Towards a joint monitoring programme for the North Sea and the Celtic Sea

CASE STUDY CHLOROPHYLL

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Contents

1. Introduction

Already for many years eutrophication has been a main issue of concern for the European regional seas in general and for the North Sea in particular. OSPAR, the Oslo Paris Convention, entered into force in 1998, after ratification by the fifteen signatories. It has been amalgamated in 1992 from the Oslo Convention of 1972 on the dumping of waste into the sea of 1972, and the Paris Convention of 1974 on the pollution of the sea from land-based sources. The aim of OSPAR is to protect the marine environment in the North Atlantic (including the North Sea) through international cooperation. To assess eutrophication within OSPAR the so-called Comprehensive Procedure (COMPP, OSPAR, 2013) has been developed. This is a harmonized, integrated assessment system of causes and consequences of eutrophication. The associated assessment parameters are divided into four categories: the causal factors, the direct effects, indirect effects and other possible effects of nutrient enrichment, each with their area-specific parameter values. Chlorophyll-a, as a proxy for phytoplankton biomass, is one of the direct effects of the eutrophication process.

In 2000 the European Water Frame Work Directive (WFD) came into force, for all freshwaters, estuarine and coastal waters. The WFD aims to improve and protect the chemical and ecological status of all water bodies from source through rivers to estuaries and coastal waters (EC-WFD, 2000).

In 2008 the Marine Strategy Framework Directive (MSFD) came into force, which requires member states to prepare national strategies to manage their seas in order to achieve Good Environmental Status (GES) by 2020. In the MSFD there is a major emphasis on international cooperation, as described in art. 11 of the MSDF directive that they *shall establish and implement coordinated monitoring programmes for the ongoing assessment of the environmental status of their marine waters ….*(EC-MSFD, 2008).

Figure 1. Map of the OSPAR areas of the greater North Sea for eutrophication assessment.

1.1 Central role of chlorophyll-a in eutrophication assessment of North Sea countries

Until now, the countries monitor their marine waters both for OSPAR and WFD. For OSPAR each country applies the Comprehensive Procedure (COMPP) to its national marine area, divided into a number of sub-

areas, known as the OSPAR areas (Figure 1) and for WFD it monitors its coastal waters. Although the procedure of the final assessment is different for OSPAR and WFD, in both assessments chlorophyll-a plays a crucial role.

As chlorophyll is one of the main indicators for the eutrophication process, it is proposed as one of the common indicators under OSPAR for the MSFD. This implies that chlorophyll will be one of the indicators that have to be monitored and assessed by all countries involved.

The terms chlorophyll and chlorophyll-a are used in a confusing way. Within OSPAR the COMPP mentions chlorophyll-a while the list of common indicators mentions chlorophyll. The recent advice by JRC on MSFD Descriptor 5 mentions chlorophyll-a as one of the indicators under criterion 5.2 *direct effects of nutrient enrichment*. In section 3.1 we describe the differences between the two terms and the consequences for the use of analytical methods. However, we can assume that all of these guidances - OSPAR, WFD and MSFD - intend to use chlorophyll/chlorophyll-a as a measure of algal biomass and have no a priori preference for a specific analytical method.

In OSPAR the parameters used for the indicator chlorophyll-a are the mean and the 90-percentile over the growing season which is defined as the period from March, 1st – September 30th for the northern Member States and March, 1st – October, 31st for the southern Member States around the Greater North Sea.

The northern Member States comprise: Belgium, The Netherlands, Germany, Denmark, Sweden and the United Kingdom. From the southern Member States only the northern part of France is involved in this study, hence the period March, 1st – September 30th has been taken as the growing season for all countries.

Monitoring is primarily carried out by ship surveys. The countries submit the results of their water-bottle and CTD observations to the ICES database, from where the data also can be downloaded via a user-friendly interface:
http://ocean.ices.dk/hydchem/hydchem.aspx. These data are all categorized as chlorophyll-a. However, North Sea countries use various analytical methodologies. The analytical methods significantly influence the detection of algal pigment(s) and cross-border comparability is low. This issue is described in section 3 of this document. The metadata of the methods used are available in the ICES database, but they can't be reached via the interface.

1.2 Towards cross-border assessments

The MSFD calls for coherent assessments of GES, using assessment areas that are delimited according to ecological and physical characteristics rather than country borders. For the Intermediate Assessment 2017 assessment units are under development in OSPAR, also taking into account the water bodies of the Water Framework Directive. A draft version of the subdivision of the North Sea is in Figure 2.

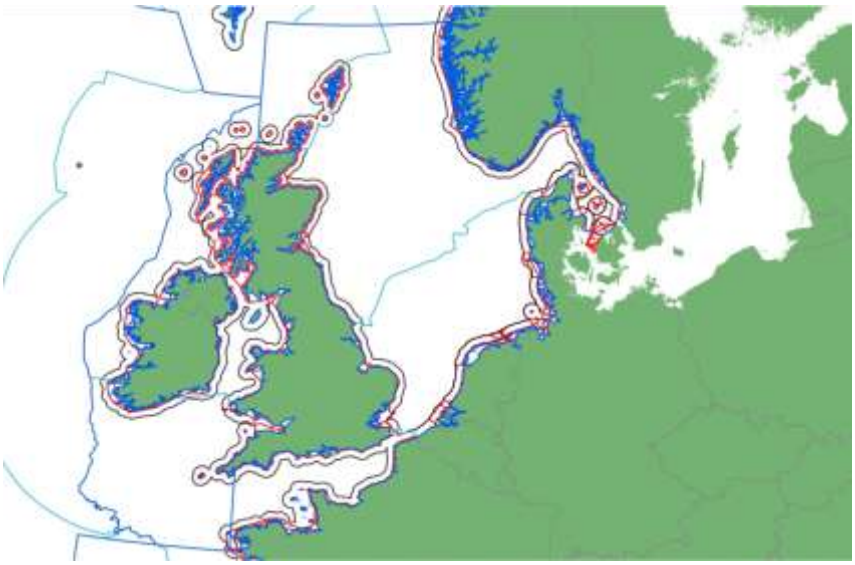

Figure 2. Level 4 assessment units, including WFD water bodies[1]

The challenge is to compile cross border assessments from national monitoring results. The project JMP NS/CS has investigated to which extend this would be possible and how alternative joint monitoring programmes can help to overcome the issue of comparability.

2 Design of chlorophyll monitoring programmes – Annex I

An inventory of all available ICES chlorophyll-a data (ICES, 2014), submitted by the countries involved, has been made and described by Baretta-Bekker et al. in Annex I. The years 2001-2006 have been chosen as the period with most data from all countries, and only the observations from the surface layer --down to a depth of 10 m-- have been selected, because these are the observations used for the OSPAR assessments.

2.1 Optimizing spatial monitoring design using joint assessment areas

The JMP NS/CS project made an attempt to investigate how monitoring could be optimized using distinct assessment areas of homogeneous ecological and physical characteristics, so-called strata. Taking into account the OSPAR areas, but looking for a subdivision that would also be applicable for other MSFD indicators we chose a stratification which is a modification of the stratification applied in the EU FP7 project VECTORS for the ecosystem model 'Atlantis' by Hufnagl *et al*. (unpublished). See Figure 3. Statistical optimization methods can show how the quality of the assessment (statistical power) relates to the density and positioning of sampling stations. The high spatial variability of chlorophyll requires relatively high sampling effort.

The stratification used in OSPAR's COMPP (Figure 1) is a subdivision of national waters and it therefore does not support joint assessments and cross-border monitoring. Aggregation of results at the level of the draft strata that are under development for the Intermediate Assessment 2017 (*cf.* Figure 2) would be cumbersome.

In order to develop a joint monitoring design using cross-border assessment areas we need to be able to bring data from several countries together in statistical analyses. This is hampered by:

a. differences in spatial design of monitoring programmes

[1] In: Preparation for Publication of the Intermediate Assessment 2017 and the QSR21, meeting document ICG-COBAM (1) 15/1/4-E , Annex 5.

b. differences in temporal design of monitoring programmes, and
c. differences in analytical methods.

The project concluded that current monitoring data held in the ICES database do not allow for such an analysis. However, data processed by comparable (or better: standardized) methods, such as data currently retrieved from satellite images, would enable development of joint assessment protocols.

The obstacles to joint monitoring and assessment are further explored below.

2.2 Differences in spatial design of monitoring hamper comparison between countries

The in-situ monitoring often has a rather skewed spatial distribution with a preponderance of stations near shore (Figure 3). It is known that the chlorophyll concentration has a rather steeply decreasing gradient from near shore to offshore. In case an assessment area covers both near shore and offshore waters averaging over the in-situ samples of the whole area will lead to an overestimation of the chlorophyll concentration. This can be seen by comparing the Remote Sensing results in Figure 6 with the size and form of the OSPAR areas in Figure 1. This is especially true for the coastal area of Germany (German Bight). A comparable issue appears when a densely sampled area is combined with a sparsely sampled area of a neighbouring country.

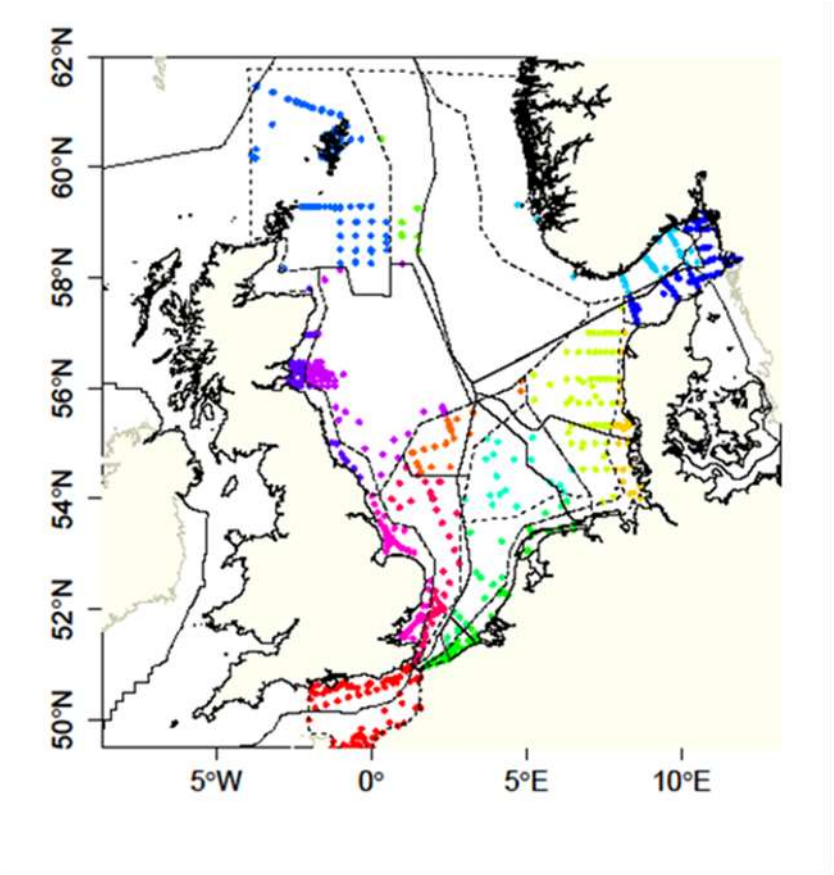

Figure 3. Stations reported for chlorophyll monitoring in the ICES database. Year 2006. Dashed black lines and colour-coding of stations: boundaries of and measurements within strata that can be used as relatively homogeneous assessment units (Atlantis Stratification, see Annex I). Solid black lines: national borders.

2.3 Differences in temporal design of monitoring hamper comparison between countries

The ICES database shows that the design of monitoring programmes is not identical for all countries. Some countries have fixed stations which they visit a number of times per year, other countries apparently monitor randomly. Furthermore, the observations are unequally divided over the growing season. In some of the national datasets the emphasis is on the spring period, in others on the late summer months, and some countries only have monitoring data for a few months. Figure 4, illustrates the distribution of monitoring effort by each North Sea country during the growing season in the years 2001-2006. It also presents the total number of observations per country and the total number of locations[2] visited by each country. This situation significantly hampers cross-border comparison and joint assessments.

It should be noted that chlorophyll is a highly variable parameter showing distinct seasonal patterns. Any assessment of eutrophication status should be able to detect changes therein against natural background levels.

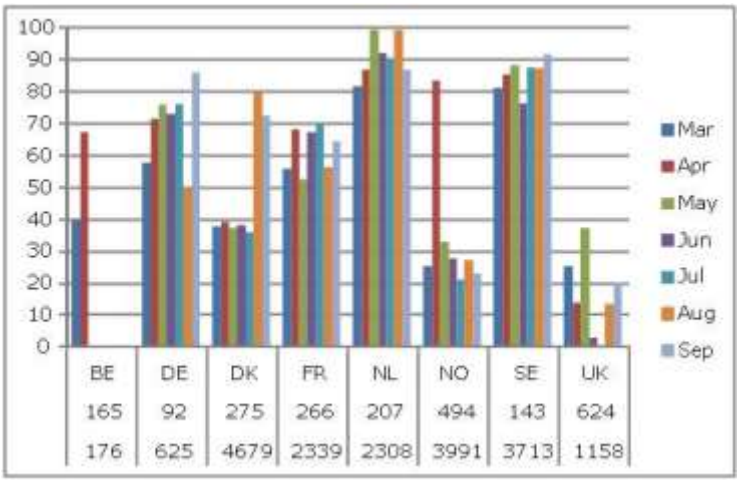

Figure 4. Distribution of chlorophyll sampling effort during growing season season in the region between Latitude N49 to 62 and Longitude E-5 to W12. For each country the percentages of stations visited per month are given. The numbers under the country acronyms are the number of different stations (upper row) and the total number of observations made in the period 2001-2006 (lower row).

3 Analytical methods to measure Chlorophyll – Annex II

The temporal and spatial coverage of monitoring programmes affect the calculated growing-season mean concentrations of chlorophyll-a. In addition, different analytical methods contribute significantly to the variance between the observations. In Annex II Walsham et al. give an overview of the various analytical methods that are currently in use, and the possible consequences for the assessment results.

3.1 Differences in analytical methods hamper comparison between countries

Aspects of measuring chlorophyll, such as the type of filter used, the way the filter is stored, the time before the sample is analysed, the solvent used for the extraction of the pigments (ethanol, methanol or acetone)

[2] NB. Locations have been defined by the unique combination of year, latitude and longitude.

and the detection method itself (fluorometry/photometry or HPLC) all influence the value of the chlorophyll concentration. Especially the extraction and the detection methods cause significant differences.

A recent comparison of extraction and detection methods by QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) indicates that ethanol may be the most efficient solvent for the extraction of chlorophylls. Furthermore, fluorometry/photometry and HPLC do not measure the same pigments.

Most of the countries use fluorometric/photometric methods to measure chlorophyll, while only two countries, NL and BE, use the HPLC method. Only with HPLC it is possible to measure Chlorophyll-a exclusively, while the other methods not only measure the chlorophyll-a component, but also other pigments, resulting in a concentration of "chlorophylls". As a consequence, concentrations determined by HPLC are lower than those determined by fluorometry/photometry. It can be argued which parameter is the best indicator for algal biomass, which ultimately drives the food web.

In OSPAR there is no single standard extraction technique recommended for the determination of chlorophylls, but they emphasize the importance of recording the method used when reporting data.

In OSPAR this never was a problem, since eutrophication assessment on the scale of the North Sea is based on the outcomes (*i.e*. problem area, non-problem area or potential problem area) of national assessments. National assessment levels are based on the method each country uses for monitoring chlorophyll. For MSFD, however, incomparability will be a problem because one of the requirements of this directive is to assess whole (sub)regions together with all involved countries.

3.2 Application of conversion factors considered not reliable

In a final attempt to bring data from North Sea countries together for the development of a joint monitoring design the project tried to estimate conversion factors between analytical methods, using QUASIMEME 2014 data. These factors varied between 1,7 and 5 for a limited set of samples. Converting chlorophyll values from one method to another is considered not reliable, because the relation between different methods depends on the phytoplankton abundance and species composition and therefore is not constant over space and time (Figure 5). We decided not to use this approach. The project concluded that monitoring design and analytical methods need to be standardized to enable cross-border comparison.

A B

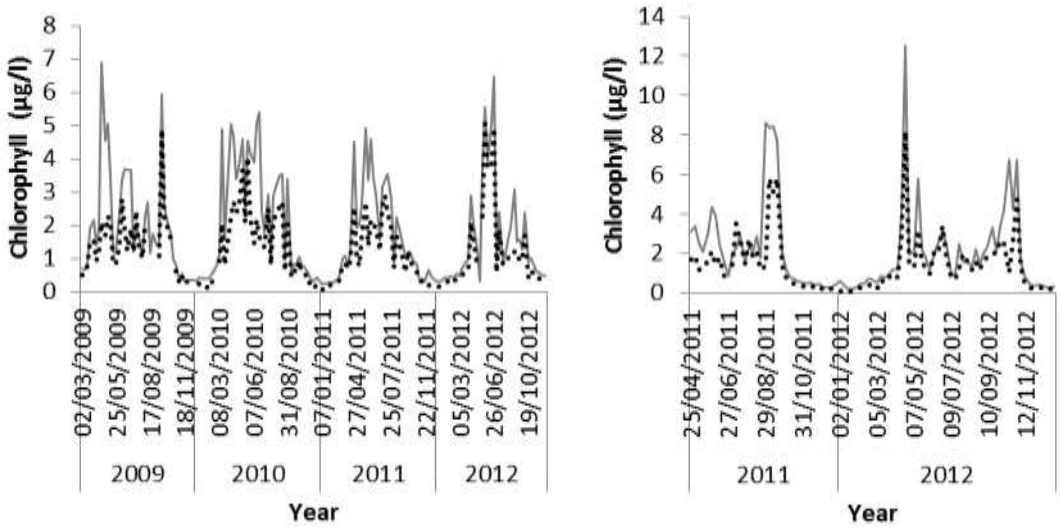

Figure 5. Comparison of weekly uncorrected chlorophyll concentrations determined by fluorometric methods (grey line) and chlorophyll 'a' determined using HPLC (dashed line) at the (A) Stonehaven and (B) Loch Ewe monitoring sites.

4 Sampling methods: alternatives for ship surveys

4.1 Costs of current ship based sampling of chlorophyll – Annex I

Data used in current assessments of chlorophyll originate from dedicated ship based sampling on a country by country basis. Looking at potential alternative approaches from a joint monitoring perspective the JMP NS/CS project assumes that the costs of monitoring and the quality of the assessment using monitoring data are key parameters to compare current monitoring with potential alternative sampling methods.

The project developed an approach to estimate the cost of ship-based monitoring of the entire North Sea. As a proxy for monitoring effort we estimated the number of ship miles travelled in one year using the 'travelling salesman' algorithm. This algorithm creates the shortest route that visits every sampling station and returns to the starting place. It assumes that one ship visits all stations and it does not include trips from and to the harbour.

Using the station information of the year 2006 in the ICES database, and taking into account repeated visits of stations within that year, the total distance covered was approximately 320.000 nautical miles. Compared to the demersal fish surveys (IBTS), that amount to approx. 160,000 nm yearly, chlorophyll monitoring can be considered a significant effort.

The countries considered in this calculation are BE, DE, DK, FR, NL, NO and UK. See Figure 3 for the distribution of sampling stations. It should be noted that surveys generally sample additional parameters next to chlorophyll, *e.g*. hazardous substances under the WFD (monthly sampling in coastal waters).

4.2 Use of alternative large-scale sampling methods to promote coherence

The relatively high sampling effort needed to determine chlorophyll relates to the variability in time and space of chlorophyll concentrations, also during the growing season. The power of the assessment depends on sampling frequency as well as precision of the analytical procedures. As described above cross-border assessments are currently hampered by differences between analytical methods and differences in the distribution of chlorophyll data in time and space. In addition, we cannot determine how many stations and how often we need to visit these in a joint monitoring design.

Therefore, the project looked into the use of alternative data sources, such as FerryBox data and especially Remote Sensing data from satellites. These monitoring platforms generate high density data at relatively low costs and are operated internationally, hence processing of samples will generally be harmonized.

It should be noted that changing from one sampling method to another will inevitably generate a discontinuity in time series of chlorophyll. Continuation of both methods in parallel for some time will enable comparison.

4.2.1 FerryBox data – Annex II

The Dutch FerryBox data of the North Sea have a limited accuracy, according to Blaas et al. (2014). The same is true of the Algaline data in Skagerrak and Kattegat (Mohlin, pers. comm.). This was a reason for not using these data in this study. Due to time constraints the use of other FerryBox data of the North Sea has not been considered.

The added value of FerryBox data value lies not so much in the assessment of Chlorophyll concentration against a fixed threshold or baseline, but more in the interpretation of any changes in them. They also do provide supporting data (such as temperature, salinity and nutrients) indicative of the state of the ecosystem. Because they often are installed on research vessels, sailing from station to station, they provide additional

information about the system state in the large areas between monitoring stations, which is extremely useful for correct interpolation, using Emperical Orthogonal Functions.

4.2.2 Towards a joint eutrophication assessment using Remote Sensing data – Annex III

Remote sensing data held by Ifremer cover a period of 17 years. In Annex III Gohin and Baretta-Bekker (2015) describe the methodology of the Remote Sensing (RS) chlorophyll-a estimates from satellite images and show a figure of the validation[3] of the RS data versus *in-situ* data of the North Sea, the western side of the English Channel, the Portuguese coast and the French part of the Mediterranean Sea, according to two different algorithms. The algorithm used in this study has an explained variance of 87%, which is a great improvement over the previous algorithm with only 31% explained variance. Using the improved comparability of RS and *in situ* data the project investigated whether RS data can deliver a reliable estimate of eutrophication status of the North Sea.

The resolution of RS images is 1.2*1.2 km^2. Gaps due to cloud cover are filled with interpolation algorithms, using data from neighbouring grid cells in space and time. This does not affect the spatial resolution. The boundaries of the Ifremer grid on which raw and interpolated products are available since January 1998 are 36N, 60N, 12W, 13E, *i.e.* including the Greater North Sea, except for the most northern part.

RS imaging yields 'big data', too numerous to handle. For further processing we used 1 pixel out of 5 from each row and column, so 1 grid cell per 25 km^2. On the basis of this data set an assessment for chlorophyll was carried out over the period 2001 to 2005 (Figure 6) to compare with the last OSPAR assessment of the same period (OSPAR 2010).

[3] From a cross-border cooperation project on coastal eutrophication, supported by the INTERREGIVa 2Seas Program (http://iseca.eu)

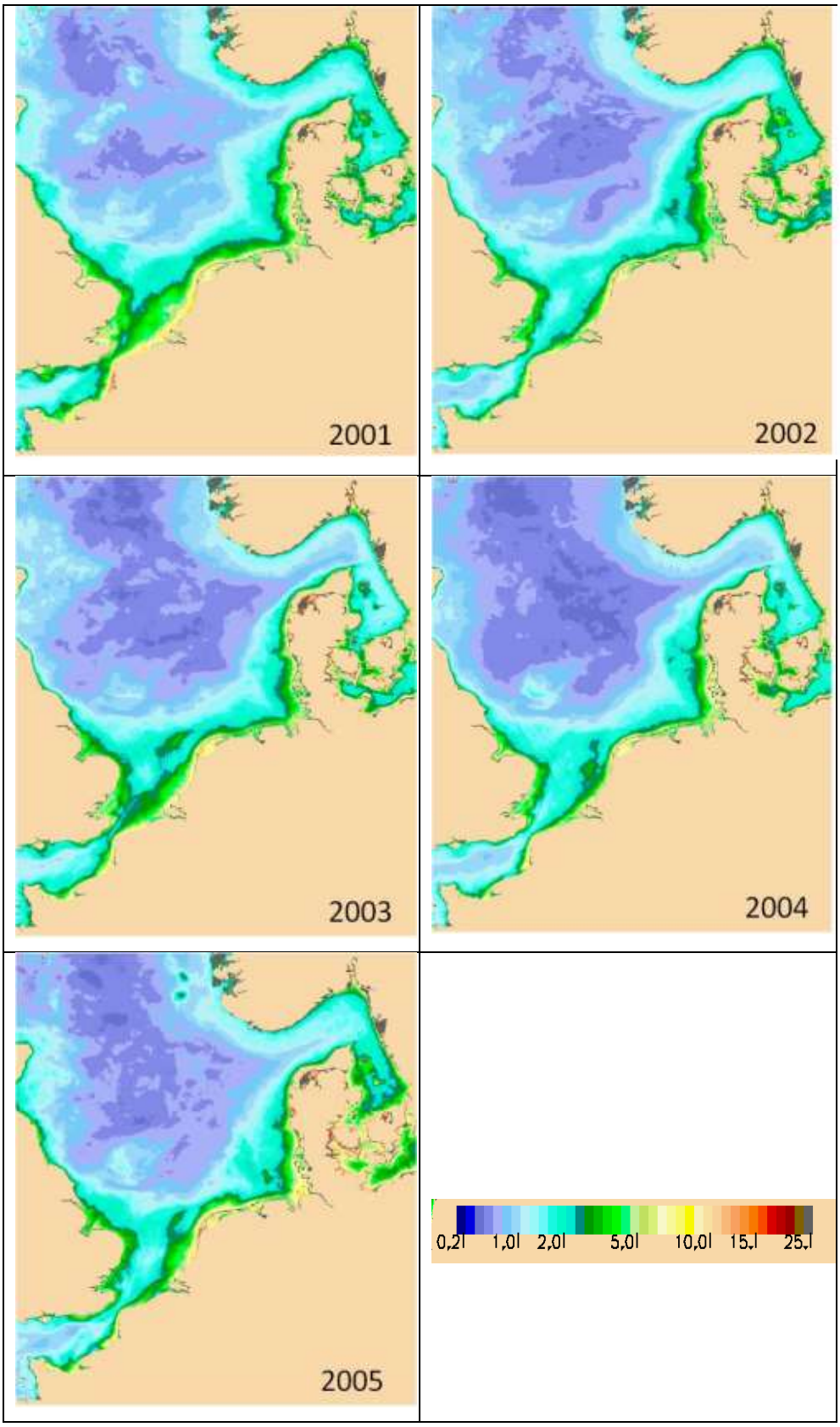

Figure 6. Mean growing-season chlorophyll-a in the North Sea, including Kattegat and the English Channel in 2001 - 2005. The growing season is the period from March to September (Incl.)

For both assessments the assessment levels from the national reports, that are the basis for the OSPAR Quality Status Report (OSPAR, 2010), have been used. The results of this comparison are shown in Table 1 with the assessment levels in the first column.

Table 1. Assessment for growing-season mean concentrations (µg/l) for all OSPAR areas in the North Sea, based on satellite observations. The colours indicate the status of the area concerning chlorophyll, depending on the corresponding assessment levels in Table 1. Red: PA - Problem Area; green: NPA -Non Problem Area; orange: PPA – Potential Problem Area. C stands for coast and O for offshore. Last column: grey cells indicate a difference between the regular OSPAR assessment and the assessment based on RS data. Dark grey cells indicate where these differences can be explained by RS measuring errors.

Assessm level (µg/l)	Area	Assessment Chl, based on Remote Sensing						Overall OSPAR assessment, based on ship surveys period 01-05	Comparison and remarks
		2001	2002	2003	2004	2005	period 01-05		
3.5	NO-Skagerrak coast						C		?[1]
1.5	SE-Inshore Kattegat						C		=
1.5	SE-Inshore Skagerrak						C		=
1.5	SE-Offshore Skagerrak						O		≠[2]
1.5	SE-Offshore Kattegat						O		=
7.5	UK-East Anglia (coast)						C		=
5.0	UK-South. North Sea						O		=
5	UK-North. North Sea						O		=
7.5	UK-NE England (coast)						C		=
7.5	UK-E English Channel						C		=
7.5	UK-E England coast						C		=
7.5	BE-Coastal area						C		≠[3]
4.2	BE-Offshore area						O		=[4]
1.5	DK-North Sea						O		=[4]
3.33**	FR-North Sea Coast						C		≠[5]
3.2*	DE-North Sea						O		=[4]
2.3*	DE-German Bight						C		≠[3]
2.25	NL-Dogger Bank						O		=
2.25	NL-Oyster Grounds						O		=
2.25	NL-Southern Bight						O		=
7.5	NL-Coastal Waters						C		≠[3]

Notes:
* The German assessment levels are not definitive.

** half the 90-percentile assessment value, valid in English Channel and southern North Sea.

1. The assessment of the NO Skagerrak as PA has been based on macroalgae and toxic algal species. Chlorophyll data were not available (National report NO).
2. This has to be investigated further. A possible explanation can be *Chlorophyll median concentrations were below or close to background concentrations*. (National report SE).
3. The coastal areas German Bight, Dutch and Belgium coast are so-called Case II waters, very turbid, which makes estimating of chlorophyll concentrations by Remote Sensing problematic.
4. The BE offshore has been defined as PPA area due to the insufficient data. The DK-North Sea area is a PPA due to increased nutrient concentrations, while chlorophyll does not form a problem; The DE-North Sea area is a PPA, due to occasional oxygen depletion in bottom waters (< 70 %) and insufficient monitoring (National reports BE, DK and DE).
5. This has to be investigated further.

The notes of Table 1 are rephrased in Table 2.

Table 2. RS and OSPAR assessments for growing-season mean concentrations (µg/l) for the OSPAR areas in the North Sea, where both assessments differ with background information from the national reports. See for the colour coding the legend of Table 2.

Remark	**Area**	**Chl - RS assessment**	**OSPAR overall assessment**	**OSPAR assessment based on *(sources: national reports)***	**Conclusion**
1	NO Skagerrak	NPA	PA	macroalgae and toxic algal species; insufficient Chl data	No issue related to RS
2	SE Offshore Skagerrak	PA	NPA	chlorophyll *median* concentrations were below or close to background concentrations	? has to be investigated further
3	BE, NL and DE Coastal areas	NPA	PA	case II waters, very turbid, which makes estimating of chlorophyll concentrations by RS problematic	Known problem, related to RS
4	BE offshore DK North Sea DE North Sea	NPA NPA NPA	PPA PPA PPA	insufficient data enhanced nutrient concentrations occasional oxygen depletion <70% in bottom waters	Possibly identical? Chl NP → Identical Chl NP → Identical No issues related to RS
5	FR North Sea Coast	PA	NPA	?	? has to be investigated further

It should be noted that the assessment of OSPAR eutrophication status not only depends on chlorophyll concentration and in some cases the outcome of the assessment is determined by another factor such as macroalgae, see notes 1 and 4 in the Table above. An assessment based on chlorophyll concentration alone would be better comparable with the RS data.

Note 3 reveals a real issue of RS: turbidity in coastal waters can mask chlorophyll levels. Methods that correct for turbidity are already in use and may be applied to coastal eutrophication assessment areas where needed.

From this comparison we conclude that in most cases RS data can be used to assess chlorophyll against the OSPAR assessment levels.

Benefits of RS for eutrophication assessment are:

- RS integrates chlorophyll levels over a 1.44 km^2 area, while in situ sampling only takes a small volume of seawater;
- Large spatial coverage and daily images;
- Yields coherent cross-border assessments;
- No need for intensive in situ (ships) sampling scheme and hence reduced monitoring costs.

Issues with RS are:

- Turbidity in coastal areas masks chlorophyll;
- Only the surface layer of approx. 10 m is measured;
- In situ sampling still needed for calibration of RS (but less intensive) and determination of toxic algae blooms (requires intensive sampling for early warning in some areas and during some periods of the year).

5 Summary and conclusions

Towards joint assessment of chlorophyll – general conclusion

Chlorophyll is a *common indicator* for eutrophication assessment. OSPAR moves towards joint assessments of common indicators at scales that generally cover sea areas of several neighbouring countries. The project investigated to which extend this would be possible for North Sea countries and how this could be supported by a joint monitoring design.

We met serious obstacles that relate to the current ship-based monitoring design and the analysis of chlorophyll. The current monitoring data do not allow for a cross-border assessment and cannot provide information on spatial and temporal variability of the indicator that is needed to design an optimized joint monitoring programme. Creating subsets of data to increase comparability and trialing conversion factors between the different analytical methods that are currently in use was considered insufficient. One option to improve the situation is to harmonise monitoring design and analysis of chlorophyll.

A second option is to gradually switch to remote sensing by satellites as the main source of chlorophyll data for the assessment of eutrophication. The high variability of this indicator in time and space calls for high sampling frequencies and dense sampling patterns, which is what satellite observation can deliver easily. Current monitoring effort using ships is comparable to one of the main demersal fish surveys (IBTS) and we expect that using satellite information can significantly reduce the costs of chlorophyll monitoring.

More specific conclusions are presented below:

Joint assessments require comparable sampling designs

- Cross-border assessment areas based on physical and ecological characteristics can increase statistical power of the assessment;
- However, differences in temporal and spatial sampling design between countries hamper joint assessments.

Joint assessments require comparable analytical methods

- Different countries use different analytical methods to measure chlorophyll. Differences in each step of the analysis can cause different results. The two most relevant differences are caused by:

    - extraction method: There is no single standard extraction technique recommended for the determination of chlorophylls. A recent investigation by QUASIMEME concluded that the most efficient extraction solvent was ethanol, although they did not indicate whether this is for cold or hot solvent;
    - measurement method: with HPLC chlorophyll-a can be measured, while other methods, such as fluorometry or photometry do not separate the pigments and measure all chlorophylls (chlorophyll-total);
- Which method individual countries are using to measure the chlorophyll concentrations is not relevant for the OSPAR assessments, as long as the national assessment levels are based on the same method. However, for MSFD Descriptor 5 assessments the choice of analytical method has implications, because it concerns cross-boundary regional assessments. For the MSFD it is recommended to harmonise the chlorophyll analytical measuring methods.
- Using fixed conversion factors for inter-comparisons between chlorophyll concentrations determined by the different analytical techniques is no option, because the relation between results of measurements by different methods is dependent on species composition and other variables, and can't be expressed as a fixed number;
- The decision which method of analysis is most appropriate must be made by the end user of the data. Both chlorophyll a and chlorophyll-total serve as proxies for algal biomass. End users must also consider the implications of changes in methodology for historical time series and if necessary maintain existing methodology for comparison for some time and/or complement with new parameters;
- Variation in chlorophyll measurements caused by differences in analytical methods should be compared with natural variability in the occurrence of algae. Limitations in temporal and spatial coverage connected to ship-based monitoring hamper an effective assessment because of natural variability.

Consequences for OSPAR's JAMP and for ICES

- Although the algal growing season is clearly defined in the JAMP Guidelines, this is not always followed in sampling programmes, hampering cross-border comparison. The reasons for this should be investigated;
- The term 'total chlorophyll-a' by fluorometric or photometric analysis, as described in the current JAMP Eutrophication guidelines is misleading. The authors recommend the JAMP guidelines are revised, replacing the term 'total chlorophyll-a' for fluorometric and photometric analysis with an alternative term e.g. chlorophylls or total chlorophyll;
- The ICES parameter codes should be revised to reflect the current JAMP Eutrophication monitoring guidelines for chlorophyll in water. Data should only be reported as chlorophyll-a if a method is used which does discern chlorophyll-a from other chlorophylls and pigments, otherwise an additional ICES code may be needed for methods measuring all chlorophylls;
- The data submitted to ICES should be of comparable quality to permit accurate assessment across all MSFD regions. It is important that any data submitted have enough methodological metadata to support data assessments. The current nomenclature used for submission of chlorophyll data to the ICES database is ambiguous and should be clarified and aligned to reflect revised OSPAR JAMP guidelines. Consideration should also be given as to whether additional method metadata are required with data submissions.

Explore increased use of Remote Sensing (RS)

- Alternative methods such as RS and ferry boxes can greatly enhance temporal and spatial coverage and calibration of the results of these methods is a priority;
- Expand comparison of assessments based on ship sampling with RS, *cf*. OSPAR's Comprehensive Procedure (involve ICG-EUT);
- Develop a joint sampling scheme for continued calibration of (new) satellites, using harmonised analytical methods;

- Develop area-dependent calibration algorithms to correct for turbidity where relevant, *eg*. in some coastal areas;
- Further investigate the costs of RS monitoring and assessment and compare these with the current practices. Investigation of the temporal and spatial requirements of other parameters that are being collected during ship surveys need to be considered as well.
- Involve RS experts!

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Case study Chlorophyll

Annex I

Inventory of the Chlorophyll-*a* data in the ICES database

first version 16 December 2014

updated June 2015

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Table of Contents

1. Introduction

In the Project Management Plan of the project "Towards a Joint Monitoring Programme" (Anonymous, 2014; Grant Agreement no. 07.0335/2013/659567/SUB/C2; in short JMP NS/CS) the aim of the project has been described as follows:

The main goal of the project is to develop a joint, integrated marine monitoring programme in the North Sea region with in parallel a monitoring programme for the Celtic Sea. These joint programmes have to meet monitoring requirements of the Marine Strategy Framework Directive (MSFD) and other environmental legislation and to support the management of human activities in these sub regions.

The aim of this pilot project is to find ways to integrate marine monitoring efforts of the partner organisations. This will be done by finding synergies in existing marine monitoring programmes and finding cost-effective ways of maximising efficiency of existing resources (e.g. multi-use of existing monitoring platforms). This will enhance efficiency and effectiveness of marine monitoring efforts. The partners wish to work towards reducing the overall monitoring costs of the implementation of the MSFD.

All countries around the North Sea and Celtic Sea are participating in the JMP pilot project: France, Belgium, The Netherlands, Germany, Denmark Sweden, UK (England and Scotland) and Ireland. The data of Norway[4]) have been used as well in the analysis.

The project has been sub divided in a number of Activities, of which Activity E is in charge *to adopt and/or develop planning and evaluation tools which will address the required precision and spatial and temporal resolution of integrated monitoring.*

Within the project three case studies have been defined: Elasmobranchs, Benthos and Eutrophication with Chlorophyll-*a* as indicator. In the following this case study is called: Chlorophyll case study. In this document the terms Chlorophyll and Chlorophyll-*a* are used interchangeably.

Chlorophyll-*a* is one of the indicators for Eutrophication used for the WFD, for regional Conventions, such as OSPAR and HELCOM, and also proposed as common indicator for the MSFD.

This working document describes the inventory and analysis of the Chlorophyll-*a* data in the ICES data. Unfortunately, the Chlorophyll data, collected by the countries around North Sea and Celtic Sea, have not been measured in the same way. There are differences in the treatment of the samples and also different measurement methods have been used, such as HPLC, spectrophotometry and fluorometry. Although these methods are not measuring exactly the same constituents of Chlorophyll, all results have been called Chlorophyll-*a* and stored as such in the ICES database. In this study the differences have of necessity been ignored, but an extensive review of the analytical methods has been prepared (Walsham al., 2015; Annex II of the final report) within the JMP project. In follow-up studies any consequences of differences in the measurements due to the use of different methods may be taken into account.

During the workshop at the Thünen Institute in Hamburg we have discussed and decided to use the growing season as annual assessment period, because this period conforms to both the WFD and OSPAR. As assessment parameter the mean value and the 90-percentile value over the growing season are in use in the WFD and in OSPAR. In this case study we have chosen to use the mean only, as using the 90-percentile makes the analysis unnecessarily complicated. Moreover, an initiative within JMP has been started to evaluate whether the 90-percentile does provide additional information.

In Activity E (TOOLS) we originally intended to develop four scenarios for the case study Chlorophyll-*a*:

Scenario 1 The statistical power and costs of the current monitoring network for each country individually and for the whole monitoring network for the North Sea and Celtic Sea.

Scenario 2 The statistical power and costs of an optimized monitoring network for each country individually and of optimized joint monitoring networks for the whole North Sea and for the Celtic Sea.

[4] As Norway is not one of the EU countries and therefore Norway is not required to comply to the MSFD.

Scenario 3 A monitoring network with the same power as scenario 2 with a minimal observational programme supplemented with remote sensing, with the estimated total costs.

Scenario 4 A monitoring network with the same power as scenario 2 with a minimal observational programme supplemented with FerryBox data, with the estimated total costs.

During the preliminary analysis of the chlorophyll data it gradually became clear that the power analysis for chlorophyll could not be performed in the same way as for macrobenthos and elasmobranchs, the other case studies in the project.

The reason for this is that the chlorophyll data not only have a spatial aspect but also a temporal aspect, which cannot be ignored. Macrobenthos and elasmobranchs are both long-lived organisms, with a fairly stable biomass throughout the year, whereas phytoplankton biomass, with chlorophyll as proxy, varies greatly over the year.

An alternative approach to use the indicator statistic (the seasonal mean concentration) was not an option either. That would only work if all countries sampled every year throughout the season at fixed stations, which is not the case. This implies that it was not possible to carry out the power analyses for each of the scenarios.

However, we investigated the comparability of national data reported to the ICES database, which is a necessary step in any effort to perform a joint assessment.

In addition, we calculated the costs of the current ship-based sampling of chlorophyll, based on the ICES database. In addition, Annex III of the Case study Chlorophyll final reports on a feasibility study comparing results of the OSPAR Comprehensive Procedure based on *in-situ* data with those based on Remote Sensing images.

Limitations of this study:

- Although a monitoring programme obviously has a temporal as well as spatial aspect, the current statistical tool developed by TI only addresses the spatial aspects. For chlorophyll this is not realistic, because the assessment statistic is the seasonal mean concentration.
- The scenarios will only be carried out for the North Sea for the reason that for the other two case studies no data of the Celtic Sea are available. Therefore, no common stratification covering both areas has been provided.

Acknowledgements

The author would like to express her gratitude to Anouk Blauw for attending the workshop in Hamburg, for her R-scripts work during the workshop and for sharing her expertise with regard to analysing Chlorophyll after the workshop.

2. Comparability of national chlorophyll data in the ICES database

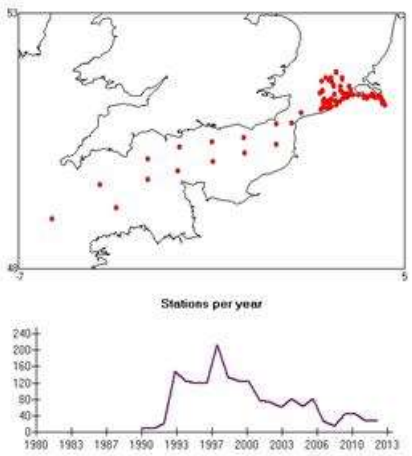
2.1 Preparing chlorophyll data for a joint assessment

The data used have been extracted from the ICES Database (ICES, 2014) for the region between Latitude N49 to 63 and Longitude E-8 to W13. These data may be results of science projects (often also outside the national areas) and/or national monitoring programmes. The start of the time series is different for all countries. From some countries data from the late 1950's onwards are present in the database.

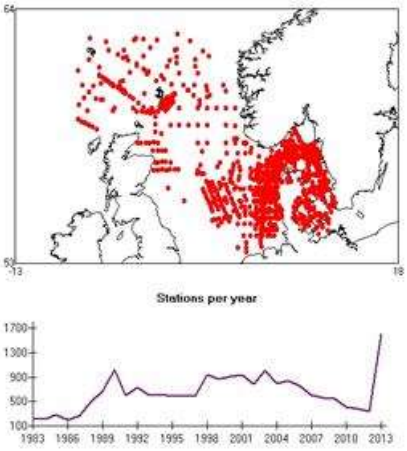
As has been mentioned before the Chlorophyll data in the ICES database, and hence also in this project, are all taken to represent Chlorophyll-*a* concentrations regardless of the measuring method.

2.1.1 Spatial allocation

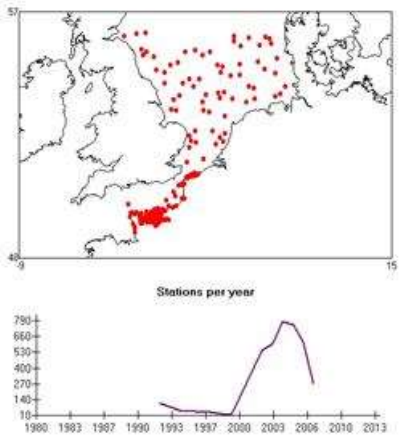
Figure 2.1 presents maps of the countries around the North Sea, the Celtic Sea and the Irish Sea with the monitoring stations where CTD and Bottle observations have been taken in the period between 1980 and 2013 (incl.). The annual number of observations made by each of the countries in the North Sea is given on the corresponding map. Among these observations are also profiles. For this study only the observations from the surface 10 m has been used. To give an impression of the number of observations in the surface layer in the period 2000-2013 the numbers per month of each year are shown in the technical Annex A, Tables XX-1 (XX stands for the country code).



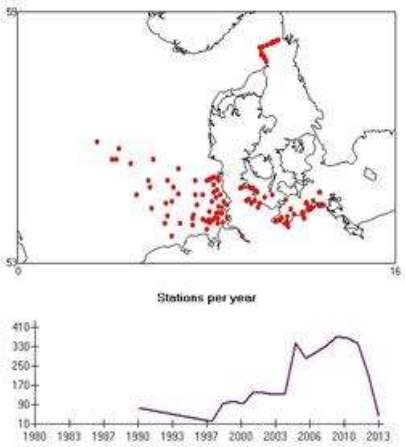
Belgium – 1767 observations



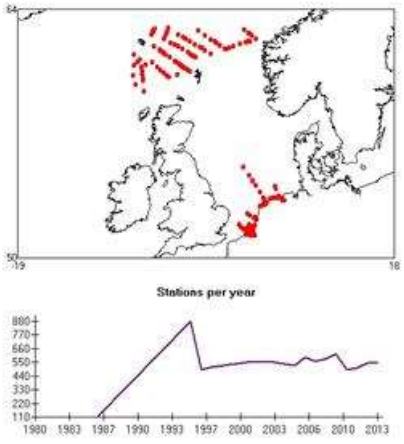
Denmark – 19898/20169 observations*



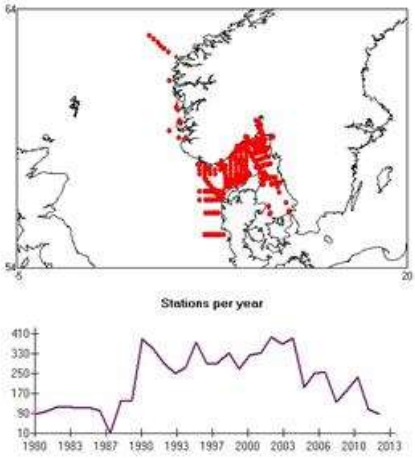
France – 3827 observations



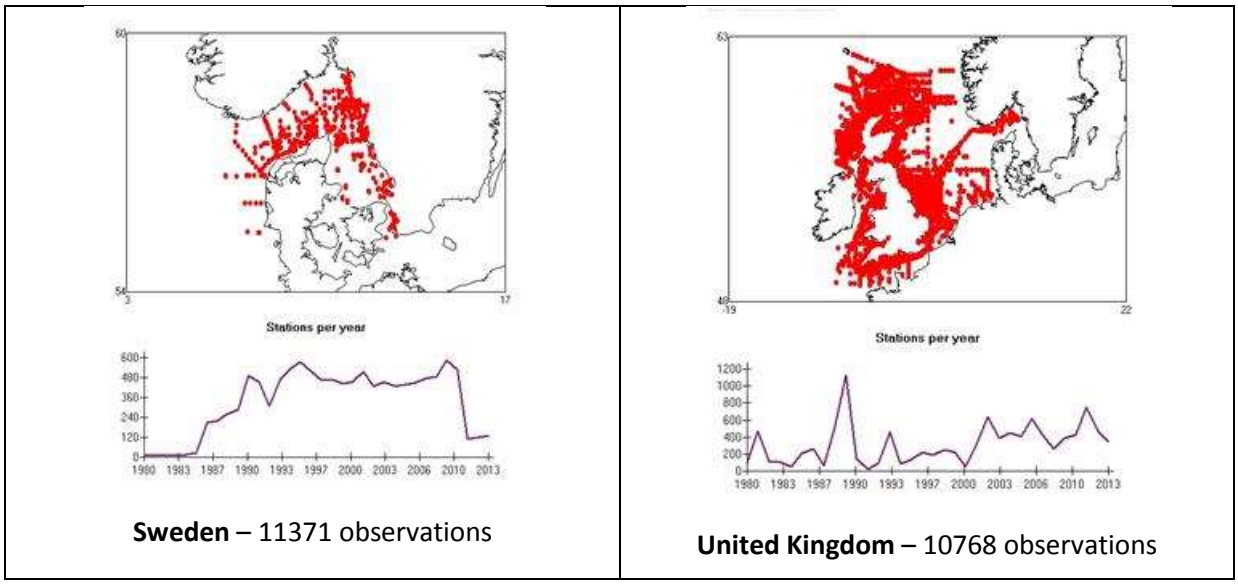
Germany – 3534 observations



The Netherlands – 9168 observations**



Norway – 7389 observations



Sweden – 11371 observations

United Kingdom – 10768 observations

Figure 2.1 Maps of all North Sea countries depicting locations where CTD and Bottle samples have been taken between 1980 and 2013 The graphs represent annual observations. Note the different ranges of the Y-axes. At the bottom of each Figure the total number of observations in the entire period is given (ICES, 2014).

* In the period 1980-2013 Denmark made 20169 observations, which exceeds the 20000 maximum that can be downloaded from the ICES database in one file. Therefore the figure *only* shows the 19898 observations in the period 1983-2013. The corresponding data file contains all data since 1980.

** In the Dutch national database monitoring data are available from 1973 onwards.

2.1.2 Temporal allocation

The Chlorophyll case study is different from the other two case studies with regard to the temporal distribution of the observations. The data files of Benthos consist of two datasets: a joint North Sea wide survey, conducted in 1986, and a collection of observations, made for several purposes, in 2000. For the Elasmobranchs data from 14 years of IBTS have been brought together in the analyses. The Chlorophyll data, on the other hand, contain long-term time-series of observations with multiple observations within years, which relates to seasonal variability. We must therefore ensure to compare data from the same season.

Because the Chlorophyll concentrations often differ considerably from year to year (Figure 2.2) the data of a 6-year period have been used. One of the most recent six year periods has been chosen for which data were available from all countries. For most countries, data are available up to 2012 or 2013, but for France only up to 2007. Although the period 2002-2007 thus is the most recent one, the period 2001-2006 has been chosen, because this period has slightly more observations.

The concentration of Chlorophyll is seasonally varying. As an example Figure 2.2 shows the typical seasonal distribution for the years 2001 – 2004 together with the climatology for Chlorophyll-*a* over that period.

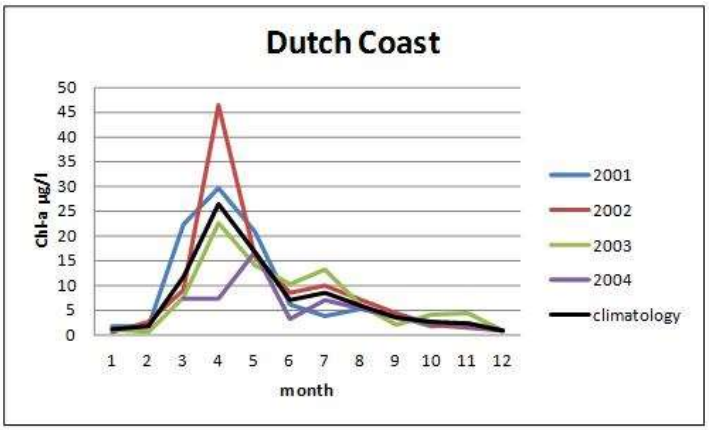


Figure 2.2 Seasonal distribution of Chlorophyll-*a* concentrations at one of the locations near the Dutch coast in the years 2001-2004 and in the climatology of this period, *ie.* the mean monthly chlorophyll concentrations over this period.

This implies that the variance of the observations will also be large due to inter- and intra-annual (seasonal) variability when we use the individual observations. Therefore, we use the seasonal average Chlorophyll-*a* concentration for each location, defined by latitude and longitude. Ideally this would be the mean over the growing season, *i.e*. the mean over seven months. Such a requirement currently cannot be met by the majority of North Sea countries. Table 2.1 shows the distribution of monitoring effort during the growing season, including the total number of locations and observations, for each North Sea country within the region between Latitude N49 to 62 and Longitude E-5 to W12.

Table 2.1. Allocation of monitoring effort during the growing season by all North Sea countries. The observations represent all chlorophyll data from the surface layer available in the ICES database for the growing season of the period 2001-2006 (six years). The numbers in column 1-7 are the percentages of locations with data of only 1 to 7 months in the growing season. The total numbers of locations and observations in this 6-year period are given as well. A high number (red) in the last column represents a fairly even distribution of monitoring effort during the growing season, while a high number in the first column indicates a peak of observations during one month only.

Country / nr months	Total nr of locations *	Total nr of observations	percentage of locations with data of 1-7 months in the growing season for each of the countries						
			1	2	3	4	5	6	7
BE	165	176	**93**	7	0	0	0	0	0
DE	92	647	5	3	1	18	45	12	15
DK	275	4679	34	29	1	1	1	3	31
FR	266	2339	15	24	6	2	4	17	32
NL	207	2308	0	8	0	5	1	7	**79**
NO	493	3991	**71**	1	0	3	7	3	14
SE	143	3713	10	1	1	1	5	16	**65**
UK	624	1158	**88**	12	0	0	0	0	0

* A location has been defined by the year in which the sample has been taken and its latitude and longitude coordinates.

With regard to the temporal design of the chlorophyll monitoring countries can be divided into three groups:

1. Countries where a "complete" set of observations is made at almost all locations. A complete set contains data in each month of the growing season (NL, SE – green)

2. Countries where a complete set of observations is made at no or few locations, and where at least 70% of the locations contain data in only one arbitrary month (BE, NO, UK – amber)

3. Countries with a monitoring strategy in between group 1 and 2 (DE, DK, FR – brown).

Figure 2.3 shows the distribution of monitoring effort over the months in the growing season. For the countries in group 1 (NL and SE) these percentages are high for all months so that for these locations a reliable mean chlorophyll concentration over the whole growing season can be calculated. This, however, is not the case for the data of the other countries. For those countries only data from one particular month or from a few consecutive months could be used to calculate a mean concentration, which moreover is valid for a different part of the growing season for each country.

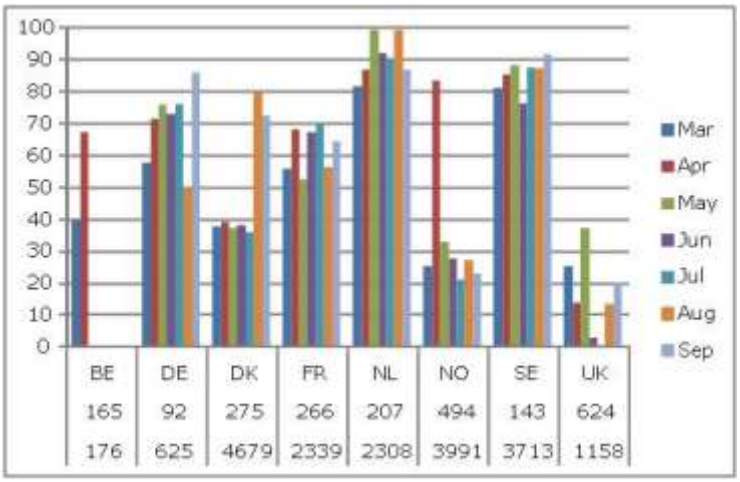


Figure 2.3 Distribution of monitoring effort over the months in the growing season. For each country the percentages of the total number of locations visited each month are given. The number under the country acronym is the total number of different locations (upper row) and the total number of observations made in the period 2001-2006 (lower row).

As can be deduced from Figure 2.3 the optimal statistical approach thus is different for each country. But what would the best approach for an analysis of chlorophyll concentration at the scale of the North Sea?

We therefore identified in which months all countries monitor chlorophyll, which appeared to be the Spring months. In order to further increase the comparability between countries we selected the observations made during March, April and May. These observations were averaged for all countries. It is disputable whether this is the best choice, because the calculated mean concentrations will not all be based on the observations of three months. Some will be based on observation(s) of only one month, while other will be based on observations of two or three months.

When using the observations of the spring months, an average of 85% of the locations per country could be used, ranging from 72 to 100%, and only 50% in one country.

Time and capacity in the JMP NS/CS project did not allow for further analyses. We merely learned that differences in temporal monitoring design are a significant obstacle for cross-border assessments and the design of a joint monitoring programme.

2.2 Common stratification

An interesting element of the approach of the Thünen Institute is to subdivide the region studied, i.e. the North Sea, into areas (in statistical language called: strata) sharing the same characteristics. During the

workshop a plea was made for a “stratification” (=division into areas) based on ecological and physical criteria and as we all felt that this makes more sense than an arbitrary factor, we agreed to use an ecosystem-based division into regions. In literature several of these divisions exist.

As a straw man for the later defined stratification, the ecohydrodynamical regions (in short ecohydro regions) as defined with the GETM-ERSEM-BFM model for the years 1958 – 2008 (Van Leeuwen et al., submitted) has been used as the hydrodynamics largely determine the spatial distribution of phytoplankton, and hence of chlorophyll. Although this stratification with five ecohydro regions has white, intermediate strata (Figure 2.4, left) it shows strong similarity to the stratification designed within the EU project VECTORS (Figure 2.4, right) which was chosen by the partners of the Thünen Institute as a common JMP stratification for all three case studies.

When all white areas are assigned to neighbouring areas except for the region Dogger Bank (including the Oyster Grounds), which is treated separately as being a sedimentation area, six areas can be distinguished:

1. Permanently stratified (red)
2. Seasonally stratified (green)
3. Permanently mixed (blue)
4. ROFI (Regions Of Fresh water Influence; yellow)
5. Intermittently stratified (purple)
6. Temporary sedimentation area (orange).

When the areas of both stratifications are colour-coded in the same way, the similarity between the two stratifications becomes clear (Figure 2.4). It is also interesting to compare this subdivision with the current assessment areas in the OSPAR eutrophication assessment, which includes national borders, but also takes into account hydrological characteristics.

Table 2.3 gives the names of the strata.

Table 2.3. Areas in the North Sea Common Stratification

code	area	color	characteristics
NorC	Norway Coast & Trench	red	Permanently stratified
UKN2	UK North offshore	green	Seasonally mixed
Ger2	Ger nearshore	green	Seasonally mixed
OSN	Orkney Shetland N	green	Seasonally mixed
NCNS	Northern Central	green	Seasonally mixed
CH1	Channel N	blue	Permanently mixed
UKS1	UK South coast	blue	Permanently mixed
UKS2	UK South offshore	blue	Permanently mixed
UKN1	UK North coast	blue	Permanently mixed
NL1	NL coast	yellow	ROFI
Sk1	Skagerrak	yellow	ROFI
DB	Dogger Bank	purple	Intermittently mixed
Ger1	Ger coast	purple	Intermittently mixed
NL2	NL nearshore	purple	Intermittently mixed
NL3	NL offshore	orange	Temp. sedimentation area

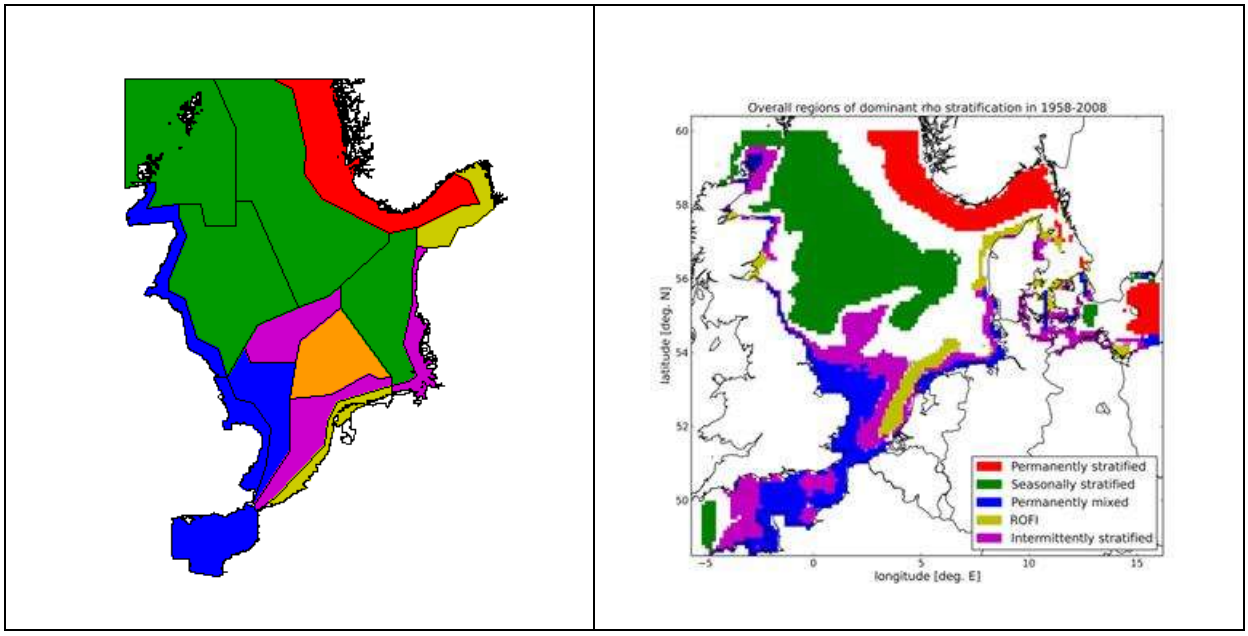


Figure 2.4 Subdivision of the North Sea. Right: The Ecohydrodynamical regions, as defined by Van Leeuwen, et al. (submitted). Left: The Common Stratification, as defined for the JMP project and coloured as the ecohydrodynamical regions of the left figure.

Figure 2.5. Map of the OSPAR areas of the greater North Sea for eutrophication assessment.

Due to the difficulties described above, the allocation of stations to these strata following the statistical method of the Thünen Institute was not successful.

3. Estimating costs of current ship-based sampling of chlorophyll

Data used in current assessments of chlorophyll originate from dedicated ship based sampling on a country by country basis. Looking at potential alternative approaches from a joint monitoring perspective the JMP NS/CS project assumes that the costs of monitoring and the quality of the assessment using monitoring data are key parameters to compare current monitoring with potential alternative sampling methods.

The project developed an approach to estimate the cost of ship-based monitoring of the entire North Sea. As a proxy for monitoring effort we estimated the number of ship miles travelled in one year using the 'travelling salesman' algorithm. This algorithm creates the shortest route that visits every sampling station and returns to the starting place. It assumes that one ship visits all stations and it does not include trips from and to the harbour.

Using the station information of the year 2006 in the ICES database, and taking into account repeated visits of stations within that year, the total distance covered was approximately 320,000 nautical miles. Compared to the demersal fish surveys (IBTS), that amount to approx. 160,000 nm yearly, chlorophyll monitoring can be considered a significant effort.

The countries considered in this calculation are BE, DE, DK, FR, NL, NO and UK. Sampling stations are mapped in Figure 2.5. It should be noted that surveys generally sample additional parameters next to chlorophyll, *e.g*. hazardous substances under the WFD (monthly sampling in coastal waters). Limitations and doubts about the present approach are discussed in Chapter 4.

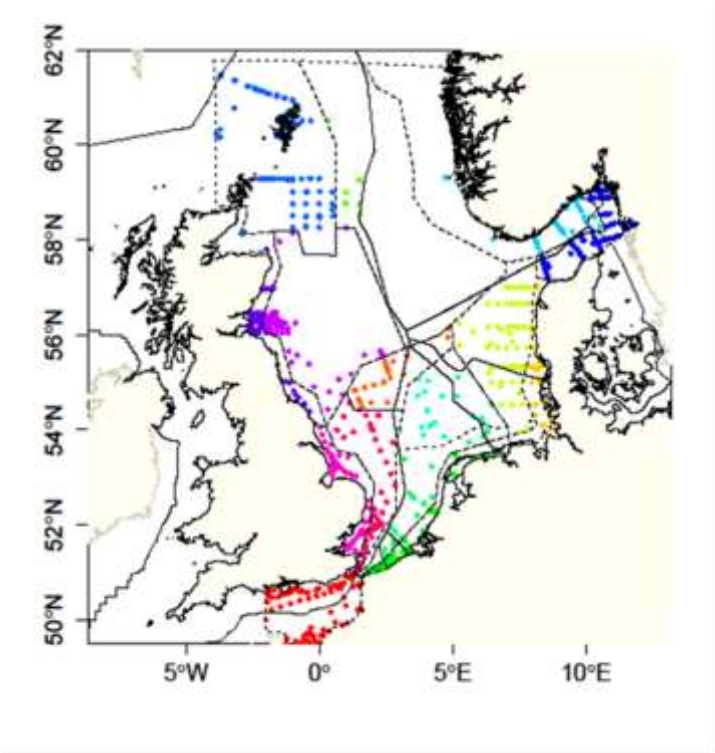


Figure 2.5. stations reported for chlorophyll monitoring in ICES database. Year 2006. Dashed black lines and color-coding of stations: boundaries of and measurements within strata that can be used as relatively homogeneous assessment units (*cf*. section 2.2).

4. Discussion and conclusions

We investigated to which extend the current, ship based monitoring data, as reported in the ICES chlorophyll database, could be used to develop a joint monitoring design. For this purpose we needed to compare data of all North Sea countries and to estimate the variability due to natural causes.

We met a series of serious obstacles that relate to the current monitoring design and the determination of chlorophyll. In this Annex the differences and similarities in temporal and spatial monitoring design of North Sea countries are investigated. Attempts have been made to improve the comparability between countries by selecting observations made in the Spring months only. In addition, we investigated how a common (spatial) stratification, based on ecohydrodynamical characteristics, would apply to the MSFD indicator chlorophyll-a.

The next step would have been a statistical analysis to determine allocation of monitoring stations for an effective sampling design, using the variances in the strata. Such an analysis requires that the data are comparable. Here, we met a second obstacle: differences in analytical treatment of chlorophyll samples. This has been further investigated in a parallel task, summarized in Annex II to the overall chlorophyll report. Application of conversion factors between analytical methods has been trialled, but the outcomes were considered unreliable.

A further difficulty was that the statistical methods applied in the project focused on spatial design of monitoring only. This is a useful approach for long-lived organisms, such as Elasmobranchs and many benthic species (the other two case studies in the project), but not for the highly variable indicator chlorophyll, that shows distinct seasonal patterns and year to year differences.

Furthermore, these methods use the current monitoring efforts as point of departure for the analysis and checks whether the number of stations can be decreased on the basis of the available data. So the implicit assumption is that the monitoring effort is over complete and properly distributed over the investigated area, and that the variances in the defined strata are representative for the ecosystem component under study. For Chlorophyll we know that there are large areas, such as the Central Northern North Sea (Foden et al., 2014), which are not monitored at all and other areas with only one station (*e.g.* Dogger Bank).

In this respect, however, Remote Sensing data could help, because the large advantages of the use of Remote Sensing to obtain Chlorophyll values are the high spatial and temporal resolution and the synopticity of the observations. A disadvantage of the use of Remote sensing is that only the chlorophyll concentration of the surface layer down to 1 optical depth can be detected. This is no problem in well-mixed waters, but it often is in stratified areas with a Deep Chlorophyll Maximum (DCM). As OSPAR only uses the data of the surface layer for the assessment anyway, which will also be the case in MFSD, this disadvantage does not matter in this case, but a real drawback remains that turbid waters (so-called Case II waters) affect the accuracy of the results negatively.

Blaas *et al.* (2013) and Baretta-Bekker (2014) conclude in their reports that the comparison between Remotely-Sensed Chlorophyll-*a* values and HPLC measurements at fixed stations is good, except for the more turbid areas in the coastal zone and during part of the year in temporary sedimentation areas such as Dogger Bank and Oyster Grounds.

As RS supplies large amounts of data it is recommended to use these data to estimate the variance between the measurements of Chlorophyll in the surface layer. If OSPAR could switch to joint chlorophyll assessments based on RS data, the acquisition of *in-situ* data is no longer necessary, other than for calibration and validation of the RS data. However, because not everyone is convinced that RS is as good as water bottle data, it is important to compare the scenarios with and without the use of RS data.

Changing to RS as the basis for assessment of algal biomass may lift a significant burden from ship-based monitoring programmes. We estimated the effort of ship-based chlorophyll sampling, expressed in nautical miles, to be about twice the distance covered yearly in the IBTS surveys that are carried out for the assessment of demersal fish stocks.

We advise to further investigate the costs of RS monitoring and assessment and compare these with the current practices. The main issue with chlorophyll monitoring, in terms of costs, is the variability of this parameter, which requires frequent sampling for a reliable assessment. Investigation of the temporal and spatial requirements of other parameters that are being collected during ship surveys need to be considered as well.

FerryBox data have not been used in this study, because the Dutch FerryBox data of the North Sea have a limited accuracy, according to Blaas et al. (2014). The same is true of the Algaline data in Skagerrak and Kattegat (Mohlin, pers. comm.). Due to time constraints the use of other FerryBox data of the North Sea has not been considered.
The added value of FerryBox data value lies not so much in the assessment of Chlorophyll, but more in the interpretation of any changes in them. They also do provide supporting data (such as temperature, salinity and nutrients) indicative of the state of the system. Because they often are installed on research vessels, sailing from station to station, they provide additional information about the system state in the large areas between monitoring stations, which is extremely useful for correct interpolation, using Empirical Orthogonal Functions.

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Annex I A: Technical Information of the data availability per country

For each country an overview is given of the data availability in the ICES database (ICES, 2014).

Belgium

Aantal van CPHL		month												
year		01	02	03	04	05	06	07	08	09	10	11	12	Total
2000			15		19	30				30			29	123
2001		18	11		19							29		77
2002			21	24									28	73
2003			30	30										60
2004			21		31							30		82
2005			31		31									62
2006			18	11	30							11	11	81
2007			9	8	2	3		3					3	28
2008		3			6		1	2					3	15
2009		12			19			9					3	43
2010		3		10	10			10					11	44
2011		3		10	10			2				3		28
2012		3		9	10			3					2	27
Total		**125**	**276**	**164**	**360**	**148**	**44**	**90**	**10**	**77**	**57**	**183**	**234**	**1768**

Table BE-1 Number of observations per month for each year from 2000-2012, from Belgium in the ICES database.

Belgium has in total a time series of 23 years from 1990-2013 in the ICES database. From 1992-1998 all years had data from each month in the growing season, while more recently the focus has been on the spring bloom.

In the period 2001-2006 there are in total 176 observations from 165 stations with in the month April 111 observations from 50 stations, which is 94% of the total number of stations.

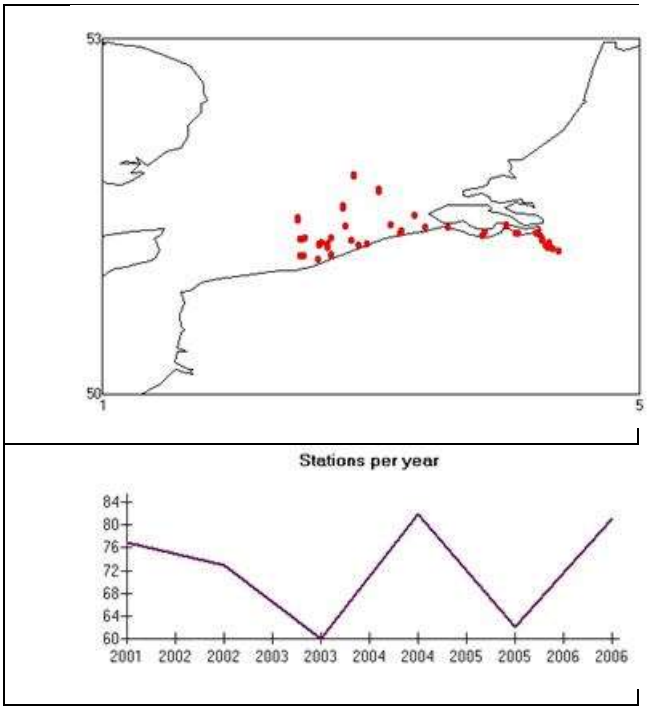

Figure BE-1 Maps with the locations of Belgium where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

Germany

Aantal van CPHL	month												
year	01	02	03	04	05	06	07	08	09	10	11	12	Total
2000	3	51	28	21	46	3	29	21	3	24	24	6	259
2001	14	13	18	17	13	14	12	4	8	13	9	7	142
2002	14	12	12	14	19	11	9	4	13	14	13	4	139
2003	11	11	8	8	13	21	16	11	14	8	7	3	131
2004	13	10	9	10	10	21	13	15	11	9	9		130
2005	22	54	25	67	62	30	66	31	29	43	45	4	478
2006	24	24	12	27	26	26	24	33	33	21	17	16	283
2007	7	30	32	27	21	37	28	32	30	29	15	19	307
2008	29	29	26	28	34	35	50	31	30	17	22	4	335
2009	49	41	51	29	56	33	81	38	34	57	31	7	507
2010	7	11	15	31	45	46	46	51	46	28	23	16	365
2011	20	21	19	18	33	48	48	43	47	21	18	7	343
2012	18	44	37	22	62	20	46	38	19	4	50	1	361
2013					96								96
Total	253	368	318	336	560	769	481	366	336	297	304	105	4493

Table DE-1 Number of observations per month for each year from 2000-2012, from Germany in the ICES database.

Germany has in total data of one single year (1990) and a time series of 17 years from 1997-2013 in the ICES database. All years of the time series, except for 2013, have data in all months of the growing season.

In the period 2001-2006 there are in total 773 observations from 45 stations with in the month June the most observations (123 from 34 stations), but in July less observations, but from more stations (119 from 40 stations), which is 76% and 89% of the total number of stations, respectively.

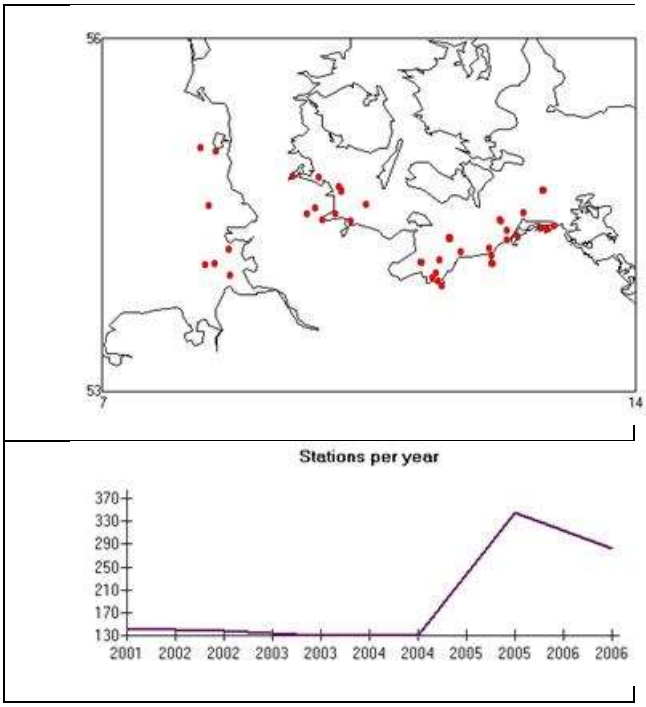

Figure DE-1 Maps with the locations of Germany where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

Denmark

Aantal van CPHL	month												
year	01	02	03	04	05	06	07	08	09	10	11	12	Total
2000	139	461	189	179	224	162	183	582	287	285	307	119	3117
2001	159	510	200	209	184	165	173	551	298	301	301	107	3158
2002	124	464	157	169	150	127	166	311	292	289	252	97	2598
2003	157	601	243	216	180	237	224	366	319	349	287	127	3306
2004	136	493	199	147	150	169	167	205	271	243	247	106	2533
2005	125	472	174	141	179	185	170	266	287	267	286	105	2657
2006	123	429	172	139	180	155	137	297	282	289	111	51	2365
2007	167	162	125	191	115	120	105	271	246	124	110	75	1811
2008	92	233	124	209	125	126	124	237	227	105	104	80	1786
2009	201	70	129	103	87	133	73	234	237	95	98	66	1526
2010	157	66	111	84	94	114	66	90	85	74	93	58	1092
2011	47	65	87	83	115	98	92	100	94	83	104	29	997
2012	65	54	86	83	87	95	97	88	81	86	87	46	955
2013	164	330	175	261	295	247	273	341	368	337	266	156	3213
Total	2970	8712	4482	3434	4347	4017	3877	7552	5693	5217	4667	1721	56689

Table DK-1 Number of observations per month for each year from 2000-2012, from Denmark in the ICES database.

Denmark has in total data of one single year (1990) and a time series of 17 years from 1997-2013 in the ICES database. All years of the time series, except for 2013, have data in all months of the growing season.

In the period 2001-2006 there are in total 5335 observations from 118 stations with in the month August the most observations (1060), which is 81% of the total number of stations.

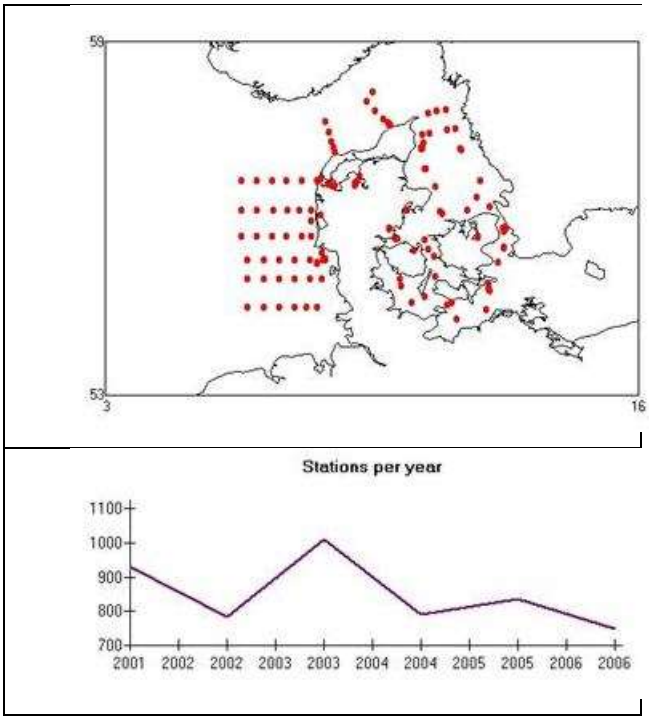

Figure DK-1 Maps with the locations of Denmark where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

France

Aantal van CPHL	month												
year	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**Total**
2002	39	23	20	60	27	44	76	68	51	51	47	38	544
2003	11	43	41	66	44	81	65	51	80	57	21	42	602
2004	77	132	54	68	70	99	93	70	69	45	42	36	855
2005	14	21	71	83	75	125	73	80	75	69	27	46	759
2006	30	12	56	70	62	69	70	73	60	78	17	20	617
2007	8	10	25	26	27	31	35	33	20	40	13	8	276
Total	179	254	275	639	346	685	460	394	552	352	167	190	4493

Table FR-1 Number of observations per month for each year from 2000-2012, from France in the ICES database.

France has two single years (1992 and 1994), a time series of 3 years and one of 6 years from 2002-2007. Only the years of the most recent time series has data in all months of the growing season.

In the period 2001-2006 there are in total 2339 observations from 82 stations with in the month June the most observations (418 from 72 stations), but in July less observations, but from more stations (377 from 840 stations), which is 88% and 98% of the total number of stations, respectively.

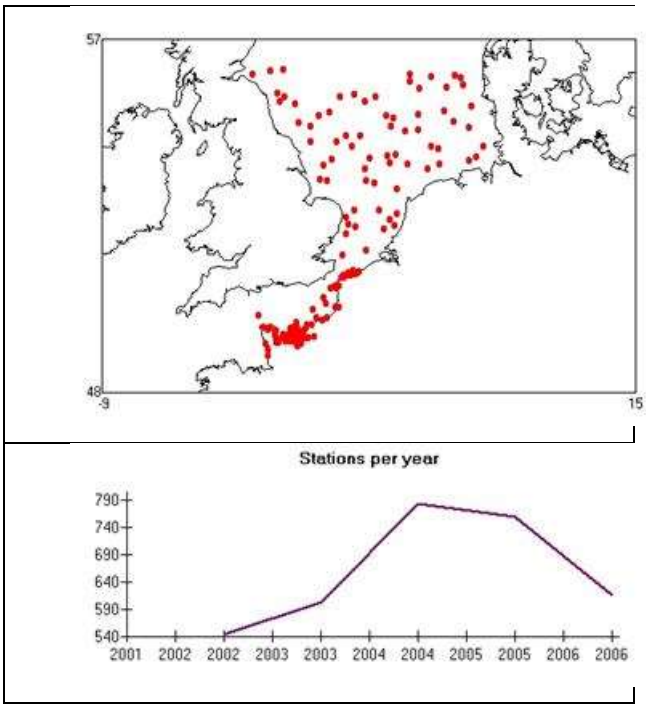

Figure FR-1 Maps with the locations of France where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

Netherlands

Aantal van CPHL	month												
year	01	02	03	04	05	06	07	08	09	10	11	12	Total
1986						504							504
1995	76	44	87	65	84	68	87	91	70	86	66	55	879
1996	25	32	30	38	71	52	53	55	43	30	36	30	495
1997	24	31	46	46	66	56	64	63	38	22	38	20	514
2001	41	36	40	37	81	56	56	74	36	41	30	32	560
2002	33	30	43	53	61	61	60	65	41	40	36	32	555
2003	38	41	52	53	51	48	56	58	50	42	38	25	552
2004	26	13	60	54	60	57	56	58	49	35	36	32	536
2005	29	26	49	43	52	68	44	59	55	34	34	31	524
2006	32	37	48	49	73	71	54	71	46	41	37	32	591
2007	34	35	43	55	76	54	60	65	40	43	30	26	561
2008	33	36	40	50	66	67	56	51	62	45	30	43	579
2009	35	40	55	53	62	76	67	54	63	38	40	36	619
2010	30	32	54	52	45	57	43	49	37	32	32	31	494
2011	35	36	49	52	57	56	43	65	35	18	37	21	504
2012	41	36	42	53	59	49	72	59	37	33	33	33	547
2013	43	38	36	53	54	57	60	57	45	33	33	34	543
Total	575	543	774	806	1018	1457	931	994	747	613	586	513	9557

Table NL-1 Number of observations per month for each year from 2000-2012, from The Netherlands in the ICES database.

The Netherlands have one single year (1986) with data in June of a special cruise, a short time series of 3 years and a time series of 13 years from 1983-2013. All years of both time series have complete sets for the growing season. [5]

In the period 2001-2006 there are in total 2308 observations from 48 stations (including estuaria). The observations have been distributed evenly between 40 and 48 stations per month, which is 83% to 100% of the total number of stations.

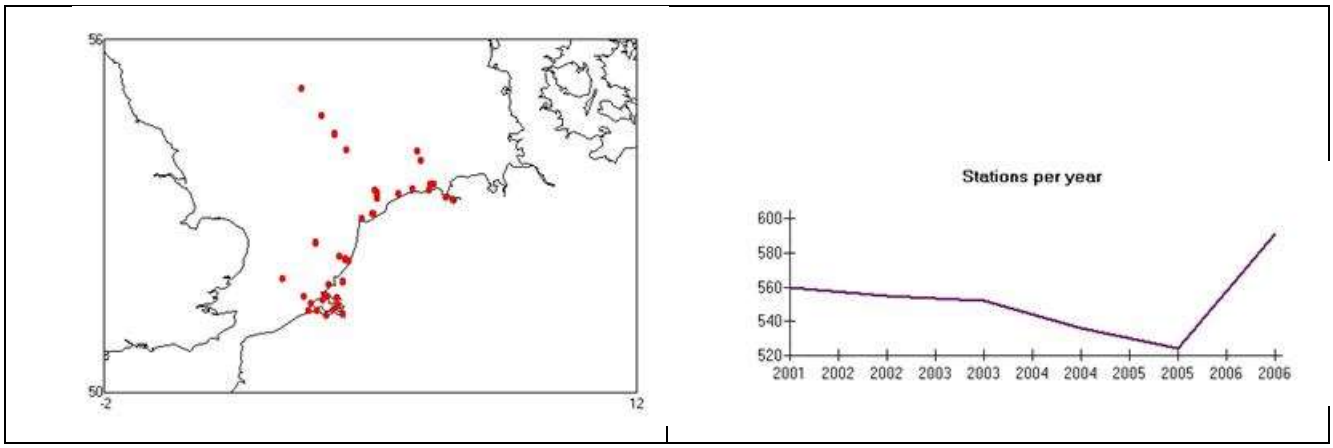

Figure NL-1 Maps with the locations of France where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

[5] In the Dutch national database, however, monitoring data are available from 1973 on.

Norway

Aantal van CPHL	month												
year	01	02	03	04	05	06	07	08	09	10	11	12	Total
2000	111	129	135	569	135	139	135	185	179	82	80	88	1967
2001	138	129	129	632	173	130	88	129	138	133	92	76	1987
2002	167	191	185	381	393	269	94	185	132	191	94	172	2454
2003	88	184	183	601	165	173	89	179	138	179	94	173	2246
2004	185	205	197	669	94	185	176	185	119	178	42	172	2407
2005	82	31	31	94	95	103	101	99	103	100	40	91	970
2006	91	114	118	98	118	114	102	109	113	104	30	38	1149
2007	92	106	111	103	112	108	108	110	107	113	56	97	1223
2008	47	48	51	52	45	52	39	54	52	38	47	28	553
2009	45	47	40	46	44	71	58	76	78	74	71	55	705
2010	65	40	85	74	83	87	62	80	73	72	73	73	867
2011	35	41	41	53	46	47	41	53	47	46	39	12	501
2012	33	50	43	39	51	45	43	45	45	39	50		483
Total	2724	2839	2977	8511	5079	4181	2523	3130	2849	3004	2204	2471	42492

Table NO-1 Number of observations per month for each year from 2000-2012, from Norway in the ICES database.

Norway has a time series of 33 years from 1983-2013. From 1989 on all years have data in all months of the growing season.

In the period 2001-2006 there are in total 2765 observations from 136 stations with in the month April the most observations (886 from 127 stations), which is 93% of the total number of stations.

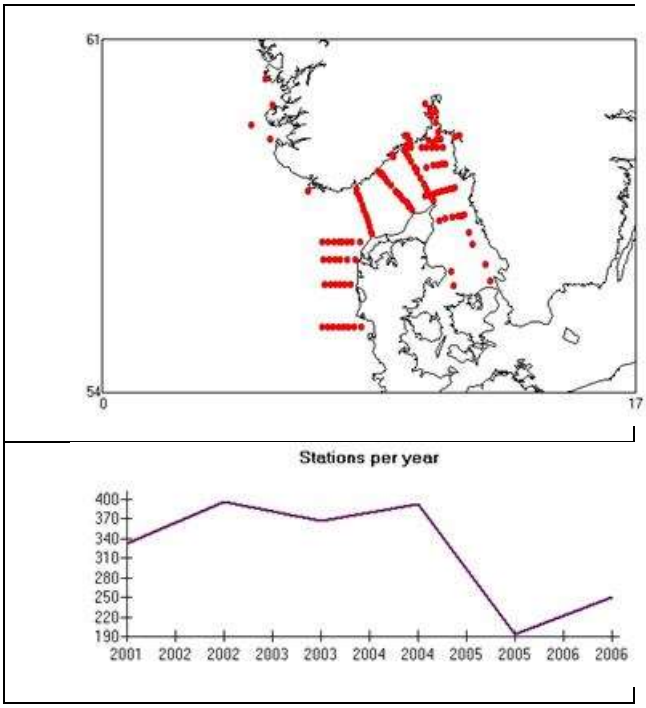

Figure NO-1 Maps with the locations of France where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

Sweden

Aantal van CPHL	month												
year	01	02	03	04	05	06	07	08	09	10	11	12	Total
2000	167	230	203	189	193	189	193	227	198	197	179	186	2351
2001	201	221	298	251	270	193	211	218	195	206	188	195	2647
2002	167	198	174	177	178	173	212	183	223	118	169	171	2143
2003	73	204	200	201	222	235	255	228	248	210	210	206	2492
2004	127	218	248	163	220	244	218	219	227	208	198	200	2490
2005	138	239	125	284	209	202	228	253	279	228	141	196	2522
2006	203	211	162	209	339	79	231	263	288	203	198	205	2591
2007	273	256	209	223	215	209	216	299	261	195	206	198	2760
2008	291	236	224	196	214	256	253	275	291	223	186	202	2847
2009	329	267	332	89	220	331	265	293	259	231	239	175	3030
2010	224	225	134	215	297	250	278	306	250	231	197	196	2803
2011	69	8	86	89	8	86	86	86	86	86	86	86	862
2012	82	69	86	84	97		83	77	84	68	84	85	899
2013	69	76	94	90	76	86	78	94	86	85	85	86	1005
Total	4531	4852	5343	5174	6213	5817	4839	5593	6109	4872	4826	4404	62573

Table SE-1 Number of observations per month for each year from 2000-2012, from Sweden in the ICES database.

Sweden has a time series of 34 years from 1980-2013. From 1989 on all years have data in all months of the growing season.

In the period 2001-2006 there are in total 3995 observations from 54 stations. The observations have been distributed rather evenly over the months, with between 37 and 48 stations per month, which is 69% to 89% of the total number of stations.

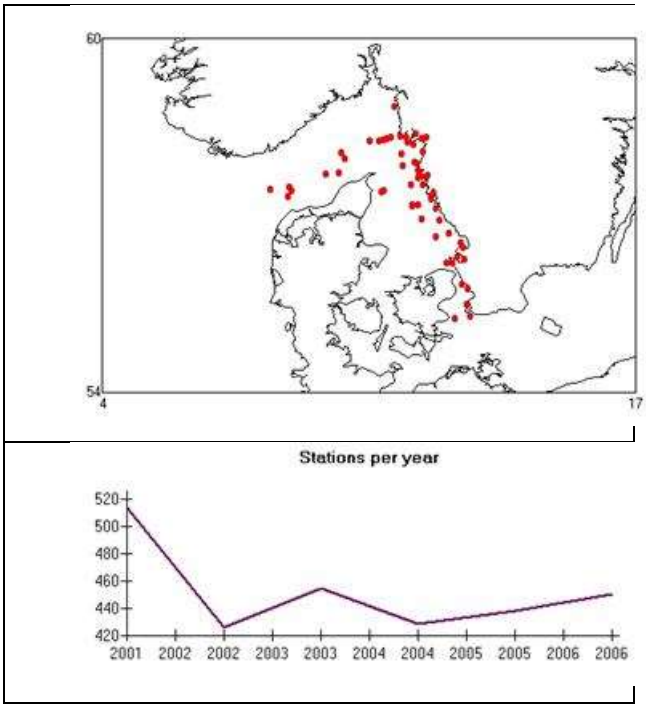

Figure SE-1 Maps with the locations of France where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014)

United Kingdom

Aantal van CPHL	month												
year	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**Total**
2000				59	46					56	16	57	234
2001	162		168	20	61	18		107		54		97	687
2002	212		1	40	145			85	57	88		124	752
2003	77	93	52	728	437	21			110	3		123	1644
2004				251	110			292		155		114	922
2005		23	8	261	631				64	59		127	1173
2006	1	108	33	115	260	18	45	29	155	119	2	109	994
2007	42			66	174	17		40	111	135		111	696
2008	44	6			132		67	32	16		30	126	453
2009	72	64			74	10		28	52	179	23	143	645
2010	107			47	173	122	142	45	45			9	690
2011	5	157	92	55	161	147	129	5	23	117	5	167	1063
2012	121		3	89	238	83	83	130	128	61		120	1056
2013	23	102	33	31	146	64	11	1	8	110	3	115	647
Total	1669	1640	1580	3096	5888	1674	3217	3170	5143	2236	1358	2466	33137

Table UK-1 Number of observations per month for each year from 2000-2012, from UK in the ICES database.

UK has a time series of 34 years from 1980-2013. All years of the time series except for 2013 have complete sets for the growing. May is the month with the most data.

In the period 2001-2006 there are in total 1219 observations from 569 stations with in the month August the most observations (220), which is only 39% of the total number of stations, respectively.

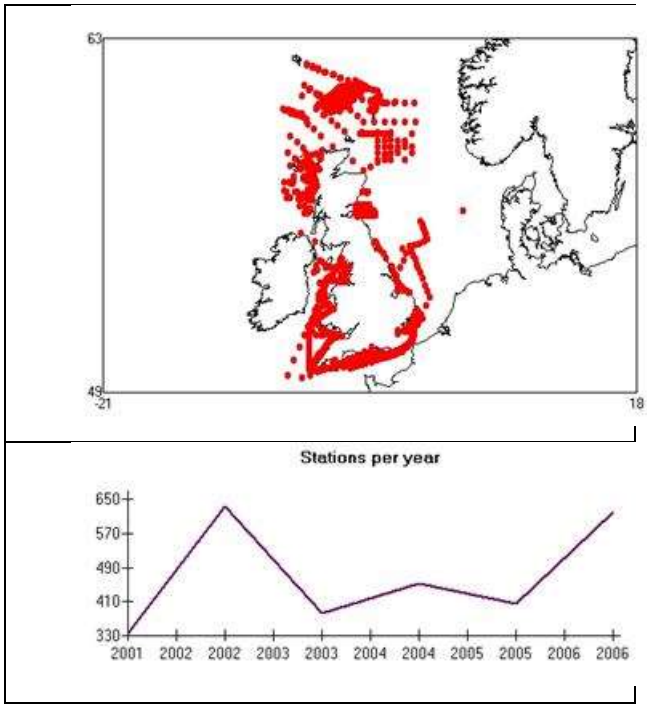

Figure UK-1 Maps with the locations of UK where CTD and Bottle samples have been taken between 2001 and 2006 (ICES, 2014).

Case study Chlorophyll
Annex II

Differences in methodologies for chlorophyll analysis and implications for data reporting and assessments under the Marine Strategy Framework Directive

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Table of Content

1 Introduction

The potential eutrophication of marine waters has been of concern for a number of years. OSPAR, the regional convention for the North-East Atlantic, have developed a Strategy to Combat Eutrophication. To assess eutrophication within OSPAR the Comprehensive Procedure (1) was developed. This is a harmonized, integrated assessment system of the causes and consequences of eutrophication. The associated assessment parameters are divided into four categories: causal factors, direct effects, indirect effects and other possible effects of nutrient enrichment, each with their area-specific parameter values. In 2000 the European Water Framework Directive (WFD) came into force, for all freshwaters, estuarine and coastal waters. The WFD aims to improve and protect the chemical and ecological status of all water bodies from source through to estuaries and coastal waters. The WFD requires the competent monitoring authority to assess the status of its water bodies by assigning them as High, Good, Moderate, Poor or Bad. All water bodies must achieve at least Good status by 2015 (2). This was followed in 2008 by the European Marine Strategy Framework Directive (MSFD). There are strong links between the MSFD and the WFD. The MSFD requires member states to prepare national strategies to manage their seas in order to achieve Good Environmental Status (GES) by 2020 and there is a major emphasis on international cooperation. Key requirements of the Directive are to provide an assessment of the current state of the seas for regions and sub-region and to provide a detailed description of what GES means along with a set of associated targets and indicators.

Assessment of eutrophication for the various directives is based on the categories developed for the OSPAR Comprehensive Procedure. The causative factors (nutrient inputs and concentrations) are used to highlight areas where further monitoring is required to assess the impact on the ecology (accelerated growth of phytoplankton and macroalgae) leading to an undesirable disturbance (excessive organic inputs leading to low dissolved oxygen and kills of fish and benthos). Assessments also need to take account of other factors affecting phytoplankton and macroalgal growth such as light availability and substrate. Chlorophyll analysis is one of the requirements to determine Eutrophication status for both MSFD and WFD. OSPAR have set criteria for chlorophyll *a*, in respect of Eutrophication monitoring, but there is no stand alone criterion for chlorophyll *a* in WFD. For WFD, chlorophyll *a* is included in the phytoplankton tool (3). MSFD Descriptor 5 (Eutrophication) requires the direct effects of nutrient enrichment to be measured. Most member states will include chlorophyll monitoring as an indicator for MSFD Descriptor 5.

Chlorophyll is the biological pigment which plants and algae use to produce food using energy from sunlight in a process known as photosynthesis. There are six known types of chlorophyll in the marine environment (4). All photosynthetic algae and higher plants contain chlorophyll *a* as a principal pigment. As a result chlorophyll *a* has been the primary pigment of interest in marine monitoring programmes and has been used as a proxy to estimate phytoplankton biomass. Traditionally, samples collected to estimate phytoplankton biomass were analysed by photometric or fluorometric techniques (4, 5). These analytical methods address specific spectral interferences but ignore others, as described in section 2.2.1. The trichromatic photometric method determines total chlorophylls in the absence of degradation products, however spectroscopic interference can result in an overestimation of chlorophyll *a* (5). Although it is widely recognised that there are issues with these techniques they are still widely used to estimate phytoplankton biomass in marine samples by many international monitoring programmes and there are long international and national time series data which have to be related to and contributed to. More recently there has been a move to methods using high performance liquid chromatography (HPLC) which have the potential to separate and measure a greater number of pigments. The Netherlands and Belgium routinely undertake HPLC determinations of chlorophylls for their monitoring purposes with datasets

of 20 and 14 years respectively. The other countries around the North Sea and the Celtic Sea use photometric or fluorometric techniques.

The use of different analytical techniques in determining chlorophylls has implications for reporting data under WFD and MSFD. Chlorophyll data, collected using all analytical methods will be submitted via International data centres, such as ICES and EMECO. Historically all data reported to these data centres have been reported as chlorophyll a which isn't strictly true in the case of fluorometric and photometric data. Clearly there is a need to ensure clarity in what is actually reported and discussed. For the purposes of clarity within this report the term chlorophyll a is used to describe the specific chlorophyll pigment data collected by the HPLC method or in the context of descriptions used within data centres and specific guidelines. Data collected using the photometric or fluorometric methods will be described as chlorophylls.

2 Analytical Methods

2.1 Extraction Methods

There is no single standard extraction technique recommended for the determination of chlorophylls. The revised OSPAR JAMP Eutrophication Monitoring Guidelines (6) lists the standard procedures of Strickland and Parsons (7), UNESCO (8), HELCOM (9), ISO 10260 (10) and Wright *et al.* (11) for the analysis of chlorophylls, stating the importance of recording the method used when reporting data.

The standard methods listed utilise a range of techniques for extracting chlorophylls from filter papers including soaking, grinding and sonicating the filter paper in the presence of an organic solvent such as acetone, ethanol or methanol. A recent comparison of extraction methods by QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) indicates that ethanol may be the most efficient solvent for the extraction of chlorophylls. Quasimeme have also found that extraction by sonication alone may result in an underestimation of chlorophylls (12). A Scottish study found that the grinding process created heat that could result in the degradation of chlorophylls and gave inconsistent results when compared with extraction by soaking (13).

2.2 Analysis Methods

2.2.1 Fluorometric and Photometric Methods

The trichromatic photometric method determines chlorophylls in the absence of degradation products. Spectroscopic interference from pheophorbides, chlorophyllide *a* and chlorophyll *a* epimers and allomers result in an overestimation of chlorophyll *a* (5). The acidification method, developed in the 1960's to correct analyses of chlorophyll *a* from interference from magnesium free chlorophyll derivatives (phaeophytins and phaeophorbides, collectively known as phaeopigments), has been used on both spectrophotometers and fluorometers, as described in Text Box 1. The presence of carotenoid compounds also interferes with the determination of chlorophyll *a* from acidified extracts as these are labile towards acid; the extent of the error is dependent on the species composition of the phytoplankton (14), which varies with nutrient availability, site seasonally and interannually. Although it is widely recognised that there are a number of issues with these techniques they are still used to estimate phytoplankton biomass in marine samples by many international monitoring programmes and there are long international and national time series data which have to be related to and contributed to. The revised OSPAR JAMP Eutrophication Monitoring

Guidelines (6) no longer recommend acidification because it is time consuming and the results are questionable.

Text Box 1. Chlorophyll Acidification Method

The sample is measured before and after acidification. It is assumed that acidification degrades all chlorophyll-like pigments into phaeopigments by eliminating the magnesium ion from the tetrapyrrole complex. Measurements of chlorophyll made before acidification are normally called 'uncorrected chlorophyll' and measurements made after acidification are 'phaeopigment-corrected chlorophyll'. The calculation of the phaeopigments assumes that all of this pigment is phaeophytin *a*, which is probably not the case (14), but the absorption coefficient of phaeophorbide is unknown. The presence of chlorophylls *b* and *c* can significantly interfere with chlorophyll *a* measurements depending on the amount present. If chlorophyll *b* is present in the sample this will result in an underestimation of chlorophyll *a* along with an overestimation of phaeophytin *a*. The degree of interference depends upon the ratio of chlorophyll *a* : chlorophyll *b*. The presence of chlorophyll *c* also causes the underestimation of phaeophytin *a*, although not as severe as the effects of chlorophyll *b* (15).

2.2.2 High performance liquid chromatography (HPLC) with Ultraviolet (UV)/ Diode Array Detector (DAD)

HPLC is an alternative method to fluorometric and photometric for the analysis of chlorophylls. HPLC is used in conjunction with either an UV or DAD. The HPLC methods have the advantage over the fluorometric methods in that interfering pigments will be separated from the chlorophyll a resulting in an accurate determination of chlorophyll a concentrations. In addition, the HPLC data can provide valuable information about the contribution of different functional groups to the biomass of the phytoplankton community.

Automated HPLC methods for the routine determination of chlorophylls, phytoplankton, degradation products and carotenoids were first developed in the late 1970s, early 1980s and have continually been improved upon (11,16). Methods were initially developed using UV detectors, but more recently DADs have become the detector of choice (Text Box 2). These methods can now separate, identify and quantify over 50 chlorophylls, cartenoids, their derivatives and isomers from marine phytoplankton.

As a result of the increasing need to accurately determine chlorophyll, and to identify a wider range of pigments and monitor changes in the phytoplankton community, there has been a move to the use of HPLC methods using either UV or DAD detectors, although DAD is more common. Pigment analysis by UV detection is made primarily on the basis of retention time and analyst experience. If concentrations of pigments are low and co-elution from other pigments occurs, identification by retention time alone is difficult. DAD detectors produce a full spectrum of each pigment peak collected to be made, without stopping the flow, greatly facilitating the identification of the pigments as the spectrum can be used to confirm or refute the presence of a particular pigment.

An argument against HPLC analysis is that it can be much more time consuming and expensive compared to fluorometric analysis and may not be necessary for routine monitoring. Dutch experience however contradicts this. Annex 1 of this document tabulates the range of extraction and detection methods used for the determination of chlorophyll by countries submitting data via the ICES database. This is by no means a definitive list of each countries submissions but highlights the lack of comparability.

Text Box 2. Development of pigment analysis by HPLC

The early pigment HPLC methods published, describe the separation of chlorophylls, cartenoids and their degradation products, and provide qualitative information only, due to limited standard availability (16). The United States Environmental Protection Agency Method 447(17) describes the quantification of chlorophylls *a* and *b* and identification of the other pigments of interest using HPLC-UV. In 2002, QUASIMEME held a workshop to discuss 'The Analysis of Chlorophyll *a*' (18). During this workshop a sub-group of participants, undertaking chlorophyll analysis by HPLC, discussed the various detection methods. Participants used both UV and DAD for detection of chlorophyll *a,* there was no information given on the quantification of the other pigments of interest and no conclusions as to detector suitability made. In 2008, Silva *et al*. *(*19) reported a HPLC-DAD method which uses a chemotaxonomic approach to compare major phytoplankton groups based on HPLC pigment analysis and cell counting by inverted microscopy, to study the seasonal variability of the phytoplankton community in Lisbon bay. This method quantifies chlorophylls *a*, *b*, *c2* and *c3*, peridinin, fucoxanthin, diadinoxanthin, diatoxanthin, 19-hexanoyloxyfucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, alloxanthin, 19-butanoyloxyfucoxanthin and zeaxanthin using commercial standards from DHI Lab Products, Hoersholm, Denmark.

3 Comparison of Methods

3.1 Quasimeme Workshop 2014

A recent (2014) QUASIMEME chlorophyll and nutrients workshop highlighted the lack of improvement in the performance of chlorophyll exercises, in contrast to other exercises such as nutrients. QUASIMEME initiated an internal investigation into methods used by participants reporting chlorophylls. No significant differences in participant results were observed using differing extraction times or solvent volumes. However, using an extraction volume less than 10 ml and/or an extraction time less than 1 minute may give poorer chlorophyll recoveries. In addition, recoveries were dependent on the method of extraction (sonication or soaking) and extraction solvents used. Ethanol seems to be the better solvent to extract chlorophylls. Samples extracted by sonication alone resulted in under estimation of chlorophylls. A clear separation was observed between participants reporting chlorophylls as chlorophyll *a* by either fluorometric or photometric methods with those using high performance liquid chromatography (HPLC). As indicated, within this report, the standard photometric and fluorometric methods for determining chlorophylls do not completely separate the different chlorophyll pigments, while this is possible by HPLC. Therefore, HPLC will provide lower but accurate concentrations of chlorophyll *a*. Future QUASIMEME exercises will assess the data from HPLC and fluorometric methods separately and the exercise may be expanded to include additional pigments. The workshop also highlighted the need for harmonisation of methods used for the analysis of chlorophylls in marine waters. To date no report of the outcomes of the study or workshop report have been published.

3.2 *In vivo* and *in situ* chlorophyll fluorescence

In vivo fluorometers mounted on conductivity, temperature, depth devices (CTD's) and other *in situ* instruments are often called *in situ* fluorometers. These may also be mounted on oceanographic buoys or in FerryBox systems on ships of opportunity, e.g. ferries. The fluorescence of chlorophyll is related to the concentration of chlorophyll which is a proxy for phytoplankton biomass. Chlorophyll fluorescence is influenced by the composition of

phytoplankton and of the light exposure history of the organisms. A Swedish study (20) reported the night time to day time chlorophyll fluorescence of the same phytoplankton community may vary with a factor of 2-3. In Fig. 1 data on hourly measurements of chlorophyll fluorescence at approximately 2 m depth in the Kattegat are presented. It should be noted that chlorophyll concentrations were low during the day and high at night, with the night data the most consistent. It is likely that the same phytoplankton community were present during both the day and night. Thus it is recommended to use night time chlorophyll fluorescence for near surface sensors. Another example of data from an *in situ* fluorometer mounted on an oceanographic buoy in the Kosterfjord in the Skagerrak is presented in Fig. 2. Reference chlorophyll data measured in discrete samples collected as part of the water sampling programme for the Water Quality Association of the Bohus Coast is also shown in Fig. 2 for comparison.

Chlorophyll fluorescence *in vivo* may also be influenced by humic substances and non-phytoplankton particles. Figure 3 shows a comparison of chlorophyll concentrations measured *in vivo* and in discrete water samples collected from Kattegat and Skagerrak area in 2012. The discrete water samples were stored at 4 degrees and analysed in the laboratory using a fluorometer within 20 hours of collection. The chlorophyll concentration measured by *in vivo* fluorescence may be overestimated as shown in Fig. 3.

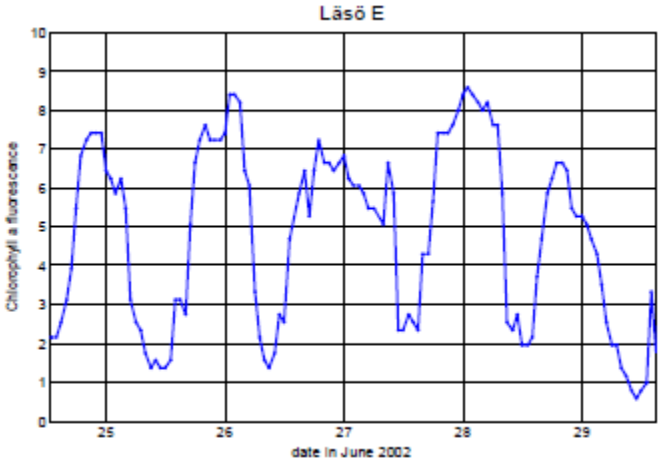

Fig. 1 Variability of *in vivo* chlorophyll fluorescence measured at approximately 2 m depth using the SMHI oceanographic buoy Läsö E. in the Kattegat in 2002. Night time to day time ratio is about 2-3.

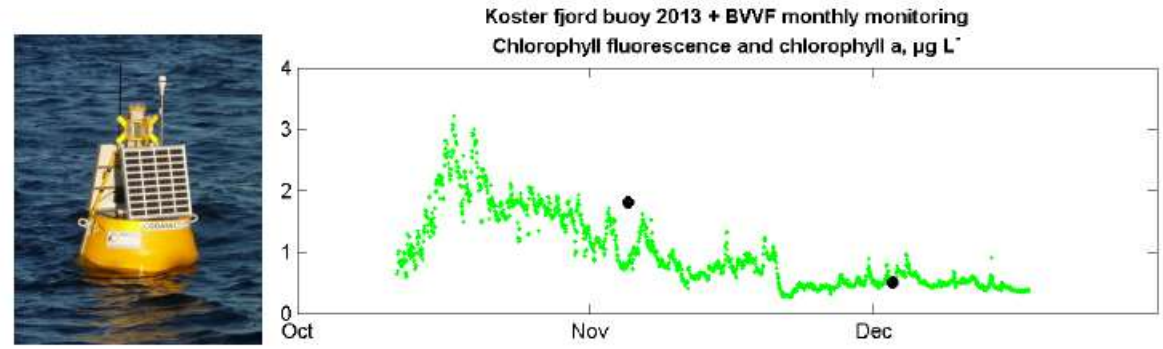

Fig. 2 *In vivo* chlorophyll fluorescence in the Kosterfjord in 2013 measured at approximately 1 m depth. Black dots represent water samples for chlorophyll *a* analysed in the laboratory as part of the regional monitoring program (BVVF).

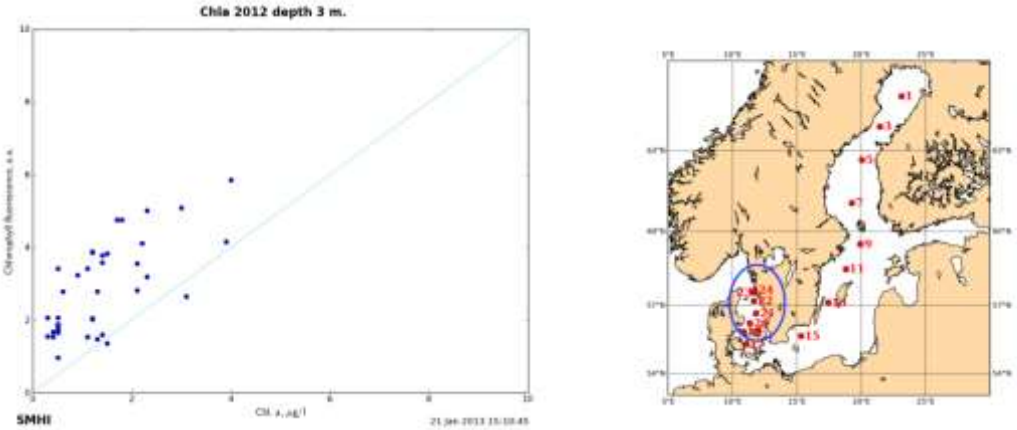

Fig. 3. The correlation between *in vivo* chlorophyll fluorescence and extracted chlorophyll from water samples, measured at approximately 3m depth on a FerryBox system in the Kattegat and Skagerrak area in 2012.

3.3 Satellites

Remote sensing of ocean colour gives the opportunity to cover large sea areas during day light and cloud free conditions. There are currently several suitable satellites available, mainly the National Aeronautics and Space Administration (NASA) satellites Aqua and Terra with the Moderate-Resolution Imaging Spectroradiometer (MODIS) sensor and the National Polar-orbiting Operational Environmental Satellite System Preparatory Project (NPP) with the Visible Infrared Imaging Radiometer Suite (VIIRS) sensor. Environmental Satellite (EnviSAT) with the Medium Resolution Imaging Spectrometer (MERIS) sensor has not been available since May 2012. The launch of the first European Space Agency (ESA) Sentinel-3 satellite is planned for late 2015. Therefore, there is the potential for further high quality satellite data suitable for work with algal blooms to become available in 2016. A second Sentinel satellite with ocean colour sensor is also planned for launch 18 months later, and a third before 2020.

Estimation of near-surface chlorophyll by satellite is obtained through algorithms based on the ratio between different wavelengths of light (colours blue and green) leaving the water surface. Normalised fluorescence line height, which is a relative measure of water-leaving radiance associated with chlorophyll fluorescence, is also available. The depth of water column to which a satellite is measuring depends on the turbidity of the water. In open ocean this may be greater than 20m, but only 1-2m in highly productive waters, or those with high suspended sediment. Other known limitations and problems associated with estimating chlorophyll using satellite remote sensing include cloud cover, non-phytoplankton particles and influence from humic substances, particularly in coastal areas, although algorithms are available which attempt to minimise the influence of these substances on the estimated chlorophyll concentration. Additionally, in very shallow waters the sea floor may influence the data. Satellite data is generally discounted within 1 km of land, to ensure that the data is truly marine, and not contaminated by land colour. It has been claimed that water body status (Water Framework Directive) can be determined using MERIS satellite data. However, since information on phytoplankton abundance and species composition is limited to genus such as *Karenia* and coccolithophores this may not be possible. However, satellite based measurements of ocean colour do give useful information that complements discrete water sampling and subsequent analysis of phytoplankton and chlorophyll. They also have the advantage in that they can cover large spatial areas with daily or near daily coverage (cloud cover allowing), which would be exorbitantly expensive and time restricted to be covered by discrete water sampling, or ferrybox schemes. Satellites can also be used to direct or focus sampling campaigns .

3.3.1 Calibration of sensors

All sensors require regular calibration against discrete water samples collected at the same location. Currently no analytical technique has been recommended for this procedure and individual laboratories use various techniques including HPLC and fluorometric and photometric techniques. If remote sensors are to be used for monitoring purposes comparability of the chlorophyll data is essential, as well as the use of standard analytical methods for calibration.

3.4 Fluorometric chlorophyll - corrected versus uncorrected concentration

As part of a Scottish study (21) the calculated concentrations of corrected chlorophyll (corrected for the presence of phaeopigments by acidification) and uncorrected chlorophyll measured by fluorometry collected at two long term monitoring sites (1999 – 2013) were compared. A regression analysis between uncorrected and corrected chlorophyll since 2009 at Stonehaven and 2011 at Loch Ewe show a strong correlation between the two (r^2 = 0.986 and 0.990 respectively – Fig 4A and 4B) and a gradient close to unity, suggesting that there is little difference between the corrected and uncorrected chlorophyll concentrations at these two sites during the period studied.

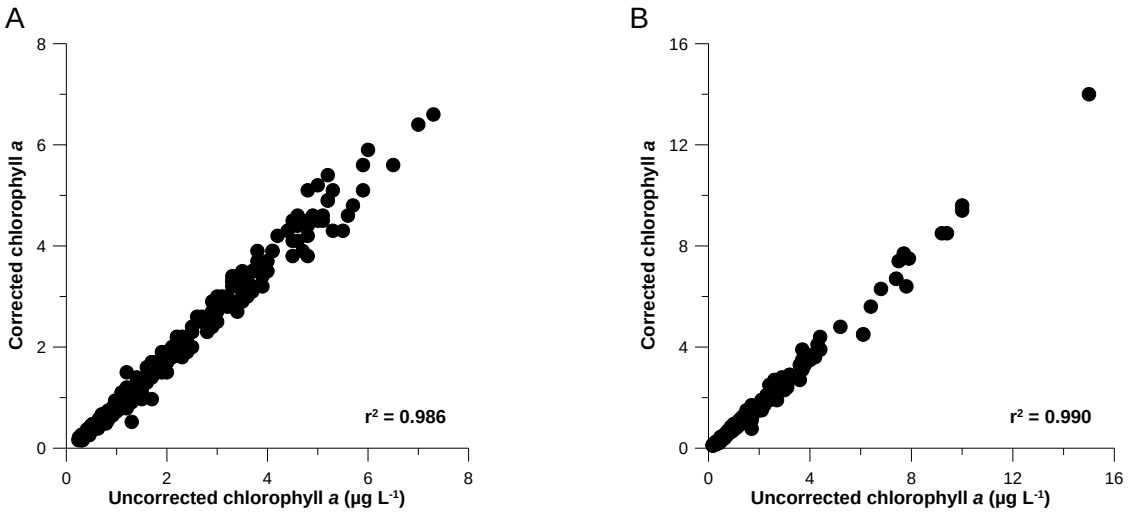

Fig 4. Plot showing the correlation between uncorrected and corrected chlorophyll *a* results at (A) Stonehaven and (B) Loch Ewe monitoring sites.

3.5 Comparison of the Fluorometric/Photometric and HPLC methods

As part of a Scottish study (23) the calculated concentrations of chlorophylls measured by fluorometry were compared with the chlorophyll *a* concentrations measured by HPLC-UV at two long term monitoring sites. Both methods showed that the chlorophyll concentrations are lowest during the winter months (November – February) with chlorophyll *a* concentrations less than 1 µg/l. The chlorophyll concentrations increased between March and April and remained elevated until autumn (Fig 5 A and B).

The fluorometric and HPLC methods show good alignment during the winter months when phytoplankton growth is low. In contrast, during growth periods the fluorometric method gives a nearly, but not always, higher result for uncorrected chlorophylls (Fig 5 A and B). The concentration of HPLC measured chlorophyll *b, c*2 and *c*3 were investigated to determine high concentrations of these pigments may be interfering with the fluorometric

detection of chlorophyll, an example of the chlorophyll *c*2 is presented in Fig 6 and highlights that chlorophyll *c*2 pigments were interfering with the fluorometric analysis .

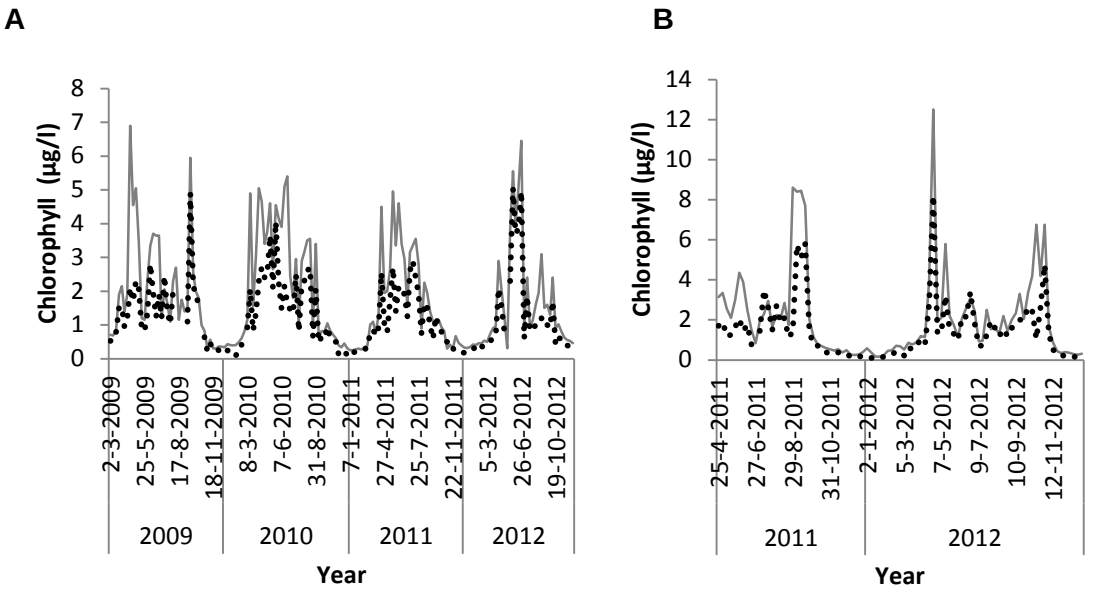

Fig 5. Comparison of weekly uncorrected chlorophyll concentrations determined by fluorometric methods (grey line) and chlorophyll '*a*' determined using HPLC (dashed line) at the (A) Stonehaven and (B) Loch Ewe monitoring sites.

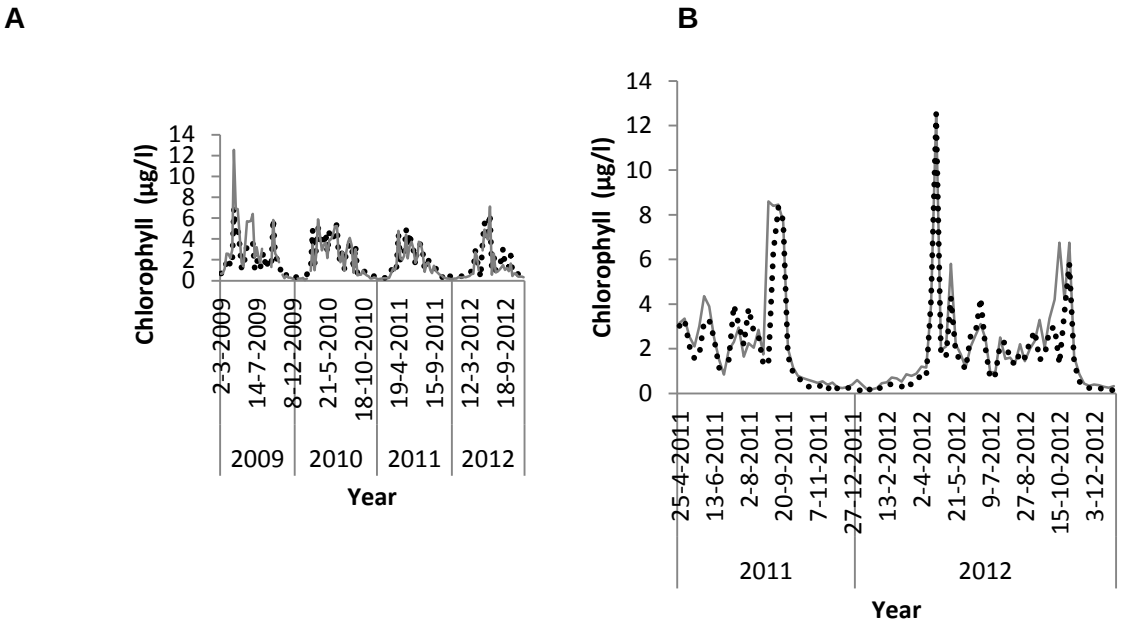

Fig 6. Comparison of weekly uncorrected chlorophyll concentrations determined by fluorometric methods (grey line) and chlorophyll '*c*2' determined using HPLC (dashed line) at the (A) Stonehaven and (B) Loch Ewe monitoring sites.

4 Reporting of Chlorophyll data

There are two main data receptacles for reporting chlorophyll data, namely the International Council for the Exploration of Seas (ICES) and the European Marine Ecosystem Observatory (EMECO).

4.1 ICES

The ICES-Data Centre (ICES-DC) is the primary repository of marine monitoring data for OSPAR. There are two routes of entry to ICES-DC the ICES environment database (ERF 3.2 format) or Oceanography (IOF free format using BODC codes). The ERF 3.2 format used in the ICES DOME database which uses the ICES vocab parameter list and accepts metadata including detailed method and Quality assurance (QA) information. Oceanography deals with a core of ca. 14 parameters which can be submitted in any format and are stored in the Ocean database. Chlorophyll is one of the core parameters. When the core parameters are submitted to DOME, they are also entered in the "Ocean" database. To avoid duplicates in extractions and in viewing on EcoSystemData, only the values in the "Ocean" database are used in extractions. When method information is required, DOME-stored meta data is extracted upon request.

Data can be submitted, to the ICES-DC, using three parameter codes; CPHL (Chlorophyll *a*), CPHLC (Chlorophyll *a*, SCOR/UNESCO method) and CPHLL (Chlorophyll *a*, Lorenz acidification method). The UNESCO protocol (6) describes the determination of chlorophyll by both the HPLC and the acidified fluorometric methods. It is not clear which of these methods the parameter code CPHLC is referencing. The parameter code CPHLL is used for the submission of flourometric data by the acidification method (corrected chlorophyll), as discussed earlier, the acidification step of the Lorenz acidification method is no longer recommended. There is no method information associated for the parameter code CPHL. In addition, when fluorometric data was submitted previously, using the three codes listed above, it is unclear whether corrected or uncorrected chlorophyll data was used in submissions. Consideration should be given as to whether additional method metadata is required with chlorophyll data submissions to the ICES-DC.

The current JAMP Eutrophication monitoring guidelines for chlorophyll in water states that "because the standard photometric and fluorometric methods used for determining chlorophyll *a* do not completely separate the different chlorophylls or distinguish between chlorophyll *a* and chlorophyllide *a* the term "total chlorophyll a" should be used when reporting results from these methods. For chlorophyll data, analysed by HPLC, which considered the other chlorophyll derivatives as well the term "chlorophyll *a*" should be used". There is currently no parameter code within the ICES-DC for "total chlorophyll a". The term "total chlorophyll *a"* itself *is* misleading, as described above, the fluorometric method cannot separate different chlorophylls, therefore a more accurate description for this is required.

4.2 EMECO

EMECO holds observational data from a number of in-situ platforms collected by 17 partner organisations in 9 countries by means of Ferryboxes, research vessels, ships of opportunity and buoys. In addition EMECO also sources data from satellites and ICES oceanographic database. Chlorophyll data is reported as Chlorophyll (µg/l) with supporting metadata. Data held within EMECO may not always be of comparable quality, for example the metadata for buoy data for some institutes indicate calibrated data is submitted while for others only indicative concentrations are submitted.

Data from countinueous monitoring platforms such as Ferryboxes and buoys will be an important part of data assessments for MSFD Descriptor 5. However, when using the EMECO chlorophyll data for assessments, consideration will have to ensure the quality of data sets prior to assessments, ideally with only quantitative, quality controlled data being used. In addition the analytical method used to calibrate in-situ platforms will also have to be known.

5 Assessment criteria

OSPAR have set assessment criteria for chlorophyll, in respect of Eutrophication monitoring, but there is no equivalent criterion for chlorophyll in WFD. For WFD, chlorophyll is included in the phytoplankton tool. MSFD Descriptor 5 (Eutrophication) requires the direct effects of nutrient enrichment to be measured, including the measurement of chlorophyll.

OSPAR contracting parties have set area specific background concentrations and assessments levels (defined as 50% above regional background concentrations) for what has been described as chlorophyll *a* as part of the common procedure for assessing Eutrophication status within the OSPAR maritime area (1). Annex 6b of the common procedure (1) includes details of the methods used to derive these background chlorophyll concentrations. A range of analytical methods have been used to derive background concentrations, including fluorometric, photometric and HPLC determinations. Only chlorophyll determined by HPLC can specifically be called chlorophyll *a* while concentrations reported for the other techniques are 'chlorophylls'.

There is no accurate conversion factor available for inter-comparisons between chlorophyll concentrations determined by the different techniques, although recent work by Noklegaard *et al.* attempted this (22). Authors investigated whether a standard conversion factor existed between two different extraction (cold acetone and hot methanol extraction) and detection methods (fluorometric and photometric) for samples collected at three sites in Irish waters during the growing season. The authors derived a conversion factor, however, they concluded that due to the variability within the samples this should not be used in practice due to differences as a consequence interfering pigments such as phaeo-pigments. Therefore, any future development of a conversion factor between the analytical techniques would require full validation and have to fully account for regional, seasonal and inter-annual variation of phytoplankton communities. However, as part of the JMP "Towards a Joint Monitoring Programme for the North Sea / Celtic Sea" an intial trial has been undertaken and is described in Text Box 3 and Fig 7 & 8. A simpler solution may be for all nations to define chlorophyll background concentrations on the basis of a single defined method.

The choice of analytical method used to derive background concentrations has implications for Descriptor 5 assessments. Contracting parties have derived their own area specific background concentrations, potentially resulting in difficulties with cross boundary regional assessments. There is greater potential for regions to fail GES if data produced was by means of fluorometric or photometric determinations whilst background concentrations were derived using HPLC. Conversely, if background concentrations were based on fluorometric or photometric determinations and assessments were based on HPLC determinations, there is potential for underestimation. Therefore, the analytical method used when deriving background concentrations needs to be known and the same method should be applied to routine monitoring if the concentrations are to be compared to the assessment criteria.

To meet statutory obligations contracting parties are increasingly relying on the use of remote sensing devices such as buoys, ferryboxes and satellites for inclusion in data sets for their assessments. There is no single analytical technique recommended for the calibration

of these devices. It is important that a consistent analytical approach is taken to calibrate such devices to ensure comparability and correct assessment of GES.

Text Box 3 – Conversion Factor Trial for JMP

Countries within the North Sea region are measuring chlorophyll with a range of different extraction and detection methods. Several attempts have been made to present conversion factors to correct for differences in analyitical techniques (23,22). Due to the variability between the data and depending on if analysis was undertaken on a culture or on a natural sea water sample, the use of correction factors have not been recommended.. However, as part of the JMP project, using Quasimeme 2014 data, a set of conversion factors was calculated to illustrate the differences. The trial used seasonal chlorophyll means (2001-2005) used in the Swedish national report for the OSPAR Assessment 2007 (24). The chlorophyll data was extracted by ethanol and analysed using fluorometry. The data was then "converted" to fluorometry/acetone, HPLC/ethanol and HPLC/acetone using the factors in Table 1. Results are presented in Fig. 7 and 8

Table 1. "Conversion factors calculated from Quasimeme test data using natural sea water and culture. Original data is here seasonal means of Swedish chlorophyll data analyzed with fluorimetry/ethanol.

"Conversion factors"	
Natural seawater	Culture
Fluorometry/acetone = original data * 1/1.14	Fluorometry/acetone = original data * 1/3.59
HPLC/ethanol = original data *1/1.17	HPLC/ethanol = original data *1/1.65
HPLC/acetone = original data * 1/1.14 *1/1.17	HPLC/acetone = original data * 1/3.59*1/1.65

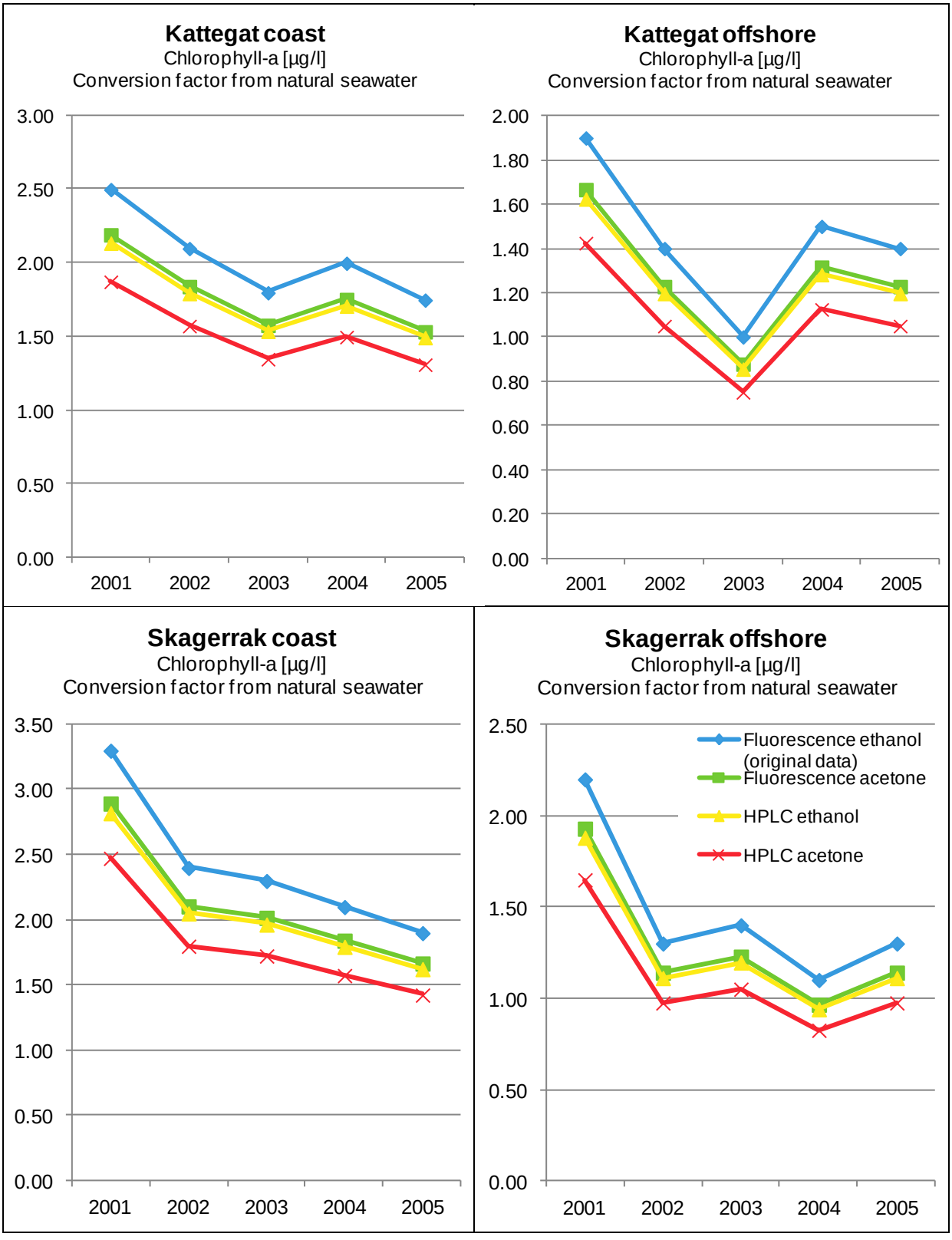

Fig 7. Seasonal means (2001-2005) of chlorophyll-a used in the Swedish national report for the OSPAR Assessment 2007. Swedish chlorophyll data are analyzed with fluorimetry and ethanol as extraction (original data). Data are then "converted" to fluorimetry/acetone, HPLC/ethanol and HPLC/acetone using Quasimeme test data from natural sea water.

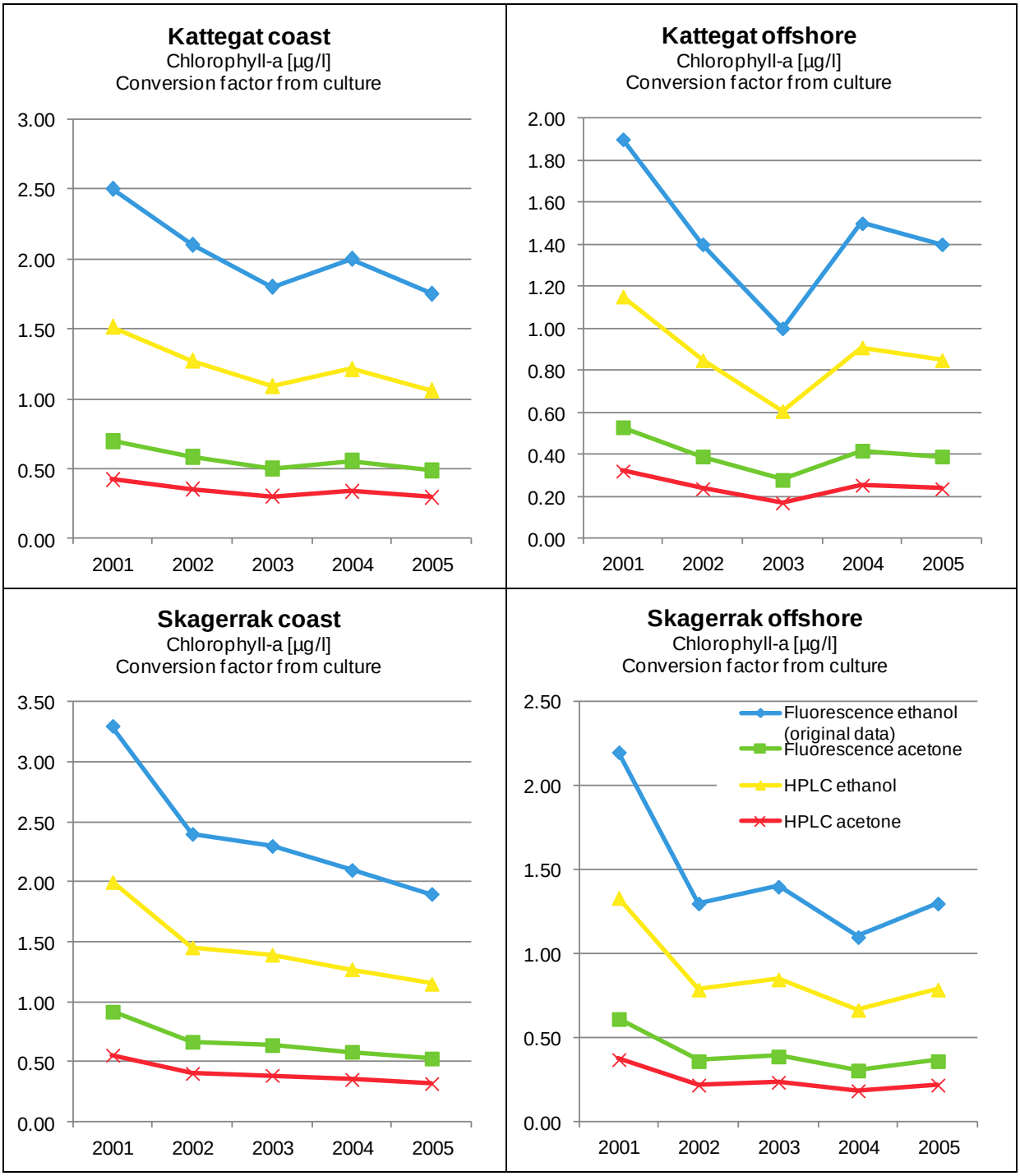

Fig 8. Seasonal means (2001-2005) of chlorophyll-a used in the Swedish national report for the OSPAR Assessment 2007. Swedish chlorophyll data are analyzed with fluorimetry and ethanol as extraction (original data). Data are then "converted" to fluorimetry/acetone, HPLC/ethanol and HPLC/acetone using Quasimeme test data from culture.

6 Conclusions and Recommendations

1. There is no single standard extraction technique recommended for the determination of chlorophylls. Although a recent investigation by QUASIMEME found chlorophyll was underestimated if extraction was by sonication alone. The most efficient extraction solvent was found to be ethanol, although they did not indicate whether this is for cold or hot solvent.

2. The fluorometric and photometric methods do not separate all pigments and therefore overestimate the chlorophyll a concentrations and should be described as chlorophylls and not chlorophyll a. Although, methods such as the Lorenz acidification method were developed to correct for overestimation resulting from interferences from phaeopigments, these methods are inaccurate in the presence of interfering algal pigments such as Chlorophyll b, and are no longer recommended.

3. Chlorophyll measurements (not corrected using the acidification method and also known as uncorrected chlorophyll) made using the fluorometric or photometric method may be sufficient if all that is required is to ensure that the chlorophyll concentrations meet the OSPAR assessment criteria, as long as the assessment levels are also based on chlorophyll total.

4. HPLC methods, using an ultraviolet (UV) or diode array detector (DAD), have the capability of separating a number of chlorophylls and other pigments. The DAD is the preferred detector as a full spectrum of each pigment peak collected to be made, greatly aiding the identification of the pigments as the spectrum can be used to confirm or refute the presence of a particular pigment. If an accurate concentration for chlorophyll a is required, for example for individual algal pigment determinations, then HPLC analysis should be used.

5. The decision on which method of analysis is most appropriate must be made by the end user of the data. End users must also consider implications for changes to methodology if programs have historical long term significance and if necessary maintain existing methodology and complement with new parameters.

6. The term ‘total chlorophyll a’ by fluorometric or photometric analysis, as described in the current JAMP Eutrophication guidelines is misleading. The authors recommend the JAMP guidelines are revised, replacing the term ‘total chlorophyll a’ for fluorometric and photometric analysis with an alternative. The ICES parameter codes should be revised to reflect the current JAMP Eutrophication monitoring guidelines for chlorophyll in water. Data should only be reported as chlorophyll a if an HPLC method is used which can separate chlorophyll a from other chlorophylls and pigments. Another ICES code may be needed, or at least for the description of the code for chlorophyll a clarified.

7. The data submitted to ICES should be of comparable quality to permit accurate assessment across all MSFD regions. It is important that any data submitted has enough methodological metadata to support data assessments. The current nomenclature used for submission of chlorophyll data to the ICES database is currently ambiguous and should be revised and aligned to reflect revised OSPAR JAMP guidelines. Consideration should also be given as to whether additional method metadata is required with data submissions.

8. Background concentrations, as listed In the OSPAR Common Procedure for assessing Eutrophication status, are area specific and set by individual contracting parties. A range of analytical methods have been used to derive these, including fluorometric, photometric and HPLC determinations. The choice of analytical method used to derive background concentrations has implications for Descriptor 5 assessments, potentially resulting in difficulties with cross boundary regional assessments. The authors recommend that background concentrations are harmonised.

9. There is no accurate conversion factor available for inter-comparisons between chlorophyll concentrations determined by the different analytical techniques. A solution would be for all nations to define chlorophyll background concentrations on the basis of a single defined method and the same method applied to routine monitoring if comparison to assessment criteria is required, such as would be the case for MSFD Descriptor 5.

10. To meet statutory requirements for drivers such as MSFD and WFD, contracting parties are increasingly relying on the use of automatic and remote devices such as buoys, ferryboxes and satellites for inclusion in data sets for their assessments. There is no single analytical technique recommended for the calibration of these devices. It is important that a consistent analytical approach is taken to calibrate such devices to ensure comparability.

11. Variation in chlorophyll measurements caused by differences in analytical methods should be compared with natural variability in the occurrence of algae. Limitations in temporal and spatial coverage connected to ship-based monitoring hamper an effective assessment because of natural variability. Alternative methods such as RS and ferry boxes can greatly enhance temporal and spatial coverage and calibration of the results of these methods is a priority.

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Acronym List

OSPAR	Oslo Paris Commission
WFD	EU Water Framework Directive
GES	Good Environmental Status
MSFD	EU Marine Strategy Directive
HPLC	High Performance Liquid Chromatography
UV	Ultra Violet
DAD	Diode array detector
NASA	National Aeronautics and Space Administration
MODIS	Moderate-Resolution Imaging Spectroradiometer
NPP	National Polar-orbiting Operational Environmental Satellite System Preparatory Project
VIIRS	Visible Infrared Imaging Radiometer Suite
EnviSAT	Environmental Satellite
MERIS	Medium Resolution Imaging Spectrometer
ESA	European Space Agency

Annex IIA Range of extraction and detection methods used for the determination of chlorophyll by countries submitting data via the ICES database.

Note: This is by no means a definitive list of each countries submissions but highlights the lack of comparability

Country	BE	NL	DE	UK	FR	SE	DK	IR	NO [6]
Filtration							Within 24 hours	500 ml	
Filter used	Whatmann GF/C	Whatmann GF/C		Whatmann GF/F	Whatmann GF/C 47mm	Whatmann GF/C	Whatman GF/C or Advantec GF75	Whatmann GF/F (Marine Institute [MI]) GF/C (EPA)	
Filter storage for short period	liquid nitrogen	4 ± 2^{o}C			for few weeks: between -18 and -20°C, at best - 25°C.	-20^{o}C	Filters kept on dry ice	-20^{o}C	
Analysed within		24 hours				Within 2 weeks	Analysed within 3 months	Within 2 weeks to 1 month	
Filter storage for longer period	at -80 ºC	Between -20 & -35 ^{o}C			Beyond a few weeks : liquid nitrogen (-196°C)		-18^{o}C	NA	
Extraction	90% acetone	acetone	acetone	90% acetone, neutralised with $NaHCO_3$	90% acetone	Ethanol 96%	Ethanol 96%	90% acetone (MI Method) Hot methanol (96.4%) (EPA Method)	
Detection	Reversed phase HPLC	HPLC & fluorescence	1. Fluorometry 2. Spectrophotometry	Fluorometry after acidification with 8% HCL	Spectrophotometry 665 and 750 mm	Fluorometry, no acidification	Spectrophotometry 665 and 750 mm	Fluoremetry (MI method) Spectrometry (EPA method)	

[6] Awaiting information from Norway

Case study Chlorophyll

Annex III

Chlorophyll assessment based on satellite observations, a feasibility study

version 8 April 2015

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Hanneke Baretta-Bekker BarettaBekker – mariene ecologie

1 Introduction

During the preparation of the chlorophyll data sets for the statistical tool in development at the Thünen Institute (see Baretta-Bekker et al., 2015; Annex I of the final report) it turned out that the national monitoring approach is not the same for all countries. Some countries have fixed stations which they visit a number of times per year, while other countries apparently monitor randomly. This would not be a problem, if the observations were more or less evenly distributed over the growing season, but this is not the case for all countries. In some of the national datasets the emphasis is on the spring period, in others on the late summer months, and some countries only have monitoring data for a few months. Next to these differences also the analytical methods in use by the various countries are different (see for details Walsham et al. (2015; annex II of final report).

The differences in the analytical methods and in the distribution of chlorophyll monitoring data in time and space between countries as sketched above imply that the calculated growing-season mean concentrations of chlorophyll-a are not comparable, making it desirable to test alternative data sources, such as Remote Sensing data from satellite (RS data).

With RS images of Ifremer, it was possible to compare assessments for mean chlorophyll concentrations in the growing season based on these images with the results of national assessments based on the OSPAR Comprehensive Procedure as used in the OSPAR Quality Report (OSPAR, 2010). Due to time constraints as the JMP project was nearly finished, it was only possible to carry out a superficial feasibility study. Recommendations for further work are given.

2 Material and Methods

Remote Sensing – satellites

The estimation of chlorophyll-a is obtained by application of three Look-Up-Tables (LUT) to the spectral remote-sensing reflectance (Rrs) of SeaWiFS (1998-2004), MODIS (2002-2014) and MERIS (2002-2012). The method, described in detail in Gohin et al. (2002), is empirical and derived from the OC4/SeaWiFS algorithm of NASA (or OC3M-547 for MODIS and OC4E for MERIS). This method gives results similar to OC4 in open waters but provides more realistic values over the continental shelf. In coastal waters, mineral SPM, absorption by CDOM (Coloured Dissolved Organic Matter) and errors in the atmospheric correction are the cause of frequent overestimations of the chlorophyll concentration by the standard procedures.

This algorithm, known as OC5, is a 5-channel algorithm for MERIS and SeaWiFS and a 4-channel one for MODIS. Calibrated on the coastal waters of the southern North Sea, the English Channel and the Bay of Biscay, method has been applied with success in the North Sea and other turbid coastal waters for years (Gohin et al., 2008, Gohin, 2011) These satellite data have also been used for validating the NEMO-ERSEM operational ecosystem model for the North West European Continental Shelf (Edwards et al., 2012). OC5 chlorophyll-a products on the North West European continental shelf are also daily provided by PML within the MyOcean2 European project. The data used here are the daily interpolated multi-temporal multi-sensor products (Saulquin et al., 2010). The interpolated products provide a well-balanced data set temporally and spatially.

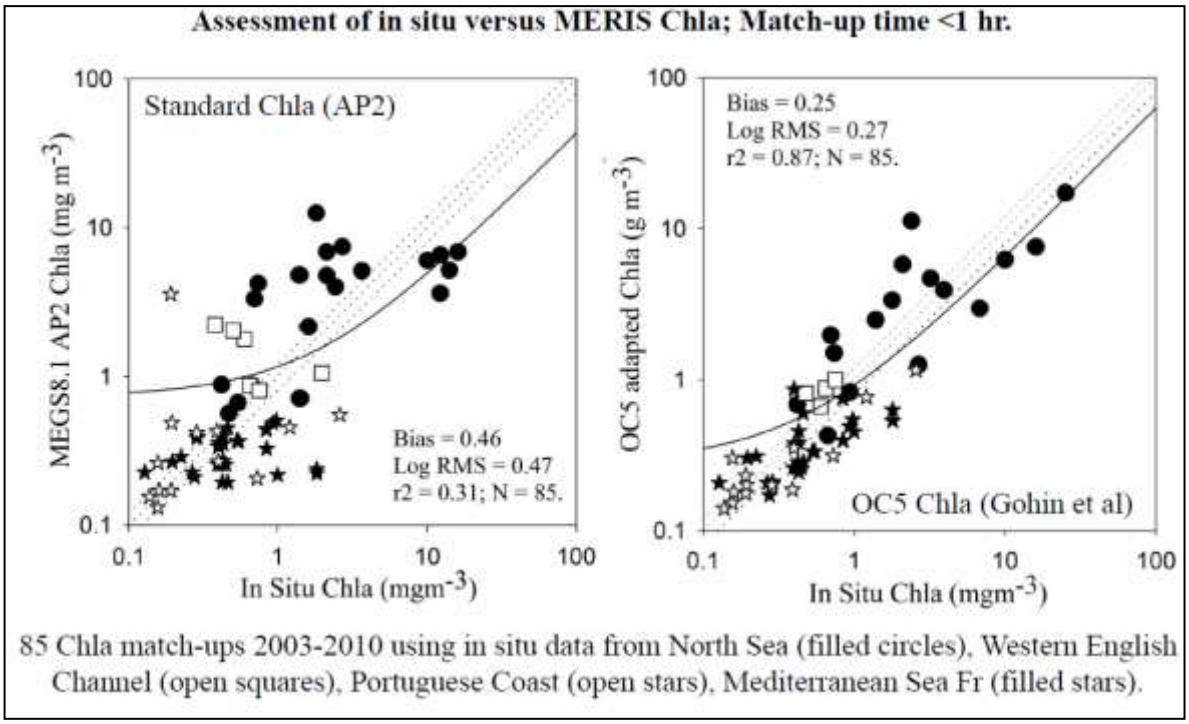

Figure 1 Validation of the OC5 versus standard MERIS Chl-a products (figure from: Tilstone et al., 2014).

Figure 1 shows an assessment of the OC5 chlorophyll-a carried out through the ISECA project which ended in September 2014. ISECA was a cross-border cooperation project on coastal eutrophication, supported by the INTERREGIVa 2Seas Program (http://iseca.eu).

The resolution of the interpolated grid is 1.2*1.2 km^2 (similar to the resolution of the raw images). The boundaries of the Ifremer grid on which raw and interpolated products are available since January 1998 until and including 2014 are 36N, 60N, 12W, 13E.

On the basis of a data set with 1 pixel out of 5 from each row and column, so 1 grid cell per 25 km^2, an assessment for chlorophyll was carried out over the period 2001 to 2005 to compare with the last OSPAR assessment of the same period (OSPAR 2010).

From these images the growing season (March to September) mean concentrations could be calculated in each of the grid cells. This resulted in a series of figures/maps for 1998-2014. Figure 2 shows the growing- season mean chlorophyll-a concentrations for 2001 – 2005, incl.

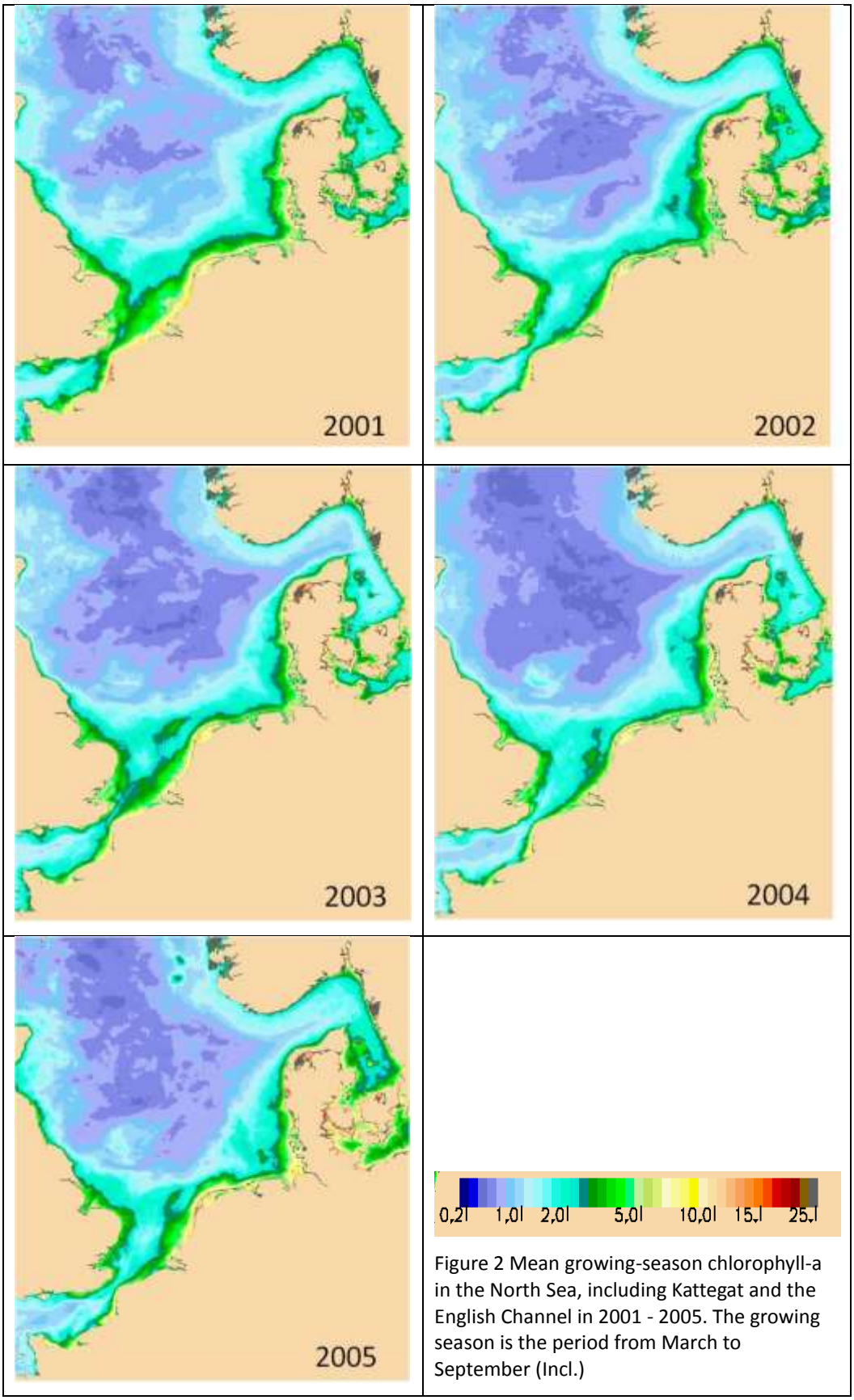

Figure 2 Mean growing-season chlorophyll-a in the North Sea, including Kattegat and the English Channel in 2001 - 2005. The growing season is the period from March to September (Incl.)

OSPAR assessment

Within OSPAR the Common Procedure has been developed,. This procedure comprises two steps. The first step is the screening procedure, a broad-brush process to identify obvious non-problem areas with regard to eutrophication. Following that step, all areas not identified as non-problem areas shall be subject to the Comprehensive Procedure (COMPP). The COMPP consists of a set of assessment criteria that may be linked to form a harmonized, integral assessment system of the main causes and effects of the eutrophication status of the maritime area.

Through this process the OSPAR maritime area is classified into areas which are considered to be problem, potential problem, or non-problem areas with regard to eutrophication. Repeated application of the Comprehensive Procedure should identify any change in the eutrophication status of a particular area.

The Contracting Parties did apply the OSPAR COMPP to each of their maritime areas, the so-called OSPAR areas (Figure 3). The results for the growing seasons of 2001-2005 have been described in the national reports (see links to national reports in the Reference list) and summarised in the Quality Status report (OSPAR, 2010). Figure 4 shows the results for the North Sea area, including Kattegat and the English Channel).

Figure 3 Map of the OSPAR areas of the greater North Sea.

Figure 4 Eutrophication status in the period 2001–2005 (OSPAR, 2010).

The national assessment levels for the growing-season mean chlorophyll concentrations used for the assessment results of Figure 4 are shown in Table 1. NB As described in Walsham et al. (2015; annex II of final report) the countries use different analytical methods to measure the chlorophyll concentrations, basing their national assessment level on the same method.

Table 1. Assessment levels for growing-season mean chlorophyll concentrations (µg/l) for coastal areas and offshore areas (Source: OSPAR, 2010).

Country	Assessment level (µg/l)	
	Coast	Offshore
BE	7.5	4.2
DE*	3.2	2.3
DK	1.5	1.5
FR	3.3**	3.3**
NL	7.5	2.25
NO	3.5	----
SE	1.5	1.5
UK	7.5	5

* The German assessment levels are provisional.

** half the 90-percentile assessment value, valid for The English Channel and southern North Sea

3 Results

The assessments for the years 2001-2005, based on the remote-sensing data of satellites have been compared with the assessments over the period 2001-2005 published in the OSPAR Quality status report (OSPAR, 2010).

In Table 2 the RS assessment results for chlorophyll are shown per OSPAR area for each of the 5 years, together with the final result over the whole period. The final result is determined by the most frequent assessment of the five years. These results are compared with the overall OSPAR results, based on the COMPP, so not only on chlorophyll, but also on other parameters such as nutrients, *Phaeocystis*, oxygen, etc.

In total the RS and OSPAR assessments of 21 OSPAR areas have been compared[7], of which 12 are identical and 9 (5 coastal areas and 4 offshore areas; the grey cells in the table) are different from each other. In Table 3 the areas with different results of both assessments are summarized with a possible explanation. In four of these cases the status of Problem Area according to the OSPAR COMPP is not based on chlorophyll (NO coast – toxic algae and macroalgae; Belgian offshore– insufficient chlorophyll data; DK offshore– too high nutrient concentrations; DE offshore– oxygen deficiency). In three very turbid coastal areas (BE, NL and DE) the status according to RS is Non-Problem, while OSPAR COMPP assessment is Problem Area, based on chlorophyll. These so-called Case II waters make estimating chlorophyll concentrations by Remote Sensing problematic. In two cases there is no explanation for the different assessments and further research is necessary.

[7] The reason that some of the OSPAR areas are missing has to do with the available shape file with the boundaries of the areas. There was no time within this project correct the shape file.

Table 2. Assessment for growing-season mean concentrations (µg/l) for all OSPAR areas in the North Sea, based on satellite observations. The colours indicate the status of the area concerning chlorophyll, depending on the corresponding assessment levels in Table 1. Red: PA - Problem Area; green: NPA -Non Problem Area; orange: PPA – Potential Problem Area. C stands for coast and O for offshore.

Assessm level (µg/l)	Area	Assessment Chl, based on Remote Sensing						Overall OSPAR assessment	Comparison and remarks
		2001	2002	2003	2004	2005	period 01-05		
3.5	NO-Skagerrak coast						C		?[1]
1.5	SE-Inshore Kattegat						C		=
1.5	SE-Inshore Skagerrak						C		=
1.5	SE-Offshore Skagerrak						O		≠[2]
1.5	SE-Offshore Kattegat						O		=
7.5	UK-East Anglia (coast)						C		=
5.0	UK-South. North Sea						O		=
5	UK-North. North Sea						O		=
7.5	UK-NE England (coast)						C		=
7.5	UK-E English Channel						C		=
7.5	UK-E England coast						C		=
7.5	BE-Coastal area						C		≠[3]
4.2	BE-Offshore area						O		=[4]
1.5	DK-North Sea						O		=[4]
3.33	FR-North Sea Coast						C		≠[5]
3.2	DE-North Sea						O		=[4]
2.3	DE-German Bight						C		≠[3]
2.25	NL-Dogger Bank						O		=
2.25	NL-Oyster Grounds						O		=
2.25	NL-Southern Bight						O		=
7.5	NL-Coastal Waters						C		≠[3]

Remarks to the table

6. The assessment of the NO Skagerrak as PA has been based on macroalgae and toxic algal species. Chlorophyll data were not available (National report NO).
7. This has to be investigated further. A possible explanation can be *Chlorophyll median concentrations were below or close to background concentrations*. (National report SE).
8. The coastal areas German Bight, Dutch and Belgium coast are so-called Case II waters, very turbid, which makes estimating of chlorophyll concentrations by Remote Sensing problematic.
9. The BE offshore has been defined as PPA area due to the insufficient data. The DK-North Sea area is a PPA due to increased nutrient concentrations, while chlorophyll does not form a problem; The DE-North Sea area is a PPA, due to occasional oxygen depletion in bottom waters (< 70 %) and insufficient monitoring (National reports BE, DK and DE).
10. This has to be investigated further.

Table 3. RS and OSPAR assessments for growing-season mean concentrations (µg/l) for the OSPAR areas in the North Sea, where both assessments differ with background information from the national reports. See for the colour coding the legend of Table 2.

Area	Chl - RS assessment	OSPAR overall assessment	OSPAR assessment based on *(sources: national reports)*	Conclusion
NO Skagerrak	NPA	PA	macroalgae and toxic algal species; insufficient Chl data	Possibly identical?
SE Offshore Skagerrak	PA	NPA	chlorophyll *median* concentrations were below or close to background concentrations	? has to be investigated further
BE, NL and DE Coastal areas	NPA	PA	case II waters, very turbid, which makes estimating of chlorophyll concentrations by RS problematic	Known problem
BE offshore	NPA	PPA	insufficient data	Possibly identical?
DK North Sea	NPA	PPA	enhanced nutrient concentrations	Chl NP → Identical
DE North Sea	NPA	PPA	occasional oxygen depletion <70% in bottom waters	Chl NP → Identical
FR North Sea Coast	PA	NPA	?	? has to be investigated further

4 Concluding remarks

When comparing different methods, such as an assessment based on in situ data and on Remote sensing images from satellite one can't expect that the results always are identical.

The differences between the RS-based assessment and the OSPAR assessment of the water bodies can be largely explained by a number of aspects:

The first one is related to the quality of the satellite Ocean Colour products. In regions of freshwater influence (ROFI's) we may encounter high levels of CDOM (Coloured Dissolved Organic Matter) which complicates a correct evaluation of the chlorophyll-a content of the water as both materials absorb in the blue part of the light. CDOM plays a large role in the Baltic Sea, but also to a lesser extent along the west coast of the European mainland in the so-called coastal river, a ROFI due to the Coriolis effect.

Another aspect, complicating the calculation of the correct value of chlorophyll-a is the effect of high turbidity. In shallow meso- and macrotidal areas, tidal mixing maintains high levels of SPM in the water column badly affecting the accuracy of RS-derived chlorophyll-a observations. These waters are called Case II waters, and the RS results from these waters are still inaccurate, but improving with every new generation of satellite. Wind-event driven turbidity also negatively affects the accuracy of RS-derived chlorophyll-a in large areas, especially in autumn, by resuspension of the benthic fluff layer.

Then there is the difference between the pin-point in-situ sampling, averaging over at most a few dm^3 of water compared with the averaging done in a satellite pixel covering 1.2 km x 1.2 km x 10 m, *i.e.* a volume of around 14.4 10^9 dm^3! The satellite images thus average out the small-scale variability which is inevitable in in-situ sampling.

Discrepancies between the RS classification and the OSPAR classification of the water bodies may also be caused by the fact that the RS only uses chlorophyll levels whereas the OSPAR classification is based on the Comprehensive Procedure, including not only chlorophyll-a as a proxy for phytoplankton, but a whole set of assessment criteria ranging from the main causes to direct and indirect effects of eutrophication (cf Table 3).

The in-situ monitoring often has a rather skewed spatial distribution with a preponderance of stations near shore even though we know that the chlorophyll concentration has a rather steeply decreasing gradient from near shore to offshore. The consequence of this is that averaging over the in-situ samples of a whole area will lead to an

overestimation of the chlorophyll concentration. This can be seen by comparing the RS results in Figure 2 with the size and form of the OSPAR areas in Figure 3. This is especially true for the coastal area of Germany (German Bight) .

However, the satellite method has two major advantages over the *in-situ* method: synopticity and resolution, both spatial and temporal.

This small feasibility study demonstrates the promise of RS for monitoring, supporting an earlier study by Blaas (2013) which also provided sophisticated error statistics.

Even when RS is chosen as the main method of monitoring , it will still be necessary to regularly get in-situ data for calibration and validation of the RS-calculated values. For this, it will still be necessary to harmonize the different *in-situ* data acquisition methods into one standard analytical method .

The main conclusion of this study is that Remote Sensing is an acceptable method to estimate chlorophyll concentrations in offshore areas, but that turbidity in coastal areas causes masking of the chlorophyll signal leading to inaccurate estimates of the chlorophyll concentration. This is also true for temporarily turbid periods in offshore areas during and after severe wind events.

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FR: http://qsr2010.ospar.org/media/assessments/p00372_supplements/00112_FR_COMP2_Full_report.pdf

DE: http://qsr2010.ospar.org/media/assessments/p00372_supplements/00108Rev1_DE_revised_COMP2_Report.pdf

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