LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 64, 2019, 1779–1789

doi: 10.1002/lno.11150

Primary production, carbon release, and respiration during spring bloom in the Baltic Sea

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Abstract

We determined the gross and net primary production (GPP and NPP) for the total community and the < 10 μm size fraction, the net release of dissolved organic carbon (DOC), and the microbial respiration in the Baltic Sea during the spring bloom. Samples (n = 126) were taken from the surface (3 m depth) covering most subbasins and different phases of the bloom, defined by the inorganic nutrient and Chlorophyll *a* (Chl *a*) concentrations. During the course of the bloom, the NPP rate (i.e., growth rate) decreased from 0.34 d^{-1} ± 0.03 (SE) to 0.15 d^{-1} ± 0.02 (SE), the contribution of the < 10 μm fraction increased from 14% ± 2.5 (SE) to 47% ± 4.0 (SE) and the percent extracellular release (PER) increased from 3.8% ± 0.7 (SE) to 8.9% ± 1.5 (SE). The assimilation number, was on average 0.13 mol C (g Chl *a*)$^{-1}$ h^{-1} ± 0.01 (SE), and the average GPP:NPP rate was 1.25. The respiration increased with growth rate and was 21% of the GPP rate. The net release of DOC was relatively constant over the bloom phases, with increasing PER compensating for the reduction in biomass, and estimated to 24–36 μmol DOC L^{-1} during the whole spring bloom period in all subbasins except in the Bay of Bothnia where it was 75% lower. The assimilation number was surprisingly stable, suggesting it is uncoupled from the inorganic nutrient concentration, likely a reflection of physiological acclimation and changing phytoplankton community.

Primary production (PP) in the ocean is approximately half the global carbon fixation and forms the basis of the marine food web and its harvestable resources. Carbon fixation is heterogeneously distributed, ranging from highly productive seasonal seas and upwelling areas to vast areas with extremely low production. The former being highly dynamic, driven by pulses of inorganic nutrients (e.g., upwelling of nutrient rich deep water) or improved light conditions for photosynthesis (e.g., seasonal changes); the latter by being close to or at steady state with little variation in the biomass concentration. Falkowski et al. (2003) described these contrasting ecosystems as being in a perturbed and balanced state, respectively, each with different characteristics in ecosystem functioning and community structure. A perturbed state is typically dominated by large phytoplankton and new production (as opposed to regenerated production). At balanced state, the production rate equals the loss rate, regenerated production prevails, and there is a dominance of small phytoplankton.

In temperate coastal seas, the ecosystem will typically be somewhere in between these extremes, with a temporal development toward a balanced state after a perturbation (e.g., a pulse of inorganic nutrients). This is the case of the spring bloom cycle that develops from a highly perturbed state after winter-time mixing toward a more balanced state after the buildup of water stratification in summer (Margalef 1978).

Understanding the relationship between PP and loss processes like respiration has been identified as a key target for better predictions of carbon fluxes in aquatic ecosystems (e.g., del Giorgio and Williams 2005). In areas close to or at balanced state, it is relatively easy to measure and model carbon fluxes when carbon fixation matches carbon respiration. It is in the areas with high productivity, governed by perturbations, where the carbon fluxes are more complex and difficult to model.

Different methods exist for measuring PP, for example, measuring incorporation of radiolabeled ^{14}C, measuring O_2 production or using O_2 isotopes (e.g., $^{16}O_2$), or though noninvasive fluorescence techniques (Falkowski and Raven 2013). Of these methods, measuring the ^{14}C incorporation has historically been most widely used due to its high sensitivity, relatively low cost, and the fact that the beta particle emitted

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Additional Supporting Information may be found in the online version of this article.

contains very little energy (i.e., low risk of radiation exposure). Although the ^{14}C has been used extensively over the years, there have been different opinions on what the method actually measures (Sakshaug et al. 1997; Marra 2009). However, recent work with cultures has started to open up the black box of intracellular carbon metabolism (Milligan et al. 2015). Long incubation times provides good estimates of net primary production (NPP), but there are species-specific and growth dependent factors that may overestimate NPP (Pei and Laws 2013, 2014). Short-term incubations is a good estimate of gross carbon production (GPP) at high growth rates, but may underestimate GPP at low growth rates (Halsey et al. 2010, 2011, 2013). In spite of its shortcomings, the ^{14}C method offers the opportunity of easily measuring carbon fixation in relation to biomass for different size fractions, which is important for understanding phytoplankton physiology and carbon cycles (Milligan et al. 2015).

The Baltic Sea is an almost land locked sea, and is functionally similar to a large estuary with a salinity gradient ranging from 20 at the Danish straits to 2 in the northernmost part of Gulf of Bothnia. The spring period, with high concentrations of inorganic nutrients after winter-time mixing and increasing irradiance, is the most productive period in the Baltic Sea, similar to other coastal seas in the temperate zone. The spring bloom of phytoplankton typically produces up to half the annual production of algal biomass during a relatively short time period (2–6 weeks), and the phytoplankton community is dominated by relatively large (> 10 μm) diatoms and dinoflagellates, with little grazing pressure (Lignell et al. 1993; Wasmund et al. 1998). The major sedimentation event following the spring bloom transports a large fraction of recently fixed carbon to the sea floor (Heiskanen 1998; Tamelander et al. 2017).

The spring bloom in the Baltic Sea is a typical example of a perturbed state marine system (Falkowski et al. 2003) with a large share of annual new production (Heiskanen 1998). The bloom itself travels from south to north in a mosaic-like pattern driven by eddy mixing (Kahru and Nõmmann 1990), and there is a long-term trend (2000–2014) of earlier and more expanded periods of spring bloom (Groetsch et al. 2016).

Unlike production, very few respiration measurements have been made during spring in the Baltic Sea, but a culture study of spring bloom species suggested respiration rates $\sim$ 25% of GPP in dinoflagellates (*Apocalathium malmogiense* syn. *Scrippsiella hangoei* and *Biecheleria baltica* syn. *Woloszynskia halophila*) and $\sim$ 10% in diatoms (*Chaetoceros wighamii*, *Melosira arctica*, and *Thalassiosira baltica*) (Spilling and Markager 2008). However, due to large variability between years in spring bloom production and between subbasins, this should be tested on a larger temporal and spatial scale with natural assemblages. Respiration is, for example, known to increase with increasing growth rate and is also influenced by other factors such as physiological state, light history, and temperature (Geider 1992; Langdon 1993).

Another loss factor is the release of dissolved organic carbon (DOC). Under optimal conditions, exudation loss of DOC is relatively low at < 5% (Lignell et al. 1993), but may be important for the increase of DOC concentration in the surface water, which is an important driver for bacterial production during summer (Hoikkala et al. 2015).

In this study, we wanted to better understand the spatial and temporal variability in PP, respiration, and net release of DOC in the Baltic Sea during the spring bloom period. As the spring bloom develops from a highly perturbed state dominated by big phytoplankton cells toward a more balanced state dominated by smaller cells, we were also interested in the share of PP in the small (< 10 μm) size fraction of phytoplankton. To do this, we did ^{14}C incubations on four research cruises covering the main subbasins of the Baltic Sea during different phases of the spring bloom.

Material and methods

The data were obtained during four research cruises on board of the R/V Aranda (April–May; 2013–2016). The cruises covered Gulf of Finland (GoF), Baltic Proper (BP), Åland Sea (ÅS), Archipelago Sea (ArS), Bothnian Sea (BS), and Bay of Bothnia (BoB). The routes were different every year (Fig. 1), for example, only one going into the Gulf of Bothnia (consisting of BS and BoB). The number of sampling stations for all cruises was GoF 48, BP 38, ÅS 12, ArS 9, BS 10, and BoB 9. Some samples were taken in the Kvarken region bordering BS and BoB, and these stations were added to the BoB stations (Fig. 1). All samples were taken from 3 m depth using a Niskin bottle with a few exceptions (n = 5) when the flow through system of the ship was used (also from 3 m depth).

For measuring PP, we collected 1 L of sample water. Subsamples were taken for measurements of total and the size fraction < 10 μm (2 h incubation). For the total production, we used two incubation periods that allowed determining GPP (2 h incubation) and NPP (24 h incubation) defined as gross and net carbon fixation (i.e., not including photosynthetic output used for other purposes, e.g., nitrate reduction). Filtering the samples before the incubation was aimed to separate < 10 μm (gravity filtration through a 10 μm polycarbonate filter) phytoplankton assemblages from the rest of the phytoplankton community. In addition, a set of samples incubated for 24 h with the total community (no filtration) was filtered after incubation using 0.2 μm pore size polycarbonate filters to determine the dissolved organic carbon (DOC) released from phytoplankton cells. We are aware that a substantial heterotrophic consumption of DOC might take place during the 24 h incubation period (Lignell 1990). For this reason, the results we report must be regarded as net DOC production rates. The percent extracellular release of ^{14}C (PER) was calculated based on the $DO^{14}C$ fraction (< 0.2 μm) of the total ^{14}C fixation. Incubations were carried out in a walk-in climate control room keeping in situ surface temperature (2–6°C). The

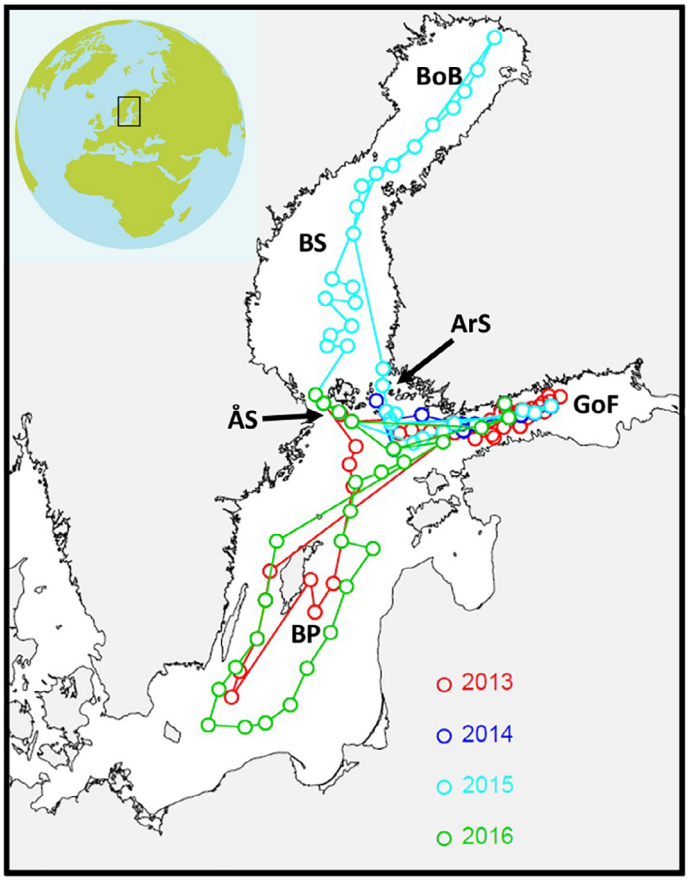

Fig. 1. Map of the Baltic Sea showing the sampling points (n = 126) during the cruises (2013–2016) held during spring bloom (April–May).

irradiance during the incubations was 50 μmol photons m^{-2} s^{-1} provided by Philips TL 20W fluorescent lamps and measured in air with a flat (cosine) Walz ULM 500 sensor. The Baltic Sea is characterized by its high turbidity and this irradiance represents an approximation to average in situ light conditions at the sampling depth (Simis et al. 2017). For the 24 h incubation, the same light conditions were used in a 12:12 h light–dark cycle and the incubation was stopped after the dark period.

In 2013 and 2014, 4 mL of sample water were distributed into scintillation vials and 20 μL of 20 μCi ^{14}C mL^{-1} labeled bicarbonate solution (DHI lab) were added (Camarena-Gómez et al. 2018). In 2015 and 2016, the stock solution (300 μL) was added to a total sample volume (60 mL) for a final specific activity of 0.1 μCi mL^{-1} and then the water was mixed before 4 mL was distributed into the different scintillation vials. Three scintillation vials were used for each combination of incubation time and filtered/non-filtered samples: one dark (obtained by wrapping the scintillation vial in aluminum foil) and two light vials. Samples were incubated directly in the vials, and after the incubation period, 100 μL of 2 M HCl was added. The vials were left without lid for 24 h in a fume hood before adding 7 mL of scintillation cocktail (Instagel Plus, Perkin Elmer). The incorporated ^{14}C was determined with a scintillation counter (Wallac 1414, Perkin Elmer). For those vials where filtration was performed after the incubation period, the filtrate was subsequently acidified and the radioactivity determined as described above. Total dissolved inorganic carbon (DIC) was determined using a high-temperature combustion IR carbon analyzer (Unicarbo, Electro Dynamo, Finland). PP was calculated from the measured uptake of ^{14}C knowing the total amount of added isotope and the DIC concentration according to Gargas (1975).

We calculated the respiration (R) according to:

$$R = (GPP*12) - (NPP*24) \quad \text{(Eq. 1)}$$

The GPP (2 h incubation) was multiplied by 12 h light, and the NPP was measured during the 24 h incubation (with 12:12 h light–dark cycle; both NPP and GPP with the units µmol C L^{-1} h^{-1}). The difference is the C loss due to respiration and assuming constant respiration rate during the light–dark cycle. Using Eq. 1, there were some samples (n = 17) that produced negative respiration values and these were not considered in further analysis.

Chlorophyll *a* (Chl *a*) and particular organic carbon (POC) was determined by filtration (50–200 mL depending on the Chl *a* concentration) onto GF/F filters (Whatman), and this was done right after the samples were taken. The filters for Chl *a* were put in scintillation vials and 10 mL ethanol was added (Jespersen and Christoffersen 1987). Chl *a* samples were stored in a freezer (–20°C), but they were acclimated to room temperature before measurement with a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies). Six concentrations of Chl *a* standard (Sigma Aldrich) were used to create the calibration curve to determine the Chl *a* concentration in the samples. The filters used for POC determination were dried and stored at room temperature until measurements using a biological sample converter (Roboprep-CN) connected to a mass spectrometer (Europa Scientific ANCA-MS 20-20, Europa Scientific Ltd.) according to the protocol from Koistinen et al. (2017). The assimilation number was calculated normalizing the carbon fixation (2 h incubation) to the Chl *a* concentration. The NPP rate (per day) was calculated by normalizing the 24 h incubation to the POC concentration. Similarly, GPP and respiration rates (per day) were calculated by normalizing (µmol C L^{-1} d^{-1}) to the POC concentration (µmol C L^{-1}). Inorganic nutrients: NO_3, NO_2, NH_4, PO_4, and dissolved silicate (DSi) were determined using standard colorimetric methods (Grasshoff et al. 1983) directly after sampling.

In most of the subbasins of the Baltic Sea, the spring bloom is terminated once dissolved inorganic nitrogen (DIN) is depleted and there is a pool of dissolved inorganic phosphorus (DIP) remaining. The exception to this is the BoB, which is primarily DIP limited and a surplus of DIN is available (Tamminen and Andersen 2007). Using the DIN for subbasins: GoF, BP, ArS, ÅS, and BS and DIP for subbasin BoB, we divided

Table 1. We divided up the spring bloom into four different phases based on the concentration of inorganic nutrients and Chl *a*. The bloom phases are the growth, the peak, the decline, and the post-bloom phases. The subbasins sampled were GoF, BP, ÅS, ArS, BS, and BoB. Chl *a* concentrations are in μg L^{-1}, $NO_2 + NO_3$ (used for all subbasins except for the BoB) and PO_4 (BoB) concentrations in μmol L^{-1}.

Stage of the spring bloom	Growth	Peak	Decline	Post-bloom
Chl *a* concentration	Low-medium	High	Medium	Low
Subbasin				
GoF	< 16	> 16	4–16	< 4
BP	< 8	> 8	3–8	< 3
ÅS, ArS, BS	< 6	> 6	2–6	< 2
BoB	< 5	> 5	1–5	< 1
Inorganic nutrient concentration	Medium-high	Low-medium	Low	Low
Subbasin				
GoF, BP, ÅS, ArS, BS ($NO_2 + NO_3$)	> 0.5	< 0.5	< 0.2	< 0.2
BoB (PO_4)	> 0.3	< 0.3	< 0.1	< 0.1

the spring bloom into four different phases based on the concentration of inorganic nutrients and Chl *a* compared with historical data (Table 1): the growth phase (growth), the peak phase (peak), the decline bloom phase (decline) and after the bloom phase (post-bloom). High Chl *a* was defined as within 20% of the average peak concentration during spring, which is different in the different subbasins (Table 1). For approximately 10% of the stations, the Chl *a* and nutrient data were not consistent (i.e., not indicating the same bloom phase). In these cases, we used the nutrient concentration to differentiate between the growth and peak phases and the Chl *a* value to differentiate between the peak, decline and post-bloom phases. This criterion was based on our assumption that inorganic nutrient concentration is more important for algal growth than the Chl *a* concentration during growth/peak phases and that Chl *a* better divides the decline/-post-bloom phases after nutrient depletion. The full data set can be found in the Supporting Information (Table S1).

Linear regressions in the graphs were fitted to the data in Sigma Plot. Results are shown as the average ± the standard error (SE) of the mean. Statistical tests were carried out by two-way ANOVA with linear regression in R. In short, a linear model with two categorical explanatory variables (bloom phase with four levels, and subbasin with six levels) were fitted to the data. A "base" level is preset for this analysis, and using the default contrast settings of linear model in R, the intercept is the predicted value of the dependent variable in the "base" subbasin during the "base" bloom phase, and the following parameter estimates are the modeled difference from the intercept. All combinations of subbasins and bloom phases were run as "base" levels to check for individual differences between bloom phases and subbasins. A summary of this two-way ANOVA analysis can be found in the Supporting Information (Tables S2 and S3).

Results

The sampling took place during all four bloom phases from early spring with cold temperature (< 1°C) to after the spring bloom with higher water temperatures (> 5°C) and low concentration of inorganic nutrients (Table 2). The mean GPP of all the samples was 1.064 μmol C L^{-1} h^{-1} ± 0.008 (SE) and normalized to Chl *a* (GPP^B): 0.13 mol C (g Chl *a*)$^{-1}$ h^{-1} ± 0.006 (SE) (Fig. 2). Comparing the different subbasins, there was some evidence suggesting that the GPP^B was slightly higher in the ArS compared with GoF and BP (two-way ANOVA; $p < 0.001$) and it was lower in the BoB compared with the rest of the subbasins (two-way ANOVA; $p \le 0.028$). In terms of bloom phase, the GPP^B decreased from the growth to the peak phase (two-way ANOVA; $p = 0.007$), but increased again in the post-bloom phase (two-way ANOVA; $p = 0.001$), and there was no difference in the GPP^B between the growth and the post-bloom phases (two-way ANOVA; $p = 0.3$).

The mean NPP rate (Fig. 2) was also lower in the BoB compared with the rest of the subbasins (two-way ANOVA; $p \le 0.047$). There was a decline in the NPP rate during the bloom progression from 0.34 d^{-1} ± 0.03 (SE) during the growth phase to 0.15 d^{-1} ± 0.02 (SE) during the post-bloom phase (Fig. 2), and the main difference was between the growth and peak phases compared with the decline and post-bloom phases (two-way ANOVA; $p \le 0.04$).

The net release of DOC was on average 0.4 μmol DOC L^{-1} d^{-1} in all subbasins, except in the BoB where it was 0.1 μmol DOC L^{-1} d^{-1} (Fig. 3), and the two-way ANOVA suggested it was lower compared with the BP and the ÅS ($p = 0.045$ and 0.011, respectively). The stage of the bloom did not affect the absolute amount of DOC release (two-way ANOVA; $p > 0.1$), but clearly affected the PER (Fig. 3).

There was both a subbasin and bloom phase effect on the PER. The PER was lower in the GoF and the BS compared with the BP (two-way ANOVA; $p \le 0.015$). The PER also increased as the bloom progressed and the average PER was 3.8% ± 0.7 (SE) and 3.6% ± 0.5 (SE) during the growth and peak phases, respectively, and increased to 6.5% ± 0.8 (SE) and 8.9% ± 1.5 (SE), during the decline and post-bloom phases, respectively. Statistically, the difference was between the growth and peak phases compared with the post-bloom phase (two-way ANOVA; $p \le 0.014$).

The GPP by the < 10 μm size fraction was similarly to PER lower in the GoF and increasing later in the bloom succession (Fig. 4). The mean contribution of the smaller size class was < 15% during the growth and peak phases (14.4% ± 2.5 [SE] and 13.9% ± 2.3 [SE], respectively), and increased to 29.2% ± 4.2 (SE) and 47.3% ± 4.0 (SE) during the decline and post-bloom

Table 2. Environmental variables, nitrite+nitrate ($NO_2 + NO_3$), ammonium (NH_4), phosphate (PO_4), dissolved silicate (DSi), and Chl *a* concentrations measured during the four cruises (2013–2016). Values represent the range between the minimum and maximum in the subbasins sampled each year. The different subbasins covered were GoF, BP, ÅS, ArS, BS, and BoB. The bloom phase corresponds to the different phytoplankton bloom stages defined in Table 1: growth (Gr), peak (Pe), decline (De), and post-bloom (PB). All the primary data are presented in the Supporting Information Table S1.

	Number of stations	Subbasins	Bloom phases covered	Temp (°C)	Salinity	NO_2+NO_3 (μmol L^{-1})	NH_4 (μmol L^{-1})	PO_4 (μmol L^{-1})	DSi (μmol L^{-1})	Chl *a* (μg L^{-1})
10–26 Apr 2013	31	GoF	Gr, Pe, De	0.76–1.98	5.09–6.38	0–5.85	0.10–0.39	0.19–0.48	7.84–19.75	10.3–22.0
	11	BP	Gr, Pe	1.62–2.72	5.97–7.24	0–2.08	0.04–0.19	0.06–0.33	8.27–14.24	2.2–11.5
	4	ÅS	Gr, Pe	1.29–1.59	5.45–6.16	0–2.59	0.07–0.13	0.02–0.35	8.92–13.10	6.1–12.7
5–10 May 2014	8	GoF	De, PB	4.25–5.18	5.03–6.01	0–0.26	0.5–0.24	0.12–0.33	2.27–9.10	2.4–10.3
	2	BP	De, PB	4.62–5.69	6.56–6.63	0	0.05	0.28	7.60–11.60	1.5–1.9
	4	ArS	De, PB	4.58–5.17	5.90–6.29	0–0.4	0.08–0.11	0.20–0.33	6.01–8.10	0.7–2.3
4–15 May 2015	5	GoF	De, PB	3.91–5.35	4.96–5.86	0–0.02	0.08–0.44	0.18–0.36	5.50–11.20	3.2–12.6
	6	ArS	Pe, PB	4.41–6.09	5.75–6.38	0–0.23	0.06–0.13	0.06–0.33	5.60–11.10	1.7–8.9
	4	ÅS	De	5.03–6.22	5.41–6.06	0	0–0.06	0.05–0.22	6.70–8.70	2.9–4.8
	4	BP	De, PB	4.52–5.69	5.72–6.56	0–0.44	0.05–0.34	0.27–0.40	7.6–11.8	2.9–6.6
	10	BS	Pe	3.59–4.16	5.12–5.54	0–0.38	0.05–0.13	0–0.21	8.10–12.70	6.4–13.0
	9	BoB	Pe, De	1.35–4.73	2.20–5.09	0–7.47	0.04–0.35	0–0.02	9.40–55.70	1.6–6.8
4–15 2016 Apr	4	GoF	Gr, Pe, De	1.56–2.89	4.95–5.20	0–6.64	0.14–0.22	0.34–0.61	12.21–19.09	9.4–26.1
	20	BP	Gr, Pe, De, PB	2.47–5.90	5.92–7.77	0–1.89	0–1.19	0.16–0.57	11.77–17.65	1.7–12.0
	4	ÅS	Pe	2.84–3.28	5.51–5.68	0–0.17	0.11–0.18	0.12–0.18	15.57–18.17	7.4–10.2

phases, respectively. Comparing the different subbasins statistically suggested a lower contribution of the < 10 μm size fraction in the GoF compared with the other subbasins (two-way ANOVA; $p \leq 0.022$), and the main difference in terms of bloom phase was between the growth and peak phases compared with the post-bloom phase (two-way ANOVA; $p < 0.001$), with a much higher contribution of the < 10 μm size fraction to the PP in the post-bloom phase.

For the total community, the NPP was 80% of the GPP on average (Fig. 5, slope = 0.8, $R^2 = 0.92$, $p < 0.0001$). The average respiration was 2.28 μmol C L^{-1} d^{-1} $\pm$ 0.31 (SE). There was no difference in the respiration between different subbasins or bloom phases (Supporting Information Fig. S1; $p = 0.6$ and 0.3, respectively), but there was a positive correlation between the calculated respiration rate and the GPP rate (Fig. 5, slope = 0.21, $R^2 = 0.45$, $p < 0.0001$).

Discussion

The temporal development of the spring bloom in the Baltic Sea is not a smooth growth curve, but rather characterized by physical heterogeneity (Stipa 2004); for example, mixing events that produce secondary Chl *a* peaks (Lips et al. 2014) and large spatial variation (Kahru et al. 1990). There are also gradients between subbasins (i.e., the inorganic nutrient and biomass concentrations used) and temporal development of environmental variables. The placement of the data points into the different phases of the bloom was consequently not unambiguous, for example, in some cases (< 10%) where the nutrient and Chl *a* concentrations indicated different bloom phases. In addition, the number of samples was not evenly distributed between subbasins. The individual measurements thus represent snapshots of the phytoplankton metabolism during the spring bloom, but the combined data give a picture of the overall variability and development in PP and R during this period.

Assimilation number

There was surprisingly little variation in the Chl *a* normalized PP, that is, the assimilation number (GPP^B). The different community composition is likely a factor that explains differences in GPP^B in addition to the physiological state. For the bloom development, the lower GPP^B during the decline phase compared with the growth phase of the bloom was likely a reflection of the deteriorating physiology of the phytoplankton community, whereas during the post-bloom phase, the community entered a more balanced state with regenerated production (Falkowski et al. 2003) and a shift in phytoplankton community to smaller species (Fig. 4), which produced a similar GPP^B to the growth phase of the bloom. The change in phytoplankton community composition toward the smaller (< 10 μm) size fraction is not surprising as inorganic nutrients become limiting for growth, and having a smaller size with lower surface to volume ratio is beneficial (Reynolds 2006). The nutrient limitation following the spring bloom creates

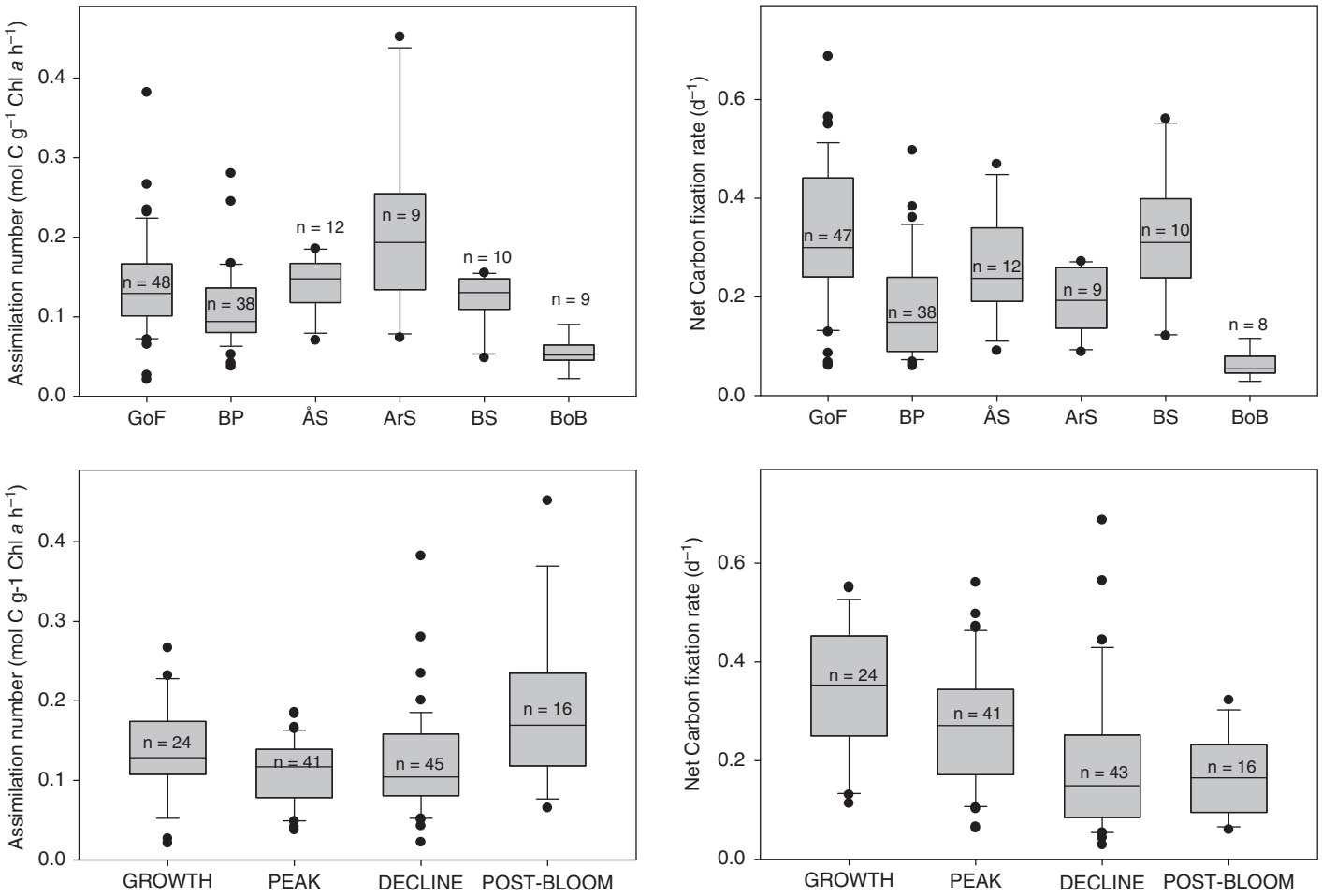

Fig. 2. The measured gross primary production normalized to Chl *a* (GPP^B), that is, the assimilation number (left), and the measured net primary production normalized to particulate organic carbon (right) for the different subbasins (top) and bloom phases (bottom). The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles and points are outliers.

physiological stress and most of the biomass sinks out of the euphotic zone (Heiskanen 1998). However, in the post-bloom phase, the physiological acclimation and the shift in community composition optimized GPP^B to levels similar as during the bloom. This is in line with Milligan et al. (2015) that argued that assimilation numbers represent net production rates, independent of nutrient-limited division rates.

The main difference in GPP^B between the different subbasins was the lower GPP^B in the BoB. The BoB is the most oligotrophic part of the Baltic Sea. It is also P-limited, as opposed to the other subbasins that are primarily N-limited (e.g., Tamminen and Andersen 2007), and it contains a different phytoplankton community composition (Kuosa et al. 2017), which likely is the main explanation for the lower GPP^B in this subbasin.

Release of DOC by the total community

A surprising finding was that the net release of DOC was more or less constant throughout the bloom in absolute numbers, and the increasing PER compensated for the reduction in biomass during the decline and post-bloom phases. The DOC pool in the Baltic Sea is relatively large at 260–480 μmol C L^{-1}, due to a lot of allochthones sources of DOC, and there is an accumulation of 20–200 μmol DOC L^{-1} in the surface water during the productive season (Hoikkala et al. 2015). With a spring period of 60–90 d (including all the phases covered in this study), the net DOC release, based on our results, would add 24–36 μmol DOC L^{-1} in all subbasins except in the BoB where the average net release of DOC was 75% lower.

A substantial part of the highly labile autochthonous DOC fraction was likely taken up already within our 24 h incubation (Lignell 1990), and not captured by our measurements. However, at least 7 d are needed for heterotrophic bacteria to utilize 50% of the DOC released by phytoplankton during summer (Hoikkala et al. 2016). In addition, part of the allochthones DOC will likely be semi-labile and available for bacterial uptake when the water temperature increases, or after photochemical mineralization (Vähätalo and Zepp 2005).

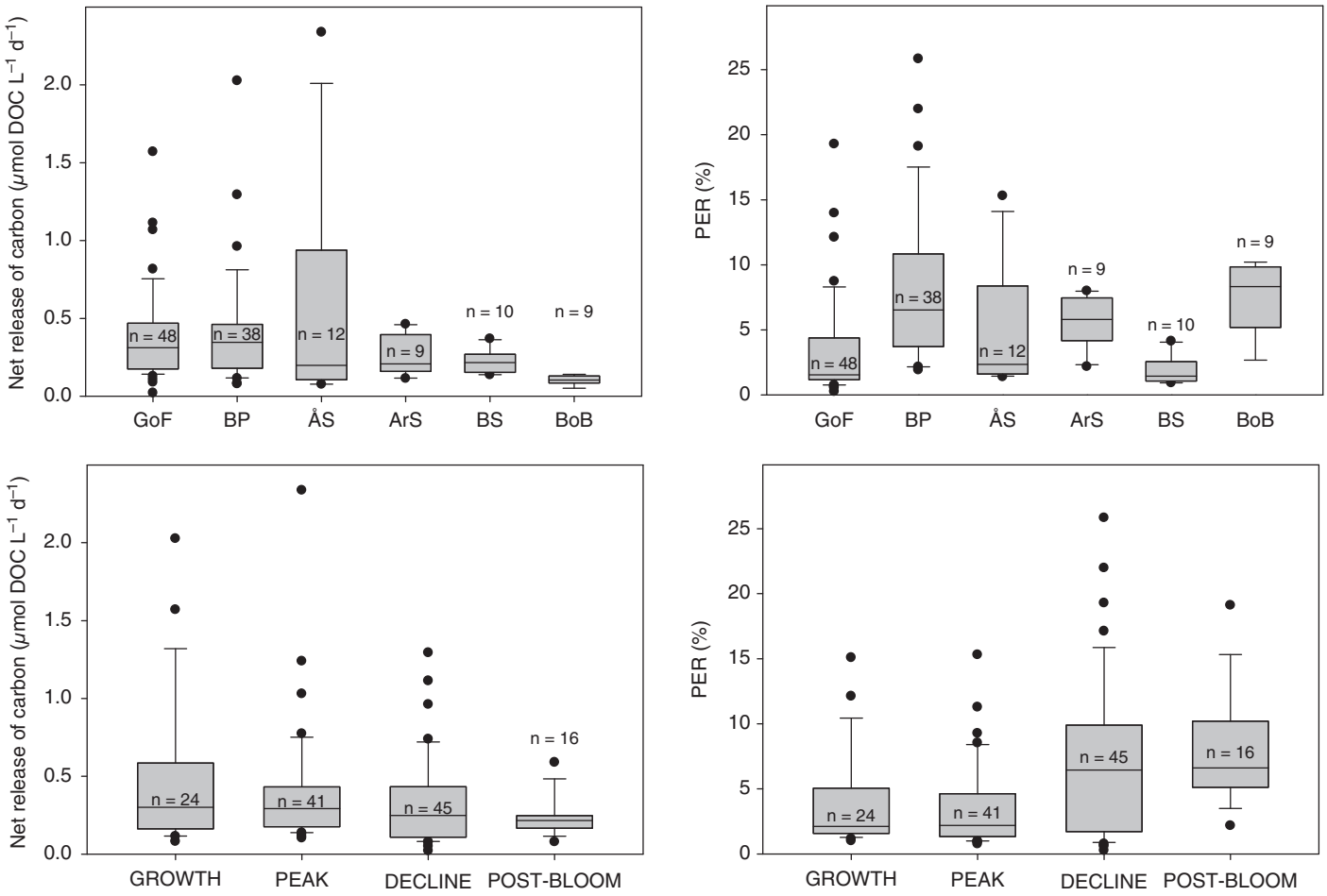

Fig. 3. Net extracellular release (total community, 24 h incubation) of DOC (left) and the net (total community, 24 h incubation) PER of carbon (right) in different subbasins (top) and bloom phases (bottom). The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles and points are outliers.

As the release of DOC stayed constant and PP decreased after the peak of the bloom, the PER increased. After nutrient depletion, photosynthesis can then either be stopped or alternatively continue fixing carbon for some time after the stop in cell division (Falkowski and Raven 2013). To some extent this extra carbon can be stored as, for example, lipids, increasing the C:N:P ratio (Spilling et al. 2014), or can alternatively be excreted as DOC.

Release of DOC can be done for several reasons, see, for example, Thornton (2014) for a review. For example, it may stimulate bacterial growth, which may provide some benefits in return, like the production of B12 vitamin (Kazamia et al. 2012), or be a way to dissipate excess light energy (Zlotnik and Dubinsky 1989). Exudation is affected by the phytoplankton community composition, where diatoms is typically the group with the highest PER of the cold water phytoplankton in the Baltic Sea (Spilling et al. 2014). This is not a general phenomenon, however, as high PER has also been found in dinoflagellates in the Mediterranean (López-Sandoval et al. 2013), and it seems to be more species than group specific in the Baltic Sea (Camarena-Gómez et al. 2018).

Gross/net production

Overall, there was a very close coupling between the short- and long-term incubation (R^2 = 0.92). If, as suggested by cultures studies, the variability in what the ^{14}C method measures (NPP to GPP) depends on growth rate (e.g., Milligan et al. 2015), we would have expected more variability as we were clearly covering different growth stages of the bloom. There is a difference in measuring ^{14}C incorporation in a community compared with a monoculture as different species are affected differently by, for example, the nutrient concentration. We argue that in our measurements the short and long incubation times are good approximations for GPP and NPP, respectively (where PP in this context is defined as carbon fixation). The average gross to net (GPP:NPP) PP ratio was 1.25, which is low but this is typical for periods with primarily new

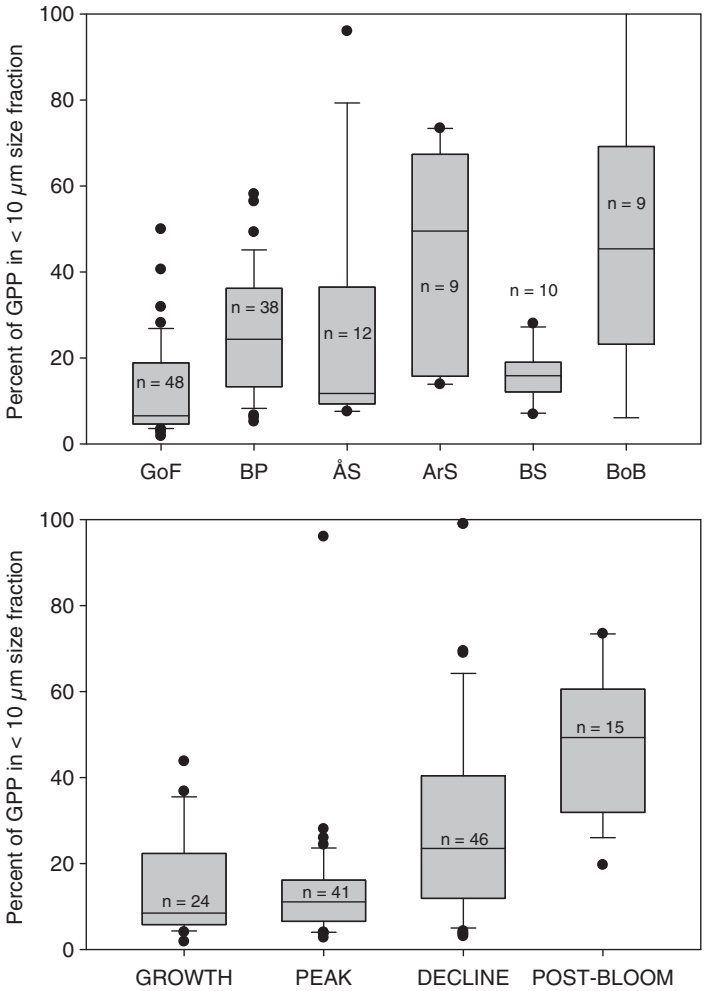

Fig. 4. The percent of GPP in the < 10 μm fraction filtered before incubation (2 h) in the different subbasins of the Baltic Sea (top) and different spring bloom phases (bottom). The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles and points are outliers.

production with minimal heterotrophic respiration (Halsey and Jones 2015). The GPP:NPP ratio measured during an Antarctic spring bloom was 1.7 during the growth phase and 2 during the post-bloom phase (Goldman et al. 2015). We did not record any difference in the GPP:NPP ratio over the bloom phases. Goldman et al. (2015) incubated the samples using natural solar irradiance, and did not have constant light as we did, which could have affected the development of the GPP:NPP ratio. Although our cruises were conducted after equinox and the day length was longer than 12 h, the dusk and dawn hours have very little light for photosynthesis, so the actual light period is close to 12 h. Keeping the incubations under constant light enabled direct comparison of all the samples, which was the focus of this study, but for estimating areal primary productivity, a full production-irradiance curve with measurement of in situ irradiance and light attenuation throughout the water column is needed.

Specific growth rate and respiration

The NPP normalized to POC, that is the NPP rate, could be viewed as the specific growth rate. The natural community POC would include particles that do not contribute to carbon fixation, for example, detritus and heterotrophic plankton, suggesting that it would be an underestimate of the true growth rate of phytoplankton. This could be the reason for the discrepancy between GPP^B and NPP in the ArS (Fig. 2), that is, the Chl *a* containing phytoplankton are effective at fixing carbon, but a high fraction of non-photosynthesizing particles reduced the NPP rate. Overall, however, detritus and heterotrophs is a relatively small and constant part of the POC in the Baltic Sea surface water during spring (Lipsewers and Spilling 2018), and the growth values we obtained (most in the range 0.05–0.5 d^{-1}) are comparable to common spring diatoms growing (0.3–0.4 d^{-1}) at similar irradiance as we used (Spilling and Markager 2008). Estimating the rate of biomass increase from observations of the spring bloom in the Baltic Sea, indicates that a growth rate at 0.1–0.3 d^{-1} is realistic under natural conditions (Lignell et al. 1993; Wasmund et al. 1998; Höglander et al. 2004).

Marra and Barber (2004) suggested that the reduction in ^{14}C during the dark period can be used as a measure of respiration assuming night and day respiration is similar. We did not do a dusk-to-dawn measurement, but estimated respiration by extrapolating the short-term GPP measurement to the full 12 h light period and subtracted the NPP. Using this approach, we calculated the average respiration rate to be 21% of the GPP rate, which is comparable to the respiration loss of 21–69% presented in the review by Langdon (1993). This estimate of respiration rate does not take into account any difference of respiration between light and dark periods. In terms of oxygen, there are well known light dependent respiration pathways (e.g., Halsey and Jones 2015), and also for carbon there is a transient carbon pool with rapid turnover (Halsey et al. 2011). This means that respiration rate could be elevated during daylight and into the start of the dark period (Mantikci et al. 2017). Our measurements represents the total respiration during the 12:12 h light-dark cycle. Bacterial respiration of released DOC and respiration by micro zooplankton would have contributed to the carbon loss in our measurements. However, the bacterial production in cold water (< 10 °C) is low (Camarena-Gómez et al. 2018) and also the grazing pressure is relatively low in the Baltic Sea during spring bloom (Lignell et al. 1993), suggesting autotrophic respiration was the dominating carbon loss factor.

The respiration rate increases with growth rate and the slope is the fraction of carbon respired per unit carbon assimilated and the intercept is the rate of maintenance respiration (Geider 1992; Langdon 1993). This is also what we found, with a maintenance respiration rate of 0.004 d^{-1} (Fig. 5), which is very low. With relativley high variability (R^2 = 0.45), there is uncertainty in this measurement.

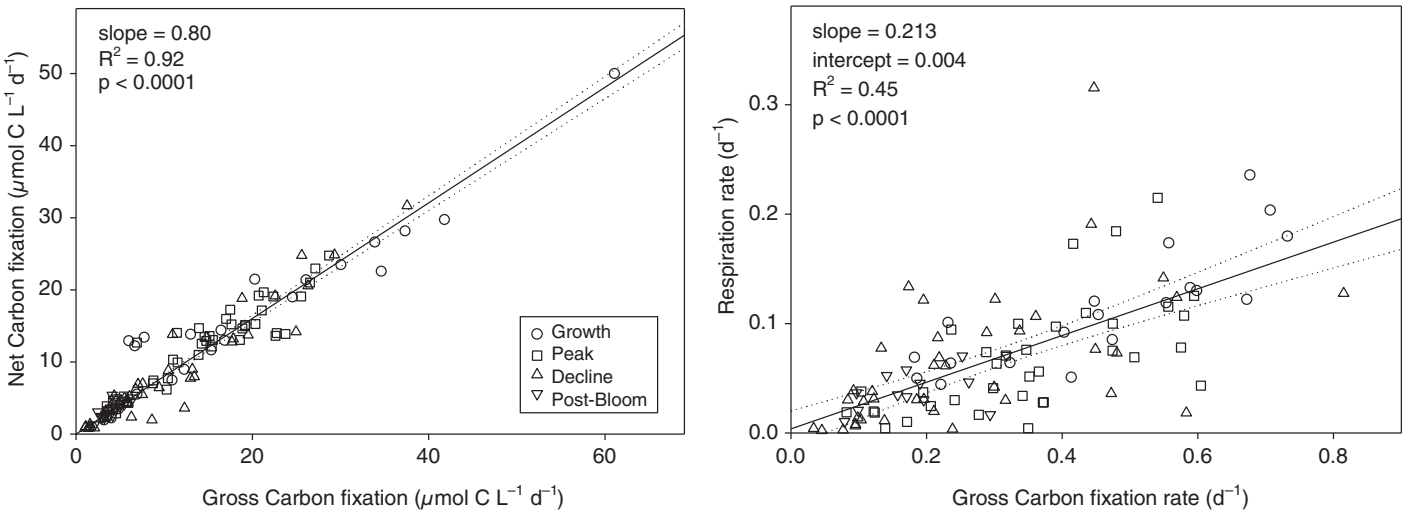

Fig. 5. The total community, GPP (2 h incubation) plotted against the NPP (24 h incubation; left) and the GPP rate (normalized to particulate organic carbon) plotted against the calculated respiration rate (right). Solid lines represent the linear regression and dotted lines the 95% confidence interval.

However, diatoms, in particular, have low maintenance respiration that could be down to 0.01 d^{-1} for some species (Geider and Osborne 1989).

In conclusion, the Baltic Sea spring bloom is characterized by a transition from a highly perturbed state toward a more balanced state after the depletion of inorganic nutrients. During this period, there is a large variation in biomass both temporally and spatially (different subbasins), but the average GPP:NPP ratio (1.25) and the net release of DOC were relatively constant over the subbasins and bloom phases. An increasing PER compensated for the reduction in biomass, and the net DOC release was estimated to 24–36 μmol DOC L^{-1} during the whole spring bloom period in all subbasins except in the BoB where it was 75% lower. The assimilation number was surprisingly stable, suggesting it is uncoupled from the inorganic nutrient concentration, likely a reflection of a changing phytoplankton community and acclimation to the ambient nutrient concentration.

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Acknowledgments

The authors would like to thank the crew and scientific staff on board R/V Aranda during the CFLUX cruises in particular T. Lipsewers and M. T. Camarena-Gómez. This work was financed by the Academy of Finland (decision number 259164) and the Walter and Andrée de Nottbeck Foundation (KS). This study utilized SYKE marine research infrastructure as a part of the national FINMARI consortium.

Conflict of Interest

None declared.

Submitted 02 July 2018
Revised 30 November 2018
Accepted 04 February 2019

Associate editor: Maren Voss