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WFD phytoplankton metric Phaeocystis

Application in the Netherlands

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Samenvatting
This report gives a description of the background of the *Phaeocystis* metric, that is used for ecological status classification as part of the Phytoplankton quality element in the Water Framework Directive. The method used by the Netherlands, and the results of the application of the metric in Dutch coastal waters is presented. Several methodological issues are discussed. Finally, a comparison is made with the methods used by Belgium, Germany and the United Kingdom. The comparison shows that application of the metric differs between countries in sampling strategy and calculation methods.

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Contents

1 Introduction

The Water Framework Directive (WFD) establishes a framework for the protection of groundwater and surface waters (inland, transitional and coastal waters). The objective of the WFD is to reach good ecological status of natural water bodies and good ecological potential of highly modified water bodies by 2015.
Good ecological status/potential in marine coastal waters is assessed using three biological quality elements, viz. phytoplankton, other aquatic flora and benthic invertebrate fauna (Annex V, WFD). An intercalibration for each biological quality element is carried out, to achieve consistency and comparability in the classification results of each member state. The first phase of the Intercalibration was carried out from 2004-2007 (Carletti & Heiskanen, 2009), and is now being continued until 2011. The Netherlands participate in the Intercalibration Group for the North-East Atlantic (NEA-GIG).
For the application of the biological quality element Phytoplankton, besides chlorophyll several other metrics are being used in the North-East Atlantic. One of these metrics is the frequency of *Phaeocystis* blooms. This metric is applied by Belgium and The Netherlands, and is being considered by Germany and the United Kingdom.
For the purpose of the Intercalibration, The Netherlands have proposed to the NEA-GIG to give an extensive description of the background and the methods of this metric. This description should enable a comparison of methods used in the four countries.

This report gives a review of the scientific background of the metric, a description of the application in Dutch coastal waters, and a comparison with the application in Belgium, Germany and the United Kingdom.

1.1 Outline

Chapter 2 describes the scientific background of the application of this metric. The description is based on a review of recent literature, with a focus on the use of *Phaeocystis* as an indicator for eutrophication.
Chapter 3 gives an overview of the methods used in the monitoring of *Phaeocystis* in Dutch coastal waters.
The results of the application of the *Phaeocystis* metric in Dutch coastal waters are presented in Chapter 4, and some of the methodological aspects are evaluated.
A comparison of the application of the metric in the Netherlands, Belgium and the United Kingdom is presented in Chapter 5.

2 Background

2.1 Development of the WFD quality element Phytoplankton

The ecological status of coastal waters in the WFD has to be determined by the status of three biological quality elements. Phytoplankton is one of these elements. The status of phytoplankton has to be assessed on the basis of its composition, its abundance and its biomass.

As the main anthropogenic pressure for phytoplankton in Dutch coastal waters is nutrient enrichment, The Netherlands has taken the OSPAR Comprehensive Procedure (OSPAR, 2005) as point of departure for the development of the WFD phytoplankton metrics. The Comprehensive Procedure is the tool, developed by OSPAR, for eutrophication assessment of the North Sea and the north-east Atlantic. It is based on a conceptual framework to assess eutrophication, distinguishing causative factors (degree of nutrient enrichment), direct effects, indirect effects and other possible effects (Figure 2.1). The direct effects of nutrient enrichment on phytoplankton are assessed by several parameters, viz. the area specific concentration of chlorophyll-a, the abundance and the bloom duration of nuisance or toxic phytoplankton indicator species. The Comprehensive Procedure provides a list of phytoplankton indicator species, containing a number of (potentially) toxic species. However, there is considerable scientific uncertainty about the link between nutrient enrichment and the presence of potentially toxic species (ICES, 2004). Consequently, these indicator species have not been included in the WFD quality element Phytoplankton.

In addition to these toxic algae there are species that form dense blooms, causing negative effects due to their high biomass.The most prominent species in the southern North Sea is the foam-forming alga *Phaeocystis* spp. (Tillmann and Rick, 2001). For *Phaeocystis* there are indications that there is a link between nutrient loads and the occurrence of dense blooms of this species. Therefore, The Netherlands included *Phaeocystis* as a metric in the WFD quality element Phytoplankton. In the Netherlands, the quality element Phytoplankton (Van der Molen and Pot, 2007) combines the area-specific concentration of chlorophyll-a as one metric and the frequency of *Phaeocystis* blooms as the second metric. The chlorophyll metric is used as a proxy for phytoplankton biomass, while the *Phaeocystis* metric describes features of phytoplankton composition and abundance related to anthropogenic disturbance of the ecosystem.

2.2 *Phaeocystis*

The genus *Phaeocystis* consists of several species, with *P. globosa* and possibly *P. pouchetii* relevant in Dutch marine waters. In general *P. globosa* occurs in temperate and tropical regions, while *P. pouchetii* prefers lower temperatures and occurs in artic waters, but they can overlap in distributional ranges (Schoemann et al., 2005). In Dutch coastal waters the main representative of *Phaeocystis* is *P. globosa*, but sometimes *P. pouchetii* is recorded (Philippart et al., 2000). Although the two species are difficult to distinguish from each other, it is most likely that the vast majority of *Phaeocystis* cells observed in Dutch coastal waters belong to *P. globosa*.

It is a multifaceted species, that occurs in three different life forms: as flagellated single cells, as unflagellated single cells and as colonies comprising any number of single cells without flagellae in a mucus matrix (Rousseau et al., 2007). Colony formation has been ascribed to several factors, like phosphate depletion, light intensity, turbulence and chemical substances. The success of *P. globosa* colonies during blooms has been related to the physiology and ecology of the gelatinous colonies. The colony acts as an energy and nutrient reservoir, and provides an additional competitive advantage as large colonies are not, or insignificantly,

grazed (for extensive reviews: see Schoemann et al., 2005; Rousseau et al., 2007; Nejstgaard et al., 2007).
It is generally the colony form of *Phaeocystis* that forms dense blooms. These blooms are a natural phenomenon, associated with nutrient enriched environments, occurring both in areas with high anthropogenic nutrient inputs (e.g. southern North Sea, Arabian Gulf) as well as in naturally enriched seas like the Greenland Sea and in the Barents Sea (Schoemann et al., 2005). Already more than a century ago *Phaeocystis* blooms along the Dutch coast have been recorded (Cadée and Hegeman, 2002).

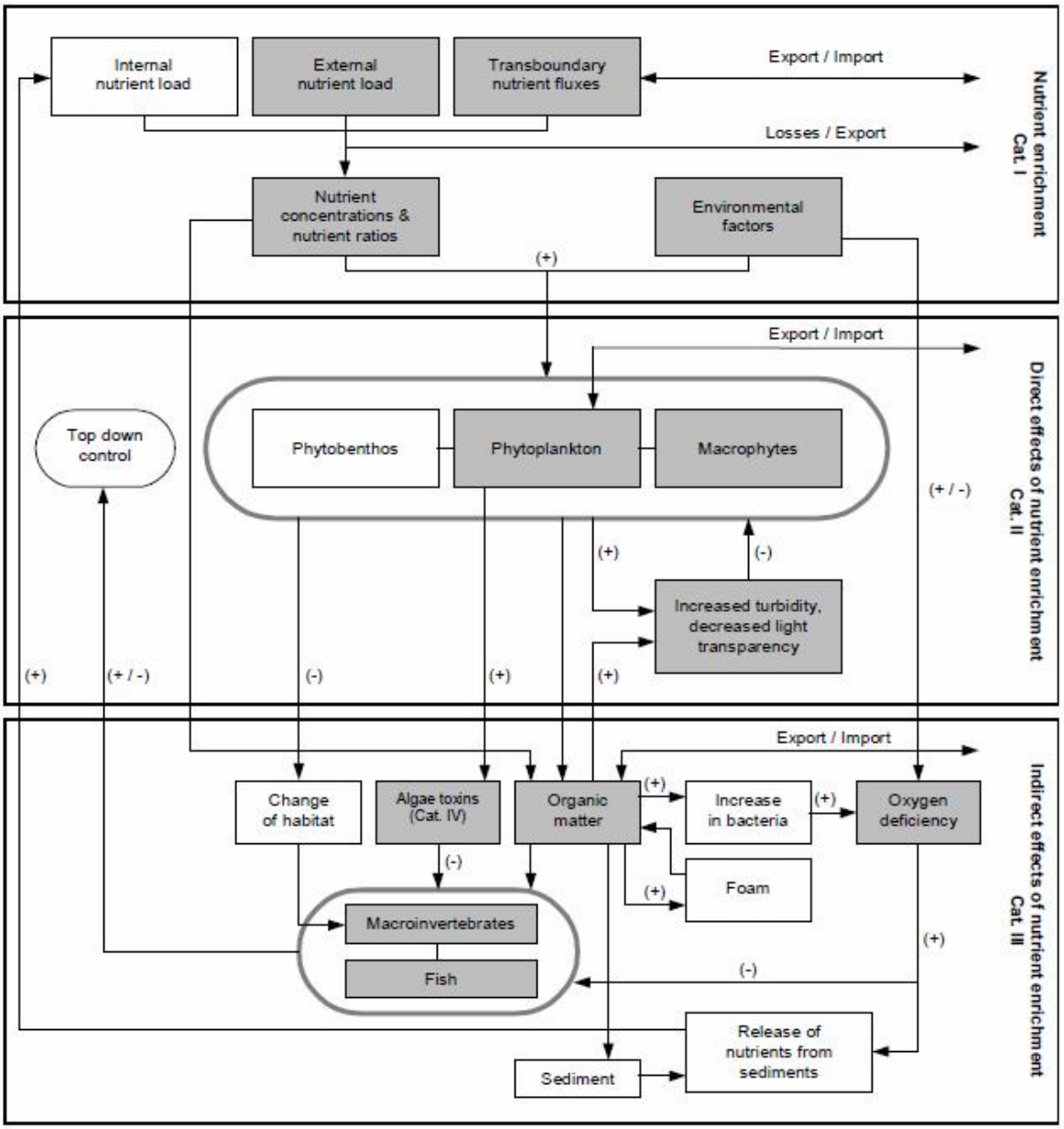

Figure 2.1 Generic conceptual framework to assess eutrophication in all categories of surface waters (OSPAR, 2005).

2.3 Relations of *Phaeocystis* blooms with environmental factors

Many papers have been published about possible relations between the occurrence of *Phaeocystis* blooms and abiotic as well as biotic environmental factors. Some papers are based on field observations and other on the results of laboratory experiments and modeling. The findings are often contradictory or inconclusive, which is also the conclusion of Tillmann and Rick (2001) in their review of North Sea phytoplankton.

Phaeocystis blooms in the southern North Sea often follow the spring diatom bloom, and in Dutch coastal waters peak levels generally occur in April/May (Veldhuis et al., 1986), as is illustrated by Figure 2.2. It was hypothesized that *Phaeocystis* can dominate the phytoplankton as soon as silicate becomes limiting for the diatom bloom, and still sufficient N and P remain for the growth of *Phaeocystis* (Egge and Aksnes, 1992). In mesocosm experiments, however, this hypothesis has been falsified (Escaravage et al., 1995), and Peperzak et al. (1998) observed *Phaeocystis* blooms in the Oosterschelde estuary at high Si concentrations as soon as the precondition of exceeding a threshold intensity of light was satisfied.

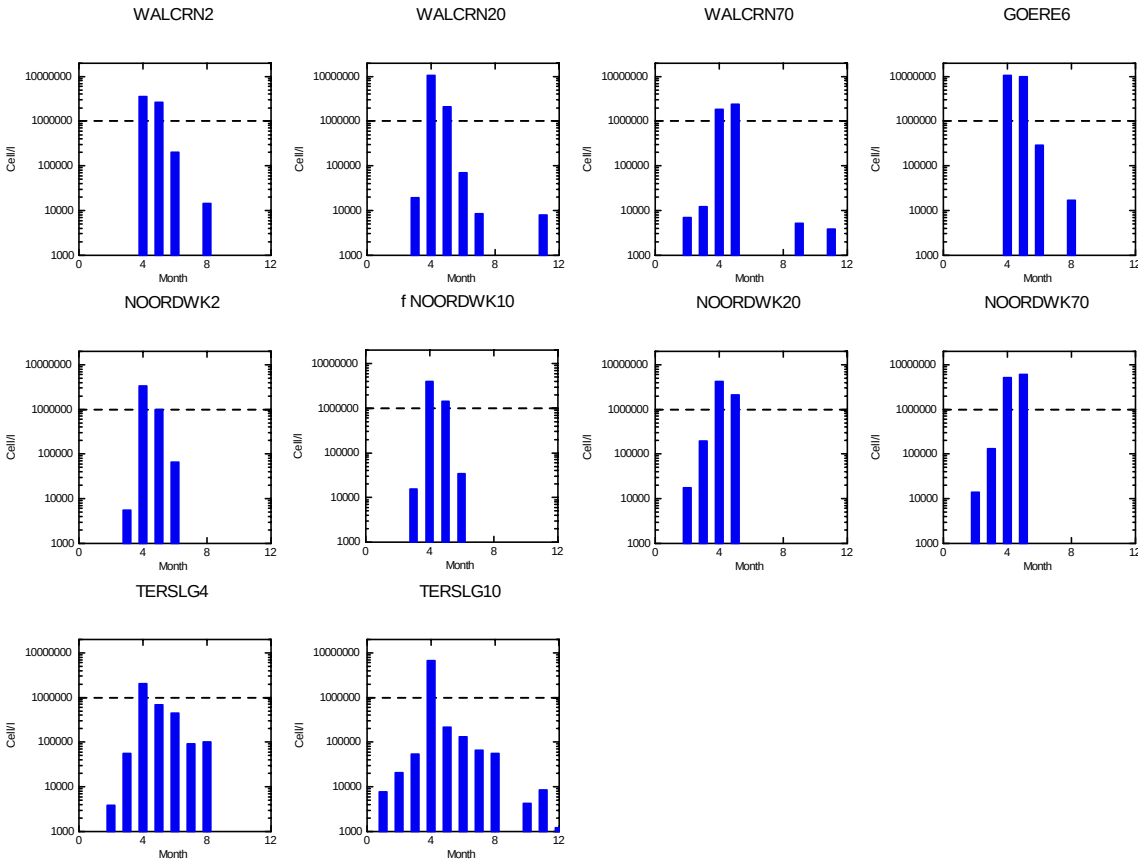

Figure 2.2 Monthly medians of cell numbers of Phaeocystis *(medians for the period 1990-2007) for ten monitoring stations in the southern North Sea (see Appendix A for map of monitoring stations). Note log scale on the vertical axis. The dashed line indicates a concentration of 10^6 cells/l.*

Light intensity clearly plays a role in the timing of the start of the bloom. According to Peperzak (1993) the spring bloom starts with the formation of the first colony stage in a non-turbulent environment when daily irradiance exceeds 100 W h m^{-2} day^{-1}. Temperature is not a condition for the beginning of the bloom, but modifies the maximum growth rate (Schoemann et al., 2005). Another factor that also may play a role in the competition between diatoms and *Phaeocystis* is the CO_2 concentration in the water (Tillmann and Rick, 2001). At low CO_2 concentrations or increased pH levels *Phaeocystis* seems to be able to outcompete diatoms (Schoemann et al., 2005). The ratio in the availability of oxidized nitrogen (NO_3^-) versus reduced nitrogen ($NH4^+$) has also been suggested to promote *Phaeocystis* dominance (Riegman et al., 1992).

It seems quite logical to expect nutrient availability to play a major role in phytoplankton bloom dynamics, but the empirical relationship between nutrient enrichment and *Phaeocystis* blooms is still not very clear.
Cadée and Hegeman (2002) studied a long-term data set from the Marsdiep, a tidal inlet in the western Wadden Sea. The data set comprised phytoplankton observations from the early 1970s to 2002. In the early 1970s phytoplankton biomass and primary production was relatively low, and doubled in size by the end of the 1970s. The duration of the *Phaeocystis* bloom increased as well. These increases where seen as an effect of eutrophication, as unchanged Secchi depth data for this period indicated that light availability did not change. From 1994 on primary production, chlorophyll-a concentrations, *Phaeocystis* cell numbers and bloom duration decreased. But, as the authors conclude from historical data on *Phaeocystis* in the Marsdiep at the end of the 19th century, also in that period, i.e. before large-scale eutrophication started, blooms of 50 to 60 days occurred (Figure 2.3).

In the Dutch part of the North Sea, the highest maximum cell numbers of *Phaeocystis* are observed at monitoring stations near the coast, and in particular at stations near the major river discharges of Haringvliet and Nieuwe Waterweg (stations GOERE6, NOORDWK2, NOORDWK10, NOORDWK20) (Figure 2.4). This illustrates the general relationship between nutrient enrichment and the occurrence of *Phaeocystis* blooms.
Van Duren (2006) showed that the number of *Phaeocystis* cells in April/May shows a positive, but weak, relation with the concentration of dissolved inorganic nitrogen (DIN) and with the N:P ratio. However, this type of comparisons combines observations from many years and many stations, disregarding spatial differences and factors causing interannual variations (e.g. meteorology). While it may give a “broad-brushed” relationship between nutrients and phytoplankton concentrations, it can not be considered a reliable dose-response relationship (Carstensen and Henriksen, 2009). An analysis of the relation between nitrogen en phosphorus concentrations and *Phaeocystis* maximum cell numbers for station GOERE6, which is the station with the strongest river influence, shows a significant correlation ($p<0.05$) between nitrogen concentration and bloom intensity. However, the data also illustrate the large interannual variation in bloom magnitude, that is not explained by differences in nitrogen concentration. For other Dutch monitoring stations the relationship between nitrogen concentration and maximum abundance is less clear, and interannual variability in the peak level of blooms is large.
The large interannual variability may be a reason why a decrease in *Phaeocystis* blooms is not apparent in the Dutch coastal North Sea waters, in contrast to the observations in the Marsdiep (Cadée and Hegeman, 2002; Baretta-Bekker et al., 2009; Prins et al., submitted).

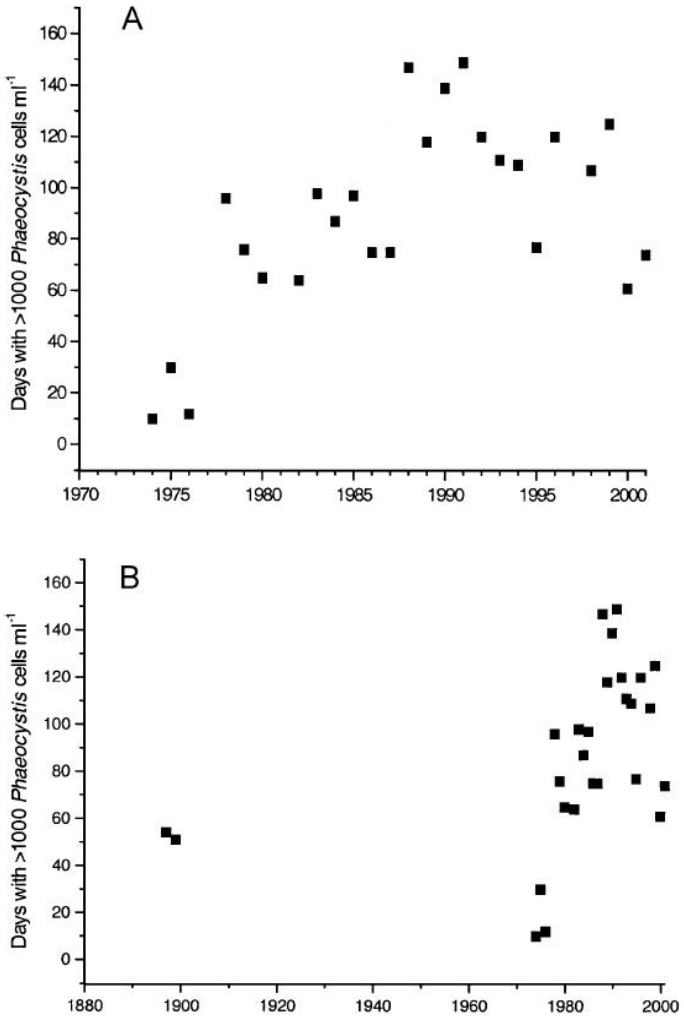

Figure 2.3 Number of days per year with a Phaeocystis *bloom of > 10^6 cells/l in the Marsdiep, in the period 1970-2002 (panel A) and data added for 1897 and 1899 (panel B). From: Cadée and Hegeman, 2002.*

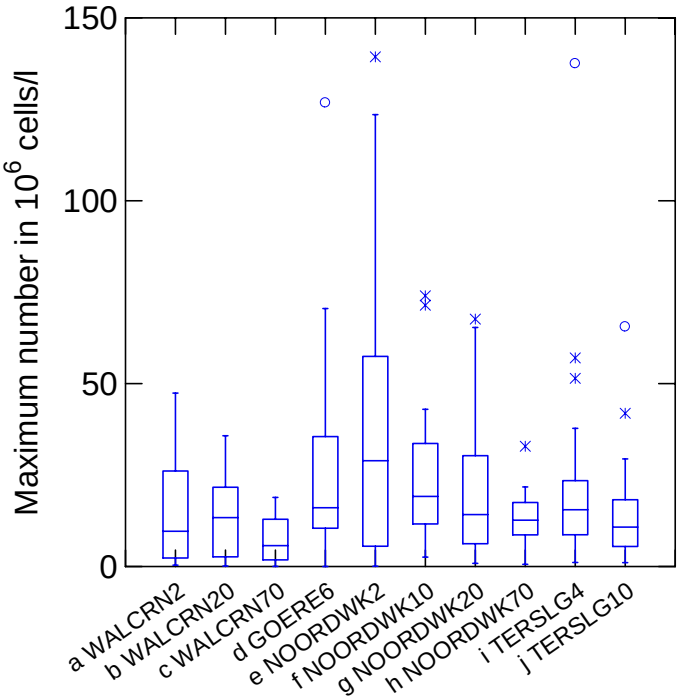

Figure 2.4 Maximum cell numbers of Phaeocystis *(period 1990-2007) at ten monitoring stations in the southern North Sea (see Appendix A for a map of monitoring stations)..*

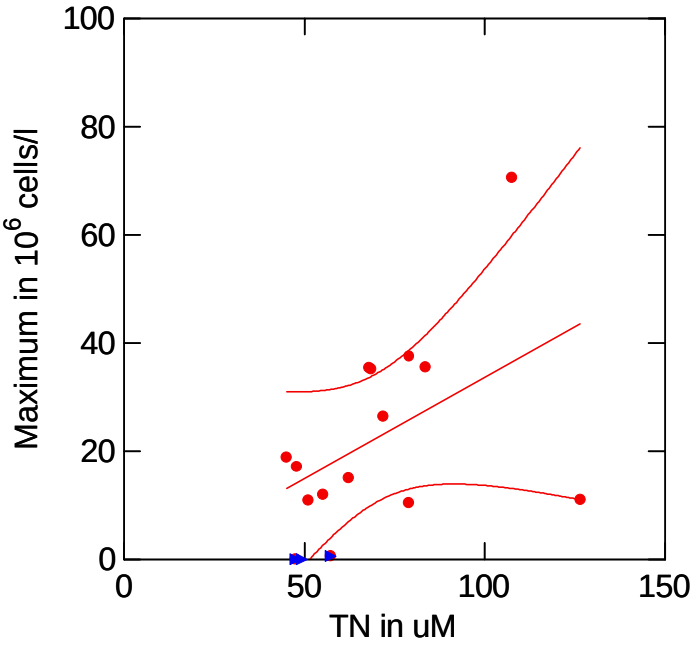

Figure 2.5 Maximum number of Phaeocystis *cells in April-May (period 1990-2007) in relation to the average concentration of total-N (April-May) at station Goeree 6.Blue symbols: years 2000, 2002 and 2006 when Phaeocystis blooms were very low in all Dutch coastal waters.*

Gieskes et al. (2007) state that eutrophication is not the explanation for the long-term variation in *Phaeocystis* abundance in the southern North Sea. They even claim that data of the Continuous Plankton Recorder survey show a higher abundance and longer growing season of *Phaeocystis* before 1965 in the open sea, when eutrophication was quite low (Philippart et al., 2000). Another puzzling aspect is their claim that *Phaeocystis* accumulates preferentially offshore. However, this inverted biomass gradient from onshore to offshore is not seen in the long-term dataset of the Dutch monitoring programme (Baretta-Bekker et al., 2009; see also Figure 2.4). Based on the same CPR data McQuatters-Gollop et al. (2007) conclude that climatic variability and water temperature may be more important than changes in nutrient concentration. However, it should be noted that the CPR data have a limited coverage of Dutch coastal waters, and mainly cover the offshore parts of the Dutch EEZ.

Ecological conditions in the southern North Sea as a whole are highly variable and dependent on the meteorological “history” (wet or dry, calm or windy, sunny or cloudy). The size of nutrient pools at the beginning of the spring bloom determines the maximum abundance of phytoplankton (Muylaert et al., 2006; Tillmann and Rick, 2001). Gypens et al. (2007) mention annual and decadal fluctuations in nutrient loads, caused by fluctuations in meteorological conditions in the drainage basins, as cause of the variability in *Phaeocystis* occurrence. Grazing by zooplankton may delay the increase of the *Phaeocystis* population. In other words, both bottom-up and top-down processes modify the onset and progression of bloom formation (Schoemann et al., 2005).

In order to explain the development and interannual variation of *Phaeocystis* blooms in Belgian coastal waters an integrated biogeochemical/ecological model (Lancelot et al., 2007) has been applied by Gypens et al. (2007). Their conclusion is that the variability of *Phaeocystis* is mainly controlled by nutrient availability and that N-reduction will decrease *Phaeocystis* blooms, whereas P reduction will decrease diatom biomass. They conclude therefore that reduction of the N loads from the Belgian rivers and from the Strait of Dover can reduce the abundance of *Phaeocystis* in the Belgian Coastal Zone, without affecting the diatoms negatively.

Despite the lack of a clear-cut cause-effect relationship between abiotic conditions and *Phaeocystis* abundance, there is a trend in *Phaeocystis* abundance, maximum peak levels and bloom duration, which closely mimics 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.

2.4 Ecological effects of *Phaeocystis* blooms

According to Smayda (1997), the subjective, differing and arbitrary criteria used to define phytoplankton blooms and their presumed negative ecological impact hamper a dispassionate evaluation of the role of *Phaeocystis* in the coastal ecosystem. However, by many authors *Phaeocystis globosa* is regarded a nuisance algal species for several reasons. *Phaeocystis* is associated with disruption of the pelagic food web, accumulation of high amounts of organic matter in sediments and foam formation on beaches. In addition, its production of dimethyl-sulphide precursors may promote acid rain (Liss et al., 1994) and *Phaeocystis* blooms have been reported to cause floating slicks on the water, to clog nets and produce bad odour.

There is conflicting information about the trophic fate of the *Phaeocystis* blooms. The accumulation of high biomass indicates that grazing on *Phaeocystis* during the colonial bloom phase Is limited. It is uncertain however, whether this is due to low nutritional value, or whether this is caused by the fact that the size of the colonies or the mucilaginous matrix

prevents grazing by zooplankton (Hamm et al., 1999; Hamm, 2000; Schoemann et al., 2005; Nejstgaard et al., 2007). Lancelot et al. (2009) stated that colonies > 400 µm diameter generally are too large to be grazed by copepods. Those colonies can be expected at concentrations above $4*10^6$ cells/l. Relatively low grazing on *Phaeocystis* colonies has been observed for smaller species of copepods (Schoemann et al., 2005; Nejstgaard et al., 2007), but also for bivalves (Kamermans, 1994; Smaal and Twisk, 1997).

The accumulation of high biomass in the water column during the colonial phase of *Phaeocystis* blooms has several consequences. The production of organic matter by *Phaeocystis* mainly fuels the microbial food web (Rousseau et al., 2000). At the end of the blooms, colony disruption results in the accumulation of mucilaginous aggregates on the sea surface, and eventually sedimentation of this organic material may increase the risk of hypoxia in sediments. Cadée (1996) showed that *Phaeocystis* colonies can accumulate in tidal sediments in the Wadden Sea. Although he suggested that this may lead to anoxic conditions, he also stated that such conditions rarely occur in the western Dutch Wadden Sea and have never been related to massive deposition of *Phaeocystis* material. An event of mussel mortality at some production sites in the Oosterschelde was linked to anoxia after sedimentation of *Phaeocystis* (Peperzak and Poelman, 2008). The authors argued that eutrophication in combination with meteorological and hydrodynamical factors was causing this event. The anoxia could not be explained from the magnitude of the bloom alone, as in other years with blooms of a similar or higher magnitude no mussel mortality was observed. Deposition of organic material and foam on intertidal sediments has been shown to increase sediment oxygen demand (Rauch et al., 2008; Spilmont et al., 2009). The increased oxygen demand and the formation of a crust on the sediment resulting from drying foam occasionally leads to anoxia, resulting in a high mortality among the benthic community (Desroy and Denis, 2004; Rauch et al., 2008; Spilmont et al., 2009). Some organisms also tended to migrate upward and were then directly accessible to the higher trophic level represented by birds (Desroy and Denis, 2004). The severity of the effects on the macrobenthic community, and the length of the recovery time (from weeks to months) are very dependant on local hydrodynamic and topographic conditions and sediment type (Desroy and Denis, 2004; Spilmont et al., 2009), and cannot be related in a straightforward manner to bloom magnitude.

Foam accumulation on beaches is a common phenomenon in spring after the collapse of the bloom. A study of foam formation on the Dutch coast showed that foam formation mainly occurs when blooms exceed the threshold of 1 million cells/l (Peperzak, 2002). Blauw et al. (2010), using a simple fuzzy logic model, showed that foam events are more frequent and intense in years with blooms of >10 million cells/l. However, even under suitable conditions foam is not always observed.
In addition to ecological impacts, foam formation on beaches could also have negative economic effects. After an enquiry under tourists and fishermen along the Belgian coast, it was concluded that *Phaeocystis* blooms are not perceived as a nuisance by these two groups and probably induce very limited economic losses (Rousseau et al., 2004). An economic analysis, using a contingent valuation method, indicated however that tourists might be willing to pay for measures to reduce adverse effects of eutrophication (Stolte et al., 2003). However, at present there is no evidence of adverse economic effects of foam formation.

2.5 Threshold value in relation to undesirable effects

The discussion on effects of *Phaeocystis* blooms in the previous paragraph, illustrates the difficulty of defining a threshold in the abundance of *Phaeocystis* for the boundary between “good” status and “moderate” ecological status.

In the Dutch *Phaeocystis* metric, the bloom definition of an abundance of 10^6 cells/l used by Cadée and Hegeman (1991) has been applied to assess the duration of the bloom (Van der Molen and Pot, 2007). The abundance threshold (10^6 cells/l) was chosen fairly arbitrarily by Cadée and Hegeman (1991), and could therefore be considered disputable as a threshold value. However, Schoemann et al. (2005) also mention this number of cells indicating the presence of colonies. Foam formation on beaches also seems to occur above this threshold of 1 million cells/l (Peperzak, 2002), although the events remain unpredictable to some extent (Blauw et al., 2010). In the Intercalibration in the NEA-GIG it was also agreed to use the threshold of 10^6 cells/l (Carletti & Heiskanen, 2009)

Recently Lancelot et al (2009) defined the maximum diameter of grazable *Phaeocystis* colonies, based on experimental results, as being 400 µm. Colonies smaller than 400 µm occurred at a *Phaeocystis* cell abundance up to $4*10^6$ cells/l, while higher abundances were associated with the occurrence of high densities of larger colonies. The authors argued that this threshold of $4*10^6$ cells/l was thus a good indicator of ecosystem disturbance, as it is based on trophic criteria, and indicates when the blooms becomes ungrazable and high *Phaeocystis* biomass will start to accumulate in the water column. Consequently, they proposed that the threshold of $4*10^6$ cells/l was better suited for the assessment of an undesirable deviation from reference levels than the threshold of 10^6 cells/l. In WFD terms, this threshold of $4*10^6$ cells/l could be considered to represent the boundary between " good" and " moderate" ecological status (see Annex B for definitions of ecological status): there is an "undesirable disturbance", and "algal biomass (…) is such as to impact upon other biological elements".
In the same paper, the authors show that application of an ecosystem model simulating the Belgian coastal zone calculated the same abundance ($4*10^6$ cells/l) of *Phaeocystis* as a maximum under pristine conditions. If this abundance is considered to represent an undisturbed ecosystem, it would, in WFD terms, be more appropriate to translate it into the boundary between *high* and *good* ecological status (see Annex B for definitions of ecological status): "The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions".
Thus, there seems to be an inconsistency in the threshold definition of Lancelot et al. (2009), as this threshold could apply to both the *High/Good* and the *Good/Moderate* boundary.

The Dutch *Phaeocystis* metric uses the threshold of 10^6 cells/l to define a bloom, and based on this definition annual bloom frequency is calculated. This metric was developed before the results of Lancelot et al. (2009) were published. The threshold proposed by Lancelot et al. (2009) applies to the annual maximum abundance, which is not analogous with bloom frequency. This point will be further discussed in Chapter 4.

2.6 Other phytoplankton indicators

The Netherlands use the area-specific concentration of chlorophyll-a as one metric and the frequency of *Phaeocystis* blooms as the second metric in the quality element Phytoplankton (Van der Molen and Pot, 2007). The chlorophyll metric is used as a proxy for phytoplankton biomass, while the *Phaeocystis* metric describes features of phytoplankton composition and abundance related to anthropogenic disturbance of the ecosystem.
In addition to these two metrics, other metrics haven been applied in the OSPAR Comprehensive Procedure, or in WFD assessments by other member states.
In the OSPAR COMPP a list of phytoplankton indicator species is used, with regionally specific threshold values for the definition of a bloom. This list contains several (potentially) toxic species. As already mentioned in §2.1, there is considerable scientific uncertainty about the link between nutrient enrichment and the presence of potentially toxic species (ICES,

2004). An analysis based on Dutch monitoring data showed that there is generally no unambiguous relation between blooms of these indicator species and nutrient enrichment, and that other, abiotic, factors like hydrodynamics are important (Van Duren, 2006). Because of the lack of clear relations between blooms of these species and nutrient enrichment, these indicator species have not been included in the WFD quality element Phytoplankton.
The use of phytoplankton species other than *Phaeocystis* or the indicator species from the OSPAR COMPP, as an indicator of elevated blooms, was not considered during the development of the Dutch WFD quality element Phytoplankton (Van der Molen and Pot, 2007). This choice was based on lack of evidence of dense blooms of other species in relation to eutrophication.

Recently, Devlin et al. (2007) developed an alternative approach, that takes into account blooms of any species (excluding *Phaeocystis*) of phytoplankton exceeding counts of 10^6 cells l^{-1}, and blooms where cell counts (of all species combined) exceed 10^7 cells l^{-1}. An index was developed that combines four metrics, viz. 1) *the occurrence of chlorophyll levels above a certain threshold*, 2) *the occurrence of Phaeocystis blooms >10^6 cells l^{-1}*, 3) *the occurrence of other species blooms >10^6 cells l^{-1}*, and 4) *the occurrence of total cell counts >10^7 cells l^{-1}*. This combined metric showed a positive correlation with an estimated risk index from nutrient enrichment for Irish and UK transitional and coastal water bodies. However, Devlin et al. (2007) do not show how the four metrics perform individually in response to this risk index.
The metric *occurrence of other species blooms >10^6 cells l^{-1}* has been proposed in the NEA GIG (Carletti & Heiskanen, 2007) as well.
A preliminary analysis of the phytoplankton data from the Dutch national monitoring programme has been done to analyze the suitability of the metrics *occurrence of other species blooms >10^6 cells l^{-1}* and *occurrence of total cell counts >10^7 cells l^{-1}*:

- Data from a selection of stations in the coastal waters of the North Sea showed that six diatom species sometimes have blooms with concentrations > 10^6 cells l^{-1}. Of these species, the diatom *Chaetoceros socialis* was by far the most dominant species, and the only species that forms blooms that last longer than two months (this duration is considered to be equivalent for *moderate* status in the case of Phaeocystis, see Chapter 4). The analysis showed that long-lasting blooms of this species occurred only in the year 2001, a year when *Phaeocystis* blooms were virtually absent. Thus, based on this preliminary analysis it could be concluded that applying the metric *occurrence of other species blooms >10^6 cells l^{-1}* would have resulted in the inclusion of *C. socialis* blooms. This bloom occurred in 2001, when *Phaeocystis* blooms were absent, and consequently only for this year (in an observation period of 18 years) the use of this metric would have given additional information. Whether the use of this metric also would have resulted in a change in the classification of the status of the coastal water bodies (which is also determined by chlorophyll concentrations) has not yet been analyzed.
- Data from the Dutch monitoring programma show that cell counts > 10^7 cells l^{-1} occur very frequently (>15% of all observations). Application of this metric would require validation for the conditions in Dutch coastal waters, as the threshold of 10^7 cells l^{-1} seems to be too low. Whether application of this metrics would supply information about the status of the coastal waters that is additional to the information derived from application of the metrics for chlorophyll-a and *Phaeocystis*, and results in adaptation of the status assessment, still needs to be analyzed.

Another approach developed by Devlin et al. (2007), is the use of seasonal succession of functional groups (diatoms, dinoflagellates, other flagellates, *Phaeocystis*). This method requires the description of a reference, based on observations in waters with a *high* status. As

the authors indicate, this approach needs further development and validation. Lack of water bodies at *high* status, as is the case in the Netherlands, complicates the application of this approach.

3 The marine phytoplankton sampling programme

3.1 Sampling and analysis

The whole sampling and analysis method of Rijkswaterstaat (RWS) is described here, from the sampling in the field to the microscopy in the laboratory. This description is based on RWS reports on the national monitoring programme, on analysis protocols, on guidelines for the WFD monitoring and on a report by Koeman et al. (2009) on the phytoplankton analysis. This description focuses on *Phaeocystis* in Dutch coastal waters.

3.1.1 Stations

Since the 1970s The Netherlands has run an extensive national programme to monitor environmental variables at fixed stations in the fresh, estuarine and marine surface waters. In the first decades of the programme the only observed phytoplankton variable was chlorophyll-a, but from 1990 on microscopical enumeration of phytoplankton was added, which delivered phytoplankton abundance data, viz. the number of cells per taxon per unit water volume.
Table 3.1 and Figure 3.1 show the stations of the national monitoring programme, that are used for the monitoring of the coastal water bodies relevant for the WFD. With the implementation of the WFD, some monitoring stations had to be adjusted, because in a few WFD water bodies monitoring stations were missing. In the coastal waters two stations were added (GOERE2 in the Northern Delta coast and DOOVBWT in the Wadden Sea) and one station was replaced (in the WFD water body Wadden coast TERSLG4 was replaced by BoOOMKDP). For the years before 2007 GOERE6, located 6 km off the coast and TERSLG4, located at 4 km off the coast, have been used.

Water body	Water type	Location	X-coord / Lat.	Y-coord / Lon.	From	To
Ems-Dollard coast	NEA 3	HUIBGOT	53°32'53"N	06°39'24"O	1990	recent
Wadden coast	NEA 1/26b	TERSLG4	53°24'55'N	05°09'02''E	1990	2006
		BOOMKDP	53°22'00"N	05°07'60"E	2007	recent
Holland coast	NEA 3	NOORDWK2	52°15'41''N	04°24'22''E	1990	recent
N. Delta coast	NEA 3	GOERE6*	51°52'11''N	03°52'25''E	1990	2006
		GOERE2	51°50'49''N	03°50'05''E	2007	recent
Zeeland coast	NEA 1/26b	WALCRN2	51°32'56''N	03°24'39''E	1990	recent
Wadden Sea	NEA 4	DANTZGT	53°23'60"N	05°43'00"E	1990	recent
		DOOVBWT	53°03'02"N	05°05'06"E	2007	recent
Oosterschelde	NEA 4	WISSKKE	51°36'09"N	03°43'10"E	1990	recent

*GOERE6 is still included in the monitoring programme, but not used for the WFD

Table 3.1. The monitoring stations in the Dutch coastal water bodies, used for the WFD phytoplankton monitoring, with beginning and end of the monitoring period.

Figure 3.1 Map of The Netherlands with the boundaries of the coastal (and transitional) water bodies. The stations, listed in Table 1 are given as red dots.

Location	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	GS Tot	Year Tot
Eems-Dollard c.														
HUIBGOT	1	2	2	2	2	2	1	2	2	1	1	1	13	19
Wadden coast														
BOOMKDP	1	1	3	2	2	2	1	3	1	1	1	1	14	19
Holland coast														
NOORDWK2	2	1	2	2	2	2	1	3	1	1	1	1	13	19
N. Delta coast														
GOERE2	1	1	1	1	1	1	1	1	1	1	1	1	7	12
GOERE6	1	1	1	1	1	1	1	1	1	1	1	1	7	12
Zeeland coast														
WALCRN2	1	1	1	1	1	1	1	1	1	1	1	1	7	12
Wadden Sea														
DANTZGT	1	1	3	2	2	2	1	3	1	1	1	1	14	19
DOOVBWT	1	1	3	2	2	2	1	3	1	1	1	1	14	19
Oosterscheldet														
WISSKKE	1	1	1	2	2	3	2	2	2	1	1	1	14	19

Table 3.2 The stations of the 2010 WFD monitoring programme in the Dutch coastal water bodies, with the monthly sampling frequency planned in 2010 (RWS, 2009). The yellow columns refer to the months in the so-called "growing season"

3.1.2 Frequency

The present Dutch WFD monitoring programme formally requires sampling of one station per WFD water body, with a frequency of monthly sampling during March-September, i.e a total of seven samples per year.

However, at present the routine national monitoring programme is (still) more extensive. In this national programme the stations are sampled year round. Table 3.2. shows the sampling frequency per month, as planned in 2010 (RWS, 2009). Sampling in the national programme is performed at least monthly, while in the growing season more frequent sampling is carried out at many locations. A number of stations have a frequency of 19 samples per year. These stations are sampled with a 4 week interval from January to March, a 2-3 week interval from April to September, and a 4 week interval from October to December. Several other stations have a frequency of 12 samples per year, and are sampled with a 4-5 week interval.

For a number of stations, the sampling frequency in the years before 2010 differed from the programme shown in Table 3.2. Station DOOVBWT was not sampled, and BOOMKDP was only sampled in 2007-2009, with a lower frequency than in 2010.

For various reasons such as bad weather, fixation errors or analytical errors, the number of elaborated samples may be lower than the scheduled number of samples per station.

3.1.3 Sampling

Water samples are taken at 1 m below the water surface. If the water column is stratified two additional samples are taken with a rosette sampler and Niskin bottles, at the pynocline and at 1 - 3.5 m above the bottom.

The phytoplankton monitoring study comprises live samples on some of the stations (WFD station NOORDWK2 and three other stations), for a qualitative inventory of phytoplankton

composition. This is done especially for those species that are difficult to recognize after fixation with Lugol, and to follow developments in the phytoplankton in near-real time. In addition, formalin-fixed samples (for determining the density of coccolithophores) and Lugol-fixed samples for a quantitative description of the species composition and density of phytoplankton throughout the year, are taken. Only the last group of samples is used for the determination of *Phaeocystis* abundance.

3.1.4 Fixation

All Lugol-fixed samples have an approximate volume of 1 liter of seawater fixed with 4 ml of acetic acid Lugol in brown glass bottles. They are checked on receipt for correct fixation, labelling and registration and stored in the dark at 4 °C until analysis.
Just before processing the samples, the fixation is checked again. The contents of the bottles have to have a cognac-like colour. Samples, which have not been fixed correctly, are discarded. After analysis, the remainder of the sample is stored in the dark at 4°C.

3.1.5 Concentrating the samples

The analysis focuses on determination of the number of phytoplankton cells per liter in the original, fixed, non-concentrated samples. In the case of turbid samples and samples which are suspected to contain species at densities of >10000 cells/l, two sub samples are prepared: one non-concentrated straight from the bottle and an approximately 10 times concentrated sub sample.
The procedure of concentrating the sample is as follows: Samples are left undisturbed for at least one week in a low-vibration, cold and dark environment. During this period all solids settle out. Without disturbing the settled material the samples are brought into the treatment room, and left at least a day for acclimatisation.
The largest part of supernatant is then removed from the sample bottle and collected in a calibrated measuring cylinder of 1000 ml. The settled residue (volume 40-200 ml) is transferred to a calibrated measuring cylinder of 250 ml after homogenization.

3.1.6 Lugol-fixed subsamples

A sub sample of of 0.2, 1.3 or 2.3 ml ml of the unconcentrated and / or 1 to 2.3 ml of the concentrated sample is examined. This subsample is taken from the homogenized sample with a calibrated pipette (Eppendorf pipette or automatic) and pipetted into one or more sedimentation chambers with a surface area of 1.25 cm^2 and a height of 1 to 2 cm. In the sedimentation chambers 0.2 to 1 ml of diluted seawater algae-free Lugol is pipetted in advance. The sedimentation chambers were covered with a cover slip and left alone in a dark area for at least four hours to allow the cells to settle, assuming a sedimentation rate of 0.25 cm / hour for nano-plankton.
If there are less than 15 observations of an “important” taxon in a sample, additional observations are done in the next sub sample or, if necessary in a 10x concentrated sub sample.
Plankton-poor samples must be concentrated to obtain a sub sample with a sufficient number of organisms. This is further complicated by the presence of inorganic sediment and detritus particles, especially in the Dutch coastal waters.

3.1.7 Analysis

The samples are analyzed using an inverted microscope (Olympus IMT-2), with a long working distance condenser (numerical aperture 0.55), 10 × WHK eyepieces, one with a calibrated ocular micrometer and the following lenses: Olympus SPlan-Apo 20x / 0.70 and 60x/1.40. Analyses are performed in bright field. To correct for any edge effects image fields

are studied in sectors of the sedimentation cuvet. The analysis is supported by epi-fluorescence microscopy.

3.1.8 Determination

Phaeocystis sp. appears as three distinct types of cells, single cells, flagellate cells and cells from colonies. In samples fixed in Lugol *Phaeocystis* colonies disintegrate easily into single cells. Therefore single cells may be natural, but also may originate from disintegrated colonies. The number of cells in small colonies are counted and in large colonies (> 100 cells) they are usually estimated. For analysis, the three types of cells are summed.

Although *Phaeocystis globosa* is the most common species of the genus *Phaeocystis* taxonomically it is difficult to distinguish this species from *P. pouchetii*. The single cells and flagellate cells of both species cannot be identified down to species level with a light microscope. In live samples colonies of both species can be distinguished, based on differences in morphology. Although the observed colonies of *Phaeocystis* in the live samples from the coastal zone station Noordwijk 2 all belonged to the species *Phaeocystis globosa*, it is not sure that all single and flagellate *Phaeocystis* cells belong to this species and therefore they all are identified as *Phaeocystis* sp.

4 Results of the application of the *Phaeocystis* metric in the Netherlands

Blooms of *Phaeocystis* in themselves are a natural phenomenon, but extremely abundant (>> 10^6 cells/l) and long-lasting blooms are considered an effect of eutrophication. The Dutch metric for *Phaeocystis* takes bloom frequency as criterion for eutrophication (Van der Molen and Pot, 2007), using the monitoring data from seven months (March to October) considered as the growing season for phytoplankton in Dutch coastal waters. It is assumed that *Phaeocystis* does not reach bloom densities during the winter months (October-February)

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. A frequency of 10% (> 1 month per year) is taken as the boundary between *high* and *good* status. A frequency of 17% (> 2 months per year) is the boundary between *good* and *moderate* status, and frequencies of 35% (>4 months per year) and 85% (>11 months per year) are the boundaries for *poor* and *bad* status (Figure 4.1).

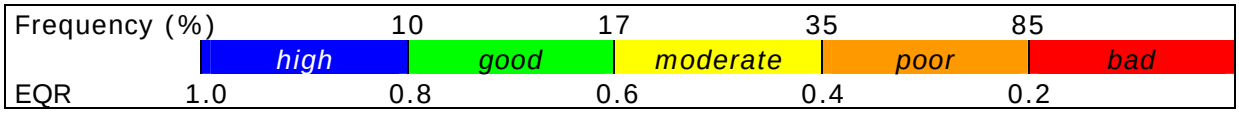

Figure 4.1Boundaries of the Phaeocystis *submetric, expressed in frequency (% of months with a bloom) and in the Ecological Quality Ratio (EQR)*

4.1 Results

Table 4.1 gives the number of months per year with blooms of more than 10^6 *Phaeocystis* cells/l in the Dutch coastal water bodies, and the value of the calculated Ecological Quality Ratio (EQR). The status classification is indicated by the colours as shown in Figure 4.1.

The graphical presentation of the *Phaeocystis* EQRs for the Dutch coastal water bodies is given in Figure 4.2. The red lines indicate the boundary between good and moderate ecological status.

	Number of months with blooms							EQR value						
Year	Ems-Dollard coast	Wadden coast	Holland coast	Northern Delta Coast	Zeeland coast	Wadden Sea	Oosterschelde	Ems-Dollard coast	Wadden coast	Holland coast	Northern Delta Coast	Zeeland coast	Wadden Sea	Oosterschelde
1990	0	2	1	0	1	0	1	**1,00**	**0,61**	**0,83**	**1,00**	**0,83**	**1,00**	**0,83**
1991	3	2	2	3	3	3	2	**0,51**	**0,61**	**0,61**	**0,51**	**0,51**	**0,51**	**0,61**
1992	2	5	3	1	1	1	2	**0,61**	**0,37**	**0,51**	**0,83**	**0,83**	**0,83**	**0,61**
1993	1	3	1	1	1	2	1	**0,83**	**0,51**	**0,83**	**0,83**	**0,83**	**0,61**	**0,83**
1994	1	4	2	1	1	3	1	**0,83**	**0,42**	**0,61**	**0,83**	**0,83**	**0,51**	**0,83**
1995	3	2	2	1	3	5	2	**0,51**	**0,61**	**0,61**	**0,83**	**0,51**	**0,37**	**0,61**
1996	3	4	3	4	4	2	2	**0,51**	**0,42**	**0,51**	**0,42**	**0,42**	**0,61**	**0,61**
1997	2	4	4	2	2	3	2	**0,61**	**0,42**	**0,42**	**0,61**	**0,61**	**0,51**	**0,61**
1998	2	1	3	2	1	1	2	**0,61**	**0,83**	**0,51**	**0,61**	**0,83**	**0,83**	**0,61**
1999	1	2	3	2	1	2	2	**0,83**	**0,61**	**0,51**	**0,61**	**0,83**	**0,61**	**0,61**
2000	4	1	1	1	2	2	0	**0,42**	**0,83**	**0,83**	**0,83**	**0,61**	**0,61**	**1,00**
2001	3	3	3	2	3	5	2	**0,51**	**0,51**	**0,51**	**0,61**	**0,51**	**0,37**	**0,61**
2002	1	2	0	1	0	2	1	**0,83**	**0,61**	**1,00**	**0,83**	**1,00**	**0,61**	**0,83**
2003	2	3	3	3	2	2	1	**0,61**	**0,51**	**0,51**	**0,51**	**0,61**	**0,61**	**0,83**
2004	3	4	1	2	2	4	2	**0,51**	**0,42**	**0,83**	**0,61**	**0,61**	**0,42**	**0,61**
2005	4	3	2	3	1	3	1	**0,42**	**0,51**	**0,61**	**0,51**	**0,83**	**0,51**	**0,83**
2006	2	1	0	0	1	2	0	**0,61**	**0,83**	**1,00**	**1,00**	**0,83**	**0,61**	**1,00**
2007	1	2	2	1	2	3	2	**0,83**	**0,61**	**0,61**	**0,83**	**0,61**	**0,51**	**0,61**
2008	3	3	3	2	2	6	3	**0,51**	**0,51**	**0,51**	**0,61**	**0,61**	**0,34**	**0,51**
Mean 2003-2008								**0.56**	**0.54**	**0.65**	**0.65**	**0.69**	**0.48**	**0.73**

Table 4.1 Number of months per year with blooms of more than 10^6 Phaeocystis cells/l, and EQR values of the Phaeocystis metric in the Dutch coastal water bodies for the years 1990-2008. The mean EQR over the last six years is also given. The colours depict the status, as shown in Figure 4.1

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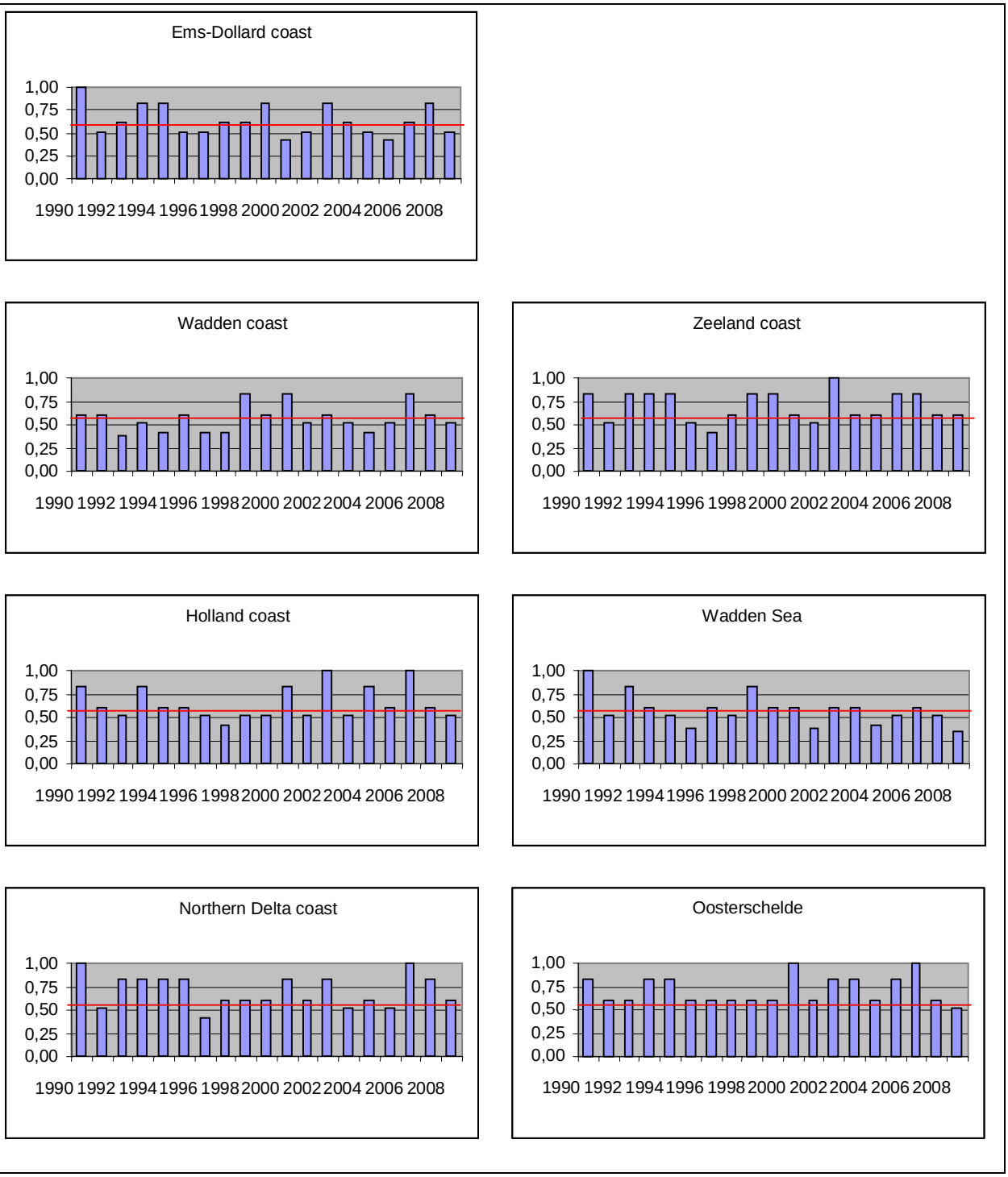
Ems-Dollard coast
1,00
0,75
0,50
0,25
0,00
1990 1992 1994 1996 1998 2000 2002 2004 2006 2008
Wadden coast
1,00
0,75
0,50
0,25
0,00
1990 1992 1994 1996 1998 2000 2002 2004 2006 2008
Zeeland coast
1,00
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0,50
0,25
0,00
1990 1992 1994 1996 1998 2000 2002 2004 2006 2008
Holland coast
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Wadden Sea
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1990 1992 1994 1996 1998 2000 2002 2004 2006 2008
Northern Delta coast
1,00
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0,50
0,25
0,00
1990 1992 1994 1996 1998 2000 2002 2004 2006 2008
Oosterschelde
1,00
0,75
0,50
0,25
0,00
1990 1992 1994 1996 1998 2000 2002 2004 2006 2008

Figure 4.2 EQR values for the Phaeocystis *submetric in the years 1990-2009 in the Dutch coastal water bodies. The red line indicates the boundary between Good and Moderate status.*

4.2 Discussion

The method used in the *Phaeocystis* metric is based on several assumptions and choices. Assumptions have been made regarding the threshold for cell abundance that defines a bloom, and regarding growing season. Choices in methodology were made with respect to using bloom frequency as metric, and with respect to sampling frequency.

4.2.1 Bloom threshold definition at 10^6 cells/l or 4 x 10^6 cells/l

The Netherlands had formulated the *Phaeocystis* metric several years before Lancelot et al. (2009) derived a threshold value for blooms causing an undesirable disturbance (see §2.5). In Figure 4.3 the EQRs calculated for the Dutch metric with thresholds of 10^6 and $4*10^6$ cells/l are compared with each other. The differences are considerable, and with the higher threshold the assessment changes from *moderate* to *good* in many cases.

However, the threshold proposed by Lancelot et al. (2009) applies to the annual maximum abundance of *Phaeocystis*, and not to bloom frequency. A comparison of bloom frequency and annual maximum abundance shows that a bloom frequency of >2 months per year (*moderate* status in the Dutch WFD metric) coincides with a maximum abundance above the “Lancelot” threshold of 4 million cells/l in all cases. At a bloom frequency of 1 or 2 months (*good* status in the Dutch WFD metric), the 4 million cells/l threshold is exceeded in less than 50% of the cases (Figure 4.4).

Concluding, using the maximum abundance threshold of 4 million cells/l for the boundary between g*ood/moderate* status would result in a more stringent classification than the Dutch WFD metric. Using the threshold of 4 million cells/l for the determination of bloom frequency logically results in a less stringent classification.

4.2.2 Monitoring seven versus twelve months per year

The present application of the metric is based on the assumption that *Phaeocystis* blooms never occur before or after the so-called growing season March-September. Data from the national monitoring programme show this assumption to be incorrect, however. In February 2005 there was a bloom in the coastal waters at Noordwijk 2. In November 1997 and 2003 there were blooms in the Wadden coast and in October 1995, 2003 and 2008 and November 2003 in the Wadden Sea. The Northern Delta coast had a bloom in November 2003. The highest number of blooms outside the growing season occurred in 2003 and in the areas Wadden coast and Wadden Sea ((Figure 4.5). In one case (Wadden Sea in 2003) there were two blooms outside the growing season, in all other cases only one.

In total, there are 7 water bodies and 19 years of monitoring data (in total 133 assessments). In 7 cases (5.3%) there were blooms outside the growing season. In 4 of these cases the assessment, taking into account the blooms outside the growing season, resulted in the same classification as the original assessment (taking into account only the growing season). In 3 cases (2.3 %) the assessment based on observations in all twelve months, resulted in a one class lower classification (in 2 cases going from good to moderate) (Table 4.2).

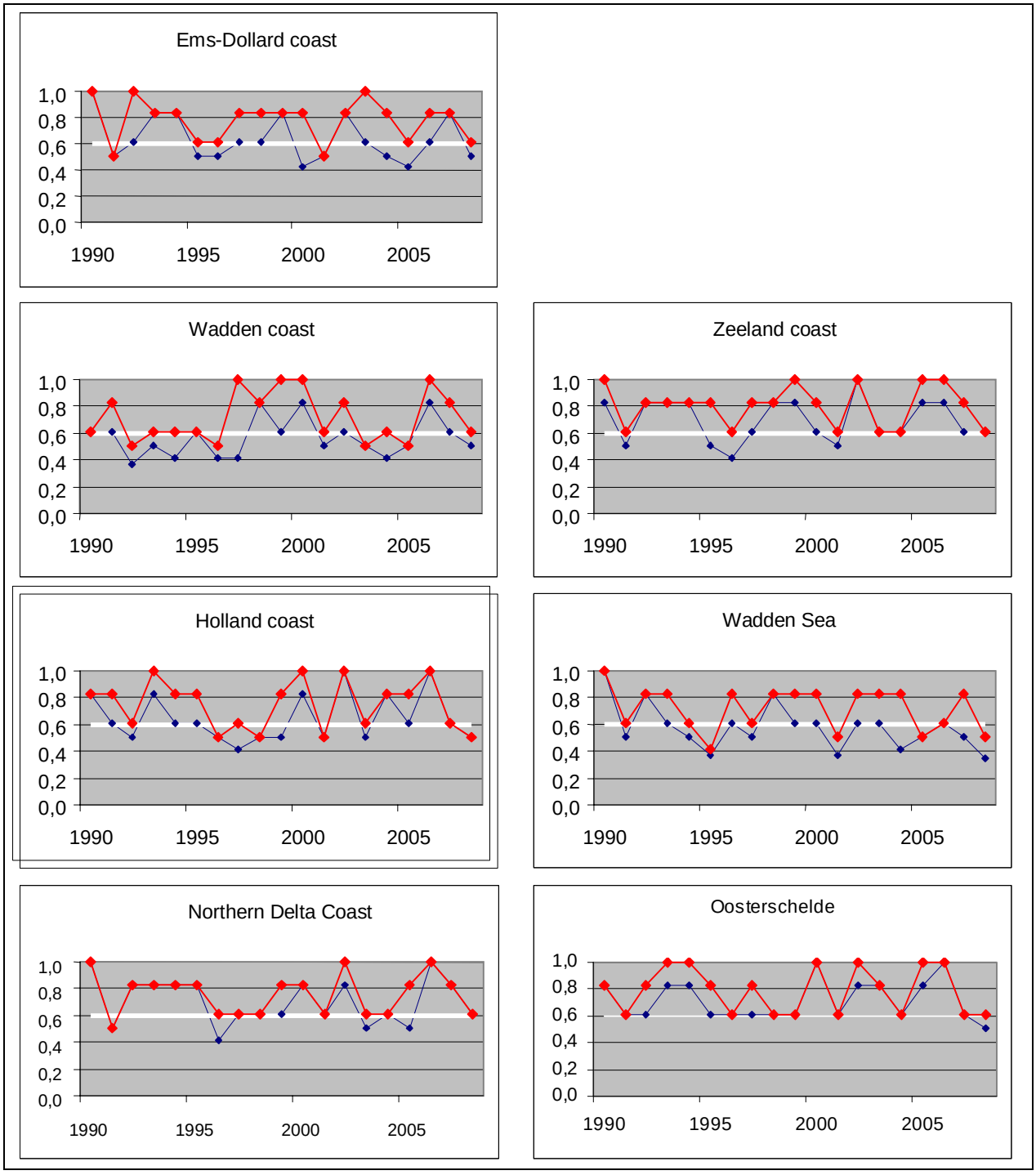

Figure 4.3 The graphical presentation of the Phaeocystis *EQRs for the Dutch coastal water bodies with two different bloom definitions: blue line: threshold at 10^6cells/l; red line: threshold at 4 x 10^6cells/l. The white line indicates the boundary between good and* moderate *status.*

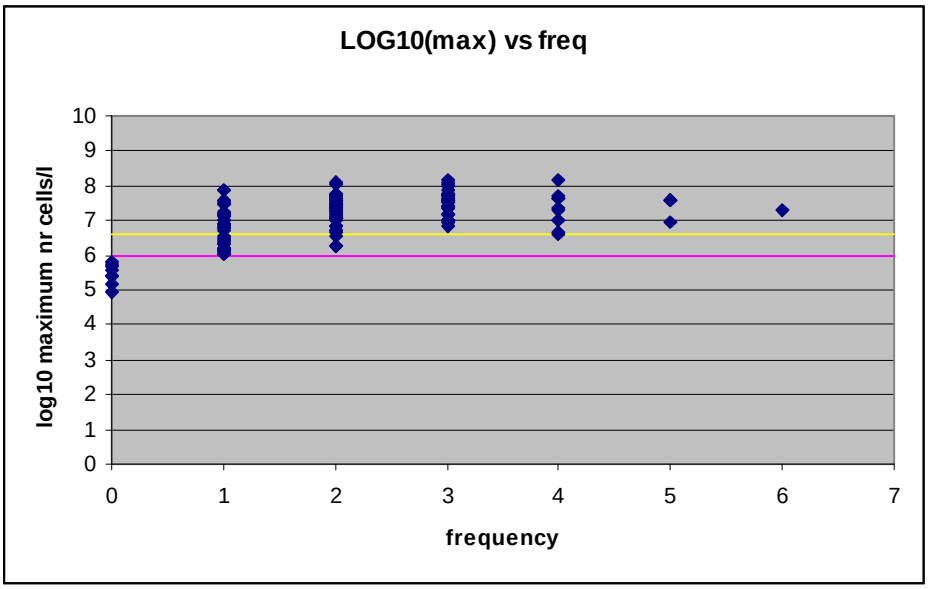

Figure 4.4 The log of the annual maximum number of cells against the frequency of months with blooms of more than 10^6 cells/l. The pink line indicates a concentration of 10^6 cells/l, the yellow line a concentration of 4.10^6 cells/l

		Assessment based on 7 months		Assessment based on 12 months		Ecological status classification	
	year	nr of blooms	EQR7	nr of blooms	EQR12	7 months	12 months
Noordwijk 2	2005	2	0,61	3	0,51	good	moderate
Wadden coast	1997	4	0,42	5	0,37	moderate	poor
Wadden coast	2003	3	0,51	4	0,42		
Wadden Sea	1995	5	0,37	6	0,34		
Wadden Sea	2003	2	0,61	4	0,42	good	moderate
Wadden Sea	2008	6	0,34	7	0,31		
N Delta coast	2003	3	0,51	4	0,42		

Table 4.2 Comparison of the assessment results based on 7 or 12 months of observations.

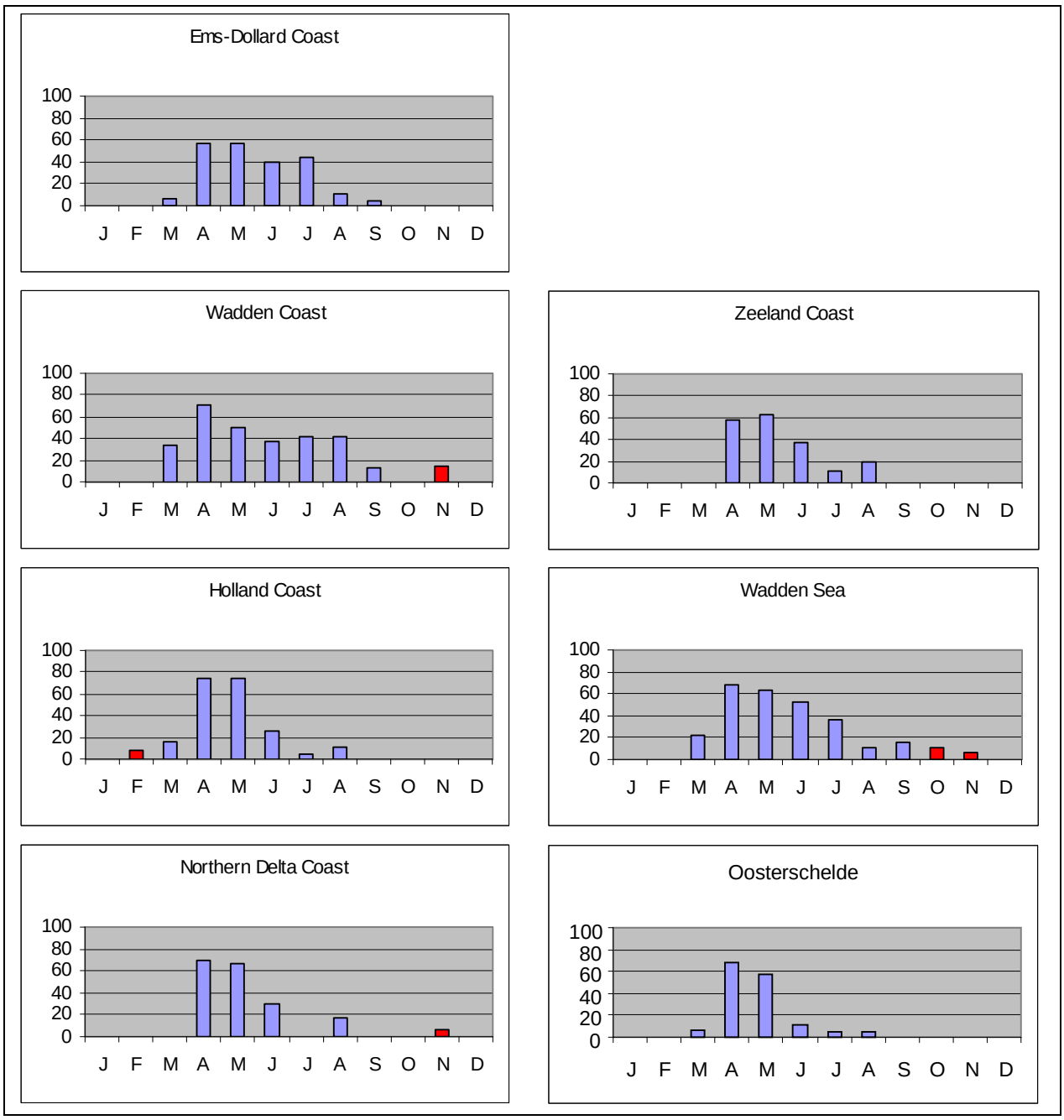

Figure 4.5 Percentage of years in the period 1990-2008 with bloom(s) (> 10^6 cells-l) for each month. The red bars lie outside the "growing season" months.

4.2.3 One or two samples per month

Wortelboer (2007) describes a statistical analysis of the *Phaeocystis* data from one of the Dutch monitoring stations, and concludes that the small number of observations per month (1-3) lead to an underestimation of the real abundance. This is due to the fact that the chance of observing a "peak" in a phytoplankton bloom is relatively small. It is, however, not feasible to intensify the labour-intensive monitoring and analysis of the phytoplankton samples because of financial and operational constraints.

Although the WFD monitoring programme specifies only one sample per month in the growing season, for a number of stations (see Table 3.2) in the national monitoring programme two or more samples per month are available.

For one of these stations, Noordwijk 2, the effect of doubling the monitoring frequency from monthly to biweekly was studied.

Three calculations have been carried out:

S1: EQR's based on all first samples in a month

S2: EQR's based on all second samples in a month

WFD: EQR's based on all available samples, using the highest abundance sample when more than one sample was available. This is the method used for the WFD assessment.

The differences in results can be considerable. Using S1, 31% of the years deviate from the WFD score and using S2 this is even 42%. Logically sets with only one sample always result in higher EQR's, which was to be expected (Table 4.3).

	S1	S2	WFD	max diff in nr blooms	max diff in classes
1990	1,00	0,83	0,83	1	0
1991	0,61	0,61	0,61	0	0
1992	0,61	0,61	0,51	1	1
1993	0,83	0,83	0,83	0	0
1994	0,83	0,61	0,61	1	1
1995	0,61	0,83	0,61	1	1
1996	0,51	0,83	0,51	2	2
1997	0,42	0,83	0,42	3	2
1998	0,51	0,83	0,51	2	2
1999	0,61	0,51	0,51	1	1
2000	0,83	1,00	0,83	1	0
2001	0,61	0,61	0,51	1	1
2002	1,00	1,00	1,00	0	0
2003	0,61	0,51	0,51	1	1
2004	0,83	0,83	0,83	0	0
2005	0,61	0,61	0,61	0	0
2006	1,00	1,00	1,00	0	0
2007	0,61	0,83	0,61	1	1
2008	0,51	0,51	0,51	0	0
Mean 90-08	0,69	0,75	0,65	0,84	0,68
Mean 03-08	0,70	0,72	0,68	0,33	0,33

Table 4.3. EQRs at the station NOORDWK2, using all available samples (WFD), or only the first (EQR1) or second (EQR2) samples in months with two samples. Max diff indicates the maximum difference in number of blooms and classes. For colour coding see Figure 4.1. The mean over the year 1990-2008 and over the last six years is also given.

5 Comparison with methods used by Belgium and the United Kingdom

Information on the methods used by the various countries was provided by S. Denayer (Gent University) for Belgium, by A. Grage (NLWKN) for Germany and by S. Milligan (CEFAS) for the UK.
The table below gives an overview of the major aspects of the application

	Belgium	Germany	The Netherlands	United Kingdom
No of sampling sites	3	1 (Norderney)	1 for each WFD water body; five WFD sites in the North Sea	No information provided
Sampling frequency	Monthly, in March-May 3 times a month	Weekly	2 to 4 weekly in the growing season (March-September); at least one sample per month	No information provided
Sampling depth	3 m below surface	No information provided	1 m below surface	No information provided
Live samples collected	Yes	Yes	At one WFD station	No information provided
Live samples counted	No	Yes, for colonies	No	No information provided
Sample fixation	Lugol	Lugol	Lugol	Lugol
Counting procedure	Utermöhl chambers, inverted microscope	Utermöhl chambers, inverted microscope	Utermöhl chambers, inverted microscope	Utermöhl chambers, inverted microscope
Separate counting of flagellate, non-flagellate cells and cells in colonies	Yes, colonies separated through inverse filtration	Yes, colonies counted in live samples	Yes, in Lugol-fixed samples (NB colonies partly disintegrate)	No, *Phaeocystis* not distinguished from other small flagellates
Counting of colony size	Yes	Yes	No	No
Calculation of total density	Sum of colonial cells, free-living flagellated cells and non-flagellated cells	Total number of cells counted in Lugol-fixed samples (NB colonies disintegrate)	Sum of colonial cells, free-living flagellated cells and non-flagellated cells	Few observations of *Phaeocystis* Counted as “small flagellates possibly *Phaeocystis*”
Threshold for	4 million cells/l	1 million	1 million cells/l	No information

bloom definition		cells/l		provided
Calculation of bloom frequency	Step 1: calculation of samples exceeding threshold Step 2: Frequency based on number of samples exceeding threshold	Step 1: calculation of monthly maximum Step 2: Frequency based on number of months exceeding threshold	Step 1: calculation of monthly maximum Step 2: Frequency based on number of months exceeding threshold	No information provided
Class boundaries and EQR values	*High* status frequency 0-10% EQR 1.0-0.9 *Good* status frequency 10-17% EQR 0.9-0.83 *Moderate* status frequency 17-35% EQR 0.83-0.65 *Poor* status frequency 35-85% EQR 0.65-0.15 *Bad* status frequency 85-100% EQR 0.15-0.0		*High* status frequency 0-10% EQR 1.0-0.8 *Good* status frequency 10-17% EQR 0.8-0.6 *Moderate* status frequency 17-35% EQR 0.6-0.4 *Poor* status frequency 35-85% EQR 0.4-0.2 *Bad* status frequency 85-100% EQR 0.2-0.0	

The main differences in methods are:

- *Phaeocystis* colonies disintegrate due to fixation in Lugol's solution; The UK does not distinguish loose cells of *Phaeocystis* from other small flagellates
- Belgium uses a higher sampling frequency during the months with (generally) the highest *Phaeocystis* concentration
- Germany uses a higher sampling frequency than the other countries
- Belgium uses a threshold of 4 million cells/l, the Netherlands and Germany use a threshold of 1 million cells/l
- Belgium calculates the frequency of blooms as the *frequency of samples* exceeding the threshold. The Netherlands and germany calculate the frequency of blooms as the *frequency of months* exceeding the threshold, based on the maximum abundance per month.
- The calculation method of Belgium gives more weight to the main bloom period March-May

6 Conclusions

Phaeocystis blooms

- *Phaeocystis* blooms are a common phenomenon in nutrient-enriched seas
- The maximum concentration of *Phaeocystis* shows large interannual variation which is only partly explained by differences in nutrient concentrations
- Blooms occur annually in the southern North Sea; while there are records of blooms occurring before 1900, present day observations and models generally indicate a relation between (nitrogen) enrichment and bloom magnitude
- Blooms of *Phaeocystis* are associated with a number of adverse ecological effects
- There is no well-established quantitative relation between *Phaeocystis* abundance and adverse ecological effects

WFD assessment

- Thresholds used to define a bloom are 1 million cells/l (NL, D), or 4 million cells/l (B)
- Using a threshold of 4 million cells/l for the maximum abundance of *Phaeocystis*, as proposed by Lancelot et al. (2009), generally leads to a lower classification than assessments based on bloom frequency
- Using a threshold of 4 million cells/l to determine bloom frequency generally leads to a higher classification than using a threshold of 1 million cells/l
- The sampling frequency influences the results of the assessment. Germany uses a high frequency (weekly), while Belgium uses a higher frequency during the spring bloom (March-May). The frequencies used by The Netherlands differ between sampling stations
- Including *Phaeocystis* blooms outside the growing season March-September in the assessments, leads in a small number of cases (< 2.5%) to a lower classification
- The calculation of the metric by the Netherlands and Germany gives equal weight to all months in the growing season. The calculation by Belgium gives equal weight to the samples, resulting in more weight to the March-May period
- The Netherlands use ‘ normalized’ EQR values, whereas Belgium derives EQR values from bloom frequencies. While the classification of ecological status is comparable at the same bloom frequency, the EQR values differ

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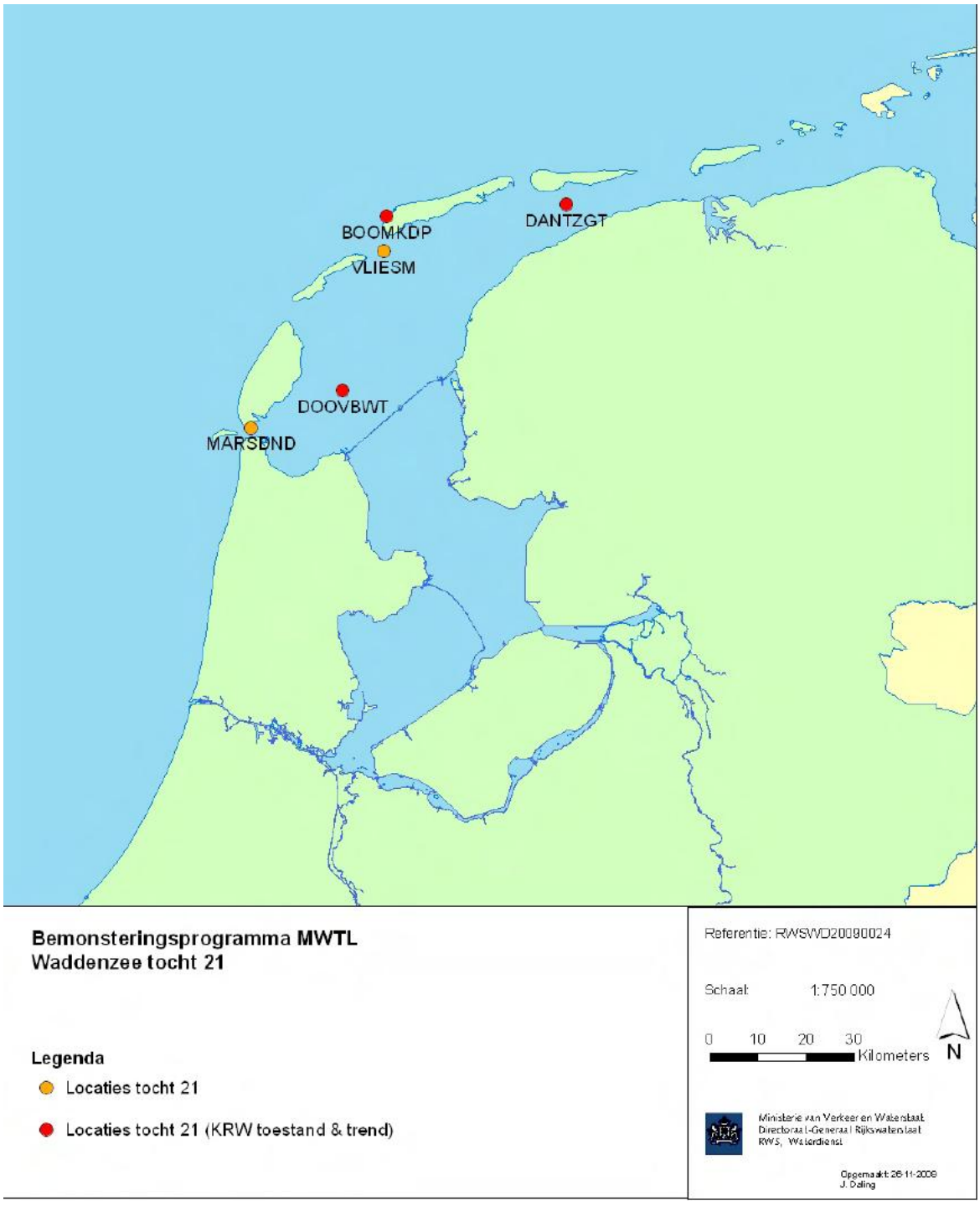
A Maps of monitoring sites in the North Sea and Wadden Sea

North Sea monitoring sites



Wadden Sea monitoring sites

B Definitions for high, good and moderate ecological status in coastal waters

High status	Good status	Moderate status
The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions.	The composition and abundance of phytoplanktonic taxa show slight signs of disturbance.	The composition and abundance of planktonic taxa show signs of moderate disturbance.
The average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions.	There are slight changes in biomass compared to type-specific conditions. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the quality of the water.	Algal biomass is substantially outside the range associated with type-specific conditions, and is such as to impact upon other biological quality elements.
Planktonic blooms occur at a frequency and intensity which is consistent with the type specific physicochemical conditions.	A slight increase in the frequency and intensity of the type-specific planktonic blooms may occur.	A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months.

Source: Annex V, WFD