

In-depth analysis of an alternate-stage *Prymnesium polylepis* (Haptophyta) bloom and long-term trends in abundance of Prymnesiales species in the Baltic Sea

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ABSTRACT: In late autumn/winter 2007, high abundances of the alternate-stage *Prymnesium polylepis* were observed in many Baltic Sea areas, attaining bloom concentrations in spring 2008. To understand long-term variability in Prymnesiales density, we analysed changes in the abundance of different size classes of Prymnesiales using a long-term time series (1985–2008) for an inshore–offshore gradient in the northern Baltic proper. Further, to understand environmental conditions contributing to the *P. polylepis* bloom, we examined environmental factors associated with the increase in Prymnesiales abundances by placing the bloom dynamics within the context of temporal and spatial environmental variability over the last decades in the Baltic Sea. Significantly increasing abundances were found for larger size classes of Prymnesiales (6–10 µm and >10 µm) but not for the smaller species (Prymnesiales < 6 µm). Our analyses indicate that high spring sea surface temperatures favor development of larger Prymnesiales (6–10 µm and >10 µm) that tolerate low annual NO₂+NO₃ concentrations. Thus, long-term trends in increase in abundance, as well as changes in the relative contributions of species within these potentially toxic haptophytes, are likely to be the result of interacting changes in temperature and nutrient conditions since the late 1980s.

KEY WORDS: *Prymnesium polylepis* bloom · Phytoplankton · Spring bloom · Baltic Sea

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INTRODUCTION

Bloom-forming Haptophyta belonging to the genera *Chrysochromulina* and *Prymnesium* have attracted increasing attention during the last decades (e.g. Edvardsen & Paasche 1998, Gjørseter et al. 2000, Granéli et al. 2012). These flagellates are intriguing, from the ecological perspective with regard to factors triggering the blooms (e.g. Lekve et al. 2006) and their toxicity to the biota (e.g. Moestrup 1994, Edvardsen & Paasche 1998), as well as from phylogenetic and taxonomic perspectives, including uncertainties associated with morphological variability and complex life cycles in many species (Edvardsen & Paasche 1992, Granéli et al. 2012).

In the last taxonomic revision, several *Chrysochromulina* species were transferred to other genera

within the order Prymnesiales (Edvardsen et al. 2011). The name *Chrysochromulina* spp. used in earlier studies includes, therefore, species from several genera and, due to this revision and lack of reliable taxonomic identification in routine phytoplankton analysis, are now referred to as Prymnesiales or Prymnesiophyceae, particularly in studies based on monitoring data (Majaneva et al. 2012, Suikkanen et al. 2013). *Chrysochromulina polylepis* was also revised to *Prymnesium polylepis* (Manton & Parke Edvardsen, Eikrem & Probert (Edvardsen et al. 2011). For consistency, here we use *Prymnesium polylepis* and Prymnesiales also when referring to studies and sources where the former names were used.

Species belonging to the order Prymnesiales are common worldwide and are known to efficiently utilize low nutrient levels (Lekve et al. 2006) and to pro-

duce harmful blooms (Granéli et al. 2012). They are also known to acquire nutrients via osmotrophy (e.g. Pintner & Provasoli 1968, Palenik & Morel 1991) and phagotrophy (e.g. Nygaard & Tobiesen 1993, Legendre et al. 2001). However, the balance between photosynthesis and phagotrophy is influenced by environmental factors (e.g. Nygaard & Tobiesen 1993) as is the magnitude of the blooms (Dahl et al. 2005, Lekve et al. 2006). *P. polylepis* is also a mixotrophic, cosmopolitan species, with high tolerance to temperature and salinity (Edvardsen & Paasche 1992, 1988, Rhodes et al. 1994).

The most extensive toxic bloom in Scandinavian waters, caused by the ichthyotoxic authentic type of *P. polylepis*, occurred in spring 1988 in the Kattegat-Skagerrak area (Dahl et al. 1989). It reached extremely high densities ($\sim 2 \times 10^8$ cells l^{-1} ; Kaas et al. 1991), killed nearly a thousand tonnes of farmed fish (Skjoldal & Dundas 1991), and caused considerable mortality in both pelagic and benthic organisms (Moestrup 1994). Since 1988, blooms of *P. polylepis* have recurrently been reported from the same region in late spring and summer, albeit without any harmful effects (Edvardsen & Paasche 1998, Dahl et al. 2005). In the Baltic Sea, Prymnesiales species, including *P. polylepis*, are common (Hajdu et al. 1996, Østergaard Jensen 1998, Suikkanen et al. 2013). Their abundances are typical of those reported for other temperate coastal areas (Østergaard Jensen 1998 and references therein), increasing in late spring and summer and north to south (Østergaard Jensen 1998, Hajdu 2002). In coastal areas, Prymnesiales species often comprise a large fraction of the nanophytoplankton and may contribute considerably (>50%) to the total phytoplankton carbon biomass (Hajdu et al. 1996, S. Hajdu unpubl). In the offshore Baltic Sea, abundances of Prymnesiales species often increase during summer (Hajdu 2002).

P. polylepis has a haploid-diploid life cycle, with 2 cell types called authentic and alternate (Paasche et al. 1990, Edvardsen & Paasche 1992, Edvardsen & Medlin 1998). The ecological significance of a haplo-diploid life cycle is unclear, but it may be an adaptation to a seasonally variable environment (Edvardsen 2002). In cultures, the alternate cells grow better at lower temperatures and irradiances and can be twice the size of the authentic ones (Edvardsen & Paasche 1992, Edvardsen & Vaultot 1996). During the winter 2007–2008, an unusual increase (up to 4×10^5 cells l^{-1} in winter 2007 and $>10^6$ cells l^{-1} in March–April 2008) of a relatively large Prymnesiales (cell size $>10 \mu m$), later identified as the alternate-type *P. polylepis*, was observed in many Baltic Sea areas (Majaneva et al.

2012). In spring 2008, a bloom dominated by this species developed in the entire Baltic Sea proper. According to Majaneva et al. (2012), high light availability and low turbulence in the fall of 2007 made it possible for *P. polylepis* to build up a considerable biomass, which persisted throughout the winter, the mildest on record in south-eastern Sweden since 1858–1859 (Hellström 2008). However, a broad evaluation of the environmental conditions governing abundances of Prymnesiales in the Baltic Sea is still lacking, which could be approached by placing the bloom dynamics during 2007–2008 within the context of the temporal and spatial variability of abiotic conditions.

The aim of our study was to explore linkages between the variations in abundances of Prymnesiales and environmental factors using monitoring data from 6 stations in the Baltic proper. We also analysed variations in temperature, salinity, the North Atlantic Oscillation (NAO), and nutrients in relation to the long-term variability in the abundances of Prymnesiales using data series (1985–2008) available from 3 stations, representing an inshore–offshore gradient in the northern Baltic Sea proper. Adopting a modelling approach for zero-inflated data, we examined factors related to presence/absence as well as abundance of different size groups of Prymnesiales and identified abiotic factors contributing to the blooms of these flagellates in the Baltic Sea.

MATERIALS AND METHODS

Study areas

The Baltic Sea is a large, semi-enclosed, non-tidal brackish water estuary comprised of a series of large basins. Our data for the years 2007 and 2008 originate from 6 monitoring stations located in the Baltic Sea proper (Fig. 1), where salinity gradually increases southward from 5 to 8 (Voipio 1981), and water temperature varies from ice cover or near $0^\circ C$ during winter to 15 – $20^\circ C$ during summer. Seasonal pycnoclines and nutriclines are closely coupled, and the depth of the upper mixed layer is usually 10 to 20 m in summer (Stigebrandt & Wulff 1987, Fennel et al. 1991).

Of the 6 stations, 3 were located in the central and southern basins (BY15, BY5, and BY2; bottom depth 249, 91 and 48 m, respectively) and visited once a month, while 3 other stations were in the northern part (H4, B1 and BY31; bottom depth 30, 40 and 459 m, respectively) and sampled 1 to 4 times a

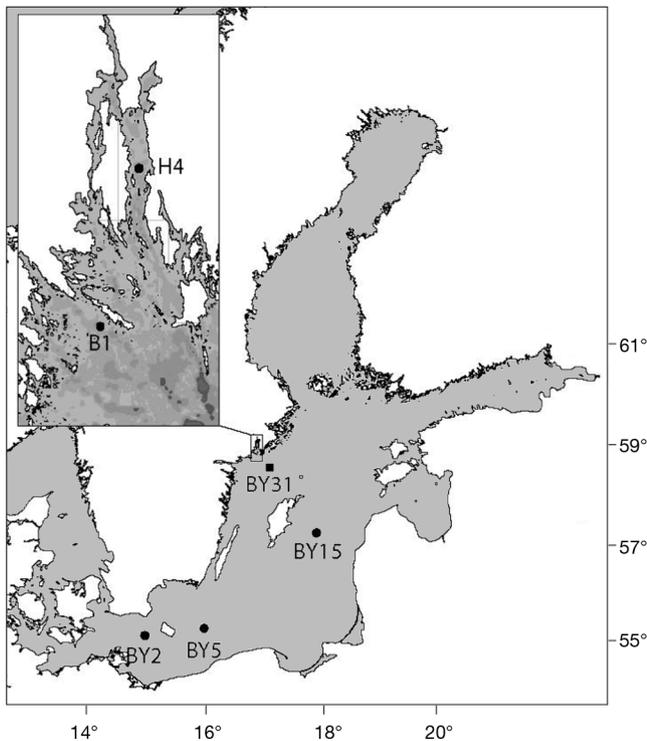


Fig. 1. Sampling locations in the Baltic Sea proper

month, depending on the season. These last 3 stations represent an inshore-offshore gradient and differ in salinity, nutrient levels, phytoplankton development, and production (Hajdu 2002). Stn H4 is located in the middle of a deep inlet (Himmerfjärden Bay), moderately eutrophied by discharges from a municipal sewage treatment plant. Stn B1 is an open coastal station, outside the area influenced by the sewage treatment plant discharge (Savage & Elmgren 2004), while Stn BY31 (the Landsort Deep) is the deepest offshore monitoring station in the Baltic Sea (Fig. 1). Some of the same stations in this study were used in Majaneva et al. (2012, often referred to in this study): BMPJ1 (our study: BY15), BMPK2 (BY5), BMPK4 (BY2), BMPH3 (BY31) and BMP12 (B1).

Sampling and sample analysis

Phytoplankton samples were collected and analyzed according to the Baltic Sea monitoring guidelines (HELCOM 2014). Briefly, integrated phytoplankton samples were collected with a plastic hose (inner diameter 19 mm, length 10, 14 or 20 m depending on station) and preserved with acidic Lugol's solution. On each sampling occasion, salinity and water temperature were measured using a CTD

(Meerestechnik Elektronik). Nutrient concentrations—dissolved inorganic phosphate (DIP) as PO_4 , and dissolved inorganic nitrogen (DIN) as the sum of ammonium (NH_4), nitrite (NO_2), and nitrate (NO_3)—were analysed at Stns B1, BY31 and H4 from every 5 m using the same basic methods (ALPKEM O. I. Analytical Flow Solution IV: DIP and DIN, methods #319528, #319526 and #319527) adjusted to various instruments. The detection limits for PO_4 , NH_4 , NO_2 , and NO_3 were at least 0.016, 0.04, 0.015, and 0.015 $\mu\text{mol l}^{-1}$, respectively. To calculate the average nutrient concentrations, we used data from 0, 5 and 10 m. Wind speed and insolation were registered at Visby station (central Baltic proper, 57° 39' N, 18° 20' E) and data were taken from the Swedish Meteorological and Hydrological Institute's database (www.smhi.se). Chl *a* was extracted from phytoplankton collected on GF/F filters which were ground in 90% acetone, stored 2 h at room temperature before being analyzed for chl *a* using a spectrophotometer (SIS-standard SS 02 81 46). Transparency was measured with a Secchi disc 25 cm in diameter.

Phytoplankton (>2 μm) were counted after sedimentation in 10 or 25 ml chambers using an inverted microscope with phase contrast. Microphytoplankton were counted on the half/whole chamber bottom or in diagonals with a 10 \times objective (total magnification 150 \times). Nanoplankton were counted in 1 or 2 diagonals with a 40 \times objective (total magnification 600 \times). In each sample, a minimum of 50 units (cells/colonies/filaments) of the dominating species were enumerated, giving a maximum counting error of $\pm 28\%$ corresponding to a 95% confidence limit for the counts (Lund et al. 1958); maximum error for total count per sample was less than $\pm 10\%$. Biovolumes of phytoplankton cells were calculated using Olenina et al. (2006) and the HELCOM taxa-specific biovolume table at www.ices.dk/marine-data/Documents/ENV/PEG_BVOL.zip.

In most cases, the species-level identification of *Prymnesiales* requires electron microscopic investigation and/or molecular approaches. As these are not an option in routine phytoplankton analysis, size-based enumeration was used; this is the recommended method in phytoplankton analysis employed by environmental monitoring in the Baltic Sea (Olenina et al. 2006). For *Prymnesiales* species, 6 size groups were considered: (1): 2–4 μm , (2): 4–6 μm (spherical), (3): 4–6 μm (flattened), (4): 6–10 μm , (5): 10–15 μm , and (6): 15–20 μm . These groups were based on cell length measurements (Olenina et al. 2006, www.ices.dk/marinedata/Documents/ENV/PEG_BVOL.zip).

In the northern Baltic proper, these size groups of Prymnesiales species have been monitored since 1985 (Stns B1 and H4) and 1990 (Stn BY31). The 2 alternating cell types of *Prymnesium polylepis*, authentic and alternate, belong to different size classes. The authentic *P. polylepis* is 6–10 μm (Hajdu et al. 1996) and thus included in the size group 4 as defined above. The alternate type of *P. polylepis*, which dominated during autumn 2007 and spring 2008, was 10–20 μm in cell size (mainly 10–15 μm) and therefore included in the size groups 5 and 6. During the bloom, these latter size groups were composed exclusively of the alternate type of *P. polylepis* (Majaneva et al. 2012). Therefore, in this study, we assumed that all cells in size groups 5 and 6 were the alternate type of *P. polylepis* (hereafter *P. polylepis* refers to alternate *P. polylepis* >10 μm). The smaller authentic type of *P. polylepis* contributed to the size group 4 (6–10 μm). This latter group, however, might also include other species (e.g. *Haptolina ericina*, *H. hirta*) and is therefore referred to as 'Prymnesiales 6–10 μm ' hereafter.

Statistics

Long-term changes in Prymnesiales abundance

To test for the presence of trends in the time series of Prymnesiales abundance (total Prymnesiales, Prymnesiales 6–10 μm and *P. polylepis*) and percentage of Prymnesiales in the total phytoplankton by biovolume at the coastal Stns B1 and H4 (years 1985–2008, with missing data for 1991), and the off-shore Stn BY31 (years 1990–2008, with missing data for 1992–93), we used the non-parametric Mann-Kendall test after testing for autocorrelation. Serial correlations were analyzed by computing the Durbin-Watson statistic on the residuals of the LOESS smoother for annual means in each time series with significant trends ($p < 0.05$); none of these data series had significant positive serial correlation ($p > 0.05$ in all cases).

Effects of environmental factors on Prymnesiales abundance

To evaluate effects of environmental factors on the presence (binomial response) and abundance (continuous response) of Prymnesiales, we applied a Hurdle regression model given that the flagellate abundances were zero-inflated and over-dispersed (Potts & Elith 2006). Hurdle regression is a 2-component

model with: (1) a presence/absence component that models the zero counts (presence/absence model), and (2) a truncated count component for positive counts (abundance model). To build these models, binomial probability regression and the truncated-at-zero count data regression are fitted separately (Welsh et al. 1996). The presence/absence model estimating probability of finding zero or positive abundance values were adjusted to a binomial distribution with a logit link, whereas the count model was fitted to a truncated at zero log-normal distribution using GLM (McCullagh & Nelder 1989) in S-plus (TIBCO Software). At each station, the abundance data of the 2 groups of interest (*P. polylepis* and Prymnesiales 6–10 μm) were normalized to zero mean and unit variance and used in all models. The relationships between the dependent variables (abundance of *P. polylepis* or Prymnesiales 6–10 μm) and environmental predictors were investigated using the same monitoring data as for the analysis of the long-term trends (Stns H4, B1 and BY31). In both presence/absence and abundance models, monthly and winter NAO index, monthly anomalies of sea surface temperature ([SST] at 0–10 m), salinity (at 0–10 m) and nutrients (NH_4 , PO_4 and NO_2+NO_3), as well as SST, salinity and nutrient anomalies averaged over different time periods (winter: January to March, spring: March to May, and late summer: August to September) were tested as environmental predictors. These time periods were chosen based on developmental cycles and seasonal variations in Prymnesiales abundances in the Baltic Sea (Hajdu et al. 1996, Hajdu 2002). The monthly NAO index values were taken from the Climate Prediction Center, Washington, DC (www.cpc.ncep.noaa.gov/data/tele-doc/nao.shtml). A high, positive winter NAO index indicates mild and rainy winters, while low, negative values occur during cold winters over Europe. To identify the best model (best subset regression), the Akaike information criterion (AIC) was used to optimize the number and combination of predictive variables included. When validating the proposed models, the Wald statistic was used to check the significance of the regression coefficients for each parameter, a likelihood ratio test was used to evaluate the statistical significance of including or not including each parameter and model goodness of fit was checked using deviance and Pearson χ^2 statistics. The change in deviance for single variables was used to estimate the contribution of individual variables to the deviance explained by the final model (Collet 1991). A variance inflation factor (VIF) was used as an indicator of multicollinearity; for each

independent variable VIF was <3 . Residual plots for each model were examined to exclude remaining unattributed structure indicative of a poor model fit.

RESULTS

Prymnesiales occurrence in 2007 and 2008

In summer 2007, Prymnesiales cells $>10\ \mu\text{m}$, later identified as the alternative type of *Prymnesium polylepis* (Majaneva et al. 2012), were found at all open sea stations (BY2, BY5, BY15, and BY31) and increased in abundance during autumn and reached 390 , 420 and $73 \times 10^3\ \text{cells l}^{-1}$ in November–December at the northern stations (BY31, B1, and H4 respectively). At this time, *P. polylepis* contributed as

much as 69 and 83% to the total phytoplankton biovolume at Stns BY31 and B1, respectively. In 2007, on an annual basis, *P. polylepis* contributed ~ 4 and 7% at the northern stations (BY31, B1, respectively) compared to a maximum of 1.6% in 1986–2006.

In spring 2008, *P. polylepis* became very abundant in the whole Baltic Sea proper, reaching its maximum from March to May (Fig. 2). On an annual basis, it contributed 30–40% of the total phytoplankton biovolume in the north (Stns BY31 and B1), 27% at the central basin (Stn BY15), and 6–8% in the south (Stns BY2 and BY5). In January–May 2008, *P. polylepis* made up 35–39% of the total phytoplankton biovolume at the northern and central stations and 23–31% at the southern stations. The proportion of Prymnesiales 6–10 μm increased considerably in May at stations BY5 and BY15 (Fig. 2) and from June

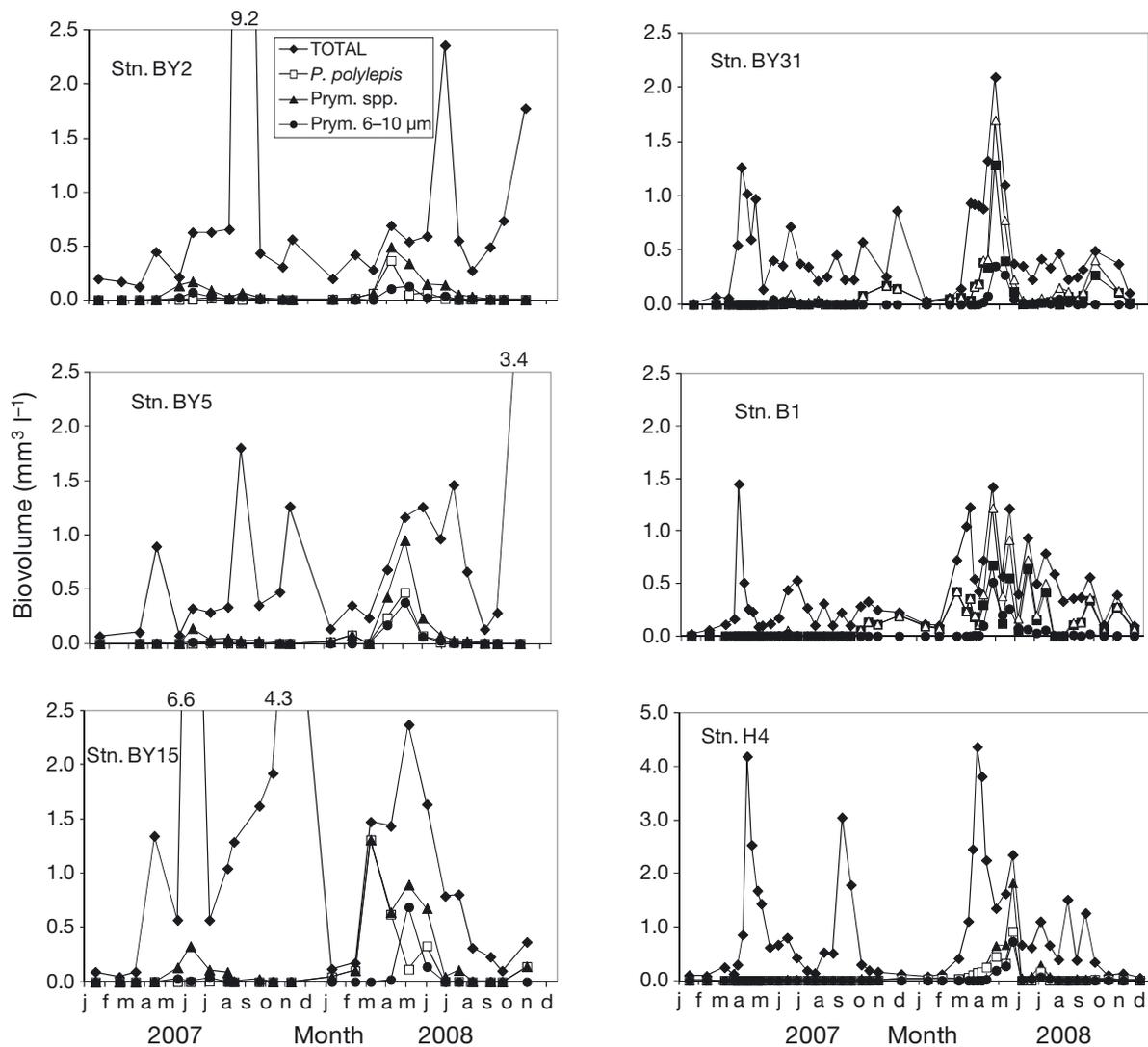


Fig. 2. Seasonal development of the total phytoplankton biovolume (TOTAL), *Prymnesium polylepis*, Prymnesiales total (Prym. spp.) and Prymnesiales 6–10 μm (Prym. 6–10 μm)

onwards, *P. polylepis* occurred only in low numbers at the central and southern stations. By contrast, at the northern stations, especially at Stn B1, *P. polylepis* occurred during the whole year, except for a short period in summer, when the dinoflagellate *Heterocapsa triquetra* (Ehrenberg) Stein dominated.

Abiotic factors in 2007–2008

When *P. polylepis* was particularly abundant (November–December 2007 and January–July 2008; Fig. 2), the SST at the open sea stations was above the long-term mean values (1985–2006; Fig. 3). Salinity did not deviate appreciably from the long-term

mean, except in December 2008 and March–April (2007 and 2008) (Fig. 3). In February–March 2008, SST at Stns BY31, B1 and H4 was among the highest on record since 1985. Salinity showed no consistent difference between stations (Fig. 4; Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m526/p055_supp.pdf). Generally, SST in 2008 was about 1°C higher compared to 2007 and 1.6–2.6°C higher compared to the long-term means at these stations.

Wind speed was very low in October 2007, and lower than the mean in November–December (Fig. 5). Following normal winds in January–March 2008, they were exceptionally low in April–May 2008 (Fig. 5). The first signs of stratification were observed mid-April in the southern and central basins (Ma-

janeva et al. 2012) and some weeks later in May in the north (data not shown). Insolation did not deviate appreciably from the mean, except for May–July 2008, when it was higher (Fig. 5).

PO₄ concentrations were higher than the mean long-term values during winter 2007–2008, especially in February (Fig. 3). By contrast, NO₂+NO₃ concentrations were very low (except at Stn H4). These nutrient dynamics resulted in low NO₂+NO₃:PO₄ ratios in both 2007 and 2008, particularly during the winter (Figs. 3 & 4; Figs. S2 & S3 in the Supplement).

Chl *a* values were above the mean values (Fig. 3; Fig. S3) and exceptionally high at Stn B1 from October 2007 to March 2008 (2.5 mg m⁻³ November–December 2007 and 2–5 mg m⁻³ in January–March 2008). Transparency was low from November 2007 to March 2008, especially at Stn B1, with 5–6 m compared to the long-term mean values of 8–11 m at Stn B1 (see <http://www2.ecology.su.se/dbhfj/index.htm>).

Long-term changes in Prymnesiales stocks and effects of environmental factors

In the coastal northern Baltic proper, the first cells of Prymnesiales 6–10 µm appeared in September 1987 in the monitoring samples (Hajdu et al.

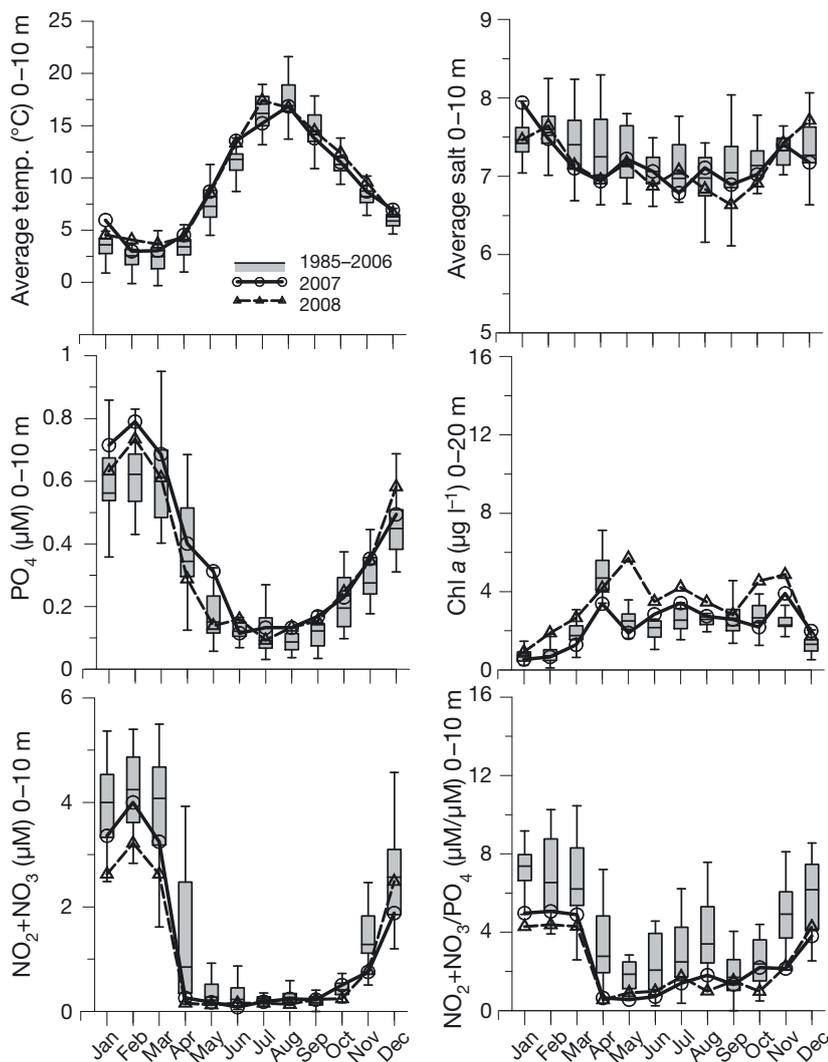


Fig. 3. Seasonal long-term variability of the abiotic parameters (box and whiskers plots: mean, 25% and 75% quartiles, max. and min. values) and values for years 2007 and 2008 at the open sea stations (pooled values for Stns BY2, BY5, BY15, and BY31, see Fig. 1)

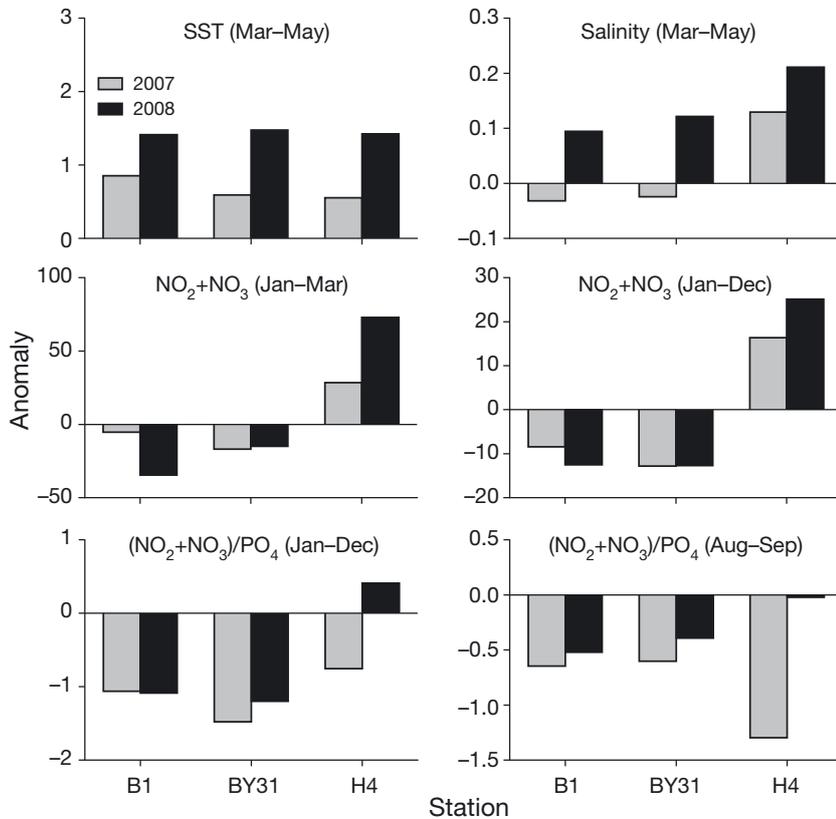


Fig. 4. Anomalies of sea surface temperature (SST, 0–10 m), salinity (0–10 m) and nutrients (NO₂+NO₃, NO₂+NO₃/PO₄) averaged over 3 time periods (winter: January to March, spring: March to May, and late summer: August to September) showing their respective anomalies compared to the long-term data (1985–2006)

1996), whereas *Prymnesiales* cells >10 µm, similar to *P. polylepis*, were first observed in November 1999. Since 1999, they have been reported sporadically, both in high (up to 1.4×10^6 cells l⁻¹, Stn H4, winter 2004–2005) and low numbers (up to 7×10^5 cells l⁻¹,

Stn B1, winter 2005–2006). The mean annual abundances of both *Prymnesiales* 6–10 µm and *P. polylepis* showed significant increasing trends over the study period (1985–2008, Fig. 6) for all time series analyzed (Table 1). The trends remained significant even when the extreme values for the year 2008 were excluded (pooled $\tau = 0.27$ and 0.60 ; $p < 0.05$ for *Prymnesiales* 6–10 µm and *P. polylepis*, respectively). There were no significant trends for the smaller species (*P. spp.* <6 µm in Fig. 6) or total *Prymnesiales* (Table 1), whereas the percentage of *Prymnesiales* in the total phytoplankton significantly increased at Stns H4 and B1 and marginally significantly at Stn BY31 (Table 1).

The presence/absence model for *P. polylepis* indicated that the probability of occurrence is positively affected by negative winter NAO index, low NO₂+NO₃ concentrations in winter and positive anomalies in spring SST (Table 2). The spring SST was again a strong positive predictor in the abundance model for *P. polylepis* together with annual mean PO₄ concentration, whereas annual NO₂+NO₃ concentrations had negative effects (Table 2). The presence/absence model for *Prymnesiales* 6–10 µm indicated opposite seasonal effects of salinity, with positive influence in spring and negative influence in late summer (Table 2). Similar to *P. polylepis*, *Prymne-*

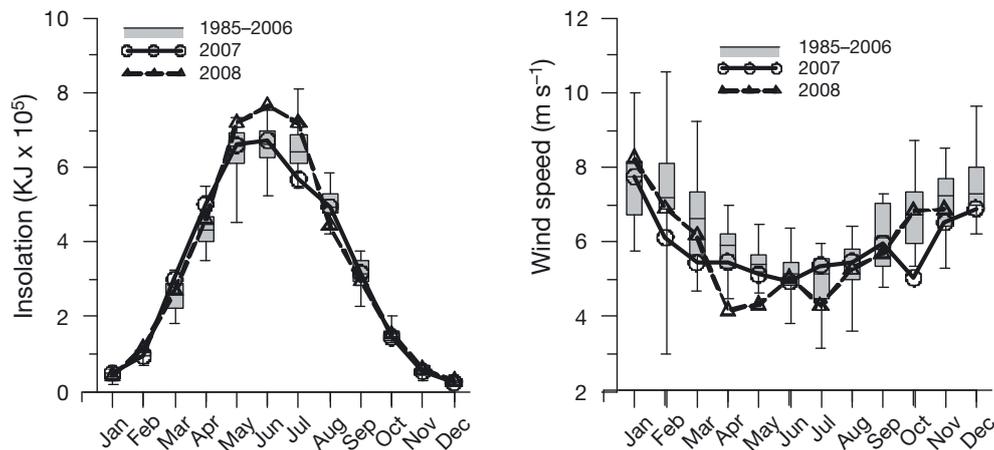


Fig. 5. Seasonal long-term variability of wind speed and insolation at Visby station (57° 39' N, 18° 20' E), and values for years 2007 and 2008. Data are presented as in Fig. 3

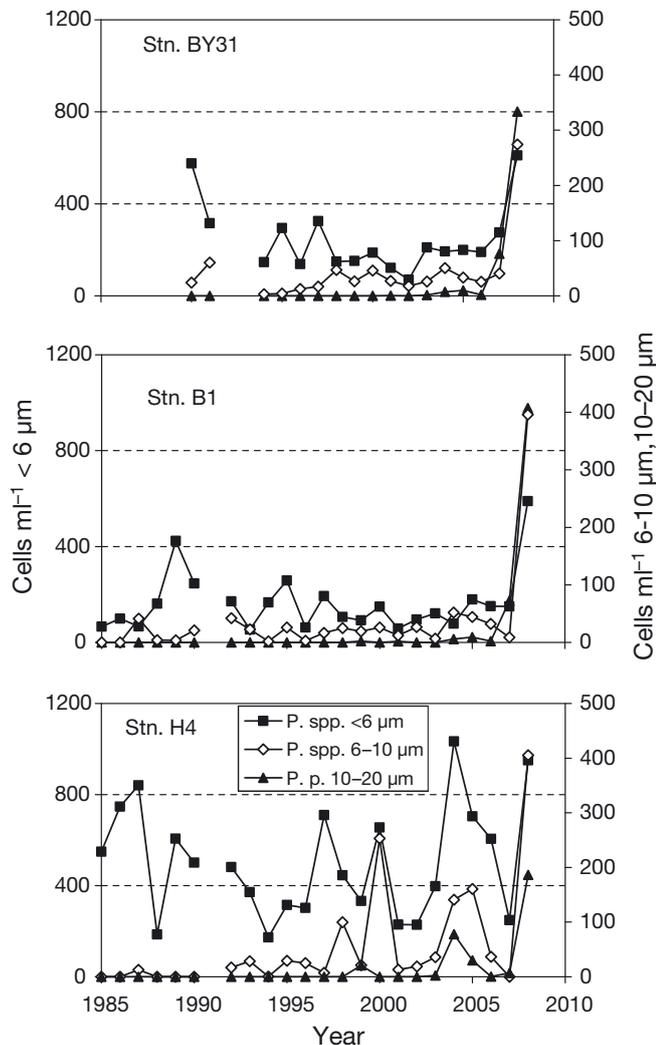


Fig. 6. Yearly mean abundances of the different size classes of Prymnesiales from 1985 to 2008. P. spp. = Prymnesiales species, P. p. = *Prymnesium polylepis*

siales 6–10 μm were favored by high spring SST and negatively related to annual NO_2+NO_3 concentrations (i.e. tolerates low nutrient levels) (Table 2). Reflecting responses to NO_2+NO_3 and PO_4 , the resulting N:P ratio assayed as the ratio of NO_2+NO_3 and PO_4 , had overall negative effects (Table 2).

DISCUSSION

The Baltic alternate-stage *Prymnesium polylepis* bloom was first reported by Hajdu et al. (2008) and later described by Majaneva et al. (2012), who suggested that calm and sunny weather in October 2007 in combination with a strong thermocline made it possible for *P. polylepis* to build up a considerable

Table 1. Results of the Mann-Kendall test for the time series of Prymnesiales abundance (total Prymnesiales, Prymnesiales 6–10 μm , and *Prymnesium polylepis*) and percentage of Prymnesiales in the total phytoplankton by biovolume (%Prym) at the coastal (B1 and H4) and offshore (BY31) stations in the northern Baltic proper from 1985 to 2008. ** $p < 0.01$, * $p < 0.05$, + $p < 0.1$, ns, not significant

Station (sample size)	Prymne-siales group/taxa	Mann-Kendall statistic (S)	Kendall's Tau	Significance
H4 (23)	Total	23	0.06	ns
	6–10 μm	149	0.53	**
	<i>P. polylepis</i>	116	0.59	**
	%Prym	78	0.34	*
B1 (23)	Total	44	0.22	ns
	6–10 μm	93	0.38	*
	<i>P. polylepis</i>	122	0.63	**
	%Prym	88	0.35	*
BY31 (17)	Total	21	0.06	ns
	6–10 μm	89	0.35	+
	<i>P. polylepis</i>	158	0.76	***
	%Prym	71	0.32	+

Table 2. Hurdle model selection results for the occurrence of *Prymnesium polylepis* (I, ab) and Prymnesiales 6–10 μm (II, ab): (a) presence/absence models and (b) abundance models. p-values are given for the explanatory variables: winter NAO and long-term anomalies of sea surface temperature (SST) and salinity in different time periods; see 'Materials and Methods' for details on data origin and time coverage. Significant effects are in **bold**. Data are for 1985–2008

Effect	Estimate	Wald statistic	p
I. <i>P. polylepis</i>			
(a) Presence/absence			
NAO, winter	–1.065	7.345	0.005
SST, spring	1.142	5.085	0.018
NO_2+NO_3 , winter	–0.026	4.789	0.027
(b) Abundance			
SST, spring	2.943	318.312	<0.0001
NO_2+NO_3 , annual	–0.052	74.359	<0.0001
PO_4 , annual	0.165	146.136	<0.0001
II. Prymnesiales 6–10 μm			
(a) Presence/absence			
Salinity, spring	6.937	7.472	0.006
Salinity, late summer	–6.498	7.541	0.006
(b) Abundance			
SST, spring	8.718	18.854	<0.0001
NO_2+NO_3 , annual	–0.464	14.147	0.0001
$\text{NO}_2+\text{NO}_3:\text{PO}_4$, annual	–3.992	11.519	0.001
$\text{NO}_2+\text{NO}_3:\text{PO}_4$, late summer	–29.105	17.921	<0.0001

population. Indeed, the mildest ever recorded winter in the Baltic Sea in December 2007–March 2008 (Hellström 2008) could have triggered the winter-

spring bloom of this species. Following fairly normal winds during the winter 2007–2008, winds were at an all-time low in April and May 2008 compared to 1985–2006 (Fig. 5). This may have aided an early increase in water mass stability in mid-April/early May, when the *P. polylepis* bloom reached its peak. At that time, the weather was also sunny, with SST being higher and salinity lower than their long-term means (Fig. 3). These climatic conditions were generally favorable for phytoplankton development in 2008, both in the south (Stn BY5) and in the north (Stn B1) (Gorokhova et al. 2014). Our results suggest that they were also favorable for Prymnesiales species, especially for *P. polylepis*, which supports findings of Lekve et al. (2006) who suggested that the abundance of Prymnesiales species is positively related to light intensity and stratification.

The influence of the climatic factors on the increased abundance of Prymnesiales 6–10 μm and *P. polylepis* was also supported by the analysis of the long-term data (Table 2). In the late 1980s, the NAO index changed from a negative to a positive phase (Edwards et al. 2013). Mild winters, increasing air temperature and SST strongly affected the physical and biological processes in the Baltic Sea (Alheit et al. 2005, Möllmann et al. 2008). An important change was the increase in spring and autumn surface mixed layer temperature by about 1.5°C (Alheit et al. 2005). These environmental changes since the late 1980s coincided with the appearance and successive increase of the mean annual abundance of both Prymnesiales 6–10 μm and *P. polylepis* since their first observations in the monitoring samples (Table 1). Moreover, the contribution of Prymnesiales to the total phytoplankton biomass has also increased significantly during this period (Table 1). The Hurdle models suggest that the probability of *P. polylepis* occurrence is higher for years with lower winter NAO index. Although we found no correlative coupling between the NAO index and abundance of Prymnesiales 6–10 μm , the abundances of both groups appear to be favored by high spring SST (Table 2). A combination of a relatively low winter NAO and high SST in spring is rather unusual, but this has actually occurred several times since 1999, as, for example, in 2004–2006, which coincided with a high abundances of Prymnesiales cells >10 μm (here classified as *P. polylepis*). Similarly, winter 2007–2008 was rather warm, albeit with relatively low NAO values (0.08 to 0.89).

There were several other anomalies in the late autumn of 2007 and winter–spring of 2008 compared to the long-term variability (1985–2006) that may

have contributed to the bloom formation. In particular, higher PO_4 and lower NO_2+NO_3 concentrations compared to their long-term means occurred during the bloom; Stn H4 was an exception due to the elevated nitrogen discharge from a sewage treatment plant and high land run-off (Fig. 4). These anomalies in nutrient concentrations resulted in an overall decrease in $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratio (again, Stn H4 being an exception); this nutrient situation may have contributed to the *P. polylepis* proliferation (Table 2). In line with this, the effects of NO_2+NO_3 on the probability of occurrence and abundance of Prymnesiales 6–10 μm and *P. polylepis* estimated by the models were found to be generally negative, i.e. low winter nitrogen stocks would increase probability of occurrence of *P. polylepis*, whereas low annual NO_2+NO_3 concentrations would increase abundances of both size groups. By contrast, high annual PO_4 concentrations and consequently low $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratios had positive effects on the abundance of *P. polylepis* and Prymnesiales 6–10 μm . These findings partially support conclusions from a 12 yr study of Lekve et al. (2006) who suggested that the abundance of Prymnesiales is inversely related to the nutrient levels and positively related to the nutrient ratios ($\text{NO}_2+\text{NO}_3:\text{PO}_4$, $\text{N}:\text{SiO}_4$). Contrary to our findings, Dahl et al. (2005) reported that annual abundance of Prymnesiales was positively related to spring and summer $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratio and negatively to summer PO_4 concentration. However, summer $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratios observed in our study were uncertain since the absolute concentrations of the nutrients were low, often down to the detection limit (Fig. 3). Hence, greater uncertainties in the calculated ratios for the summer months may have resulted in spurious effects. One can also speculate that the low $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratio after the spring bloom may eventually lead to extreme nitrogen deficiency in the phytoplankton community, which would negatively affect *P. polylepis* abundance (Table 2). Such an effect could be further speculated to be driven by higher nitrogen removal in spring and early summer of 2008, due to the higher phytoplankton stocks compared to the same period in 2007. Factors other than nutrient uptake by either total phytoplankton or Prymnesiales species, such as competition in phytoplankton communities and/or grazing pressure in the nutrient-limited conditions, may also contribute. It should be also noted that direct comparisons between our study and those conducted in the Skagerrak (Dahl et al. 2005, Lekve et al. 2006) are difficult, because the responses may be species- and stage-specific; in addition, no reliable identification

system for different Prymnesiales species was implemented in these 2 studies. Nevertheless, the similarities in abiotic factors during the blooms suggest that such synergy of climatic and nutrient conditions are favorable to promote Prymnesiales blooms.

The nutrient effects are most likely explained by competitive advantage of Prymnesiales species that can rely on mixotrophic feeding at low nitrogen levels (e.g. Pintner & Provasoli 1968, Jones et al. 1993, Nygaard & Tobiesen 1993). In the Baltic Sea, Prymnesiales cells are often observed with ingested prey (phagotrophic nutritional mode) when N and P are nearly co-limiting for non-diazotrophs at the culmination of the cyanobacterial bloom (Hajdu 2002, Walve & Larsson 2010). Moreover, Prymnesiales, like other small algal flagellates, are also able to use dissolved organic nitrogen (Lindehoff et al. 2011), which conveys a competitive advantage when inorganic nitrogen is limited. An experimental study of Errera et al. (2008) with *P. parvum*, a closely related prymnesiophyte species, albeit with a different distribution and salinity tolerance, provides support for this mechanism of the observed effects of nutrients. When both N and P were limiting, *P. parvum* populations dominated the community, with the best performance in the treatment with N limitation only. In contrast, when none of the nutrients were deficient, *P. parvum* tended to lose its competitive edge to other taxa such as chlorophytes, euglenophytes and diatoms, with these latter groups then dominating the community. Finally, Lekve et al. (2006) also suggested that low NO_2+NO_3 concentrations may be associated with other favourable conditions for Prymnesiales, such as high light availability, strong stratification, low turbulence and availability of regenerated NH_4 and possibly micronutrients.

To summarise, significant increases in the abundance of *P. polylepis* and contribution of Prymnesiales to total phytoplankton biomass have been observed during the past decades. These trends are likely to result from interactions of changed environmental conditions (i.e. increasing temperature) and a changed nutrient balance in the Baltic Sea, where nitrogen concentrations have decreased significantly since the 1990s (Savchuk & Wulff 2009). Together, our observations on the variability of the environmental factors across the Baltic proper during the bloom in 2007–2008 and the analysis of the long-term variability in the inshore-offshore gradient in the northern Baltic proper, suggest that the abundances of *P. polylepis* are favored by high spring SST, possible intrusions of saline water during winter, and low annual NO_2+NO_3 concentrations. In line with this

conclusion, Suikkanen et al. (2013) suggested that a combination of changes in salinity, temperature and possibly $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratios during the last 3 decades have favoured an increasing abundance of Prymnesiophyceae in the northern Baltic Sea. Therefore, the following 2 circumstances may stimulate even more frequent *P. polylepis* blooms in the Baltic Sea: first, the projected further increase in temperature due to climate change, and second, a trend of decreased nutrient loading (e.g. due to continued efforts to improve sewage treatment that since the 1980s has increased load $\text{NO}_2+\text{NO}_3:\text{PO}_4$ ratio from <40 to ca. 50—half what is needed to restore levels to mid-19th century conditions; Gustafsson et al. 2012). More research is, however, needed to establish effects (if any) of these factors on toxin production by the *Prymnesium* cells and food web effects of these blooms in specific ecosystems. Another area of concern with respect to the composition of the blooms is the poor taxonomic resolution of the current methodology (i.e. exclusive reliance on light microscopy) in the monitoring analysis. The analysis of the monitoring samples should be complemented with alternative, e.g. molecular, methods for species identification, if we are to understand species-specific responses to particular environmental factors as well as seasonal dynamics of this potentially harmful algae that appear to respond positively to the temperature increase in the Baltic Sea and thus contribute to the climate-driven changes in the pelagic food webs.

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