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Phytoplankton in NEA 3/4 coastal waters

WFD Class boundary values for chlorophyll-a

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Title
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Summary
This document gives an overview of the coastal water bodies in the Netherlands defined under the Water Framework Directive (WFD) and the monitoring of chlorophyll in those water bodies. The Biological Quality Element phytoplankton and the derivation of reference conditions is described, and the phytoplankton growth conditions in the water bodies are characterised. The Dutch assessment method of phytoplankton in coastal waters is reviewed against WFD requirements.
This document provides background information relevant for the review of the intercalibration of chlorophyll-a, carried out recently by JPI Oceans.

References
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	March 2017	dr. T.C. Prins		dr. A.N. Blauw		F.M.J. Hoozemans MSc	
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Appendices

1 Introduction

1.1 Background

The main objective of the Water Framework Directive (WFD) intercalibration procedure is to set harmonised ecological quality criteria to meet the protection and restoration targets for all surface waters throughout the European Union. Therefore one aim is to get comparable assessment results of different Member States and a harmonized classification based on Ecological Quality Ratios (EQR).
The NEA GIG type NEA 3/4 consists of exposed polyhaline waters in the North Sea and Wadden Sea type polyhaline waters. This NEA GIG type is shared by the Netherlands and Germany. For the Dutch and German coastal waters of NEA GIG type 3/4, a report on the intercalibration of Chlorophyll for this type was finished in December 2016 (Bonne & Desmit 2016).

This document is intended to provide background information on the approach by the Netherlands regarding the setting of reference conditions and class boundaries under the WFD for the type NEA3/4. It also provides information on the monitoring program and on the conditions in the coastal water bodies of the Netherlands. The information in this document addresses several issues, relevant in view of the Intercalibration approach described in the JPI Oceans report by Bonne & Desmit (2016), and provides the necessary background for evaluating the Dutch approach in WFD.

1.2 Outline

Chapter 2 gives information on the Dutch coastal water bodies and their characteristics, and on the monitoring of phytoplankton in those water bodies. Chapter 3 describes the submetrics used by the Netherlands for the assessment of the biological quality element Phytoplankton. Chapter 4 gives a description of the characteristics of the coastal water bodies with respect to nutrient loadings, as nutrient loadings were used as the main pressure in the intercalibration. The methods and underlying data that were used to derive reference conditions and class boundaries for the biological quality element phytoplankton are described in Chapter 5.
Chapter 6 gives a synthesis of the main issues that are relevant for the intercalibration. Chapter 7 summarizes the Dutch assessment method and evaluates this approach with respect to the requirements of the WFD.

2 WFD Coastal water bodies in the Netherlands

2.1 General description of coastal water body types

The coastal water bodies of the Netherlands consisted originally of three types (Table 2.1), that were distinguished in the intercalibration in the Northeast Atlantic Geographical Intercalibration Group (NEA GIG). The Common intercalibration types were agreed based on the obligatory factors salinity and tidal range, plus optional factors, depth, current velocity, exposure, mixing and residence time (Carletti & Heiskanen 2009).

Table 2.1. WFD coastal water types in the Netherlands, defined in the NEA GIG intercalibration (Carletti & Heiskanen 2009)

Characteristics	CW NEA 1/26b	CW NEA 3	CW NEA 4
Name	Enclosed seas, exposed or sheltered, euhaline, shallow	Enclosed seas, exposed or sheltered, polyhaline, shallow	Sheltered, polyhaline, shallow (Wadden Sea type)
Salinity	Fully saline (>30)	Polyhaline (18-30)	Polyhaline (18-30)
Tidal range	Mesotidal (1-5)	Mesotidal (1-5)	Mesotidal (1-5)
Depth	Shallow (<30)	Shallow (<30)	Shallow (<30)
Current velocity	Medium (1 – 3 knots)	Medium (1 – 3 knots)	Medium (1 – 3 knots)
Exposure	Exposed or sheltered	Exposed or sheltered	Exposed or sheltered
Mixing	Fully mixed	Fully mixed	Fully mixed
Residence time	Days	Days	Days

Figure 2.1 shows a map with the coastal water bodies in the Netherlands.

- The coastal water bodies of NEA type 1/26b are euhaline, exposed, coastal waters. The water bodies are found in the southwest (“Zeeland coast”) and to the north of the barrier islands of the Wadden Sea (“Wadden coast”).
- The water bodies of NEA type 3 are polyhaline, exposed, coastal water bodies that are strongly influenced by freshwater discharges, and are found near and downstream from the outflows of the rivers Rhine and Meuse (“Northern Delta coast”, “Holland coast”) or the river Ems (“Ems-Dollar coast”).
- The water bodies of NEA type 4 are polyhaline, sheltered, water bodies. These water bodies are also influenced by freshwater discharges in the Rhine/Meuse delta (“Eastern Scheldt”) or through Lake Ijssel (“Wadden Sea”).

After consideration of the relevance of the original types within the NE Atlantic complex, based solely on the above factors, it was decided that in some cases there was no biological difference between types in relation to the chosen quality element or metric(s) being intercalibrated and that some could be merged together (Carletti & Heiskanen 2009). For The Netherlands this resulted in the merging of the water types NEA 3 and NEA 4 into NEA type 3/4 that is shared with Germany.
Annex A gives an overview of the Dutch and German coastal water bodies in NEA type 1/26b and NEA type 3/4.

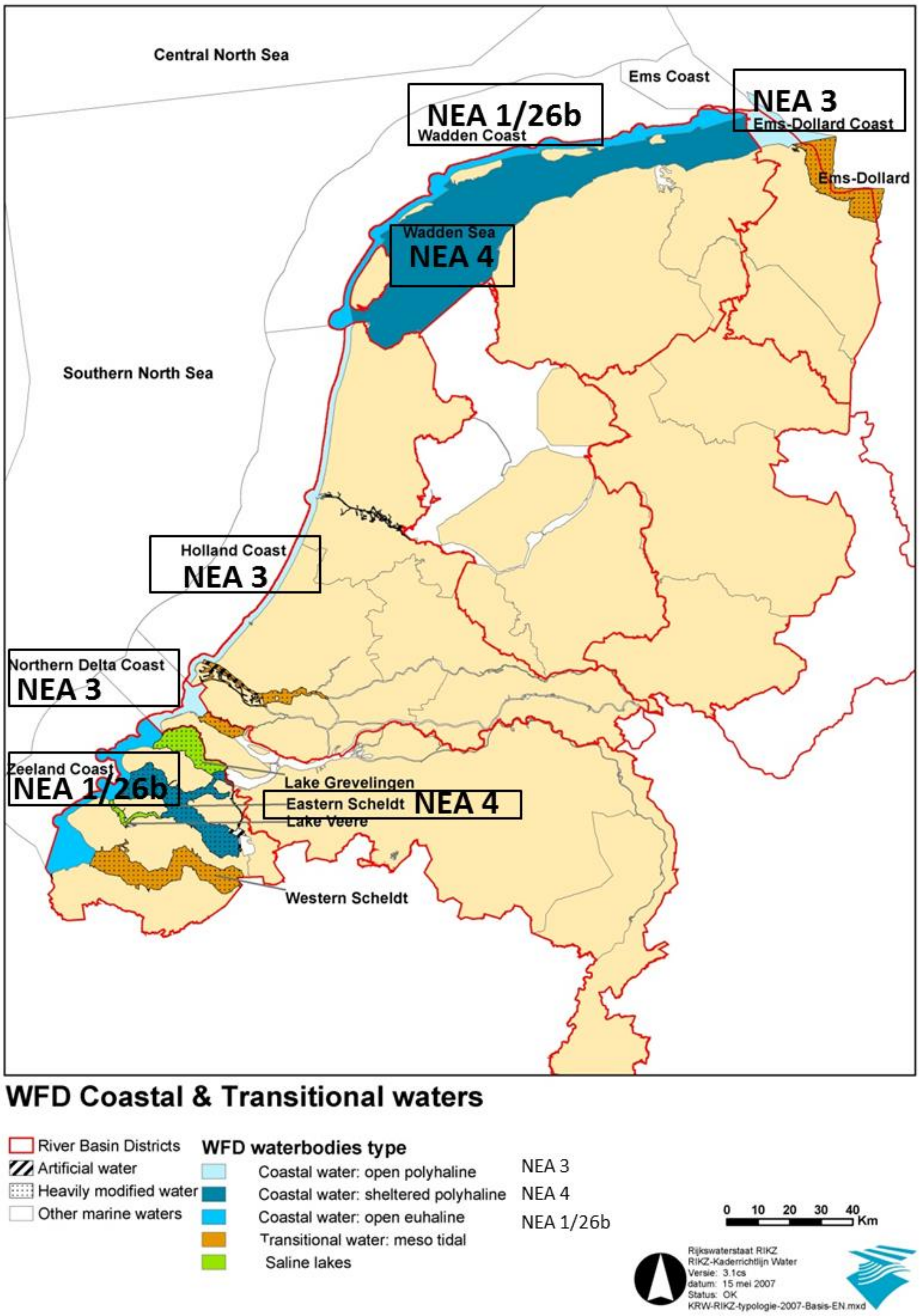

Figure 2.1. WFD coastal water types in the Netherland. The coastal water types NEA 3 and NEA 4 were merged into type NEA 3/4 (see §2.1).

2.2 Characteristics of the water bodies

2.2.1 Salinity

Dutch coastal waters show strong gradients in salinity due to freshwater discharges and mixing with seawater. There is a general pattern with the highest salinities in NEA type 1/26b, lower salinities in the coastal waters of NEA type 3 and lower and highly variable salinities in the Wadden Sea (NEA type 4). However, the boundary of salinity 30 between polyhaline waters (type 3/4) and euhaline waters (type 1/26b) does not give a sharp distinction between these water types in Dutch coastal waters of the North Sea. Figure 2.2 shows the winter averaged salinities at the monitoring stations in Dutch coastal waters.

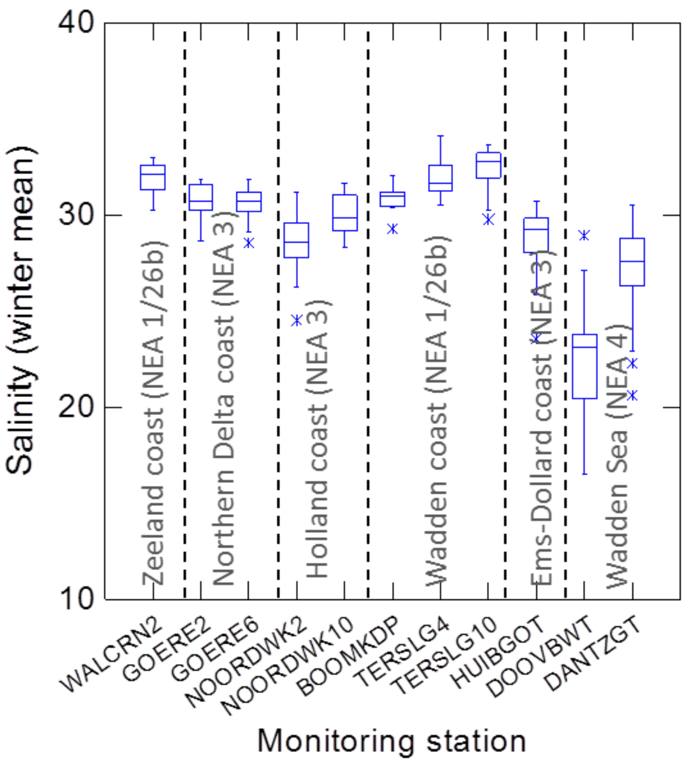

Figure 2.2. Box plots of the annual winter averages of salinity (December-February) at WFD monitoring stations (data for 1990-2015). Water bodies and type are indicated. Note that coastal water types NEA 3 and NEA 4 were merged into type NEA 3/4 (see §2.1).

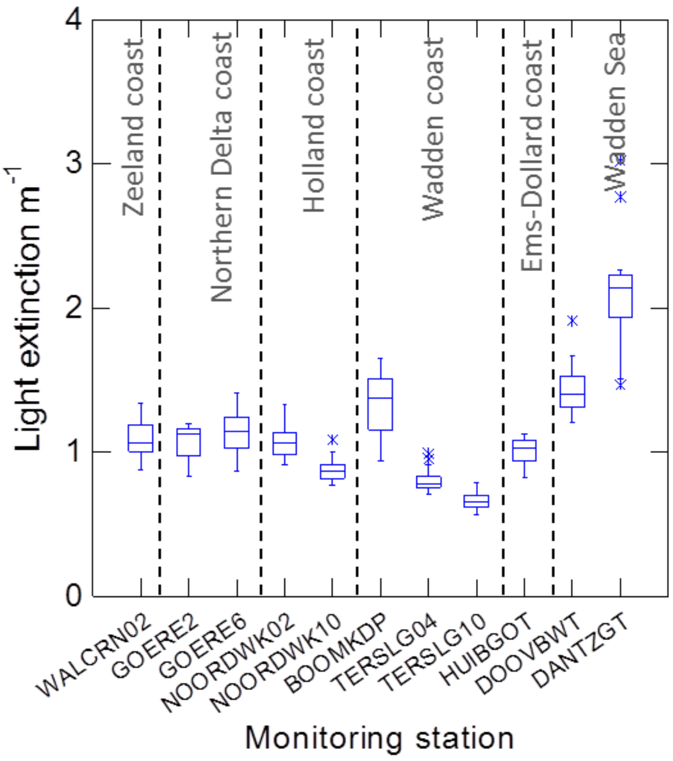

Figure 2.3. Box plots of the annual averages of light extinction during the phytoplankton growing season (March-September) at WFD monitoring stations (data for 1990-2015). Water bodies are indicated. Note that coastal water types NEA 3 and NEA 4 were merged into type NEA 3/4 (see §2.1).

2.2.2 SPM levels and light conditions

Dutch coastal waters also show strong gradients in the levels of suspended particulate matter (SPM) due to silt transport by rivers, tide- and wind-driven erosion and sedimentation of silt and transport of silt along the continental coast in the southern North Sea. This results in highly variable turbidity and also large spatial gradients in turbidity. The overall pattern shows high SPM levels and consequently high light extinction near the coast and in the Wadden Sea (Figure 2.3). Water depth increases with distance from the coast and is highly variable in the Wadden Sea where tidal flats, shallow depths and deeper tidal channels intertwine. Light conditions, important for phytoplankton growth, are a combination of light extinction and mixing depth and show a complex pattern in coastal waters.

2.3 Conclusions

- the coastal water bodies of NEA types 1/26b, 3 and 4 are positioned in a transect, going from sites near freshwater discharge points (NEA 3/4) to sites at a larger distance or with lower freshwater discharges
- as a consequence, abiotic conditions in terms of salinity, SPM levels and light conditions are highly variable in time and space

3 Description of the Dutch Biological Quality Element Phytoplankton

The Dutch Biological Quality Element (BQE) Phytoplankton consists of two sub-metrics. One sub-metric is focussed on phytoplankton abundance and uses chlorophyll-a concentrations. The other sub-metric is focussed on phytoplankton species composition, and uses concentrations of the nuisance alga *Phaeocystis globosa* as an indicator species for eutrophication effects. The calculation of the metric Ecological Quality Ratio (EQR) is always based on the combination of the two sub-metric EQR values.

3.1 Definition of relevant season

The BQE Phytoplankton is determined for the months March-September, which is defined as the phytoplankton growing season and which captures both the phytoplankton spring bloom and summer blooms (Figure 3.1). The general pattern shows a spring bloom starting in March and reaching peak levels in April or early May. After the spring bloom, phytoplankton blooms occur throughout the months May-September but mostly at lower levels than the spring bloom due to nutrient limitation. From September to March, light limitation generally prevents the occurrence of blooms.

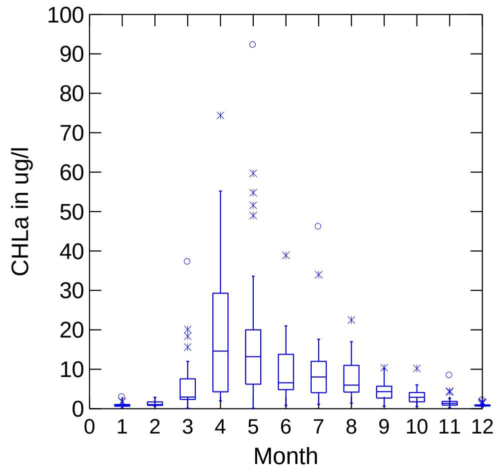

Figure 3.1. Box-plot of chlorophyll concentrations per month at monitoring station Noordwijk-2 in the years 1990-2014

3.2 Description of the metrics

3.2.1 Sub-metric Chlorophyll-a

The sub-metric Chlorophyll-a uses the chlorophyll-a concentrations calculated as 90-percentile values from monitoring data which are collected at a bi-weekly or monthly frequency (depending on the monitoring station).

3.2.2 Sub-metric Phaeocystis

The sub-metric *Phaeocystis* is based on the concentrations of *Phaeocystis globosa* (cells/l). This alga forms high-biomass blooms in spring in the southern North Sea. These blooms are a natural phenomenon, associated with nutrient enriched environments, occurring both in areas with high anthropogenic nutrient inputs like the southern North Sea (e.g. Lancelot *et al.* 2014) as well as in naturally enriched seas like the Greenland Sea and in the Barents Sea (Schoemann *et al.* 2005). Trends and spatial gradients in *Phaeocystis* abundance, bloom strength and duration, closely mimic the spatial gradient in nutrient concentrations and the interannual changes in nutrient loads to the Dutch coastal waters (Cadée and Hegeman, 2002). This supports the use of *Phaeocystis* as indicator for the eutrophication status of Dutch coastal waters (see Prins & Baretta-Bekker 2010 for a more elaborate discussion).

The Dutch metric for *Phaeocystis* uses bloom frequency as parameter (Van der Molen *et al.* 2012), calculated from monitoring data for the months March–September. As *Phaeocystis* only rarely reaches bloom densities during the winter months (October-February), the assumption is that it does not bloom during winter. Bloom frequency was defined as parameter based on the observation that bloom duration had increased in the western Wadden Sea following the increase in riverine nutrient loads (Cadée & Hegeman 1986).
The bloom frequency is determined by looking at the number of months in a year with more than 10^6 *Phaeocystis* cells/l. The frequency is expressed as a percentage of 12 months. This threshold level of 10^6 cells/l was based on Cadée & Hegeman (1986).

3.3 Calculation of the metric

The EQR for the Phytoplankton BQE is calculated from a combination of both sub-metrics. The sub-metrics are expressed in an EQR value between 0 and 1.

$$EQR_{phyto} = \text{Minimum}((EQR_{chl}+EQR_{Phaeo})/2,\ EQR_{chl})$$

Or in words: the final assessment is the smallest of
1) the average of the two assessments and
2) the assessment that is based on chlorophyll-a alone;

In other words, the *Phaeocystis* sub-metric can lower the value of the Phytoplankton EQR in comparison to the sub-metric Chlorophyll-a, but cannot improve it. The rationale behind this approach is that, in the case of very low *Phaeocystis* concentrations the Phytoplankton EQR will not result in a higher classification than the classification based on chlorophyll-a alone.

3.4 WFD monitoring of phytoplankton

An overview of the monitoring stations and monitoring frequency for each of the coastal water bodies is given in Table 3.1. Figure 3.2 shows a map of the monitoring stations including several stations that are not used for WFD monitoring and reporting but are part of the routine water quality monitoring program of the Netherlands.

Table 3.1.Overview of water bodies and monitoring stations where nutrients, chlorophyll-a and phytoplankton are monitored for WFD assessments.

Waterbody	WFD monitoring station	Monitoring frequency per year	Additional information
Zeeland coast (NEA 1/26b)	Walcheren 2	Monthly	
	Schouwen 10	Monthly	Since 2012
Northern Delta coast (NEA 3/4)	Goeree 2	Monthly	Since 2007
	Goeree 6	Monthly	
Holland coast (NEA 3/4)	Noordwijk 2	Bi-weekly	
	Noordwijk 10	Bi-weekly	
Wadden coast (NEA 1/26b)	Terschelling 4	Bi-weekly	Until 2007
	Boomkensdiep	Bi-weekly	Since 2008
	Terschelling 10	Bi-weekly	
Ems-Dollard coast (NEA 3/4)	Huibertgat Oost	Bi-weekly	
Wadden Sea (NEA 3/4)	Doove Balg west	Bi-weekly	
	Dantziggat	Bi-weekly	
	Marsdiep noord	Bi-weekly	No WFD station
	Vliestroom	Monthly	No WFD station
Oosterschelde (NEA 3/4)	Wissenkerke	Bi-weekly	

Figure 3.2.Map showing the WFD monitoring stations (in yellow) and other monitoring stations (in green) in the North Sea coastal waters and Wadden Sea.

4 Position of water bodies in the nutrient pressure gradient

4.1 Nutrient loads to Dutch coastal waters

Elevated levels of the nutrients nitrogen (N) and phosphorus (P) are pressures in Dutch coastal waters that are important for the biological quality element Phytoplankton. The main anthropogenic sources of nutrients are direct and diffuse emissions into freshwater systems in the various river basins, resulting in nutrient loads into the coastal waters through river-borne transport.

The river basins that have a direct impact on nutrient levels in Dutch coastal waters are Rhine, Meuse, Scheldt and Ems. The Rhine river basin is by far the largest river basin district and the largest source of anthropogenic nutrients for Dutch coastal waters (>80%), directly discharging into the North Sea at Haringvliet, Nieuwe Waterweg and Noordzeekanaal. Figure 4.1 gives an example for the year 2006 showing the contribution of the various discharge points to the total loads of N and P to coastal waters. The biggest source for the North Sea coastal waters is the discharge of the river Rhine through the “Nieuwe Waterweg”. For the Wadden Sea, discharges from Lake IJssel are a major source (Phillipart et al. 2000) with additionally import from the North Sea coastal zone.

Riverine nutrient loads to the North Sea have decreased significantly since the 1980s. The total-N load from the rivers Rhine and Meuse combined, has decreased with approximately 45% since 1990, and the total-P load with approximately 70%. In the other river basins, nutrient loads (in particular total-P) have decreased significantly as well.

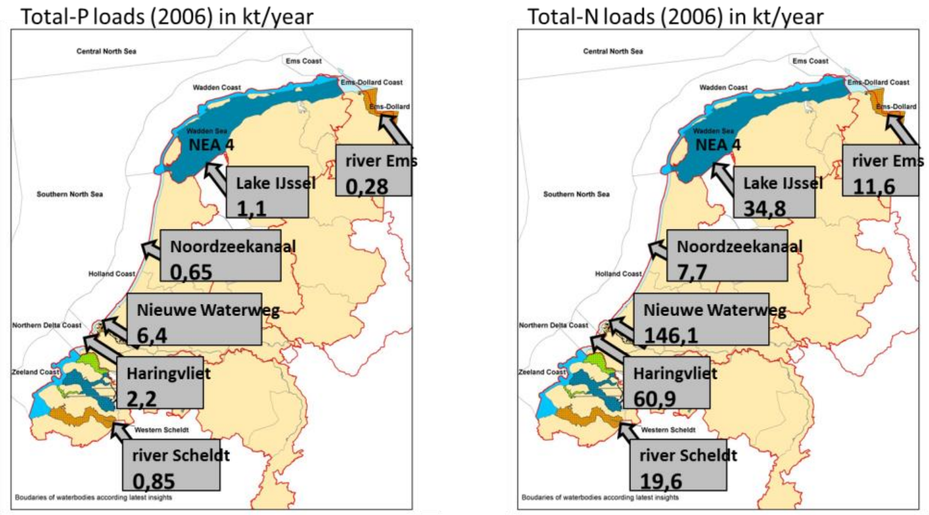

Figure 4.1 Main discharge points of nutrients into Dutch coastal waters with the total-P and total-N loads in 2006.

4.2 Description of nutrient pressure gradient

4.2.1 Parameters to express nutrient concentrations

The generally accepted procedure in the NE Atlantic to describe the level of nutrient concentrations, is to use the winter averaged concentrations of dissolved inorganic nitrogen (DIN = NO_2^-+NO_3^-+NH_4^+) and dissolved inorganic phosphate (DIP = PO_4^{3-}) (OSPAR 2013).

The rationale behind this is that, during winter, biological processes are at a minimum level and consequently the concentrations of inorganic nutrients in the water column are at a maximum level.
Alternatively, concentrations of total-N and total-P can be used, as is the case in most freshwaters. However, the latter concentrations are to some extent influenced by SPM levels and are therefore less useful to describe the amount of N and P available for primary production. The relation between the concentrations of total nutrients and dissolved inorganic nutrients is different between water bodies. In particular, the relation in the Wadden Sea deviates from the relation for the North Sea water bodies (Figure 4.2). Hence, characterization of the water bodies in terms of nutrient levels differs, depending on whether total nutrients or dissolved inorganic nutrients are used.

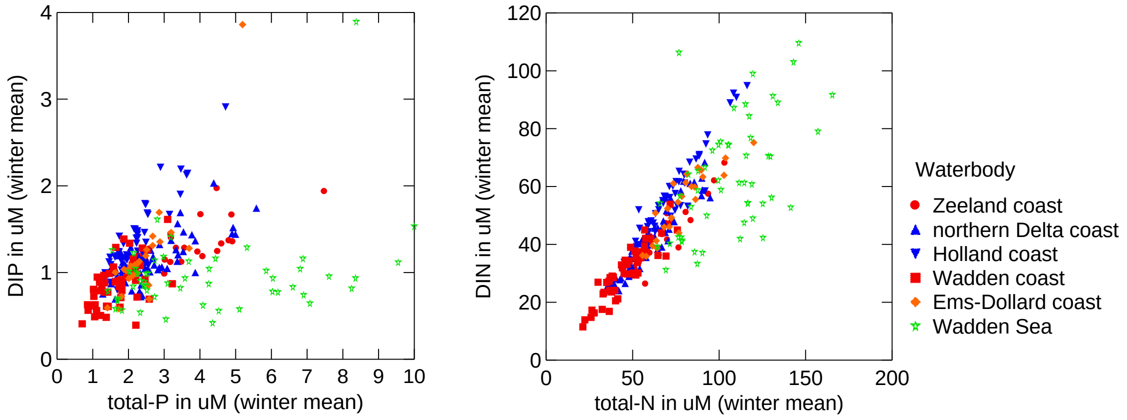

Figure 4.2 Relation between the winter averaged concentrations of total-P and DIP (left) and total-N and DIN (right). Data for 1990-2015.

4.2.2 Positioning of the water bodies in the nutrient pressure gradient

Salinity can be used as a tracer of freshwater influence. As freshwater discharges are the main source of anthropogenic nutrient loads to Dutch coastal waters, an inverse relation between salinity and winter nutrient concentrations is to be expected.
This is generally the case for DIP and DIN, but the Wadden Sea deviates from the overall pattern (Figure 4.3, Figure 4.4). This is probably due to the fact that the nutrient concentrations in North Sea coastal waters are to a large extent determined by the freshwater discharges of the rivers Rhine and Meuse through Haringvliet and Nieuwe Waterweg. In the Wadden Sea the nutrient concentrations are to a large extent determined by nutrient discharges through Lake IJssel (which are strongly influenced by retention processes in the lake) and internal (= within the Wadden Sea) processes (in particular sediment-water exchange (Jung *et al.* in press). With the exception of the Wadden Sea, the coastal water bodies with the strongest freshwater influence (e.g. northern Delta coast, Holland coast) have the lowest salinities and highest nutrient concentrations.

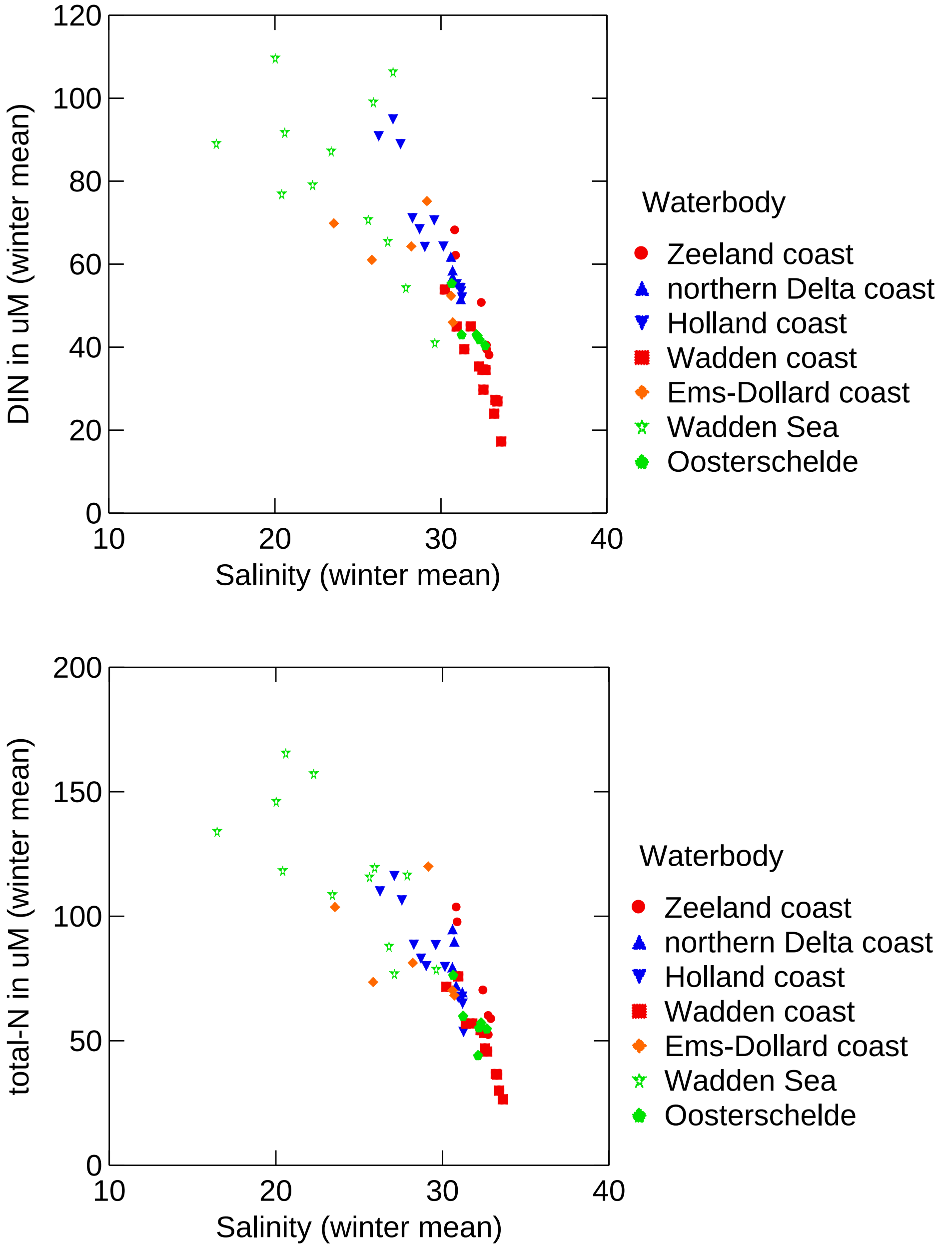

Figure 4.3 Relation between salinity and the winter averaged DIN concentration (top panel) and total-N concentration (bottom panel). Data for 1990-1995.

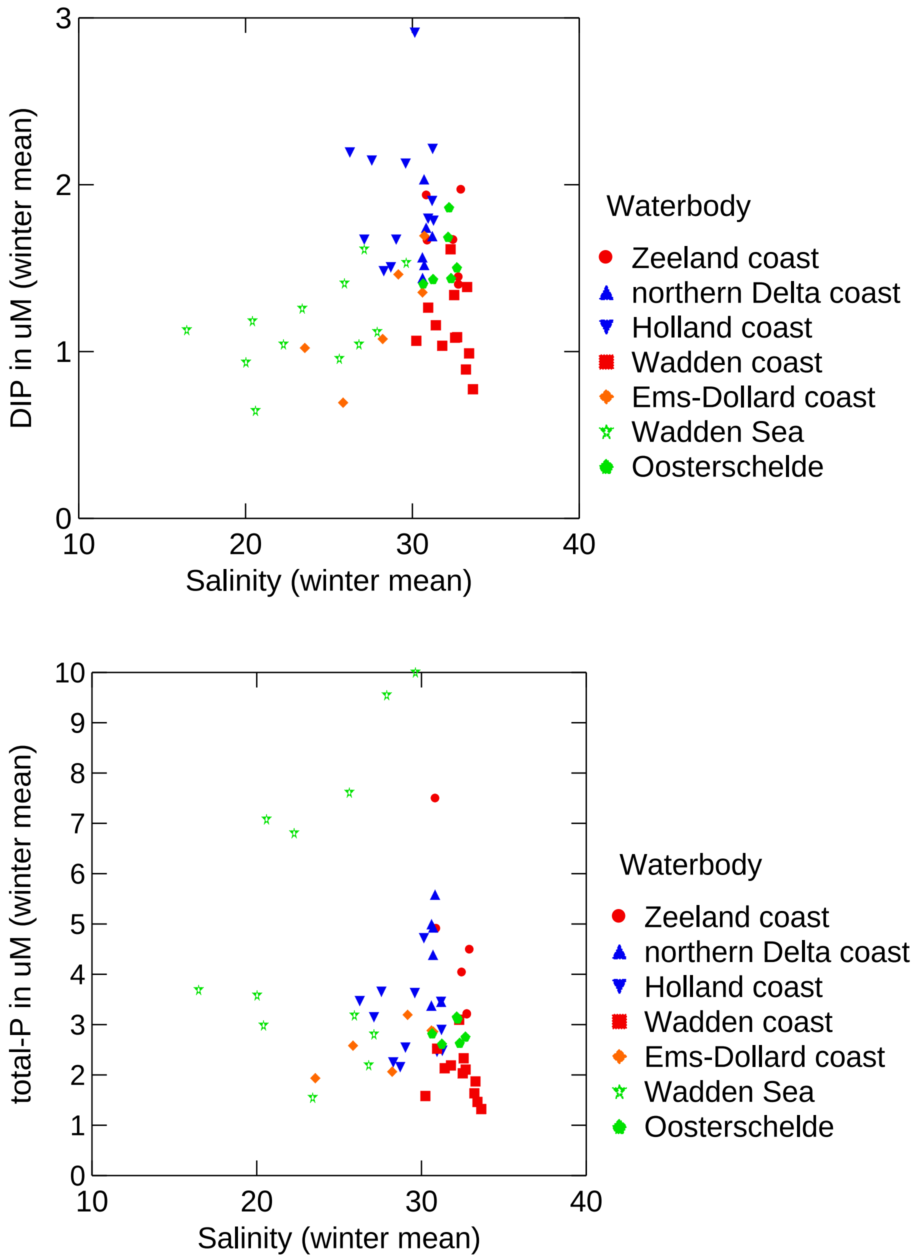

Figure 4.4 Relation between salinity and the winter averaged DIP concentration (top panel) and total-P concentration (bottom panel). Data for 1990-1995.

4.2.3 Limiting factors for phytoplankton growth

In the Dutch coastal zone of the North Sea, both light limitation and nutrient limitation have an effect on phytoplankton growth (Peeters & Peperzak 1990, Colijn & Cadée 2003, Loebl *et al.* 2009, Ly *et al.* 2014, Burson *et al.* 2016, Leote *et al.* 2016). There is a spatial pattern with P-limitation occurring mainly in coastal waters whereas N-limitation becomes more important in marine waters further offshore. Light limitation can be strong in a narrow band along the coast due to elevated SPM levels. Figures 4.5 and 4.6 show the spatial pattern and the changes over years in the relative importance of limiting factors. There is also a seasonal pattern, with P-limitation mainly occurring at the end of the spring bloom, together with Si-limitation for

diatoms, whereas N-limitation only occurs later in summer. Due to the more strongly reduced riverine P-loads, as compared to nitrogen, P-limitation most likely has become more dominant in coastal waters (Figures 4.6, 4.7, 4.8).
The spatial and temporal pattern of limiting factors (Figure 4.5) shows that P-limitation is the most important factor in coastal waters followed by light limitation and N-limitation, while in offshore waters N-limitation is the most important factor (Troost *et al.* 2014). In the Wadden Sea, P-limitation may be even more important than in North Sea coastal waters (Ly *et al.* 2014, Leote *et al.* 2016), particularly in the spring period (Figure 4.7).
In addition to light and nutrient limitation, grazing by benthic filterfeeders (e.g. shellfish) has strong impacts on phytoplankton biomass in the Wadden Sea (Philippart *et al.* 2007) and is probably also a significant factor in the shallow coastal waters of the North Sea (Van Duren *et al.* 2017).

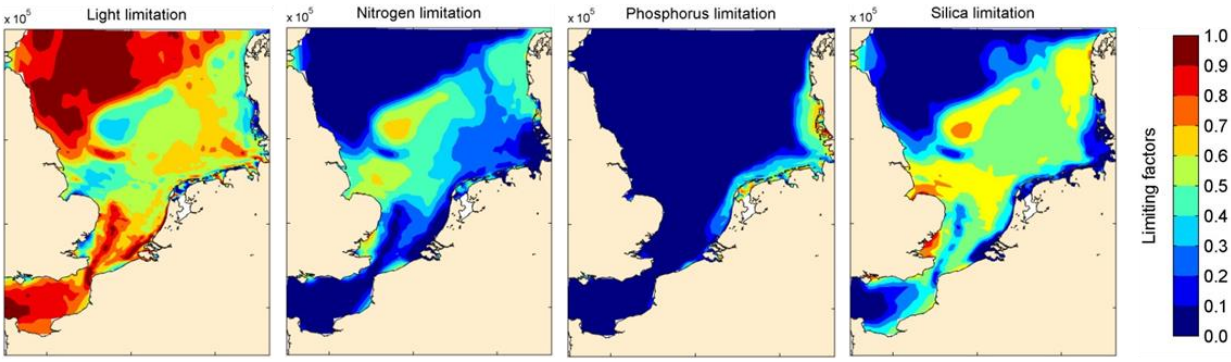

Figure 4.5. Spatial distribution of limiting factors for phytoplankton growth in the North Sea. The strength of limitation increases on a scale from 0 (no limitation) to 1 (limitation all-year round).

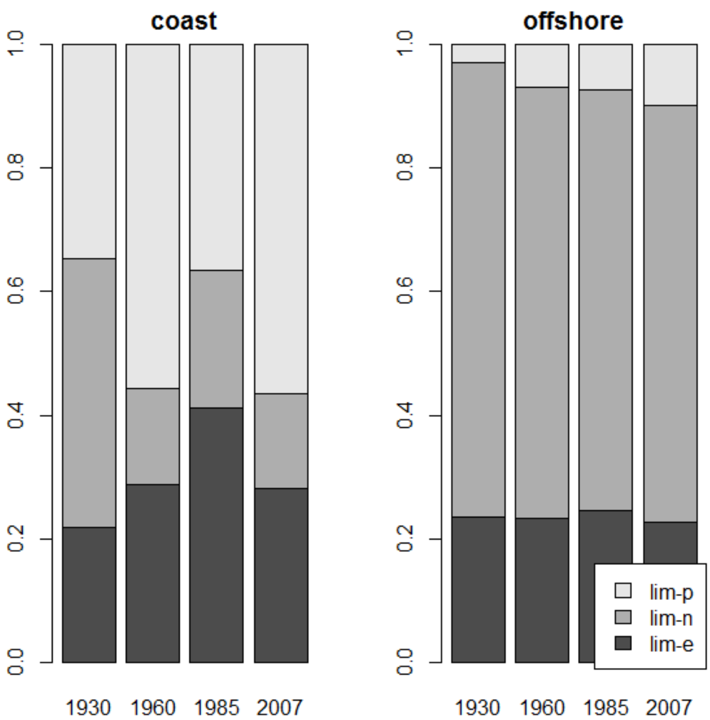

Figure 4.6. Relative importance of phytoplankton growth limitation by P (lim-p), N (lim-n) or light (lim-e) in coastal and offshore waters. Modeled results for 1930, 1960, 1985 and 2007 (Troost et al. *2014).*

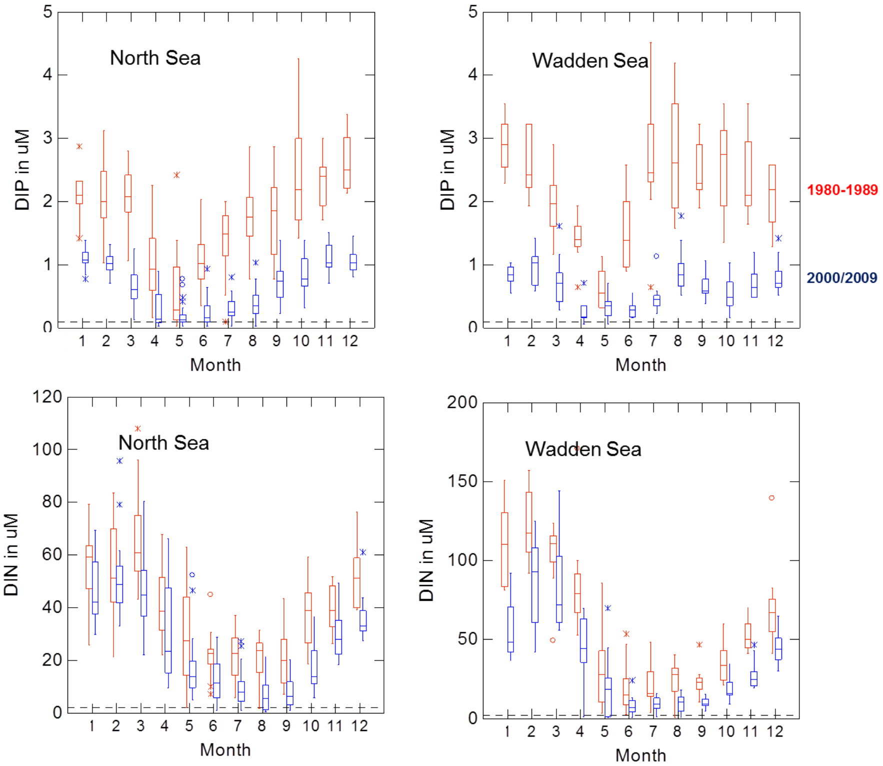

Figure 4.7. Box plots of the DIP (top panel) and DIN (bottom panel) concentrations per month. Data for station Noordwijk 10 in the North Sea (left) and for station Doove Balg west in the Wadden Sea (right), for the years 1980-1989 (red) and 2000-2009 (blue). The dotted line shows the approximate concentration where N or P becomes limiting for phytoplankton production.

4.3 Trends in nutrients and chlorophyll

The reduction in nutrient emissions and consequently riverine nutrient loads during the years 1990-2015 (Figure 4.8) is reflected in the changes in nutrient concentrations in the coastal waters of the North Sea. This is of course most clearly the case at the monitoring stations with lower salinity close to the river discharge points. This is illustrated in Figure 4.9, that shows the inverse relation between DIP and DIN concentrations and salinity on a transect from monitoring stations near Haringvliet / Nieuwe Waterweg (see Figure 3.2) to offshore North Sea waters. Due to the reduction in riverine nutrient loads over the last 3 decades, the DIP concentration at salinity 30 has decreased with approximately 50%, and the DIN concentration with approximately 25%, on the transect shown in Figure 4.9.

A trend analysis showed statistically significant decreases over the years 1990-2015 for DIP at each specific monitoring site in the coastal waters (Table 4.1), For DIN however, at fewer monitoring stations significant decreases are observed. This is probably due to the fact that the change in N loads is smaller than the change in P loads. The significant decreases in DIP and sometimes DIN concentrations, coincide with significant decreases in chlorophyll-a concentrations. However, not in all cases where nutrient concentrations show a significant decreasing trend, significant trends in chlorophyll are observed. The lack of statistically significant decreases in chlorophyll-a, in spite of the decreases in N and P, is probably caused by the large interannual variation in chlorophyll-a concentrations, that makes trend detection more difficult.

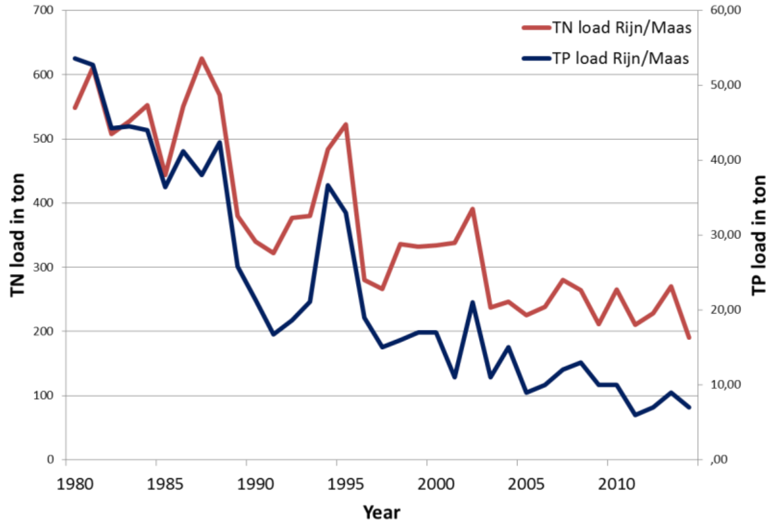

Figure 4.8 Loads of Total Nitrogen (TN) and Total Phosphorus (TP) to the North Sea. Sum of the discharges of Rhine and Meuse through Haringvliet and Nieuwe Waterweg.

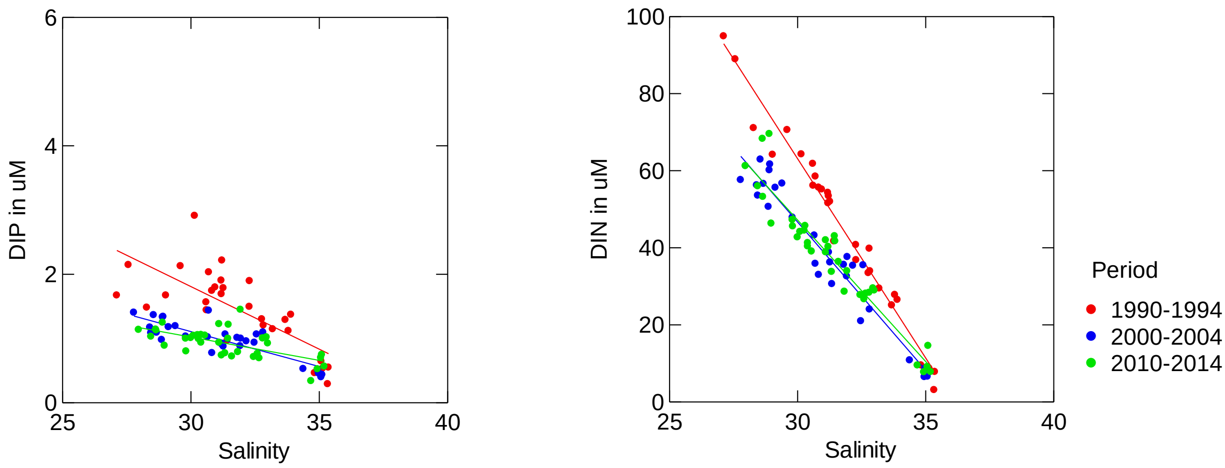

Figure 4.9 Relation between the winter averaged DIP (left panel) and DIN (right panel) concentration and salinity at a transect from Haringvliet/Nieuwe Waterweg to offshore waters, for three different 5-year periods..

Table 4.1 Results of a trend analysis for the period 1990-2015 (Mann-Kendall test) of winter means of DIN and DIP and 90-percentiles and means of chlorophyll.
** p<0.050*
*** p<0.010*
**** p<0.001*

Waterbody	**WFD monitoring station**	**Salinity**	**Trend 1990-2015 (Mann-Kendall test)**			
			DIN (winter mean)	DIP (winter mean)	CHL-a (mean)	CHLa (90-percentile)
Zeeland coast (NEA 1/26b)	Walcheren 2	32.0		-29%***	-27%*	
	Schouwen 10	32.6		-29%**		
Northern Delta coast (NEA 3/4)	Goeree 2 (2007-2015)	30.7		-41%*		
	Goeree 6	30.5	-33%***	-46%***	-47%*	
Holland coast (NEA 3/4)	Noordwijk 2	29.0	-25%*	-49%**		
	Noordwijk 10	30.4	-26%*	-40%**		
Wadden coast (NEA 1/26b)	Terschelling 4 (1990-2007)	32.1		-47%**		
	Boomkensdiep (2008-2015)	31.4				
	Terschelling 10	32.6		-50%**	-51%*	-59%**
Ems-Dollard coast (NEA 3/4)	Huibertgat Oost	29.6	-26%*	-40%**		
Wadden Sea (NEA 3/4)	Doove Balg west	24.3		-29%***	-27%*	
	Dantziggat	28.9	-33%*	-44%**	+25%*	+41%*
	Marsdiep noord	28.5		-46%***	-31%*	
	Vliestroom	29.8	-33%*	-52%***	-32%*	
Oosterschelde (NEA 3/4)	Wissenkerke	31.5		-30%***		

4.4 Correlations between DIN, DIP and chlorophyll-a

Since freshwater discharges are a major source for both DIP and DIN, there is a spatial correlation between DIP and DIN that is related to salinity, although the Wadden Sea deviates from this pattern as discussed in §4.2.2. There is also a correlation over time, as both DIN and DIP loadings to coastal waters have decreased since 1990. As a consequence, when we want to explore correlations between chlorophyll-a concentrations and nutrient concentrations in Dutch coastal waters, the covariation between the explanatory factors DIN and DIP needs to be taken into account (Table 4.2).

The correlation between nutrient concentrations and chlorophyll-a concentrations at the level of individual monitoring stations is relatively weak. Only few stations show a significant correlation with either DIN or DIP or with both nutrients (Table 4.2). In particular in the Wadden Sea, a correlation of chlorophyll-a 90-percentiles with nutrient concentrations is absent (Figure 4.10). When the time series for all stations in the North Sea coastal waters (excluding Wadden Sea and Oosterschelde) are combined, chlorophyll-a shows a significant correlation with both DIP and DIN concentrations (last row Table 4.2). This correlation is to a large extent caused by a correlation in space (stations with higher nutrient concentrations tend to have higher chlorophyll concentrations).

Table 4.2 Analysis of the linear correlations (Pearson correlation) between DIN, DIP and chlorophyll-a (expressed as growing season 90-percentiles and means) for each monitoring station and for the North Sea stations combined. Correlations were also analysed for log-transformed data (column log-log). Data for the period 1990-2015

**   p<0.050; ** p<0.010; *** p<0.001*

Waterbody	**WFD monitoring station**	**Pearson correlation**								
		DIN-DIP	**DIN-CHL 90-percentile**		**DIN-CHL mean**		**DIP-CHL 90-percentile**		**DIP-CHL mean**	
			-	**Log-Log.**	**-**	**Log-Log**	**-**	**Log-Log**	**-**	**Log-Log**
Zeeland coast (NEA 1/26b)	Walcheren 2	**								
	Schouwen 10						*	*	*	*
Northern Delta coast (NEA 3/4)	Goeree 2 (2007-2015)	*								
	Goeree 6	***								
Holland coast (NEA 3/4)	Noordwijk 2	**								
	Noordwijk 10	**	**	*	***	**		*	*	**
Wadden coast (NEA 1/26b)	Terschelling 4	*								
	Boomkensdiep (2008-2015)									
	Terschelling 10	***	*		*					
Ems-Dollard coast (NEA 3/4)	Huibertgat Oost									
Wadden Sea (NEA 3/4)	Doove Balg west			*		*				
	Dantziggat	***								
	Marsdiep noord	*								*
	Vliestroom									
Oosterschelde (NEA 3/4)	Wissenkerke		*							
Zeeland coast, Northern Delta coast, Holland coast, Wadden coast, Ems-Dollard coast	Walcheren 2, Schouwen 10, Goeree 2, Goeree 6, Noordwijk 2, Noordwijk 10, Terschelling 4, Boomkensdiep, Terschelling 10, Huibertgat Oost	***	***	***	***	***	***	***	***	***

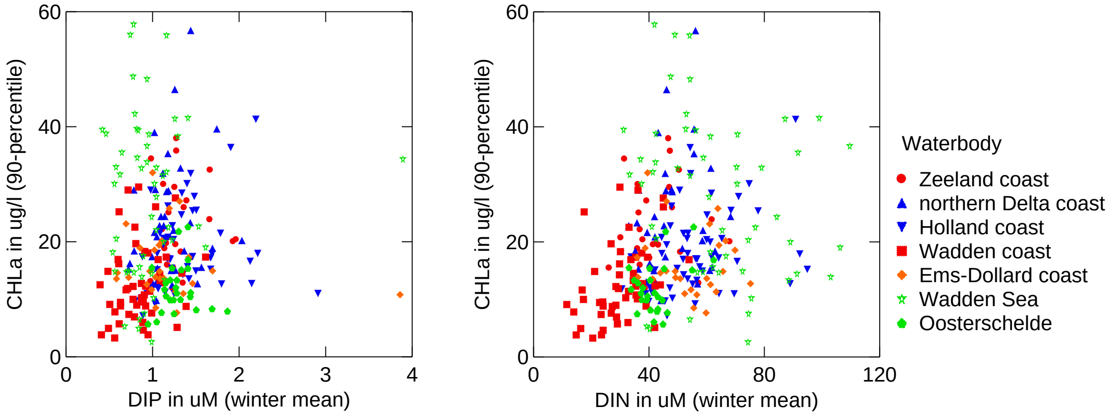

Figure 4.10 Chlorophyll-a concentrations in relation to winter means of DIP (left panel) and DIN (right panel).

When looking at all monitoring stations separately, only on a few occasions significant correlations between nutrient concentrations and chlorophyll-a concentrations are found (Table 4.2). In a GLM (General Linear Model) analysis with the data from all North Sea coastal waters monitoring stations for 1990-2015 combined, monitoring stations and either DIN or DIP concentrations were used as independent factors. Chlorophyll-a 90-percentiles and nutrient concentrations were log-transformed as this gave slightly better results than untransformed data. This combined model analyses to what extent chlorophyll-a concentration can be explained by location (monitoring station) and by DIN or DIP concentration (nutrient concentrations different between years).
The combined models with “monitoring station” and either DIP or DIN as factors were both significant. However, the percentage of variation in chlorophyll-a that was explained by nutrient concentrations was relatively low: for DIP r^2=0.31 and for DIN r^2=0.32, while the factor “monitoring station” is more important. The results are shown in Annex B. The GLM analysis shows that both DIP and DIN concentrations have limited value for predicting chlorophyll-a concentrations at the monitoring stations in the North Sea coastal waters, as most of the variation is explained by the factor “monitoring station”. The spatial pattern in chlorophyll concentrations can be explained relatively well (stations with high concentrations are distinguished from stations from low concentrations), but the interannual variation in chlorophyll-a concentrations can hardly be explained by differences in nutrient concentrations (weak relation between DIN or DIP concentration and chlorophyll-a concentration).

4.5 Conclusions

- the main source of anthropogenic nutrients for Dutch coastal waters are emissions in the freshwater parts of the river basins, in particular the Rhine
- dissolved inorganic nutrients (DIN, DIP) give a different spatial pattern of nutrient concentrations than total-P and total-N concentrations
- phytoplankton growth in Dutch coastal waters is limited by P, N and light
- changes in nutrient emissions have resulted in a significant reduction in nutrient concentrations at the monitoring stations in coastal waters in the period 1990-2015, most clearly for DIP and at some stations for DIN
- the reduction in nutrient concentrations has resulted in statistically significant downward trends for chlorophyll at few monitoring stations in the period 1990-2015
- chlorophyll concentrations in coastal waters show a statistical significant but relatively weak correlation with both DIP and DIN concentrations

5 Definition of reference values

5.1 Sub-metric Chlorophyll-a

The reference concentration for chlorophyll in the Dutch coastal waters is based on the AMOEBE approach elaborated in Baptist & Jagtman (1997), but also the values used by OSPAR, expert judgement and the EU intercalibration procedure (Carletti & Heiskanen 2009) have been taken into account.

In the framework of the “Watersysteemverkenning” (Water System Exploration), so called reference values, representing the upper boundary of the good status, for a number of functional groups and individual species (including chlorophyll-a) were calculated (Baptist & Jagtman 1997). For the calculation of these reference values the year 1930 was chosen as being illustrative for a situation with limited anthropogenic disturbance and at the same time, some availability of historical data (Baptist & Jagtman 1997). There were not sufficient data available to describe riverine nutrient loads and concentrations in the North Sea for this reference year 1930. Therefore, these data were derived from estimates of the anthropogenic fraction in the nutrient loads to the North Sea (De Vries *et al.* 1993). This anthropogenic fraction was subtracted from the actual loads to establish the natural background loads.
Consequently, the calculated reference situation represents a situation with no anthropogenic nutrient loads. Uncertainty in the model results is caused by model formulations, calibration, weather conditions and hydrodynamic conditions used in the model calculation, etc., but also by the assumptions on the anthropogenic fraction of the nutrient loads. The natural reference loads were assumed to be 10-15 % of riverine nutrient loads in 1987.

The natural reference loads were derived from multi-annual average river discharges combined with estimates of natural background concentrations for total-N and total-P (Wulffraat *et al.* 1993). Ranges for natural background concentrations had been established in an international workshop on background concentrations of natural compounds in the North Sea (Laane 1992). Estimated ranges were 20-71 µM for total-N and 0.7-4.5 µM for total-P. Those ranges were derived from studies of nutrient data in Swedish rivers (Ahl 1988; 1994). The lowest value represents the estimated upper limit for pristine conditions, whereas the highest value represents the upper limit for unpolluted conditions (Laane 1992, Ahl 1994, Laane *et al.* 2005). Using specific models for the various water systems and the estimated reference conditions for nutrients, reference values for chlorophyll-a (90-percentiles) were calculated (Baptist & Jagtman 1997; Lorenz *et al.* 2004). In conclusion, the calculated reference values for chlorophyll-a represent a situation with (nearly) pristine total-P and total-N loads.
For a 50-km wide zone of coastal waters in the North Sea, the calculated reference value for chlorophyll-a was 14.3 µg/l. This value agrees well with the value deduced from Cadée & Hegeman (2002), see Carletti & Heiskanen (2009).
The calculated chlorophyll-a reference values for the western Wadden Sea was 20 µg/l, and for the eastern Wadden Sea 17.3 µg/l. As the Wadden Sea is considered to be one water body in the WFD, the mean of the two estimates (18 µg/l) was chosen.

The Dutch coastal water bodies were divided into two groups, based on their salinity ranges during the growing season: the polyhaline and the euhaline type (see Figure 2.1). The Holland coast and the Northern Delta coast at the mouth and downstream of the main outflows of Rhine and Meuse have larger salinity ranges and lower salinities, and belong to the polyhaline type (NEA-GIG type NEA3). The other water bodies in the coastal waters (Zeeland coast, Wadden coast, Eems-Dollard coast) have smaller salinity ranges and are of

the euhaline type (NEA-GIG type NEA1/26b (see Figure 2.2). The Wadden Sea belongs to NEA-GIG type NEA4.
As both the Wadden Sea (NEA-GIG type NEA4: sheltered, polyhaline coastal water) and the water bodies Holland coast and Northern Delta coast (NEA-GIG type NEA3: open, polyhaline coastal water) were characterized by large freshwater discharges and reduced salinities, it was concluded that there was no reason to use different reference values for both water types. Therefore, the 90-percentile of chlorophyll-*a* in the growing season as calculated by Baptist & Jagtman (1997) for the Dutch coastal zone (14 µg/l) was used for both water types (that were later merged into NEA type 3/4).
Another adaptation was to interpret the model estimates of Baptist & Jagtman (1997) as the boundary between High and Good Ecological Status in the WFD and not as the WFD reference value (Carletti & Heiskanen, 2008). This is more consistent with the definitions of ecological status in the WFD, where the reference represents undisturbed conditions (High status) and Good status is characterised by "a slight deviation from reference conditions".
The High/Good boundary is 1.5 times the reference value (Carletti & Heiskanen, 2008). Thus, the 90-percentile value of 14 µg/l derived from Baptist & Jagtman (1997) results in a reference value for the Wadden Sea of 9.3 µg/l (Van der Molen & Pot, 2007).
For the coastal water body southward of the Rhine/Meuse outflow (Zeeland coast) and for the coastal water body to the north of the Wadden Sea islands (Wadden coast) the reference value was set at 6.7 µg/l. These values were established after intercalibration for this type with UK waters, based on the lower freshwater influence and higher salinity in these water bodies (Carletti & Heiskanen 2009).
The water body Ems-Dollard coast belongs to NEA-GIG type NEA3. After intercalibration with Germany, the reference value for this water body was set at 6.7 µg/l, similar to the value for NEA-GIG type 1/26b.

5.2 Sub-metric *Phaeocystis*

Observations in the Dutch western Wadden Sea showed that the duration of *Phaeocystis* blooms (>10^6 cells/l) increased in the 1970s (Cadée & Hegeman 1986; 2002) to a length of more than 30 days. To establish the duration of a bloom, high-frequency monitoring is required, and it was therefore decided to use bloom frequency as a parameter for the WFD. A bloom duration of one month and hence a bloom frequency of one month per year is assumed to be the boundary between “high” and “good” status, in all transitional and coastal water bodies. The bloom frequency is calculated from monthly or b-weekly sampling and assessment of the number of sample dates with concentrations >10^6 cells/l.

5.3 Definition of class boundaries

5.3.1 Sub-metric Chlorophyll-a

For all Dutch water bodies, the High/Good boundary is 1.5 times the reference value. The Good/Moderate boundary is 1.5 times the H/G boundary (Carletti & Heiskanen, 2008). The steps for the Moderate/Poor and Poor/Bad boundaries are a factor 2 (Table 5.1).

	90-percentile of concentration Chl-a				
	Reference	H/G	G/M	M/P	P/B
NEA type 3 / 4	9.3	14	21	42	84
NEA type 1/26b	6.7	10	15	30	60
Ems-Dollard coast		10	15	30	60
	high	good	moderate	Poor	Bad
EQR		0.8	0.6	0.4	0.2

Table 5.1 Chlorophyll reference value (µg/l), class boundaries (µg/l) and standardisation to achieve the EQR.

5.3.2 Sub-metric *Phaeocystis*

For all Dutch water bodies, a frequency of two months per year with a bloom >10^6 cells/l (frequency > 17%) is assumed to be the boundary between "high" and "good" status. More than two months per year (frequency > 35%) is the boundary between "good" and "moderate" status (Carletti & Heiskanen, 2008) (Table 5.2).

Frequency (%)			10		17		35		85	
		high		Good		moderate		Poor		Bad
EQR	1.0		0.8		0.6		0.4		0.2	

Table 5.2. Phaeocystis class boundaries and standardisation to achieve the EQR for the coastal waters.

5.4 German approach to the definition of reference values for chlorophyll-a

With the model MONERIS that simulates nutrient emissions and pathways in river basins, pristine concentrations of total nitrogen and total phosphorus in the German rivers entering the North Sea, were calculated (Behrendt *et al.* 2003, Topcu *et al.* 2011). The pristine values ranged from 14-22 µM TN and 0.25-0.80 µM TP. Those values compared well with literature values and data for natural river systems (Topcu *et al.* 2011), and are also comparable to the values used by the Netherlands (see §5.1).

Natural background concentrations in North Sea water were estimated from data from offshore monitoring. Gradients of pristine concentrations of TP and TN in the Wadden Sea and German Bight were calculated using nutrient-salinity mixing relations (Topcu *et al.* 2009, Topcu *et al.* 2011). The relative contribution of the various German rivers discharging into the North Sea to freshwater content and nutrients at each location within the Wadden Sea and German Bight was not based on hydrodynamical models but estimated from the mean salinity gradients.

Recent monitoring data were used to establish statistical relations between chlorophyll-a concentrations and TN concentrations. These relations were then used to derive pristine chlorophyll-a concentrations from the estimated pristine TN concentrations (Topcu *et al.* 2006). In this exercise, estuaries with severe light limitation due to high SPM levels were excluded. Mean natural background concentrations of chlorophyll-a during the growing season in the Wadden Sea were estimated between 2-2.5 µg/l.

As a rule of thumb, a factor 2 can be used to roughly calculate 90-percentile values from means. Using this calculation, the mean natural background concentration of 2-2.5 µg/l chlorophyll is equal to a 90-percentile of 4-5 µg/l. This is slightly lower than the reference value of 6.7 µg/l that the Netherlands derived for the Wadden Sea. The difference is assumed to be due to the difference in methods for deriving reference concentrations.

6 Synthesis

6.1 Typology of coastal water bodies

The water body types NEA 1/26b and NEA 3/4 are distinguished mainly on the basis of salinity, at the salinity boundary 30. The range in salinities in the combined type NEA 3/4 is large, between 15-30, representing a range in the percentage of freshwater at the monitoring stations of approximately 40-90%. Consequently, the nutrient concentrations within NEA 3/4 show a large variation between sites. The water bodies in NEA 1/26b have slightly higher salinities but can be considered part of the transect of coastal water bodies from river mouth to further downstream.

In addition, other abiotic conditions that are relevant for phytoplankton growth show large spatial variability as well, like mixing depth and SPM levels that in combination determine light conditions. Within NEA 3/4 there is also a large difference between the North Sea coastal waters and the Wadden Sea, with respect to depth and interaction with the benthic system. The impact of grazing by benthic filter feeders is a major factor influencing phytoplankton biomass in the Wadden Sea, and a smaller but not negligible factor in the North Sea coastal zone.

As a result, growth conditions for phytoplankton within the NEA types 1/26b and 3/4 show large differences, creating large differences between monitoring stations. Multiple factors need to be taken into account to understand the dynamics of phytoplankton growth and hence, the level of chlorophyll-a concentrations.

6.2 Pressure-state relations

The coastal waters are a highly variable environment, with spatial differences in growth conditions due to differences in abiotic conditions and biological factors. In addition, interannual differences in light conditions and freshwater discharges add to the variability in growth conditions at a multi-annual scale. Limitation of phytoplankton growth is determined by multiple factors, that co-occur during the growing season, and include various nutrients (N, P, Si), light and grazing. At each monitoring station, the interaction between multiple limiting factors results in specific growth conditions for each site.

There is a broad-brush relation between nutrient loadings, nutrient concentrations and chlorophyll concentrations in the North Sea. However, with the available monitoring data it is not possible to establish straightforward and robust statistical relations between one specific nutrient and chlorophyll concentrations in Dutch coastal waters. One reason for this is the covariation between DIP and DIN, making it impossible to decide if the relations found are mere correlations or indicative of a causal relationship with either DIN or DIP. Another reason is the simultaneous occurrence of multiple limitations of phytoplankton growth. As a consequence, correlations between chlorophyll and a single factor cannot be used to extrapolate the effects of changes in one factor on chlorophyll.

6.3 Definition of reference values and class boundaries

The reference values for chlorophyll-a in the water bodies of NEA 1/26b and NEA 3/4 are based on estimates with a coupled physical-biological numerical model. The model calculated chlorophyll-a concentrations in coastal waters using natural background concentrations of both phosphorus (total-P) and nitrogen (total-N) in the rivers discharging into the North Sea. These background concentrations were derived from historical data (Laane 1992, Laane *et al.* 2005). For total-N a concentration of 20 µM was used as a natural background concentration for pristine conditions, and for total-P 0.7 µM. In a recent German study, an overview is given of literature values for pristine concentrations of nutrients in rivers (Hirt *et al.* 2014). This

overview shows that the values of Laane (1992) fall well within the range of estimates for European lowland rivers.
The calculation of High/Good and Good/Moderate class boundaries for chlorophyll was done according to the methods applied for the implementation of the WFD in the NE Atlantic (Carletti & Heiskanen 2009).
In conclusion, at the implementation of the WFD reference values for coastal waters were derived for pristine conditions of both phosphorus and nitrogen concentrations, using established methods and state-of-the-art ecosystem models. The class boundaries were derived according to the methods established in the intercalibration.
Table 6.1 gives an overview of the reference values and H/G and G/M class boundaries.

Table 6.1 Reference values, High/Good and Good/Moderate boundary classes for the water bodies in the coastal waters of NEA type 3/4. Table adapted from Table 17 in Bonne & Desmit (2016)

Waterlichaam	**Zeeuwse kust, Wadden kust**	**Noordelijke Deltakust, Hollandse kust**	**Waddenzee**	**Eems-Dollard Kust**
Water body	***Zeeland coast, Wadden coast***	***Northern Delta coast, Holland coast***	***Wadden Sea***	***Ems-Dollard coast***
Type	**NL NEA 1/26b North Sea**	**NL NEA 3/4 North Sea**	**NL NEA 3/4 Wadden Sea**	**NL NEA 3/4 Eems-Dollard***
Reference (CHLA 90 percentile in µg/l))	6.67	9.3	9.3	6.67
H/G boundary (CHLA 90 percentile in µg/l))	10	14	14	10
G/M boundary (CHLA 90 percentile in µg/l))	15	21	21	15
H/G boundary EQR	0.8	0.8	0.8	0.8
G/M boundary EQR	0.6	0.6	0.6	0.6

* Water body shared by NL and DE

7 WFD compliance checking of the Dutch assessment method

The WFD-compliance describes the quality of a of a national assessment method resulting from an evaluation of selected method features against the classification requirements laid down in the Water Framework Directive. WFD compliance checking represents the first step in the intercalibration process as “only results from WFD-compliant assessment methods can be intercalibrated” (EC 2010, p. 10). These method features cover all steps of biological assessment, including (amongst others) sampling design, taxonomical identification level, definition of reference conditions, and status class setting. A list of relevant WFD-compliance criteria is provided in EC (2010). The following WFD-compliance checking of the Dutch assessment method is performed against these criteria, using the information from the previous chapters.

Criterion 1:
Ecological status is classified by one of five classes (high, good, moderate, poor and bad).

✓ The Dutch assessment method classifies the ecological status by of five classes (Chapter 5).

Criterion 2:
High, good and moderate ecological status are set in line with the WFD’s normative definitions.

The WFD’s normative definitions address the following biological parameters on which to base the ecological classification (WFD Annex V, Section 1.2.4): Composition and abundance of phytoplanktonic taxa; phytoplankton biomass; frequency and intensity of planktonic blooms.

At good status, composition and abundance of phytoplanktonic taxa show *slight signs of disturbance*. Phytoplankton biomass shows slight changes (compared to high status) that *do not indicate any accelerated growth resulting in undesirable disturbance to the balance of organisms present in the water body or to the quality of the water*. A *slight increase* in the frequency and intensity of the natural planktonic blooms may occur.

At moderate status, composition and abundance of phytoplanktonic taxa show *signs of moderate disturbance*. Algal biomass is *substantially outside the range associated with high status, impacting upon other biological quality elements*. *A moderate increase* in the frequency and intensity of planktonic blooms may occur. *Persistent blooms* may occur during summer months.

The Dutch assessment method pursues a ‘statistical approach’ in boundary setting (Birk *et al.* 2012), referring to the first intercalibration exercise of coastal phytoplankton (Carletti & Heiskanen 2009). The good status boundaries of *Chlorophyll a* represent the reference concentrations multiplied by a factor of 1.5 (high-good boundary) and 2.25 (good-moderate boundary), respectively. The ecological status according to *Phaeocystis* blooms (i.e. > 10^6 cells/l) is high when occurring in only one month per year, good when occurring in two month per year and moderate when occurring in three months per year.

The ‘statistical approach’ in boundary setting allows for a convenient mapping of the ecosystem status. The downside of this approach is its ambiguous ecological relevance. The normative definitions refer to secondary effects caused by accelerated phytoplankton growth, resulting in undesirable disturbance to the balance of organisms present in the water body or to the quality of the water. These effects are not explicitly addressed in the boundary setting for *Chlorophyll a*, but considered by expert opinion when judging on the relevance of acceptable levels of phytoplankton biomass in coastal water bodies. The Dutch method is similar to the methods of other countries in this regard. With the moderate status being reached when planktonic blooms occur in more than two months per year, the *Phaeocystis* metric refers to 'persistent blooms' mentioned in the normative definitions.

✓ Despite the mere 'statistical approach' followed in status boundary setting, the Dutch assessment method defines high, good and moderate status in line with the WFD's normative definitions. Note that the biological parameter 'composition and abundance of planktonic taxa' is not covered by the Dutch assessment method (but see next criterion).

Criterion 3:
All relevant parameters indicative of the biological quality element are covered. A combination rule to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the BQE as a whole.

As written above, the WFD's normative definitions address the following biological parameters on which to base the ecological classification (WFD Annex V, Section 1.2.4): Composition and abundance of phytoplanktonic taxa; phytoplankton biomass; frequency and intensity of planktonic blooms. The Dutch assessment method covers phytoplankton biomass (using growing season mean P90 *Chlorophyll a* concentration) and frequency and intensity of planktonic blooms (using *Phaeocystis* cell count threshold and frequency of exceeding this threshold). Both parameters are combined at the level of the Ecological Quality Ratios (with the planktonic bloom parameter can only downgrade the overall assessment if showing lower EQRs than the biomass parameter).

In 2015, ECOSTAT agreed that for the assessment of the biological quality element phytoplankton in transitional and coastal waters *Chlorophyll a*, used as a proxy for phytoplankton biomass, is the most useful indicator and most sensitive to nutrient pressure. In this regard, national assessment methods only classifying the ecological status of phytoplankton in coastal water can be considered as WFD-compliant (Anonymous 2015).

✓ The Dutch assessment method covers both *Chlorophyll a* (proxy for planktonic biomass) and a submetric for planktonic blooms, being indicative of the biological quality element phytoplankton as a whole. A combination rule has been devised (Chapter 5).

Criterion 4:
Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT.

✓ The Dutch assessment method is adapted to intercalibration common types that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT (Chapter 2)

Criterion 5:
The water body is assessed against type-specific near-natural reference conditions.

✓ The Dutch assessment method assesses the water body against type-specific near-natural reference conditions (Chapter 5).

Criterion 6:
Assessment results are expressed as Ecological Quality Ratios (EQRs).

✓ The assessment results of the Dutch assessment method are expressed as Ecological Quality Ratios (Chapter 5).

Criterion 7:
Sampling procedure allows for representative information about water body quality/ ecological status in space and time.

✓ The Dutch monitoring program has at least one monitoring station per water body with a sufficiently high monitoring frequency (at least once a month) to adequately assess the Biological Quality Element phytoplankton (Chapter 3).

Criterion 8:
All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure.

✓ *Chlorophyll a* is commonly used as parameter and considered to be an appropriate proxy for phytoplankton biomass (Anonymous 2015).

Criterion 9:
Selected taxonomic level achieves adequate confidence and precision in classification.

✓ For *Chlorophyll a* this is not a relevant issue. The submetric for *Phaeocystis* blooms that is used by the Netherlands is assessed at species level (Chapter 5).

Overall conclusion
The Dutch assessment method using phytoplankton in coastal waters fulfils all criteria and can thus be regarded as fully WFD-compliant.

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A Water bodies typology, monitoring and reference conditions

Table 1. Overview of IC water type, WFD water bodies and monitoring stations, and reference conditions for the 90-percentile value of chlorophyll-a in the Dutch NEA3/4 water bodies (adapted from: Bonne & Desmit 2016), German NEA3/4 water bodies and Dutch NEA 1/26B water bodies

IC typology (*NL type*)	Water body	Monitoring stations	Reference condition P90 CHLa µg/l
Polyhaline exposed NL NEA 3/4 (*K1*) North Sea	NL95_2A Noordelijke Deltakust	Goeree 2, Goeree 6	9.30
	NL95_3A Hollandse kust	Noordwijk 2, Noordwijk 10	9.30
Polyhaline exposed NL NEA 3/4 (*K1*) Eems-Dollard	NL 81_3 Eems-Dollard (kustwater)	Huibertgat Oost	6.67
Polyhaline Wadden Sea type NL NEA 3/4 (*K2*)	NL81_1 Wadden Zee	Doove Balg West, Dantziggat	9.30
	NL81_10 Waddenzee Vastelandskust	-	9.30
DE NEA 3/4 N3 Polyhaline Open Coast Ems	Polyhaline Open Coastal Ems	Bork	6.67
DE NEA 3/4	Polyhaline open coastal Weser, Aussenelbe Nord, Eider Tidebecken, Piep Tidebecken, Dithmarscher Bucht, Polyhaline Wadden Sea Ems, Polyhaline Wadden Sea Weser West, Polyhaline Wadden Sea Weser East.	AuWe, WeMu, Norderelbe, Osee, Eider Fiegenplate, Süderpiep, Büsum, Norderney low-tide, Wuku, WeMu	4.80
DE NEA 1/26C	Euhaline open coastal Ems, Euhaline Wadden Sea Weser, Euhaline open coastal Eider	Norderney high-tide, Jadebusen, Amrum	3.33
Euhaline exposed NL NEA 1/26B North Sea	NL95_1A Zeeuwse kust	Walcheren 2	6.67
	NL95_4A Waddenkust	Boomkensdiep, Terschelling 10	6.67

B Results of the GLM analysis of chlorophyll-a in relation to nutrient concentration and monitoring station

GLM analysis of log (CHL-a 90-percentile) with log(DIP) as independent factor and monitoring station as random factor

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
LOG(DIP)	0.137	1	0.137	5.083	0.025
MONITORING STATION	2.477	8	0.310	11.493	0.000
Error	4.796	178	0.027		

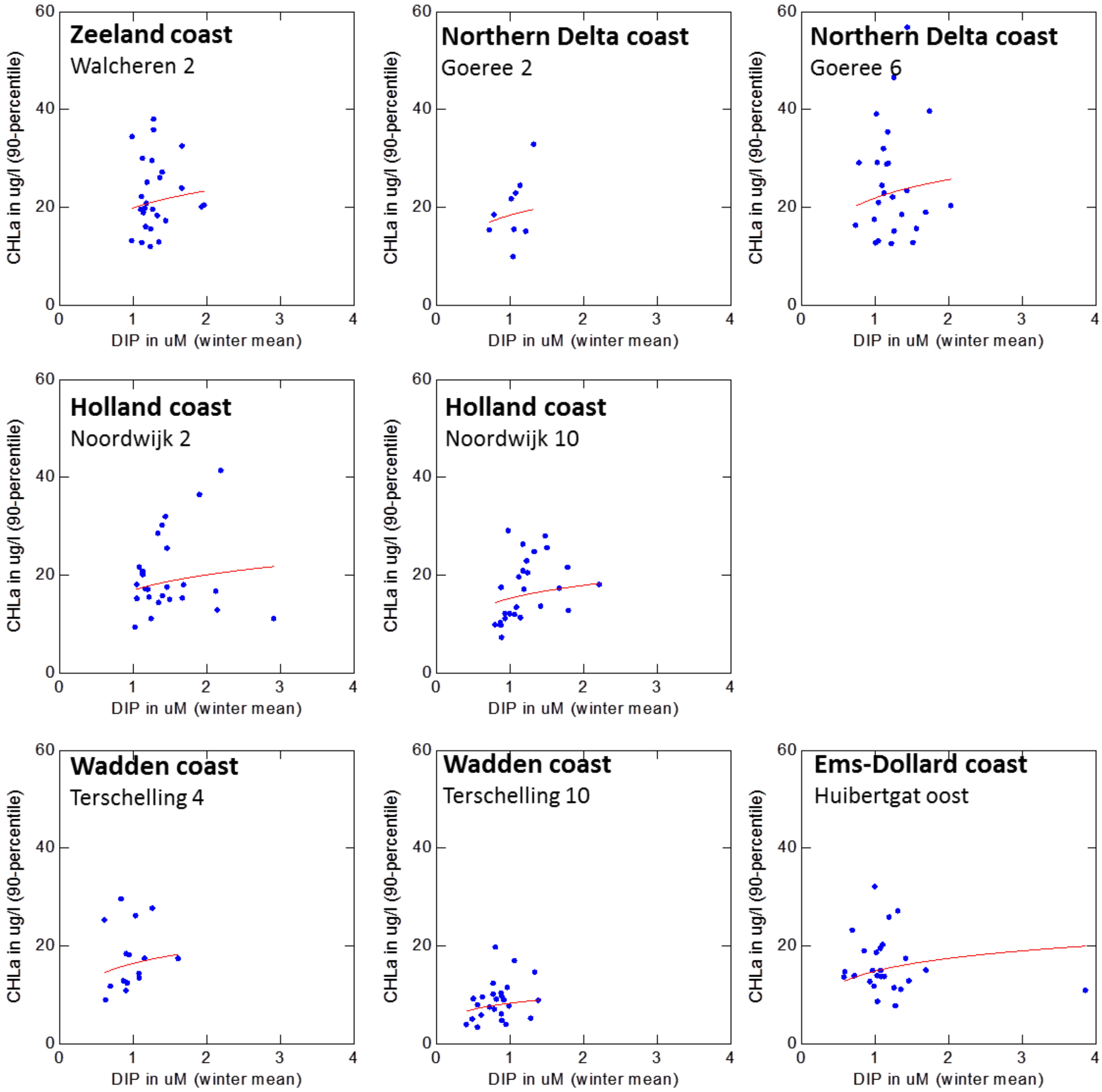

Figure B.1 Chlorophyll-a 90-percentile values (blue dots) and regression line (red) of chlorophyll-a against DIP. Results of a GLM analysis with DIP and monitoring station as independent factors.

GLM analysis of log (CHL-a 90-percentile) with log(DIN) as independent factor and monitoring station as random factor

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
LOG(DIN)	0.181	1	0.181	6.774	0.010
MONITORING STATION	2.170	8	0.271	10.162	0.000
Error	4.752	178	0.027		

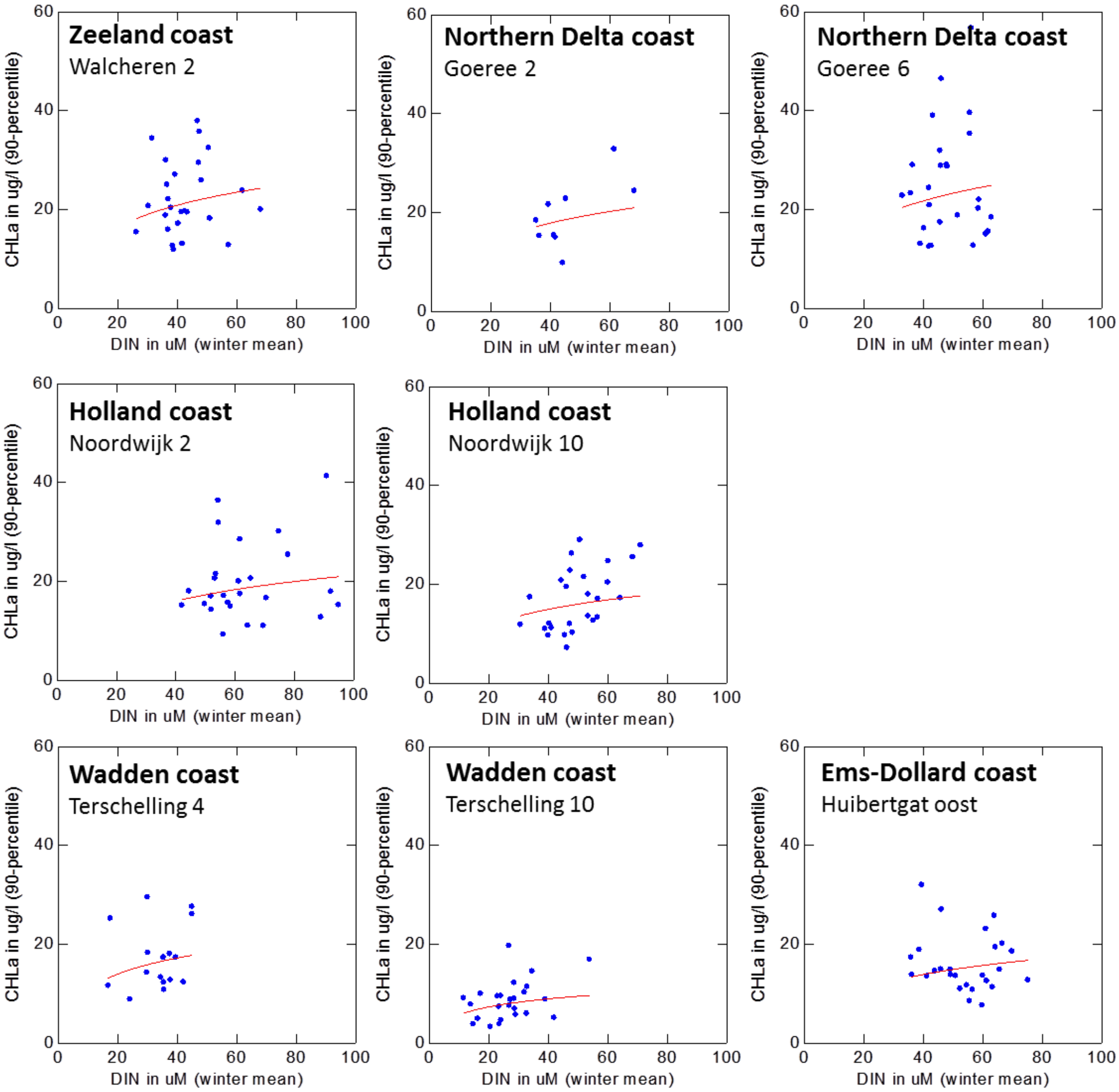

Figure B.2 Chlorophyll-a 90-percentile values (blue dots) and regression line (red) of chlorophyll-a against DIN. Results of a GLM analysis with DIN and monitoring station as independent factors.