

Changes in the vertical distribution of primary production in response to land-based nitrogen loading

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Abstract

Anthropogenic nitrogen (N)-loading has decreased significantly in the Baltic Sea Transition Zone over the past two decades. We show that the vertical distribution of primary production (PP) changed as a function of land-based N-loading using 1385 water column photosynthesis estimates, in which photosynthetic parameters were determined both in the surface water layer and in the pycnocline-bottom layer (PBL) at six stations near the Danish coast between 1998 and 2012. Total annual PP and surface layer PP (SPP) correlate positively with land-based N-loading from Denmark ($p < 0.003$). The percentage of annual PP occurring in the PBL (denoted as deep primary production, DPP) varied annually between 6% and 30% (mean = 17%). The absolute magnitude of the DPP, as well as its relative proportion of total water column PP, correlates negatively with N-loading ($p < 0.009$ and $p < 0.0003$, respectively). Thus, SPP decreases in response to decreased N-loading, while DPP increases. Land-based N-loadings also correlate positively with the light attenuation coefficient ($R^2 = 0.39$, $p < 0.05$), which may in part explain the response in DPP to changes in N-loading. DPP occurs in active phytoplankton communities acclimated and/or adapted to low light and producing oxygen in the PBL water.

Primary production (PP) is an important factor in structuring marine ecosystems, and changes in PP in response to increased nutrient loading have been identified as being responsible for symptoms of eutrophication in coastal marine systems (Nixon 1995; Cloern 2001; Smith 2003). After recognizing the relationship between anthropogenic nutrient loading and eutrophication, many countries have initiated programs to reduce nutrient enrichment (Boesch 2002) with the expectation that marine PP will respond to a reduction in land-based nutrient loading. In some areas, enough data have now been collected to allow researchers to examine the extent to which this expectation has been realized.

The Baltic Sea Transition Zone (BSTZ), which comprises the Kattegat and the Belt Seas and thus forms the connection between the Baltic Sea and the Skagerrak, is such an area. It is a shallow, stratified, and temperate marine system with dynamic hydrography. Surface salinity varies in the region from 10 to 14 in the southern part of the region and from 20 to 25 in the northern Kattegat (Gustafsson 2000). Bottom water salinity generally varies between 32 and 34, and an essentially permanent pycnocline is present. The area can be characterized as a frontal system in which the low-saline surface water from the Baltic Sea mixes with the more saline waters coming from the Skagerrak. The system demonstrates clear estuarine circulation where water transport is mainly driven by the water level difference between the Arkona Sea and the Northern Kattegat and, ultimately, by the freshwater surplus to the Baltic Sea of $559 \text{ km}^2 \text{ yr}^{-1}$ (Savchuk 2005). Mixing with surface water, especially in the Little and Great Belts (see Fig. 1), ventilates the bottom water of the BSTZ (Bendtsen et al. 2009).

The seasonal distribution of PP here is typical for temperate coastal waters: Elevated production occurs in association with the spring phytoplankton bloom, but peak PP occurs during the summer months (Petersen and Hjorth 2010), as is also seen in other temperate estuarine regions such as the Chesapeake Bay (Kemp and Boynton 1984). Winter PP is low and limited by light availability.

Anoxia and hypoxia events in the BSTZ became more widespread over the 20th century (Conley et al. 2007). As these were believed to be a consequence of increases in anthropogenic nutrient loading, legislation was established in the late 1980s to control and reduce land-based nutrient loading (Conley et al. 2002). Following the establishment of this legislation, Denmark has maintained an extensive marine monitoring program. The data set resulting from this monitoring program provides a unique resource for identifying relationships between PP and changing nutrient loadings, which may be of potential relevance in understanding eutrophication responses in other coastal areas.

The purpose of this study was to examine these monitoring data for evidence of a response in annual PP in this region to changing nitrogen conditions. Because it is well known that the BSTZ, as well as other seasonally or semi-permanently stratified areas (Richardson et al. 2003; Lehrter et al. 2009; Strom et al. 2010), are characterized by a significant amount of annual PP taking place in association with subsurface phytoplankton peaks (Cullen 1982; Richardson and Christoffersen 1991; Karlson et al. 1996), we chose not only to examine total water column PP but also the seasonal and interannual variability in the vertical distribution of PP. We hypothesized that PP occurring in the pycnocline-bottom layer (referred to as deep primary production, or DPP) may respond differently than PP in the surface waters (SPP) to changes in land-based nitrogen (N)-loading.

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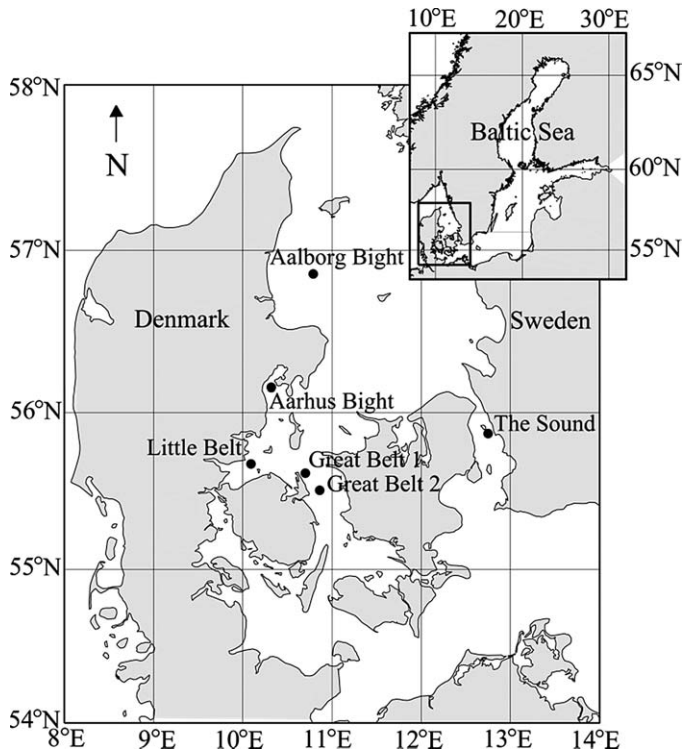


Fig. 1. The Baltic Sea Transition Zone (BSTZ) and location of the six stations.

Methods

Danish National Aquatic Monitoring and Assessment Program—Data from the 1998–2012 time period were obtained from the database of the Danish National Aquatic Monitoring and Assessment Program (MADS; Conley et al. 2002). This database is publicly available (http://www2.dmu.dk/1_viden/2_Miljoe-tilstand/3_vand/4_mads_ny/default_en.asp) and contains physical, chemical, and biological data collected in BSTZ waters since the 1980s. Sampling and measurements were done by different laboratories but followed a common set of technical guidelines and procedures (Kaas and Markager 1998), and inter-comparisons of results were frequently carried out by the former Danish National Environmental Research Institute (NERI), now Aarhus University. For the parameters used in this study, the inter-comparisons were supervised by one of the authors (S.M.).

Six study stations were selected based on (1) a minimum depth of 10 m and (2) the amount of data available. Study station depths vary from 14 m (Aalborg Bight) to 51 m in The Sound (Fig. 1). The four stations of intermediate depths are located in Aarhus Bight, the Little Belt, and the Great Belt (two stations). For each station, the following data were extracted: conductivity, temperature, and depth (CTD) profile data with 0.2 m resolution for the vertical distribution of temperature, salinity, chlorophyll fluorescence, and photosynthetically active radiation (PAR; 4π sensor). In addition, measurements of chlorophyll *a* (Chl *a*) and photosynthetic parameters (*see below*) were extracted

for the discrete depths at which sampling had been conducted.

Data for total nitrogen (TN) loadings from Denmark to the BSTZ were also taken from the MADS database. The values are based on a three-dimensional Mike She (Windolf et al. 2011) groundwater resource model validated on measured TN concentrations in Danish streams. The hydrological area chosen for this study covered the inner Danish waters and ranged from the Kattegat Sea in the north to the Belt Seas in the south.

The daily surface photosynthetic active radiation (SPAR) was determined by averaging measurements made continuously at several different localities in Denmark within approximately 30–150 km of the sample locations. Daily insolation was estimated using average SPAR (between the different locations) calculated for every half hour, and these average values were used to calculate the 24 h depth-integrated PP for all six stations. This data set is not publically available.

Only data from 1998 through 2012 were used. This period was chosen as the protocol for measuring photosynthesis parameters was altered in 1998 so that parameters were measured at two depths rather than one. These two depths were an integrated ‘surface’ sample from 0 to 10 m (unless visual examination of the density profile indicated a major density difference or pycnocline within this depth range) collected using a hose inserted to the desired depth or with Niskin bottles at 1, 5, and 10 m in depth (or to the depth of the pycnocline) and the depth of the deep chlorophyll maximum, defined as the depth with the highest chlorophyll fluorescence and where fluorescence was greater than twice the average for the surface layer (sample collected using Niskin bottle). Chl *a* concentrations were also determined in these two samples. The protocol for the monitoring program prescribed that if no deep chlorophyll maximum was encountered, the second sample was taken at the depth of 2% surface light penetration.

In addition to the two depths described above, Chl *a* was analyzed at standard depths (every 5 m) throughout the water column starting at 1 m below the surface and ending 1 m above the sediment. Chl *a* determinations were made by filtering samples onto Whatman GF/F or GF 75 Advantec filters. Filters were extracted in ethanol (96%) for 6–20 h and samples analyzed spectrophotometrically according to the method described by Strickland and Parsons (1972) and modified by Danish Standards (1986).

Photosynthetic carbon assimilation was estimated based on the carbon-14 method modified (Markager 1998) after Steemann Nielsen (1952). Photosynthesis vs. irradiance curves (P vs. E curves) were calculated from incubations made under artificial light (Osram HQI-T or high-pressure halogen lamps), where the samples were incubated at seven different light intensities for 2 h with metal grids providing approximately 35% light attenuation between each bottle. Thereafter, the samples were filtered (GF/F or GF 75 Advantec filters) and the carbon incorporation stopped with acid (200 μ L 0.1 mol L⁻¹ HCl). The amount of incorporated carbon-14 in the phytoplankton was determined by liquid scintillation counting resulting in disintegrations per

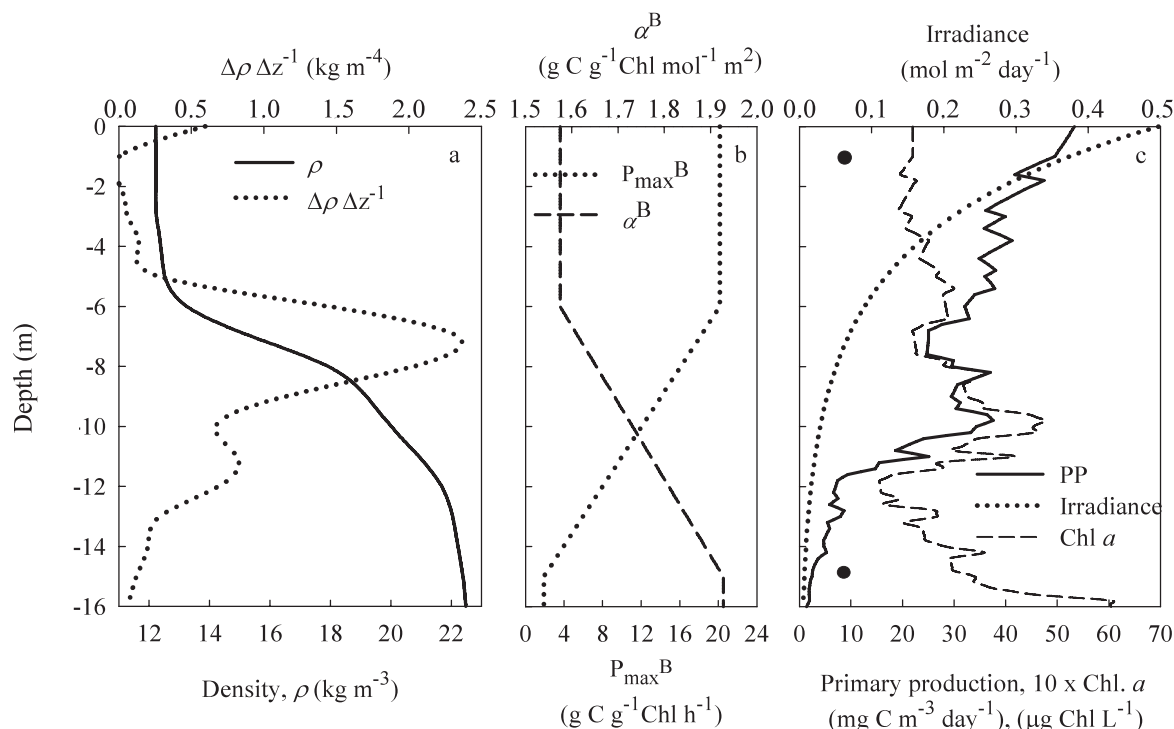


Fig. 2. Example of how parameters were derived for the primary production estimates: Profiles from 19 July 2010 in Aarhus Bight of (a) density (ρ) and density difference m^{-1} ($\Delta\rho/\Delta z$, kg m^{-4}). The starting depth of the pycnocline-bottom layer (PBL) was defined as the first depth at which $\Delta\rho/\Delta z > 1 \text{ kg m}^{-4}$ and, in the example here, was found at 6 m. (b) Interpolation of the chlorophyll-specific photosynthetic parameters, α^B and P_{\max}^B . Actual determinations of these parameters at 1 and 15 m are indicated by black dots. (c) Primary production, irradiance, and chlorophyll *a*. Irradiance is the sum over 24 h in 20 cm depth intervals.

minute (DPM). The equation used to calculate the carbon assimilation in each sample was as follows:

$$P = \frac{\text{Tot CO}_2 \times (\text{DPM}_{\text{tot}} - \text{DPM}_{\text{dark}}) \times 1.05 \times 60}{(\text{DPM}_{\text{start}} \times \text{Light minutes})} \quad (1)$$

where P is the measured production ($\text{mg C m}^{-3} \text{t}^{-1}$), Tot CO_2 is the total dissolved inorganic carbon concentration determined by Gran-titration, and Light minutes is the time (in minutes) the phytoplankton have been exposed to light. The ^{14}C isotope discrimination factor is 1.05, and the value 60 is used to calculate from minutes to hours. Light attenuation at each station was determined by estimating the diffuse light attenuation coefficient (K_d) from the CTD profile using a deck sensor as a reference.

Data analyses—The division of the water column into two layers: Density (ρ) was calculated from salinity and temperature profiles (Fofonoff 1985). These density profiles were used to calculate the depth separating the surface from the layers below (i.e., the pycnocline-bottom layer [PBL]). This was defined as being at the first vertical density gradient of $> 1 \text{ kg m}^{-4}$ (see Fig. 2a for an example). For each day with a distinct pycnocline (during which this density criteria was met), every depth in the water column was assigned as being in one of two layers (i.e., in the surface layer or in the PBL). This gave the possibility of considering the PP occurring in the surface layer (surface primary production, SPP) and below the surface (i.e., in the PBL [deep primary production, DPP]),

respectively. When the density criteria of $\Delta\rho/\Delta z > 1 \text{ kg m}^{-4}$ was not met in the water column, the total water column PP was considered as being SPP.

Two hundred fifty-six visual inspections of density profiles were made to confirm that the density criterion was effective at determining the starting depth of the PBL. The depths found by visual inspection matched the depths found by the criterion in 90% of the cases. The density criterion chosen (i.e., 1 kg m^{-4}) overestimated the starting depth of the PBL (i.e., identified a deeper [1–2 m] depth than identified visually) in the remaining 10%. Thus, our estimate of the fraction of DPP as a percentage of total water column PP (see below) is conservative.

Estimating Chl *a* from fluorescence—Fluorescence per unit of Chl *a* changed systematically with depth in the data set. Therefore, a fluorescence factor ($F_{\text{Chl}} = F/[\text{Chl}]$) was calculated whereby the chlorophyll concentration recorded in the discrete sample (Chl) and the fluorometer measurement from the corresponding depth (F) were related. Values for F_{Chl} between sampling depths were assigned by linear interpolation. This was done with 0.2 m resolution and the resulting profile of $F_{\text{Chl}}(z)$ used to estimate a continuous Chl *a* profile ($\text{Chl}(z) = F(z)/F_{\text{Chl}}(z)$) (see Fig. 2c for an example of an estimated Chl *a* profile).

Estimating PP through the water column—To estimate water column PP, chlorophyll-specific photosynthesis rates were calculated from the P vs. E curves and Chl *a*

Table 1. Number of depth-integrated primary production measurements per year at the six sampling locations. In all, there were 1385 primary production measurements from year 1998 to 2012.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Aalborg Bight						19	15	17	18	20	24		16	18	18
Aarhus Bight		26	25	25	25	28		25	29	27	25		20	19	20
Little Belt			22		21		24	24	22						
Great Belt 1					20		23	23							
Great Belt 2	49	49	48	48	39	48	49	45	42	23	19		19	19	17
The Sound	12	18	21	17	22	23	22	22	19			18		20	19

concentrations for the two samples, according to the method of Markager et al. (1999). A light matrix representing the light intensity at 0.2 m intervals throughout the water column and hourly intervals over the entire day was constructed using the attenuation coefficient and the surface light. This light matrix and the photosynthetic characteristics derived from the P vs. E curves (i.e., α , P_{\max} , and offset (c) for the two sampling depths normalized to Chl *a* concentrations [α^B , P_{\max}^B , and c^B]) were combined to estimate daily PP at 0.2 m intervals throughout the water column for all stations except the Great Belt 1 Sta., at which only P vs. E curve parameters from the surface layer were available. The P vs. E parameters were obtained by a non-linear fitting procedure (Statistical Analysis System, SAS, version 9.4) on the carbon uptake from Eq. 2 divided by the chlorophyll concentration. The P vs. E model was taken from Webb et al. (1974), modified by the inclusion of an offset (c), thus:

$$P^B = P_{\max}^B \left(1 - \exp \left(- \frac{\alpha^B E}{P_{\max}^B} \right) \right) + c \quad (2)$$

where α^B is the slope for the light-limited part of the P vs. E curve ($\text{g C g}^{-1} \text{ Chl } a \text{ m}^2 \text{ mol}^{-1}$), and P_{\max}^B the light-saturated photosynthetic rate ($\text{g C g}^{-1} \text{ Chl } a \text{ h}^{-1}$). The offset (c) was included in order to avoid a bias in α^B as a result of the fact that ^{14}C -uptake rates are always positive. See also Markager et al. (1999) for details about the curve fitting procedure.

It was assumed that the P vs. E parameters from the surface sample represented an average value for the entire surface layer, and these values were therefore extracted from the surface to the starting depth of the PBL (see Fig. 2 for an example). The reason for using the same P vs. E parameter values throughout the surface layer was that turbulence within this layer was assumed to be too high to allow further depth-specific photo adaptation (Lewis et al. 1984). Turbulence in the PBL was assumed to be lower, thus allowing more variation in the P vs. E parameter values with depth. Therefore, these were interpolated from the starting depth of the PBL (= surface values) to the second sampling depth. From the second sampling depth and downward photosynthetic parameters were assumed to be constant (Fig. 2b). The volumetric PP at each 0.2 m interval, P_{vol} , ($\text{mg C m}^{-3} \text{ d}^{-1}$) was calculated from Eq. 3 with the following parameters: $P_{\max}^{\text{vol}} = P_{\max}^B [\text{Chl}]$, $\alpha^{\text{vol}} = \alpha^B [\text{Chl}]$, and $c^{\text{vol}} = c^B [\text{Chl}]$.

$$P_{\text{vol}} = P_{\max}^{\text{vol}} \left(1 - \exp \left(- \alpha^{\text{vol}} \frac{\text{PAR}}{P_{\max}^{\text{vol}}} \right) \right) + c^{\text{vol}} \quad (3)$$

where PAR is the product of surface irradiance and the PAR fraction left at a given depth [PAR fraction = $(1 - \text{reflection}) \exp(-K_d (\text{depth} - 0.05))$]. The surface reflection was assumed to be 6%.

In this manner, estimates of total water column PP as well as the contribution made by DPP to the total were made for the 1385 d of sampling. Data availability varied between stations and, for each of the six stations, ranged from 3 to 14 yr, resulting in a final time series of 15 yr (Table 1). The sampling frequency at each station ranged from 12 to 49 measurements per year.

The average annual values of PP were calculated for all six stations. Long-term average monthly values were calculated for each station (including all available years), and these were used to fill months during which there were no measurements. If data for entire years were missing, these years were excluded from the analysis. This procedure allowed us to examine the temporal development in PP without the influence of missing values in the data set. We used the same approach with respect to missing values for the P vs. E parameters and the light attenuation coefficient (K_d) when analyzing their interannual and seasonal variations.

Statistical analyses—Multiple linear regressions were constructed to examine the degree to which the estimated PP could be explained by irradiance and N-loading. Annual PP, annual DPP ($\text{g C m}^{-2} \text{ yr}^{-1}$), DPP in percentage of total, annual land-based N-loading to the BSTZ from Denmark ($10^3 \text{ kg of nitrogen}$), irradiance, and the light attenuation coefficient (K_d) were normalized to their mean values over the study period. In this way the unit of the coefficients for the regressions performed becomes the percentage change in dependent variable (PP, SPP, DPP, DPP in %, and K_d) per percentage change in forcing variables (N-loading and surface irradiance).

The annual land-based N-loading was calculated on a monthly basis and summed over a period of consecutive months. When relating PP parameters to N-loading, we first regressed the relationships between the parameters for a given calendar year against total N-loading in the same calendar year. In recognition of the fact that loading might affect PP differently over the season and that there likely will be a lag between loading and PP response, we then tested different periods for loading against the annual PP parameters. A systematic approach was used in choosing the periods to be tested. We started with 24 months covering the whole calendar year before and the year of the given annual PP. We then excluded and included months so

Table 2. Statistics for multiple linear regressions between primary production and the diffuse light attenuation coefficient (K_d) vs. land-based N-loading from Denmark and surface irradiance. The time period extends from 1998 to 2012. In addition, the temporal development in K_d is analyzed from 1990 to 2012. Both dependent and independent variables are scaled to the mean value $\times 100$; thus, the unit of the coefficients becomes % change in dependent variable per % change in the independent variable (i.e., % change in PP/% change in N-loading). Coefficients and intercepts are given \pm standard errors, and values in brackets are p -values for significance. Finally, each dependent parameter is tested against time (annual change). Significant changes ($p < 0.05$) are indicated in bold type.

Dependent variable	Period for sum of N-loading	Coefficient (C_1) for N-loading from Denmark	Period for sum of surface irradiance	Coefficient (C_2) for surface irradiance	R^2	Intercept	Annual change, %
Surface primary production	Feb-Sep	0.61 \pm 0.12(0.0002)	Apr-Dec	0.99 \pm 0.64(0.15)	0.69	-60 \pm 70	-2.0(0.09)
Deep primary production	Mar*-Feb	-0.66 \pm 0.21(0.0090)	Apr-Dec	4.4 \pm 1.96(0.0006)	0.72	-276 \pm 98	3.6(0.08)
Total water column production	Feb-Sep	0.43 \pm 0.11(0.0025)	Apr-Dec	1.48 \pm 0.61(0.035)	0.56	-92 \pm 67	-1.0(0.33)
Deep primary production in % of total	Mar*-Feb	-0.73 \pm 0.14(0.0003)	Apr-Dec	3.2 \pm 0.66(0.0004)	0.80	-144 \pm 68	3.6(0.026)
Diffuse attenuation coefficient (K_d ; 1998–2012)	Nov*-Mar	0.10 \pm 0.05(0.0495)	—	Not significant	0.27	89 \pm 5	-0.2(0.66)
Diffuse attenuation coefficient (K_d ; 1990–2012)	Nov*-Mar	0.09 \pm 0.03(0.0121)	—	Not significant	0.39	90 \pm 4	-0.9(0.017)

* Indicates months in the year before the calendar year for which primary production is calculated.

that all possible periods having a minimum length of 2 months were tested. In all, 276 different periods of N-loading were tested against annual PP, SPP, and DPP. The periods giving the highest explanatory power (R^2) are shown in Table 2.

The irradiance values used in the statistical analyses represent the actual SPAR at the surface from April to December as in all cases this period was that with highest predictive value. A similar procedure to the one described above was applied when the N-loading was considered in relation to K_d .

An analysis of the temporal development in the depth separating the surface and PBL was carried out on normalized depths (i.e., to the average depth of the PBL for the individual station). When the normalized starting depth of PBL was calculated for each station, a linear regression was used on the normalized values as a function of years per station and then for an average of the six stations.

Results

Total and vertical distribution of PP—The mean annual PP from 1998 to 2012 for the six stations was 189, with an interannual variation in the annual mean of ± 17.2 g C m⁻² yr⁻¹. The lowest production estimates were found in Aalborg Bight, Aarhus Bight, and The Sound (155–175 g C m⁻² yr⁻¹), while the production in Great and Little Belts was higher (209–226 g C m⁻² yr⁻¹; Fig. 3). The higher values at these stations likely reflect nutrient inputs from pycnocline-bottom to surface waters through more intense mixing in the narrow straits (Lund-Hansen et al. 2008). The highest water column PP was found during summer, and the seasonal pattern in production largely followed irradiance and temperature (data not shown).

When all stations in the entire study period are considered, the DPP, on average, contributed 17% to the annual PP. The contribution of the DPP to the total water column production varied from 6% in the Little Belt to 30% in Aalborg Bight (Fig. 3). The rest of the stations exhibited contributions of DPP to annual production of between 13% and 23% (Fig. 3). The highest average monthly contribution from DPP to total was observed in Aalborg Bight in May, where the DPP contributed 48% to total water column production (Fig. 3c). DPP was low or, in some cases, nonexistent from October to February, when the phytoplankton community was well mixed throughout the water column and surface irradiance was too low to support production in the PBL (Fig. 3). Overall, the importance of DPP was slightly higher in summer from April to August (8–40% of summer production) than for the year as a whole.

Photosynthetic parameters—The seasonal pattern of the light intensity at which photosynthesis initially is saturated, I_k , (P_{max}/α , $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) is shown in Fig. 4a as the average for all six stations. Samples from the surface layer show increasing I_k values from January (68 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) to a peak in August (128 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), followed by a decline to 78 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

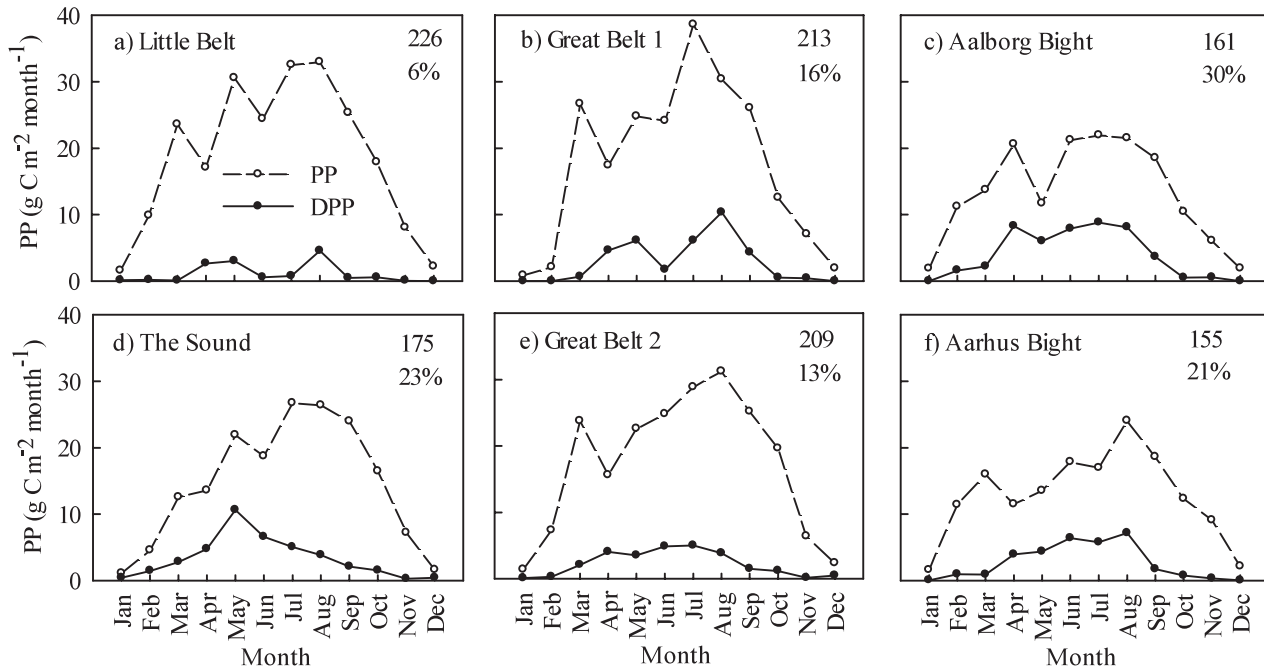


Fig. 3. Monthly average values for total water column primary production (PP) and deep PP (DPP) for six different stations in the BSTZ (1998–2012). The annual PP and the contribution (in %) from DPP to PP are given in the upper right corner of each graph.

in December. For the deep samples, the seasonal variation was smaller. I_k increased from $62 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in May to $89 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in November and then dropped to $55 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in January. A *t*-test showed significantly higher average monthly I_k values in the surface layer than in the PBL (*t*-test, $t = 3.56$, degrees of freedom [df] = 22, $p < 0.01$, $n = 24$). In addition to the characteristic seasonal pattern seen for the I_k values from the surface layer, a significant and positive relationship was found between I_k values from the surface layer and the daily surface irradiance ($r = 0.28$, $p < 0.0001$, $n = 1080$). Thus, the I_k data indicate that the phytoplankton communities in and below the surface layer are acclimated to the prevailing light levels they encounter.

Not surprisingly, this conclusion is also supported by the general patterns observed for maximum chlorophyll-specific photosynthesis rate ($P_{\text{max}}^{\text{B}}$), as this parameter controls a large part of the variation in I_k . Average monthly values (including data from 15 yr) for $P_{\text{max}}^{\text{B}}$ showed significantly higher values for phytoplankton populations found in the surface layer ($P_{\text{max}}^{\text{B}} = 3.6 \pm 1.4 \text{ g C g}^{-1} \text{ Chl}^{-1} \text{ h}^{-1}$) than in the PBL ($P_{\text{max}}^{\text{B}} = 2.3 \pm 0.4 \text{ g C g}^{-1} \text{ Chl}^{-1} \text{ h}^{-1}$; *t*-test, $t = 3.06$, $df = 22$, $p < 0.01$, $n = 24$). An exception to this general pattern was observed for the Sound, where $P_{\text{max}}^{\text{B}}$ values from the two depths were similar.

Monthly averages of $P_{\text{max}}^{\text{B}}$ values in the surface layer for all six stations for the 1998–2012 period exhibited a seasonal pattern in which $P_{\text{max}}^{\text{B}}$ increased from $2.4 \text{ g C g}^{-1} \text{ Chl h}^{-1}$ in March to $6.4 \text{ g C g}^{-1} \text{ Chl h}^{-1}$ in August and then decreased to $2.2 \text{ g C g}^{-1} \text{ Chl h}^{-1}$ in December (Fig. 4). $P_{\text{max}}^{\text{B}}$ values from surface and PBL waters were not significantly different (*t*-test, $t = 1.83$, $df = 734$, $p = 0.07$, $n = 736$) between the months of December and March. This can also be seen from the average monthly

values (Fig. 4). During this period, the water column was often well mixed and the solar irradiance so low that photosynthesis was likely light limited most of the time.

The general pattern in the chlorophyll-specific initial slope (α^{B}) of the P vs. E curves exhibited no significant difference for phytoplankton populations found in the surface layer and those found in the PBL (Fig. 4c). In addition, the variability, expressed as the coefficient of variance (CV), of α^{B} was lower than that of $P_{\text{max}}^{\text{B}}$ (CV $\alpha^{\text{B}} = 0.16$ compared to CV for $P_{\text{max}}^{\text{B}} = 0.42$). This is to be expected, as α is equal to absorption times quantum yield (Markager and Vincent 2001), and light absorption is tightly coupled to the chlorophyll content in the cells (Markager 1993). A weak seasonal pattern in α^{B} values was detected that resembled that found for I_k^{B} and $P_{\text{max}}^{\text{B}}$. The α^{B} values increased from $10.2 \text{ g C g}^{-1} \text{ Chl mol photons}^{-1} \text{ m}^2$ in March to the peak value in August ($14.2 \text{ g C g}^{-1} \text{ Chl mol}^{-1} \text{ m}^2$), and decreased thereafter to $8.2 \text{ g C g}^{-1} \text{ Chl mol}^{-1} \text{ m}^2$ in December. Higher values were found in January and February than in March and April.

PP and land-based N-loading—The concentration of nitrogen in land runoff from Denmark (mg N L^{-1}) decreased significantly between 1998 and 2012 (linear regression: $p < 0.0001$, $R^2 = 0.84$, $n = 15$; Fig. 5), primarily as a result of the establishment of water quality regulation (Windolf et al. 2012). Although there were large fluctuations in the absolute loading due to interannual variation in runoff, the absolute delivery of nitrogen from Denmark (10^3 kg N) also showed a significant decrease over the study period (linear regression: $p < 0.016$, $R^2 = 0.37$, $n = 15$; see Fig. 5).

Total PP, SPP, and DPP, as well as the ratio of SPP and DPP to total PP, were all significantly correlated to

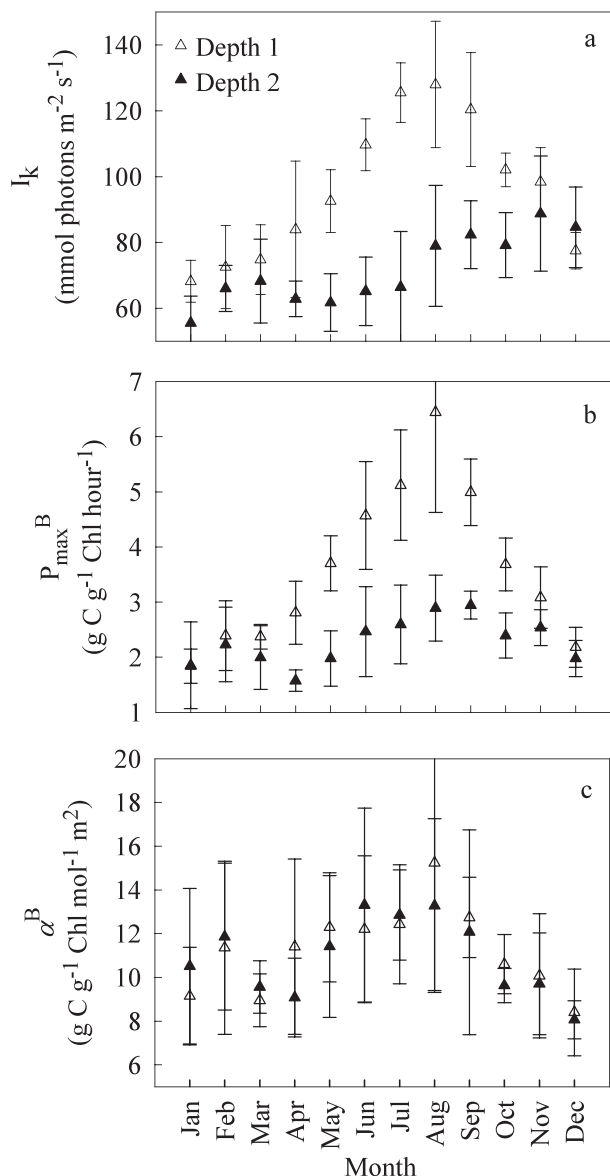


Fig. 4. Seasonal variation of chlorophyll *a*-specific P vs. E parameters: (a) the light intensity at which photosynthesis is initially saturated, I_k ; (b) maximum chlorophyll-specific photosynthesis rate, P_{max}^B ; and (c) the initial slope of the P vs. E curve, α^B . Parameters are shown for 1–10 m (depth 1) and for depths in the pycnocline-bottom layer (depth 2). The parameters are average values of six stations in the Baltic Sea Transition Zone (1998–2012), and the error bars indicate the variation in average monthly values among stations.

land-based N-loading (Table 2). However, while SPP was positively correlated to N-loading (see Fig. 6), the opposite was true for DPP.

The relationship between SPP and N-loading can be seen in Fig. 6, where both SPP and N-loading are calculated for the calendar year. The relationship is significant, with a p -value < 0.01 and a coefficient of 0.47% per % change in N-loading. Thus, when N-loading changed by 1%, the SPP changed by 0.47% in the same direction. It is, however, doubtful that N-loading calculated over the calendar year is

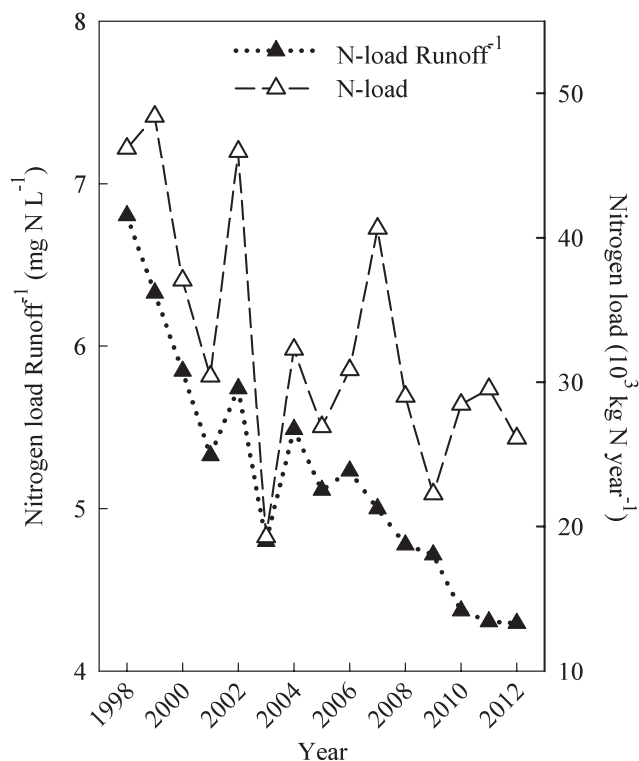


Fig. 5. Land-based annual N-loading (right y-axis) and land-based annual N-loading normalized to runoff (left y-axis) from 1998 to 2012. The numbers are based on average values of Danish N-loading to the study area.

the best descriptor in terms of relating N-loading to PP, as N-loading late in the calendar year obviously cannot affect the period of peak production during the same year. Instead, it can be argued that nitrogen has a residence time in the system. This would argue for including N-loading from the previous year when considering PP in a given year, especially given the fact that phytoplankton growth is only believed to be N-limited during the summer period in the region (Conley 1999). Following this line of reasoning, the N-loading during the summer might be most important in controlling PP. We therefore tested a number of different loading periods extending back to January of the year prior to the test year in relation to the PP variables.

A change in N-loading was the best predictor of changes in SPP when the 8 months from February to September of the study year were included (coefficient of 0.61; $p < 0.001$; Table 2). Surface irradiance was also positively related to SPP, but the coefficient was not significant. Figure 7 shows the effect on the R^2 value (explained variance) by varying the timing of an 8 month loading period. All 8 month periods with a midpoint from 01 November in the year prior to the study year to 01 July of the study year yielded significant ($p < 0.05$) coefficients for the correlation between SPP and N-loading. The value of the coefficient varied from 0.42 to 0.61. This suggests that the N-loading from the previous winter and during the period in which phytoplankton activity is greatest are the most important drivers of SPP. Calculating the N-loading over periods with different lengths gave similar results.

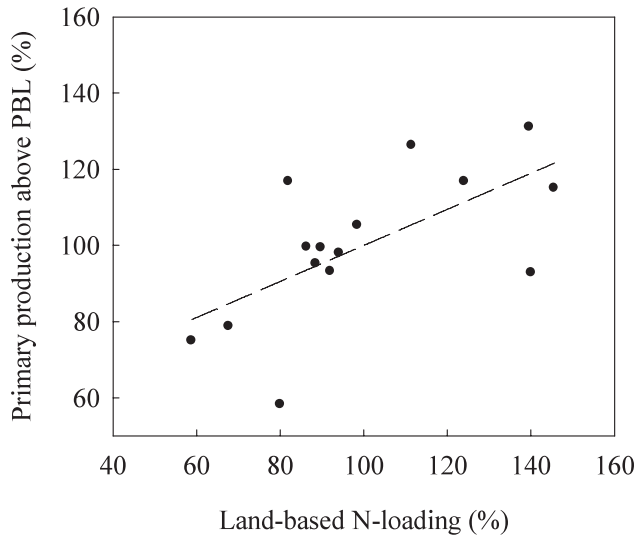


Fig. 6. Changes in annual primary production in the surface layer as a function of changes in N-loading. Change in each parameter is expressed as a percentage of the average value for the study period (1998–2012) as a whole. A significant linear relationship between the two parameters ($p < 0.01$ and $R^2 = 0.41$) is noted on the graph with a dashed line.

The DPP was strongly and positively correlated with surface irradiance (coefficient = 4.4%/%, $p < 0.001$) but negatively related to the N-loading (Table 2). In this case, the most significant relationship was found when N-loading was calculated over a 12 month period starting in March of the year prior to the study year to February of the study year (midpoint between August and September of the year prior to the study year). The coefficient to N-loading was negative for all periods but was only significant for 12 month periods with midpoints between August and October of the year prior to the study year (Table 2), which means that changes in DPP were best predicted by a period of N-loadings occurring prior to the actual DPP.

Total PP was also significantly and positively related to N-loading (summed from February to September of the study year; i.e., similar to the case for SPP). However, the relationship was weaker than the relationship between loading and SPP. This can easily be explained as the total water column PP is the sum of SPP and DPP and these two layers exhibited opposite responses to N-loading.

The most significant model relating PP parameters to N-loading was that describing the fraction of DPP (as a percentage of total water column production) as a function of N-loading. This indicates that N-loading potentially redistributes PP in the water column so it shifts from the surface layer to the PBL as loadings are reduced. This result cannot be attributed to changes in the depth at which the surface and PBL diverge as there was no significant temporal development in the starting depth of the PBL when the stations were analyzed by linear regression separately ($p > 0.05$) as well as when they were averaged into one value (normalized to the average depth for the study period as a whole) per year ($p > 0.05$). A possible explanation for the identified negative relationship between DPP and N-loading is the positive relationship found between N-loading and

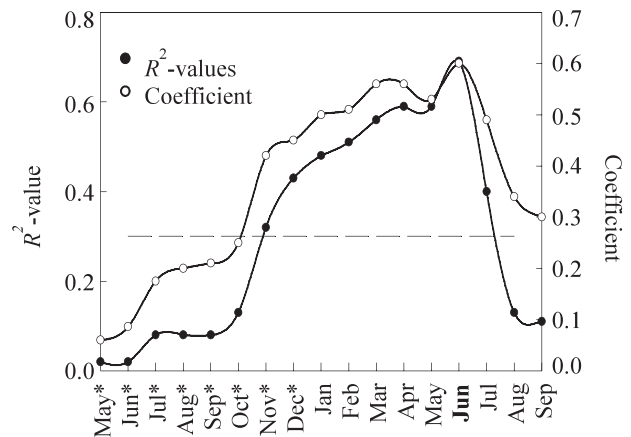


Fig. 7. An example of the relationships between periods over which the N-loading is calculated as well as the resulting R^2 values and coefficients. The month is the midpoint of the period (an asterisk indicates the year before the calendar year during which primary production is calculated), and the period from Table 2 is indicated in bold. The horizontal dashed line indicates when the p -value is below 0.05. The example shown here is for surface primary production vs. N-loading calculated over an 8 month period.

light attenuation in the water column. We therefore also tested the relationship between N-loading and the diffuse attenuation coefficient (K_d). A significant positive relationship was found with a coefficient of 0.10% in K_d per percent change in N-loading (Table 2). K_d data were available from 1990. Using this longer time series gave the same coefficient but with a lower p -value (Table 2). There was a positive relationship between the interannual variation (relative to the long-term average) in K_d and surface layer chlorophyll ($R^2 = 0.30$, $p < 0.05$, $n = 15$). Thus, the 'extra' PP stimulated by a higher N-loading increases the chlorophyll concentration in the surface layer, contributing to the drop in water transparency.

Temporal development in biological and physical parameters—The temporal trends of the studied parameters in response to changes in land-based N-loading are masked by the large interannual variability in runoff. Nevertheless, we also regressed all of the parameters studied against year (Table 2, last column). While the trends expected in light of the overall reduction in N-loadings were found, two more parameters showed a significant temporal development. The K_d decreased over time (for the time period from 1990 to 2012) and the fraction of DPP to total PP showed an increase from 1998 to 2012 (see Table 2). No temporal trends were found for PP, SPP, or DPP (as absolute carbon values). Thus, over the 1998–2012 period, there has been a significant vertical redistribution of PP in the BSTZ, whereby PP has moved from the surface waters to the PBL and water clarity has increased.

Discussion

PP in relation to land-based N-loading—This study demonstrates a redistribution of PP in the water column

with decreasing land-based N-loading from Denmark, whereby the proportion of PP taking place beneath the surface layer increases with decreasing loading. This finding is potentially important for the management of eutrophication induced by human activities. From our general understanding of eutrophication (Cloern 2001), such a redistribution of PP in response to anthropogenic eutrophication might have been predicted. However, to our knowledge, this is the first time it has been demonstrated for a coastal ecosystem on a decadal timescale. That it has not been documented earlier with field observations is likely due to the fact that there are few locations with substantial time series of data describing phytoplankton photosynthetic activity over a period with concurrent reduction in nutrient loading.

The coefficient of the regression relating SPP and N-loading (Table 2) indicates that SPP changes 0.61% per percent change in N-loading (using load period from February to September of the study year). The intercept for this regression (intercept = 60%) indicates that more than half of the production is based on regenerated N or N from other sources rather than land-based loading (*see below*). In the case of the DPP, a 1% increase in land-based N-loading leads to a 0.66% decrease in DPP. An interesting consequence of the fact that opposite responses to the reduction of N-loading are seen in the two depth layers is that the total water column production is not as sensitive to changes in N-loading as are the two individual layers when examined independently. Thus, total water column PP may not be the ideal parameter to use in assessing system responses to changes in nutrient loading.

That we see a greater shift in the distribution of PP in the water column than in total PP is reminiscent of the pattern observed for more shallow aquatic systems, in which an increase in N-loading leads to a redistribution of PP from benthic macrophytes to phytoplankton (*i.e.*, higher loading results in the production occurring closer to the surface; Borum and Sand-Jensen 1996; Krause-Jensen *et al.* 2012).

As no temporal development in the starting depth of the PBL was recorded, the most likely explanation for the patterns observed in both cases must be that higher loading leads to a reduction in water clarity. This is supported by the relationship found in this study between the N-loading and K_d . That lower R^2 values are found for K_d (0.27–0.39) compared to PP (0.56–0.80) in relation to nutrient loading agrees well with earlier studies, in which it has been demonstrated that the relationship between N-loading and ecosystem effects becomes weaker when the response of the parameter is not directly coupled to the loading being examined (Timmermann *et al.* 2010; Carstensen *et al.* 2011).

The best fits between land-based N-loading and PP were found when loading was summed over periods other than the calendar year for which the PP was calculated. For SPP and PP, the period best describing the relationship to N-loading (judged on the basis of the value of R^2) was February to September of the study year, but the winter months prior to this period were also shown to be important (Fig. 7). Thus, loading during the period when PP is high has a large effect on the PP occurring. In

contrast, both the DPP and K_d values were better described by N-loading occurring farther back in time (Table 2). Assuming that the negative coefficient for N-loading vs. DPP derives, ultimately, from the positive effect on light attenuation, both observations would suggest the occurrence of a lag period from when the loadings reach the marine system until the effects are observed.

Light attenuation is governed by several processes and components in the water column (*i.e.*, chlorophyll and accumulated organic matter [CDOM or detritus]; Siegel and Michaels 1996). CDOM is, in turn, derived partly from the PP that has occurred earlier in the system. The positive correlation found between chlorophyll in the surface layer and K_d indicates that light attenuation also is directly affected by the amount of phytoplankton present in the surface layer. However, as only 30% of the interannual variation in K_d in this study can be explained by variation in surface layer chlorophyll, we attribute a major part of the variation in K_d occurring here to be related to the amount of dissolved organic and particulate matter and the light attenuation caused by water itself. It has earlier been shown for this region that less than 30% (and often much less) of the light attenuation is caused directly by chlorophyll (Markager *et al.* 2010; Timmermann *et al.* 2010; Krause-Jensen *et al.* 2012). Thus, the interannual relationship between N-loading and K_d is directly affected by the amount of chlorophyll in the surface layer and further strengthened by the dissolved organic and particulate matter that comes with runoff water from land.

PP in the PBL—The estimates of PP made with the assumptions used here suggest that a significant proportion of the PP in the study area occurs in the PBL. The average contribution of this DPP to the annual PP for the six stations ranged from 6% to 30%. This estimate agrees well with previous findings in the region (Richardson and Christoffersen 1991; Richardson *et al.* 2000, 2003). Furthermore, the water column PP estimates indicate that photosynthesis in the PBL is a consistent feature during the period extending from April to October.

The chlorophyll-specific P vs. E parameters give support to the conclusion derived from the PP estimates that a photosynthetically active phytoplankton population is found in the PBL. These parameters also suggest that the phytoplankton population in the PBL is physiologically distinct from populations in the surface layer. Both I_k and P_{max}^B are lower in samples taken in the PBL than for samples in the surface layer. This suggests that light is a limiting factor in the PBL. The obvious adaptation and acclimatization to low light would be higher α values. However, chlorophyll-specific α values cannot be used to detect this as α and chlorophyll content per cell will covary. Chl *a* concentration within a single phytoplankton cell increases during the process of acclimatization to lower light intensities (Cullen 1982; Richardson *et al.* 1983). Therefore, the lack of difference in α^B between depths may be due to higher chlorophyll contents per cell for phytoplankton from depth 2. Unfortunately, cell counts were not made for the deep samples so we could not calculate α per cell.

The data did not allow us to differentiate between adaptation occurring at the population level (i.e., through changing species composition) and that occurring as acclimatization at the cellular level. Given, however, the very different light and nutrient conditions found in the surface layer and PBL, respectively, it seems likely that different species may have populated the two environments. Earlier studies have documented significant vertical heterogeneity in the distribution of phytoplankton species in the southern Kattegat (Mouritsen and Richardson 2003). Further evidence that the populations found in the PBL are photosynthetically active is found in the seasonal changes (highest values being found during summer months, when light intensities are greatest) noted for the P vs. E parameters derived from populations from the PBL.

Other nitrogen sources—The fact that we identify such a clear relationship between the land-based N-loadings originating from Denmark and the vertical distribution of PP occurring at six stations in the BSTZ may, initially, seem surprising, as the BSTZ receives nutrients from several sources (e.g., advection from surrounding seas, atmospheric deposition, and land-based runoff from several countries). Nitrogen budgets for the area (Jørgensen et al. 2013) show that land-based N-loading only constitute 14% of the total nitrogen input to the region and 21% of the bioavailable N input. In addition, only about half of the land-based loadings come from Denmark. The explanation for the relationship recorded may possibly be found in the fact that all of the stations in this study are located close to the Danish coast. As a result, land-based loading from Germany and Sweden is largely processed prior to its reaching the sample locations. We attempted to include the German and Swedish loadings in the regression analyses (data not shown) but this gave poorer fits than when we only included the Danish land-based loadings.

Nitrogen entering the BSTZ from adjacent seas constitutes 58% of the bioavailable N (Jørgensen et al. 2013) introduced to the region. However, these inputs probably constitute a rather-constant background that leaves the changes in land-based loading as a likely potential candidate in generating interannual variability in PP. Furthermore, some of those inputs may not be reaching the surface layer during the productive season (*see* arguments in Jørgensen et al. 2013).

The strong coupling between local loading and PP means that local load reductions also can be effective in improving the marine environment in the area. Previous studies (as discussed in Duarte 2009) suggest that a return to the previous condition after load reductions is not to be expected based on data for phytoplankton biomass. However, the results in Table 2 and Fig. 6 indicate a linear response between PP and N-loading in the BSTZ. The relationship found in this study between PP and N-loading suggests that eutrophication may, indeed, be reversible and that it can be abated by reduction in local N-loading, as phytoplankton PP is the key process starting the cascade of effects in marine nutrient eutrophication.

Oxygen production in the PBL—The vertical redistribution of PP in relation to land-based N-loading demonstrated

here for the BSTZ has potentially important implications for the production of oxygen in the PBL. Converting the average DPP from this study (17% of annual production) to oxygen equivalents ($O_2: CO_2 = 1$) yields an oxygen production in the PBL of about $89 \text{ g } O_2 \text{ m}^{-2} \text{ yr}^{-1}$. Jørgensen and Richardson (1996) examined data from the Southern Kattegat and estimated the oxygen demand in the bottom layer to be $202 \text{ g } O_2 \text{ m}^{-2} \text{ yr}^{-1}$. Hansen and Bendtsen (2013) argue that 28% of PP ($\sim 54 \text{ g } C \text{ m}^{-2} \text{ yr}^{-1}$ based on data from this study) is remineralized in the bottom waters. This would be equivalent to an approximate oxygen demand of $144 \text{ g } O_2 \text{ m}^{-2} \text{ yr}^{-1}$. Thus, the present study suggests that oxygen produced by DPP in the BSTZ may compensate for a considerable fraction of the oxygen demand in this layer.

Subsurface PP has also been shown to counteract, although not eliminate, hypoxia in other areas where light penetrates into the PBL (Lehrter et al. 2009; Murrell et al. 2009; Strom et al. 2010). Studies on the importance of oxygen production below the surface layer for the Louisiana continental shelf showed that up to 41% of the bottom water oxygen demand may be resupplied by deep PP, and when benthic production is included, this oxygen contribution may be even higher (Dortch et al. 1994; Lehrter et al. 2009).

Implications for understanding ecosystem effects of anthropogenic nutrient loading—Nutrient enrichment is well known to influence both the magnitude of the production of organic material in a system (Nixon 1995) and light attenuation in the water column (Nielsen et al. 2002). Until now, most studies of eutrophication in marine systems have, therefore, focused on the influence of nutrient enrichment on the total water column PP. The current study suggests, however, that for the BSTZ, changes in land-based N-loading influence the vertical distribution of PP more than the total magnitude of PP. We see no reason to believe that this should not be the case for other stratified coastal systems as well.

This vertical redistribution of PP is potentially important for the function of the system as a whole, as photosynthesis occurring in the PBL introduces oxygen into the deeper regions of the water column, regions that are not directly able to exchange oxygen with the atmosphere and, thus, ameliorate hypoxia. In addition, a change in the vertical distribution of PP, as reported here, will likely have consequences for the relative occurrence of different phytoplankton species. This can potentially have consequences both for food webs and for the amount of organic material reaching bottom waters. How this vertical redistribution of PP may affect ecosystem function remains, however, to be quantified.

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