg wet weight m^{-3} and 71.0, 222.0 and 174.6 mg wet weight m^{-3}.

Table 7: Reference mean (RM) and GES-threshold values for mean size (MS, µg wet weight ind.$^{-1}$) and total zooplankton biomass (TZB, mg wet weight m^{-3}) for the good environmental conditions regarding eutrophication (RefCon$_{Chla}$) and fish feeding conditions (RefCon$_{Fish}$) during spring and summer as well as on basis of the annual mean in the Arkona Sea (AS) and the Bornholm Basin (BB).

Area	Pressure	Indicator	Spring RM/GES	Summer RM/GES	Annual RM/GES	Reference years
AS	Eutrophication	MS	8.6/7.0	10.3/8.2	9.2/7.6	1984 -1989/1990
		TZB	152.7/67.5	238.1/157.2	195.2/119.9	
	Fish feeding	MS	8.3/6.6	10.6/8.8	9.2/7.9	1984 - 1993
		TZB	178.3/87.9	226.7/156.7	203.8/141.1	
	Historical	MS	-	5.8	-	1903
		TZB	-	130.8	-	
BB	Eutrophication	MS	9.2/7.3	14.4/12.3	11.8/10.6	1984 - 1990
		TZB	117.9/71.0	267.7/222.0	198.4/174.6	
	Fish feeding	MS	7.9/5.9	13.9/12.2	10.9/9.6	1984 - 1993
		TZB	125.1/80.3	232.3/177.9	186.7/161.9	
	Historical	MS	-	9.1	-	1903
		TZB	-	81.8	-	

RefCon$_{Fish}$

Following the assessment of the mean size and total stock core indicator protocol, the regional estimates of weight at age of sprat stocks (WAA$_{sprat}$) were used to define the reference conditions for fish feeding. However, for the Arkona Sea (ICES assessment area SD 24) no regional data was available and, therefore, the periods similar to the Bornholm Basin were chosen. The Bornholm data (ICES assessment area SD 25) largely resembles that of the ICES assessment for the Baltic Sea for which the ICES areas SD 22-32 are integrated (ICES 2018). In the Bornholm Basin, high values of WAA$_{sprat}$ were observed from 1980 to 1993; thus, 1984 - 1993 were chosen as a reference period for fish condition. RefCon$_{Fish}$ for mean size for spring, summer and the annual mean were 6.6, 8.8 and 7.9 µg wet weight ind.$^{-1}$ and 5.9, 12.2 and 9.6 µg wet weight ind.$^{-1}$ for the Arkona Sea and the Bornholm Basin, respectively (Tab. 7). Accordingly, RefCon$_{Fish}$ for the total zooplankton biomass were 87.9, 156.7 and 141.1 mg wet weight m^{-3} and 80.3, 177.9 and 161.9 mg wet weight m^{-3}.

3.4.3 Historical data

In total, 32 publications were identified that provide historical zooplankton data (see supplemented references). They were published in the years 1892 - 1992 and were scanned for quantitative data suitable for the calculation of historical indicator values. Most publications did not contain the required data. They were either descriptive without quantitative data or restricted to a specific zooplankton group and could not be combined in order to provide the zooplankton taxa necessary to calculate the indicator values. Four publications included sufficient data. These were Apstein (1906), Driver (1907), Kraefft (1908) and Büse (1915). The data, however, differed with regard to the geographic and seasonal coverage (Table 8). While the Kiel Bight and the Bay of Mecklenburg are generally well represented in all publications, data for the Arkona Sea and Bornholm Basis for comparison with the long-term data from 1984 - 2017 was largely restricted to August 1903 (Apstein 1906).

The sample analysis in the identified publications did not completely follow the guidelines for the zooplankton analysis in the monitoring programmes of the Baltic Sea (HELCOM 2015). This concern largely the diverse groups of copepods. Here, copepodites stages were not separated into species and stage specific groups (Copepodites 1 - 3 and 4 - 5) as required in these guidelines but integrated instead.

Table 8: Years, areas and months for which data on zooplankton composition suitable for the evaluation of historical indicator values was available.

Year	Area	Month(s)	Reference
1903	Kiel Bight Bay of Mecklenburg Arkona Sea Bornholm Basin	2,3,8,11 2,3,8,11 2,3,8,11 8, 11	Apstein (1906)
1905	Kiel Bight Bay of Mecklenburg Arkona Sea Bornholm Basin	2, 5, 8, 11 2, 5, 8, 11 - -	Driver (1907)
1906	Kiel Bight Bay of Mecklenburg Arkona Sea Bornholm Basin	3 3 3 3	Kraefft (1908)
1910	Kiel Bight Bay of Mecklenburg Arkona Sea Bornholm Basin	3 - 12 - - -	Büse (1915)
1911	Kiel Bight Bay of Mecklenburg Arkona Sea Bornholm Basin	2, 3 - - -	Büse (1915)

Therefore, a common mean individual mass of 10.9 µg wet weight ind.$^{-1}$ for copepodites, which integrates the species and stages, was used for the calculation of the indicator values for mean size and total zooplankton biomass. In consequence, biomass and mean size would be overestimated in case young copepodites stages dominate and vice versa in the case older stages dominate. The monitoring data from 1984 - 2017, however, did not show a consistent trend in this respect and the stage distributions were largely species-specific. The copepod sexes were furthermore not differentiated within the copepod species in the historical data, and again a mean species-specific value was used in calculations.

The calculated indicator values for mean size and total zooplankton biomass in August 1903 were 5.8 µg wet weight ind.$^{-1}$/131 mg wet weight m^{-3} and 9.1 µg wet weight ind.$^{-1}$/82 mg wet weight m^{-3} for the Arkona Sea and the Bornholm Basin, respectively. In the Arkona Sea, these historical values were lower than the minimum values observed for mean size (7.8 - 8.7 µg wet weight ind.$^{-1}$) or in the range of the lowest values with regard to biomass (100 - 150 mg wet weight ind.$^{-1}$) observed during 1984 - 2017. They were also considerably lower than the estimated threshold values for the GES-indicator (Tab. 7). In the Bornholm Basin, in contrast, the historical values for mean size were in the range of the lowest values of the contemporary time series (6.8 - 9.9 µg wet weight ind.$^{-1}$), while the biomass value was considerably below the minimum (108 - 170 mg wet weight ind.$^{-1}$). Differences in the composition of the zooplankton likely account for the observed differences in indicator values. Most important, rotifers contributed considerably to the low mean size in August 1903. They occurred in high concentrations ranging from 3.900 to 8.000 ind. m^{-3}. This high abundance is primarily observed during May in the actual long-term time series. *Bosmina* spp., however, contributed only little to the zooplankton in 1903, which contrast with its dominance during summer observed in the present monitoring.

4 Discussion

4.1 Implications of the seasonal dynamics and inter-annual variation for the monitoring and indicator assessment

The biennial study based on monthly estimates of zooplankton abundance, biomass and composition revealed that the seasonal development of the community is principally dynamic and irregular. Important area-specific and inter-annual differences in the productive phase, in the timing of indicator-relevant groups and in the composition of the zooplankton were observed. These can be summarized as follows:

- The ***productive phase*** in the zooplankton community starts already early in the western Baltic Sea. A significant increase in stocks and biomass occurs in early spring (March-May). The start of the seasonal development shows a general delay from March-April in the Kiel Bight and the Bay of Mecklenburg to April-May in the Arkona Sea and the Bornholm Basin.
- Associated with the longer productive phase is the ***seasonal separation*** of the important zooplankton taxonomic groups in the western Baltic. Rotifers are largely confined to the spring period during March to May, while cladocerans appear primarily during summer. Copepods, in contrast, show a rather wide temporal window of high abundance and biomass ranging from March to September. A gradient from the Kiel Bight to the Bornholm Basin regarding the temporal appearance of these major groups exist.
- The major zooplankton groups display a considerable ***interannual variation*** in their temporal occurrence and in stock size. The peak concentrations of rotifers and cladocera can vary by about a month in their seasonal appearance.
- The study confirms a strong ***gradient in the composition*** of the zooplankton. While calanoid copepods are well represented in all areas, rotifers and cladocera are generally rare in the Kiel Bight but increase considerably in abundance to the Arkona Sea and Bornholm Basin. The small cyclopoid copepod *Oithona* spp. shows a reversal pattern, with high abundance in the Kiel Bight and decreasing stocks towards the Bornholm Basin.

These features have significant implications for the application of the core indicator MSTS in the western Baltic Sea with regard to the quantitative record of the major zooplankton groups.

The assessment protocol of the core indicator suggests the period ranging from June to September as most relevant for the capture of indicator relevant groups in the Baltic Proper (HELCOM 2018a). This is based on the observation that the major increase in zooplankton biomass occurs generally during May-June (Eriksson et al. 1977, Hernroth & Ackefors 1979, Simm et al. 2014) and that rotifers, cladocera, nauplii and later development stages of copepods display their seasonal maxima during summer (Eriksson et al. 1977, Hernroth & Ackefors 1979, Simm et al. 2014).

The seasonal patterns observed in the western Baltic Sea differ from those in the Baltic Proper, particularly with regard to the temporal occurrence of rotifers and cladocerans. Here, rotifers generally account for the increase in zooplankton abundance in spring, while cladoceran were responsible for the summer maxima. In addition, the stocks of the ecologically important group of calanoid copepods increased already during March to May. These patterns largely reflect the early start of the phytoplankton production (Wasmund et al. 1998, Wasmund & Uhlig 2003, Wasmund & Siegel 2008) but also the occurrence of planktivorous fish and their larvae as early as April - May in the western Baltic Sea (Voss et al. 2006, Paulsen et al. 2016, Bernreuther et al. 2018). The assessment of the zooplankton community based on summer indicator values is, therefore, insufficient and needs to be adjusted. Considering the variable temporal occurrence of the major groups (see below), the extension of the assessment period to March/April-September in the western Baltic (Kiel Bight-Bornholm Basin) appears reasonable.

The present monitoring programme in the western Baltic is based on fixed sampling in the months February, March, May, August and September. This frequency can only partly account for the timing in the occurrence of the indicator-relevant groups and for its interannual variability. In the Kiel Bight and the Bay of Mecklenburg, the sampling in 2015 - 2016 sketches the general seasonal development better than in the Arkona Sea and Bornholm Basin. This is likely related to the earlier start of spring season, which is then represented in a regular sampling (3 out of 5 sampling events take place during February - May). Nevertheless, the seasonal timing of the rotifers was variable during March - April in the Bay of Mecklenburg, which is only partly covered by the monitoring.

In the Arkona Sea and Bornholm Basin, in contrast, the sampling is less well adapted to catch the occurrence of the main zooplankton groups. Here, rotifers and cladocera were recorded in higher abundance than in the Kiel Bight and Bay of Mecklenburg, but their peak concentrations occurring during April, July and September 2015/2016 were basically missed. Similarly, the maximum abundance of the calanoid copepods was recorded in May 2015 in the Arkona Sea, but the peak concentrations in June-July in the Arkona Sea during 2016 and in the Bornholm Basin in 2015/2016 are not recorded by the present monitoring. The ephemeral nature of the mass occurrence of rotifers, but also cladocerans, and the variability in the timing of the main groups during spring and summer argue for a more frequent sampling including at least April, June and July that would allow a better quantitative record of their abundance.

Due to the relatively infrequent sampling, it is presently unclear whether all indicator groups are sufficiently represented in the western Baltic Sea to be able to apply the core indicator 'mean size and total stock'. This question particularly applies to the Kiel Bight and Bay of Mecklenburg for which the records of rotifers and cladocera are rather infrequent. The results obtained for the years 2015 - 2016 with a regular monthly sampling schedule generally confirm the considerable gradient in the species inventory with low numbers of rotifers and cladocera in the Kiel Bight and Bay of Mecklenburg and a strong increase towards the Arkona Sea. Furthermore, a considerable interannual variability in the stock size of rotifers exist, particularly in the Kiel Bight.

The high concentrations of rotifers recorded in the Kiel Bight in March 2016 appear rather exceptional since no substantial concentrations were recorded here during 2010 - 2017. However, some outstanding peak concentrations have been recorded in March/May during the period 2000 - 2008. Thus, it cannot be unequivocally ruled out that the low concentrations or absence is based on a lacking detection of peak concentrations in the present sampling. This applies also to the Bay of Mecklenburg, in which the rotifer concentrations were on average higher than in the Kiel Bight. The irregular occurrence and low concentrations of rotifers recorded in these areas have, however, important implications for the assessment of the mean size due to the high abundance of the cyclopoid copepod *Oithona* spp. in the Kiel Bight and the Bay of Mecklenburg (see next chapter).

4.2 Gradient in zooplankton community composition and 'mean size'

The mean size in the zooplankton community results from the relation of the abundance of small sized organisms (rotifers, copepod nauplii, copepodite stages of *Oithona* spp.) and large sized organisms (calanoid copepodites and adults, some cladocera). Large seasonal differences were observed in the investigated areas that reflect the seasonal shift in community composition. The general pattern in the western Baltic Sea can be described as a winter-spring minimum caused by the high abundance of rotifers and nauplii and an increase in mean size during summer due to maturing copepod populations. The timing of the increase in early summer and of the summer maximum reflect interannual differences in the phenology in the occurrence of major zooplankton groups.

Apart from these generalizations, some regional differences in the patterns of mean size variation were identified between the Kiel Bight and the Bay of Mecklenburg on the one hand and the Arkona Sea and Bornholm Basin on the other hand. In the first two areas, a more irregular seasonal pattern in the variation of mean size, particularly in the Kiel Bight, and interannual differences in the annual mean of the indicator were observed. These reflect a considerable interannual variability in the presence of rotifers and a large variability in the timing of abundance peaks of the small cyclopoid copepod *Oithona* spp. A decrease in mean size associated with rotifers was observed only in spring 2016, while the spring minimum of mean size in 2015 was based on nauplii and *Oithona* spp. In addition, high concentrations of *Oithona* spp. during autumn 2016, caused a considerable decrease in the mean size that was only partly buffered by the cladocera (Evadne, Podon) due to their rather low concentrations. This illustrates that *Oithona* spp. had a significant effect on the indicator value of mean size, which in turn was seasonally rather unpredictable. While peak concentrations were observed in spring and summer in 2015, the species was restricted to summer in 2016. Recent monitoring data in Kiel Bight emphasizes this considerable variation. While a high abundance of *Oithona* spp. was recorded during winter and autumn in 2013 or spring in 2014, the species dominated the copepod community during the entire year in 2016.

The influence of *Oithona* spp. on the indicator value is similar to that of rotifers. It is presently unclear whether the abundance of *Oithona* spp. can be accordingly related to different degrees of eutrophication or to fish feeding conditions. An increase in the contribution of *Oithona* to the zooplankton community was observed in an eutrophic estuary compared to a mesotrophic estuary (Uye 1994). However, the predominance of the species might as well result from the considerable ability of the species to avoid predation by gelatinous predators in comparison to other zooplankton (Tanaka & Akiba 2015). Thus, it is presently unclear whether a decrease in mean size due to increasing concentrations of *Oithona* spp. can indicate the deterioration of the food web structure in consequence of eutrophication.

In contrast, the function of mean size in indicating deteriorating fish feeding conditions might still prevail under the high abundance of *Oithona* spp. Stomach analyses revealed the presence of large copepod species in the gut of sprat and sprat larvae in the Baltic Sea, but not of *Oithona* spp. (Casini et al. 2004, Bernreuther et al. 2018). Laboratory experiments have, however, shown that herring larvae are principally capable of feeding on *Oithona* spp. (Checkley et al. 1982). The present seasonal data indicate that during high abundance of *Oithona* the stocks of calanoid copepods were reduced. Thus, a low mean weight could reflect diminishing food resources, which needs further study.

In contrast to the Kiel Bight and the Bay of Mecklenburg, the abundance of *Oithona* spp. decreased towards the Arkona Sea and Bornholm Basin. Rotifers and nauplii, in turn, accounted for considerable maxima during spring and summer reducing its significance in steering mean size. In addition, the large concentrations of cladocerans with a higher individual mass than *Oithona* dampened considerably the effect of the small cyclopoid. Therefore, the interannual differences in the seasonal variation of mean size and the annual mean in 2015 - 2016 were generally small. The *Oithona* spp. occurred also more regular with elevated concentrations in the period April-September but without irregular peaks. Thus, the species has a limited influence on food web structure.

The seasonal variation in mean size in the four areas further reinforces the need for more frequent data during the summer periods. The values observed in August reflected the conditions during the summer months only insufficiently and were often lower than those observed in June - July and September. This is particularly obvious in the Kiel Bight, Bay of Mecklenburg and Arkona Sea in which high concentrations of the main zooplankton groups were missed.

4.3 Sampling variability

Zooplankton is generally not randomly distributed and varies over different vertical and horizontal scales (Mackas et al. 1985, Harding 2001). Various mechanisms account for an overdispersion including physical features such as turbulence or flow-induced instabilities or biological drivers such as predation or vertical migration (Folt & Burns 1999, Brentnall et al. 2003). Thus, some variation in the estimates of abundance and derived variables is to be expected. This can have potential implications on estimates of the indicator values when the data is generally limited and variability is high. In the western Baltic Sea, a seasonal estimate is represented by 1 - 2 samples only due to the low frequency in sampling scheme and the gradient in biological timing that prevent a joint assessment from the Kiel Bight to the Arkona Sea and Bornholm Basin. Thus, knowing the variation in the estimates of abundance, biomass and mean size is important.

The triplicate analysis of samples in 2016 in all four areas revealed a low variability among replicate sampling events. The coefficients of variation were generally below 20%. Larger variation was mostly associated with a low abundance outside the major period of the main occurrence and was related to specific taxa, which benefits the assessment of structural indicators such as mean size. In a few exceptional cases, a larger variation coefficient of 22 – 50 %was observed in nauplii and calanoid copepods. These values, however, indicate a rather low sampling variability because coefficients of variation range normally from 23 – 53 % and variation larger than 100 % is common (Cassie 1979).

With regard to the assessment of the environmental status, variation in the indicator parameters mean size (MS) and total zooplankton biomass (TZB) is of prime interest. Estimates of TZB were generally more variable than those of MS. This indicates that the abundance of zooplankton in replicated samples varied stronger than the composition of the community variable. In addition, a gradient of decreasing variation from the Kiel Bight to the Bornholm Basin was observed probably related to depth and more stable hydrographic conditions in the deeper Basins. Nevertheless, the coefficient of variation for MS and TZB were low and accounted for less than 11 and 22 %. Consequently, the confidence intervals for the estimates of the parameter were narrow. Most important, standard variation and the coefficient of variation for both MS and TZB for samples in May and August were smaller than the variation of the indicators during the reference periods of the long-term data set in the same months. For instance, the reference values for MS and TZB in the Arkona Sea and Bornholm Sea in August ($RefCon_{Chla}$) were 10.3 ± 3.1 µg wet weight $ind.^{-1}$ (VarCoeff 13%) and 238.1 ± 91.1 mg wet weight m^{-3} (VarCoeff 16%) and 14.4 ± 42.1 µg wet weight $ind.^{-1}$ (VarCoeff 6 %) and 272.1 ± 56.7 mg wet weight m^{-3} (VarCoeff 8%), respectively. The estimates in 2016, actual values for MS and TZB in the Arkona Sea and Bornholm Sea in August were 12.3 ± 0.8 µg wet weight $ind.^{-1}$ (VarCoeff 4 %) and 223.3 ± 23.6 mg wet weight m^{-3} (VarCoeff 6 %) and 9.7 ± 0.3 µg wet weight $ind.^{-1}$ (VarCoeff 2 %) and 209.3 ± 13.7 mg wet weight m^{-3} (VarCoeff 4 %), respectively. Thus, single samples appear sufficiently robust for the parameter estimation. However, this accounts only for the sample variation and not for overdispersion and patchiness over lager spatial scales.

4.4 Recommendation for the monitoring

- The occurrence of the major zooplankton groups from March to September requires quantitative data during both spring and summer for the evaluation of the indicator. In the Kiel Bight and Mecklenburg Bight the assessment period should cover the period from ***March to September*** and in the Arkona Sea and Bornholm Basin from ***April to September***, respectively, to account for the early start of the season and the dynamic seasonal development compared to the Baltic Proper.
- Due to the considerable seasonal and interannual variation in the timing of occurrence of the indicator-relevant groups in the western Baltic Sea, a ***monthly sampling*** that can depict the variability in timing and the appearance of peak concentrations of rotifers, cladocerans and

copepods is recommended. In particular, data during April, June and July is highly warranted but presently not collected. Data originating from different national and international monitoring authorities might be combined to increase the seasonal coverage. International coordination could increase data availability and reduce costs. It is advisable that the sample collection, data analysis and data quality assessment follow a common protocol (e.g., HELCOM 2018a). This is presently not the case.

- The assessment should be based on a ***regular, fixed number of months***. Variation might be introduced using a variable number of assessed months due to the interannual variability in timing and should be avoided. Procedures to fill data gaps should be established.
- In the case that data is combined from different sources, the assessment of the seasonal/annual indicator values for mean size and total stock should be based on ***monthly averages*** to avoid bias by variable sample number.
- Replication of sampling appears not to be necessary due to low variation. Preference should be given to increase the spatial and temporal coverage.

4.5 Assessment of GES

The assessment of the environmental status of the zooplankton in the Arkona Sea and the Bornholm Basin is guided by the definition of reference conditions. For both $RefCond_{Chla}$ and $RefCond_{Fish}$, these periods lie at the beginning of the time series because Chl*a* and the eutrophication ratio were lowest and weight-at-age of sprat was highest. Due to the shortness of the time-series from 1986 to the present, however, $RefCond_{Chla}$ do not represent oligotrophic conditions. At present, no estimates of weight-at-age are available for the Arkona Sea. The definition of the $RefCond_{Fish}$ is, thus, based on those of the Bornholm Basin and should be judged as preliminary. However, because the variation of weight-at-age in the Bornholm Basin reflects that of the integrated weight-at-age in the combined ICES subdivisions, the present estimate of the $RefCond_{Fish}$ in the Arkona Sea appears rather robust.

Differences between $RefCon_{Chla}$ and $RefCon_{Fish}$ in each area are largely based on the different lengths of the defined periods, which were shorter with regard to eutrophication (6-7 years) than for fish feeding conditions (9 - 10 years). The differences were, however, generally small. In contrast, differences between the areas were clearly discernible (Table 7). While the reference conditions with regard to mean size and biomass were rather comparable in the Arkona Sea and the Bornholm Basin in spring, they diverged considerably during autumn and, thus, in the annual mean. The reference conditions for mean size were generally larger in the Bornholm Basin (13.9 - 14.4 µg wet weight $ind.^{-1}$) than in the Arkona Sea (10.3 - 10.6 µg wet weight $ind.^{-1}$). Therefore, Arkona Sea and Bornholm Basin should be assessed as separate units.

Arkona Sea

In the Arkona Sea, the thresholds of $RefCon_{Chla}$ and $RefCon_{Fish}$ for mean size and biomass in August were rather similar. Mean size exceeded the lower threshold for $RefCon_{Chla}$ and $RefCon_{Fish}$ in the years 2013 and 1984, 1986, 1997, 2013, respectively (Fig. 11a). However, they did not pass the lower confidence limits in the respective CuSum control charts. Thresholds for the total zooplankton biomass were passed in 1985, 1998, 2000, 2012 ($RefCon_{Chla}$) and 1985, 1992, 1998, 2000, 2004, 2012 ($RefCon_{Fish}$). Again, the deviations did not exceed the lower confidence limits in the CuSum control charts. Thus, all years were in-control with regard to mean size and total zooplankton biomass and the area was characterised by good environmental status, which reflects the general trend to larger mean size and higher biomass since the early 2000s.

Figure 11 Control charts for mean size (MS) and total zooplankton biomass (TZB) with thresholds for RefCon$_{Chla}$ (left panel) and RefCon$_{Fish}$ (right panel) for August (a) and the annual mean (b) in the Arkona Sea. Lower DI-CuSums (red thick line) and MS/TZB-values (black circles) are shown on left and right axes, respectively. Red solid/dashed lines represent lower CuSum limits. Black solid/dashed lines represent lower MS/TZB thresholds.

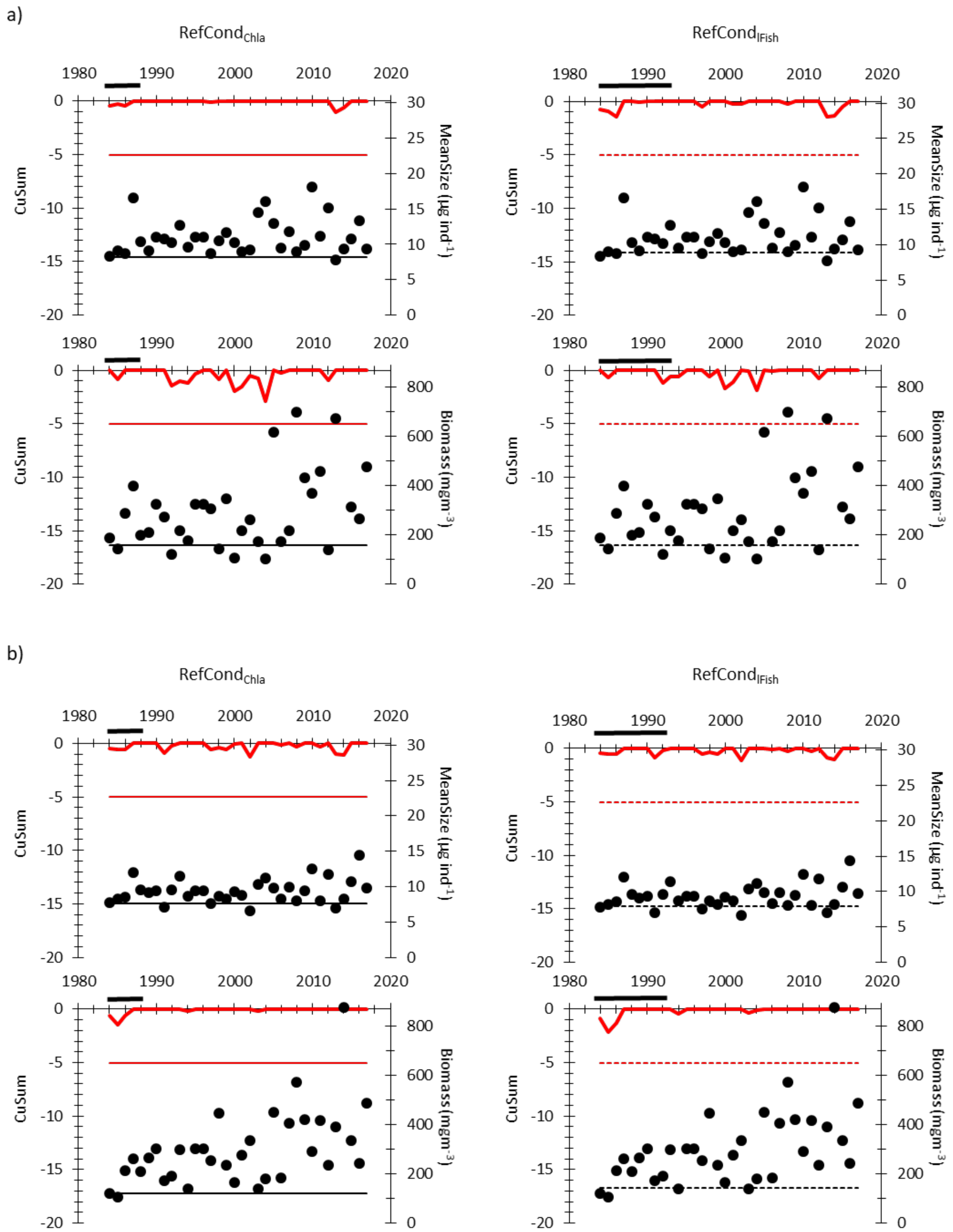
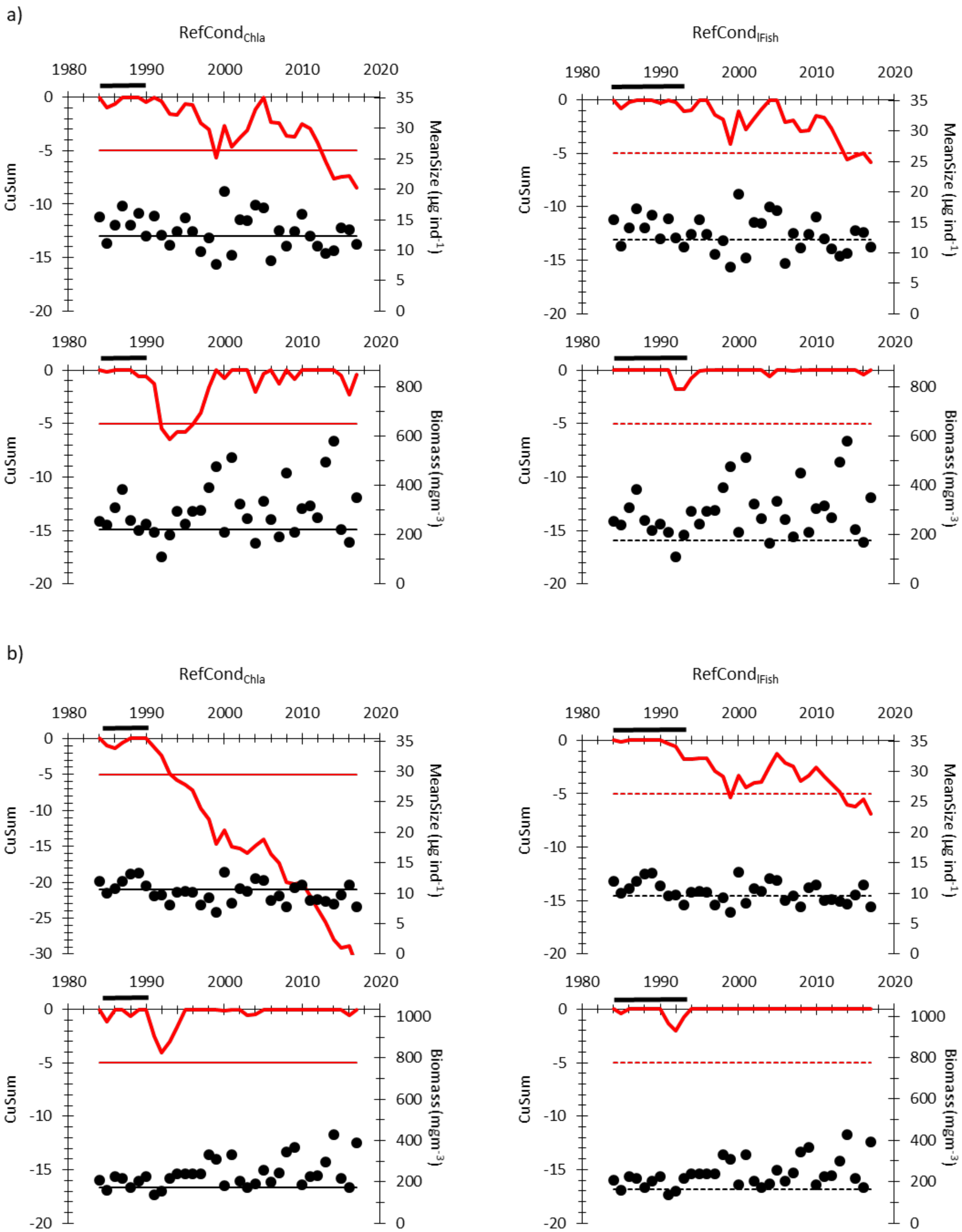


Figure 12 Control charts for mean size (MS) and total zooplankton biomass (TZB) with thresholds for RefCon$_{Chla}$ (left panel) and RefCon$_{Fish}$ (right panel) for August (a) and the annual mean (b) in the Bornholm Basin. Lower DI-CuSums (red thick line) and MS/TZB-values (black circles) are shown on left and right axes, respectively. Red solid/ dashed lines represent lower CuSum limits. Black solid/dashed lines represent lower MS/TZB thresholds.



The annual mean of combined spring and summer conditions did not differ substantially from the summer conditions. Due to averaging, $RefCond_{Chla}$ and $RefCond_{Fish}$ had lower threshold values (Fig. 11b). The interannual variation in MS and TZB, however, remained similar to August with an apparent long-term increase in mean-size and increasing biomass. Thresholds for mean size were passed during 1984 for $RefCon_{Fish}$ and 1991, 1997, 2002, 2013 for both $RefCon_{Chla}$ and $RefCon_{Fish}$, but the deviations were not substantial enough to exceed the lower confidence limits in the CuSum control charts. The lower threshold for biomass were passed 1984 - 1985 ($RefCon_{Chla}$ and $RefCon_{Fish}$) and 1994, 2003 ($RefCon_{Fish}$). Again, these violations were not part of a systematic trend, and the CuSum control charts revealed a good environmental status throughout the time series.

Bornholm Basin

In the Bornholm Basin the thresholds for $RefCon_{Chla}$ for mean size and total zooplankton biomass were slightly higher than for $RefCon_{Fish}$. The long-term negative pattern in the CuSum control charts was therefore more pronounced for $RefCon_{Chla}$ than for $RefCon_{Fish}$.

The threshold for mean size in August was passed in 12 years of the time series, particularly in the periods 1993 - 2001 and 2006 - 2014, for both $RefCond_{Chla}$ and $RefCond_{Fish}$ (Fig. 12a). During both periods the trends were systematic and the CuSum control charts detected an out of control status in 1999 and from 2013 onwards for $RefCond_{Chla}$ and from 2013 $RefCond_{Fish}$. Total zooplankton biomass exceeded the lower threshold for $RefCond_{Chla}$ during 1989, 1991 - 1993, 2000, 2004, 2007, 2009 and 2016. The CuSum controls charts reflect these negative deflections, but a systematic out of control period was detected for the 1991 - 1996 reflecting the overall increasing trend in biomass. In contrast, the threshold for $RefCon_{Fish}$ was passed in a few years because of the generally lower threshold value for total zooplankton biomass.

The assessment of the environmental status based on the annual mean differed largely from the pattern observed in August, particularly with regard to $RefCon_{Chla}$ (Fig. 12b). The threshold for mean size was passed regularly from 1991 onwards with the exception in 2000, 2004 - 2005 and 2010 - 2011. Thus, the negative trend observed in August was reinforced by the similar trend in May. The CuSum control charts revealed out-of control conditions from 1994 onwards. Due to the lower threshold for mean size for $RefCon_{Fish}$, out of control were detected in 1999 and from 2014 onwards. In contrast to mean size, no out of control conditions were detected by the CuSum control charts for total zooplankton biomass for both $RefCond_{Chla}$ and $RefCond_{Fish}$. The thresholds were mainly passed during the beginning of the time series, but did not systematically affect the status as revealed by the CuSum control charts.

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