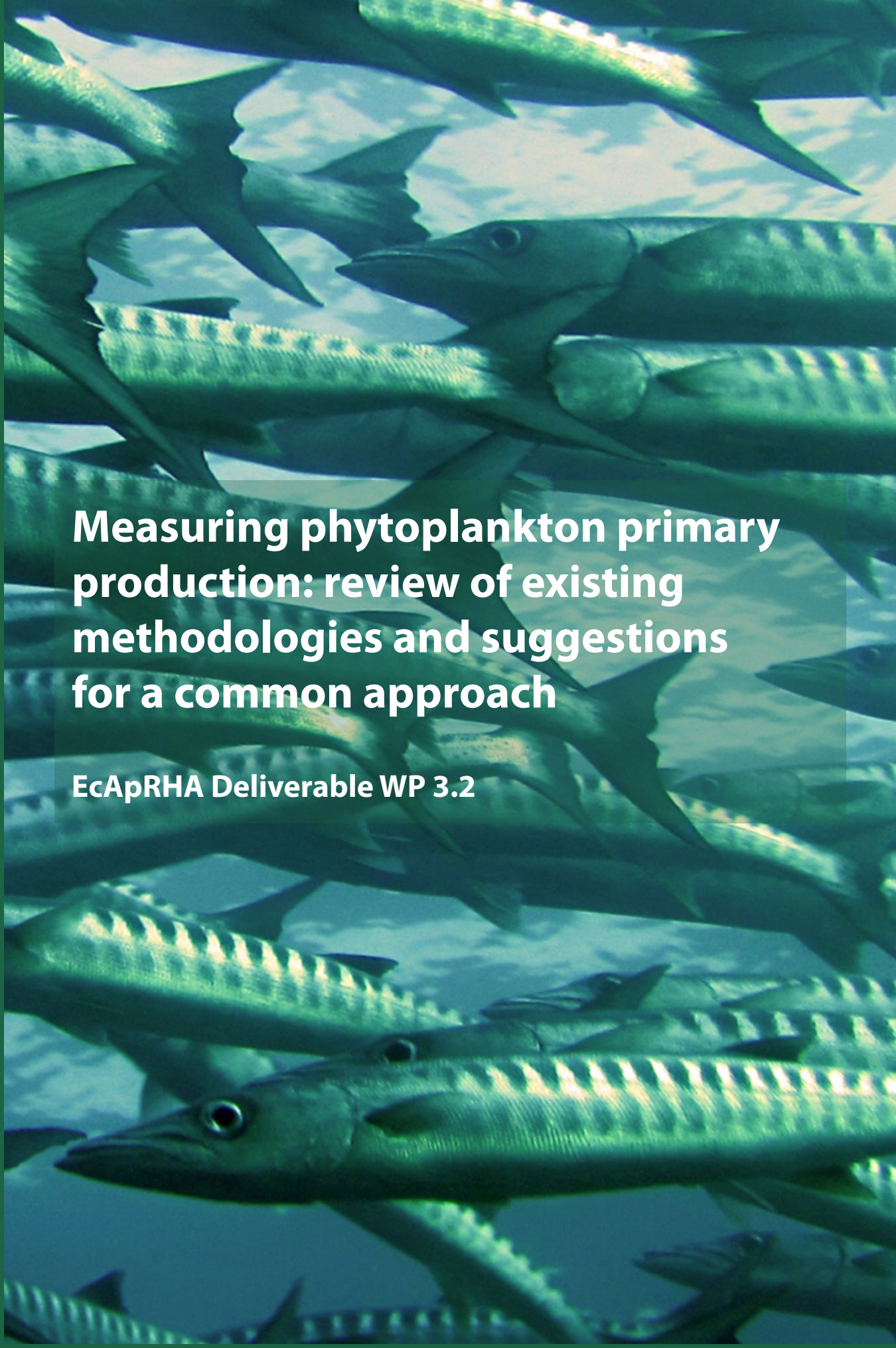


Measuring phytoplankton primary production: review of existing methodologies and suggestions for a common approach

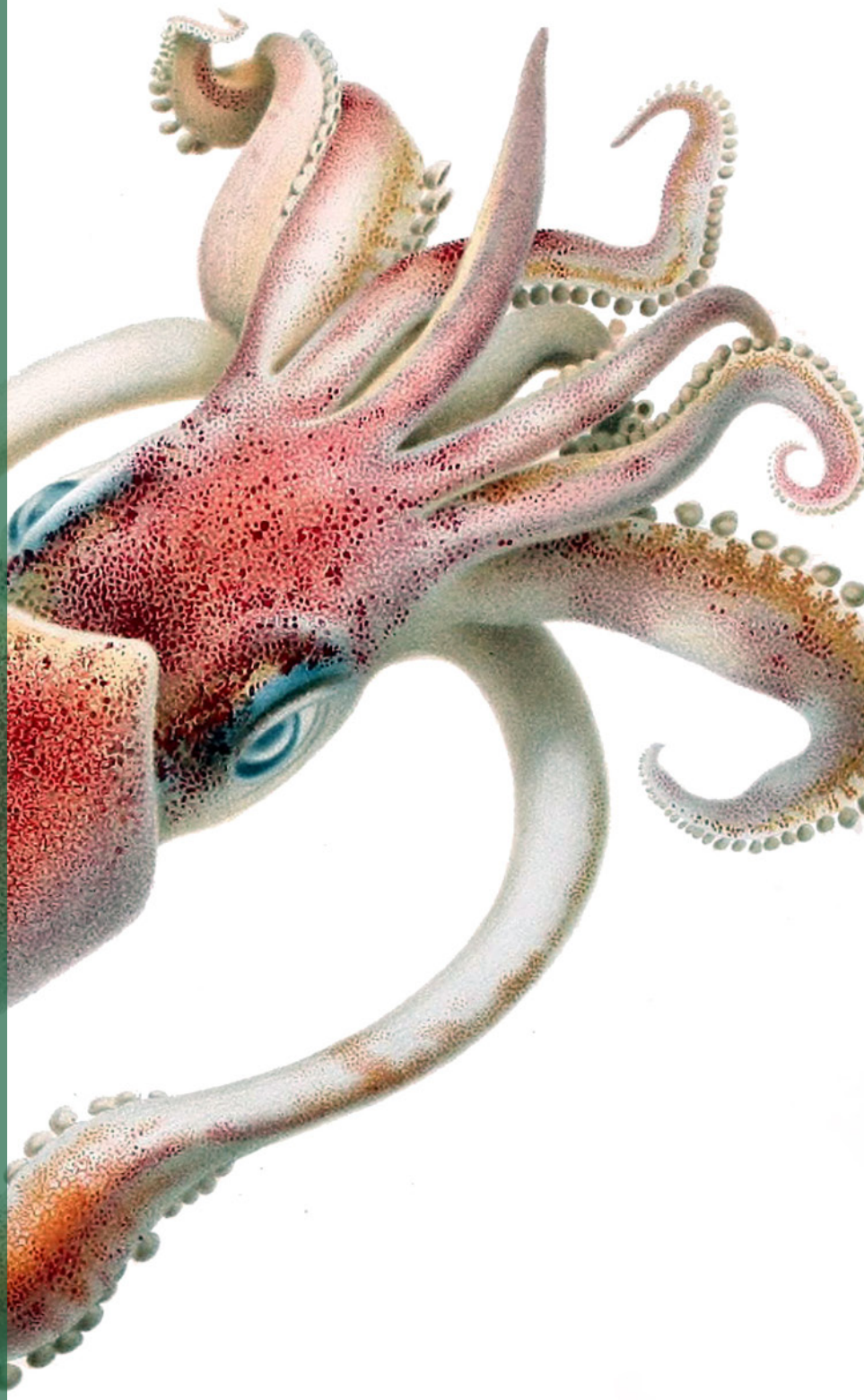
EcApRHA Deliverable WP 3.2

Co-financed by the European Union

EcApRHA an Ecosystem Approach to Regional Habitat Assessments

2017

Contents



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EcApRHA

The EcApRHA project (Applying an Ecosystem Approach to (sub) Regional Habitat Assessment) aims to address gaps in the development of biodiversity indicators for the OSPAR Regions. In particular, the project aims to overcome challenges in the development of indicators relating to the MSFD (Marine Strategy Framework Directive 56/2008/EU), such as Descriptor D1 (Biodiversity), D4 (Food webs) and D6 (Seafloor integrity), and to deliver an action plan to OSPAR that will enable monitoring and assessment at the (sub) regional scale, to contribute to OSPAR Intermediate Assessment 2017.

Indicators related to the benthic and pelagic habitats, as well as food webs, are investigated within the project at different levels (from data to indicator; from indicator to habitat assessment; from habitat to ecosystem assessment).

Acknowledgment

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Executive Summary

The importance of the phytoplankton production indicator is clearly stated in the MSFD-Foodweb home page[1]: "*the phytoplankton production indicator can reflect several pressures (e. g. hydrological changes, contaminants, nutrient inputs or climate changes). Hence, this indicator is highly sensitive and can be useful as an early warning indicator for direct pressure on food webs. In a trophic context, primary production is probably the most accurate metric for phytoplankton. Indeed, it is an indicator of potential matter flow needed by higher trophic levels to produce*".

However, Primary Production (PP) is not part yet of the OSPAR's Joint Assessment and Monitoring Programme (JAMP)(OSPAR Agreement 2014-03), the Agreement which describes the strategy, themes and products that OSPAR Contracting Parties are committed to deliver. There are several reasons for this, but most likely the method for measuring PP (e.g. the ^{14}C-technique) has been time-consuming, expensive, labour intensive and limited by health-and-safety regulations.

In this technical background document, part of EcApRHA Deliverable WP3.2, we give a short overview of current and new methods for measuring primary production. This document supports FW2 (Production of phytoplankton) as a candidate indicator and proposes options for a common approach to measuring primary production for OSPAR Contracting Parties.

Currently, most primary production measurements are based on variations of the ^{14}C-technique which measures the uptake of $^{14}CO_2$ by algal biomass. This document highlights the pros and cons of this technique as well as of techniques based on measurements of changes in the oxygen concentration in the water (either by using bottles or by measuring changes in the ambient O_2-concentration in the water). New high resolution techniques, described in this report, are very promising: the fourier based oxygen method and Fast Repetition Rate Fluorometry (FRRF). The former needs more testing, but the latter can already be used and is currently applied within the framework of the EU-H2020 project Jericho-next and the Dutch MONEOS and IN-PLACE programs. Measurements by FRRF are automated; however, as this method measures the production of electron, calibration against C-uptake measurements is necessary.

Optical methods to measure PP are also described in this document. Application of remote sensing is advised as it provides synoptic information about PP in the different OSPAR waters. We describe two approaches to estimate PP from remote sensing based on chlorophyll concentration (Biomass), photic depth (P) and daily incident irradiance (I) (BPI models). Parametrization of the BPI model is necessary and can be done using any technique to measure PP.

It is advised that a combination of FRRF and remote sensing should be used by the OSPAR member states for developing a uniform monitoring strategy of phytoplankton production across OSPAR waters.

[1] (http://www.dcsmm-d4.fr/fw2-production-du-phytoplancton?lang=en)

Acronyms

Acronym	Explanation
NIOZ	Royal Netherlands Institute for Sea Research
Cefas	Centre for Environment, Fisheries and Aquaculture Science
MSFD	Marine Strategy Framework Directive
WP	Work Package
PP	Primary production
chla	Chlorophyll-a
P:B ratio	Primary production to biomass ratio of the entire water column
EU	European Union
N	Nitrogen
P	Phosphorous
C	Carbon
GPP	Gross primary production
NPP	Net primary production
NCP	Net community production
FRRF	Fast Repetition Rate Fluorometer
PAM	Pulse Amplitude Modulated (fluorometer)
PQ	Photosynthetic Quotient (mol CO_2 fixed/mol O_2 produced)
P/E	Photosynthesis / light (curve)
DIC	Dissolved Inorganic Carbon
PAP	Porcupine Abyssal Plane
NOC	National Oceanography Centre

1 Introduction

In particular, the objectives of this report are:

1. to summarize the different methods used to measure production of phytoplankton;
2. to highlight the pros and cons of these methods, as well as their spatial and temporal scales;
3. to develop a hierarchical approach of the methods, based on simplicity / complexity and on costs (from simple technologies with limited spatial or temporal resolution to more complicated (combination of) methodologies with the potential to bridge space and time scales);
4. to formulate a comprehensive approach to measure production of phytoplankton that can be used by OSPAR member states.

The latter point is particularly important as adoption of a similar approach for measuring production by the different OSPAR member states will allow to generate comparable data. This would facilitate comparisons of results, as well as to integration and combination of data (resulting in a higher number of observations for a better assessment).

2 Background

2.1 Phytoplankton biomass and primary production

Marine primary production (PP) is the process by which algae use sunlight and dissolved nutrients in the water, to fix carbon dioxide (CO_2) and produce new organic matter. Hence primary production forms the basis of the food web and sets the upper boundary to the carrying capacity of the marine ecosystem.

PP in water bodies can be carried out by microalgae (phytoplankton and benthic micro-algae), macro-algae (seaweeds) and seagrass. In this report, *we will only consider production by phytoplankton*, as marine PP in OSPAR Regions (II, III, IV) occurs mainly in the water column (pelagic) and is carried out by phytoplankton. Primary production by macroalgae, seagrasses or benthic microalgae is also important, but it is limited to the coastal fringe (and to some offshore shallow areas such as the Dogger Bank; Reiss et al. 2007).

Phytoplankton unicellular organisms can grow and divide rapidly. Division times can vary between <1 day to 1-2 weeks, depending on the availability of resources such as nutrients, light, temperature and hydrodynamic conditions. In very turbid mixed water columns, or at low temperatures, growth rates are slow, while in relatively clear waters with sufficient resources, microalgae can grow at a rate near to the maximum.

One of the consequences of this fast growing rates is that the sampling frequency of most monitoring programmes (generally monthly) is not able to resolve the variability in phytoplankton biomass and production, resulting in an undersampling (and potentially underestimate) of these variables. This is clearly demonstrated in **Figure 1** (from Kromkamp and Van Engeland 2010) where chlorophyll-a (chla) data, a proxy for phytoplankton biomass, from two databases (CEME-LIMS and Waterbase) is compared. The spring bloom, evident in the Waterbase dataset, is missing in the CEME-LIMS database, due to the lower sampling frequency in the latter database (**Figure 1**). Consequently, the resulting chlorophyll weighted annual average (the final cumulative chla divided by 365) of the CEME-LIMS dataset was half the value of the chlorophyll weighted annual average of the Waterbase dataset (**Figure 1**).

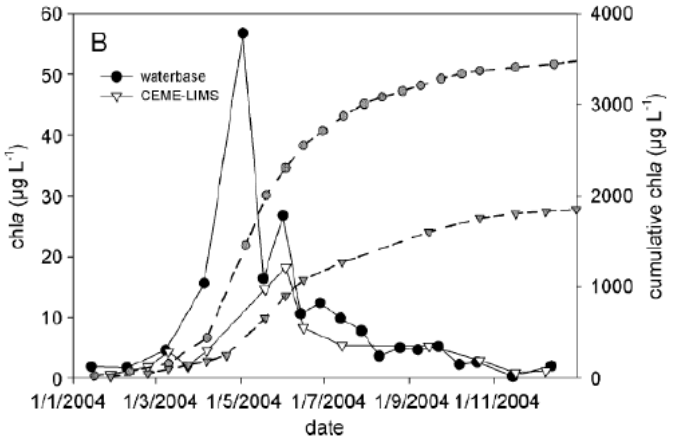

Figure 1: Comparisons between datasets of chla concentrations (µg l^{-1}), from the CENE-LIMS database (station Vlissingen at the mouth of the Westerschelde estuary, open triangles) and from the Waterbase database (full circles). Cumulative chla concentrations for the two time series are also plotted (grey full circles for the Waterbase dataset and full grey triangles for CEME-LIMS).

Knowledge of phytoplankton primary production is necessary to understand the functioning and changes of the food web. PP is affected by the availability of resources such as nutrients, light (which itself is a function of incident irradiance, turbidity, water mixing and the depth of mixing of the water column), temperature, residence times, hydrodynamic of the water column etc. These environmental factors can be collectively summarized as *bottom-up factors* controlling PP. However, *top-down factors* are also important, and these are: rates of grazing by benthic organisms (e.g. bivalves, worms) and by zooplankton, and (viral) lysis. Both bottom-up and top-down factors act simultaneously on PP.

An example of top-down control is given by a phytoplankton population that have high primary production but low phytoplankton biomass (measured as chla), as most of the produced new algal biomass is grazed away by other organisms. This will result in a high primary production / biomass ratio (or P:B ratio) in the water column. An example of bottom-up control is provided in turbid, nutrients enriched, waters, where phytoplankton production is not limited by nutrients but light availability, resulting in low P:B ratios. Relatively low P:B ratio can also be observed in highly eutrophic lakes / brackish systems, dominated by cyanobacteria, characterised by high PP and very high biomass.

A general consideration that follows from these examples, is that phytoplankton biomass (expressed as chla) and phytoplankton production can behave differently in a given phytoplankton population; therefore, there are limitations in using biomass / chla as predictor of PP, as demonstrated in **Figure 2**. The biomass of a particular group of organisms is the result of the biomass produced and imported and the biomass lost (by grazing, lysis and export). Therefore, chla concentration (phytoplankton biomass) is the result of PP minus losses and is thus the result of a flux. An analogous example is represented by the amount of money in a wallet at a given time, which does not provide indication of the wallet's owner income or spending capacity.

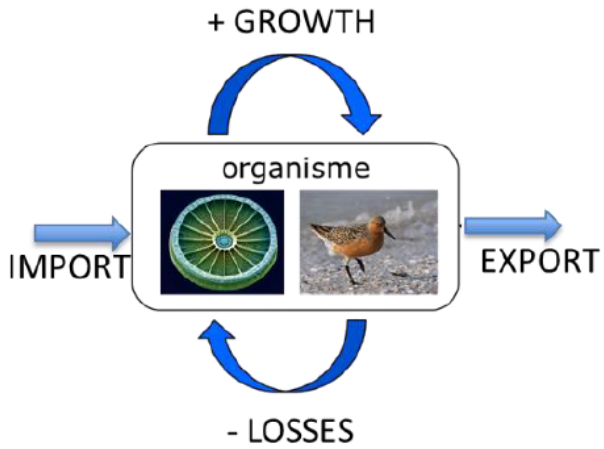

Figure 2: Conceptual diagram showing that the biomass of any group of living beings is the result of growth and import, and losses and export.

Phytoplankton PP can serve as a useful indicator of the ecosystem functioning as it responds to multiple pressures, as seen in the previous paragraphs. Particularly, as phytoplankton organisms grow rapidly, they can respond quickly to changes in the surrounding environment, caused directly (or indirectly) by human activities. Changes in land-use and nutrient enrichment have been shown to increase PP in many systems (e.g. Cloern 2001; Underwood and Kromkamp 1999), with the exception of light-limited systems such as very turbid estuaries (Heip et al. 1995). Contaminants (e.g. herbicides (Buma et al. 2009; Komenda et al. 2000; Mason et al. 2003; Snel et al. 1998), antifouling (Buma et al. 2009; Jellali et al. 2013), and heavy metals (Debelius et al. 2011; Machado et al. 2016) have been shown to influence primary production as well.

High nutrient concentration in freshwater bodies can lead to huge harmful blooms of cyanobacteria (blue-green algae). To mitigate the adverse effects of nutrient enrichment, two directives were introduced by the EU (Urban Waste Water Directive 91/271/EEC and the Nitrates Directive 91/686/EEC), in the late 80s, to reduce nitrogen (N) and phosphorus (P) loading to water bodies. The reduction of P-loads was more effective than the reduction in N-loads and this led to a serious imbalance in the N:P inorganic nutrient ratio (**Figure 3**).

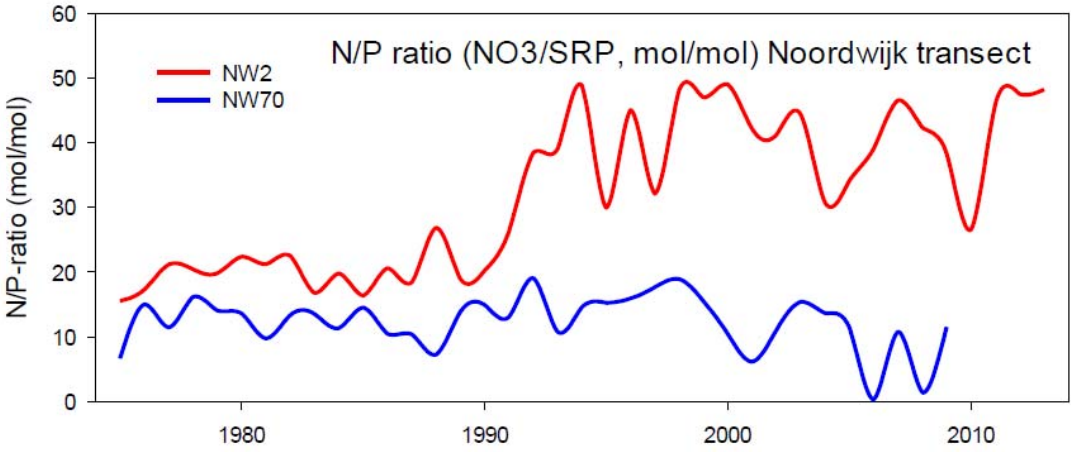

Figure 3: Changes in the annual average nitrate to phosphate ratio near the coast of Noordwijk (the Netherlands) at 2 (red) and 70 (blue) km from the coast.

The molar N:P ratio in marine waters is generally around 17:1 (close to the Redfield Ratio of 16 which is thought to represent the optical nutrient stoichiometry in algae, Redfield, 1934; Falkowski and Raven, 2007); however in a recent publication Burton and co-authors (2016) reported values ranging from 375:1 (near the Terschelling Island in the Frisian Wadden), to 1:1 (in the central North Sea). Along this N:P gradient, phytoplankton species experience different limiting nutrient conditions. Burton and co-authors (2016) speculated that a further reduction in P-loads, without a concomitant reduction in N-load, might lead to the dominance of harmful algal dinoflagellate blooms. This indicates that also "restoration" of water bodies from high to low nutrient concentration (de-eutrophication) can potentially cause problems with water quality.

Climate change can also affect PP, in two ways: ocean acidification (OA) and warming. OA is affecting ecosystem functioning and algal biodiversity (Al et al. 2015; Beardall et al. 2009; Hurd et al. 2009; Kottmeier et al. 2014) although the effects are at a large extent still unsure. The effects of global warming on the timing of algal blooms (phenology) are also unclear. The Helgoland Road time series shows that phytoplankton population in the German Bight are quite resilient with no evident long term trends (Wiltshire et al 2008). However, relatively mild autumn / winters are known to reduce the onset of the phytoplankton bloom, because zooplankton becomes active earlier in the growing season (Wiltshire et al. 2008). In the Westerschelde estuary, the phytoplankton bloom period has shifted by 1-2 months earlier and the duration of the bloom has also been affected (Kromkamp and Van Engeland 2010). Effects of these changes on annual primary production are still unknown.

2.2 Gross and net primary production

There are no generally accepted definitions of gross and net photosynthesis and production (Williams 1993b). Steemann Nielsen (1963, cited by Williams 1993a) defined gross primary production as the real rate of photosynthesis and net primary production as the rate of real photosynthesis less the rate of respiration by algae, but "real photosynthesis" itself was not defined. Williams (1993a) suggested to define photosynthesis as the conversion of light into metabolic energy, and respiration as the conversion of metabolic energy into heat. However, photosynthesis can also be viewed as the fixation of CO_2, and respiration as the production of CO_2 or of organic matter. Therefore Williams (1993a) gave the following carbon-based (C) definitions:

> *Gross primary C-production (P_G^C): is the organic carbon produced by the reduction of carbon as a consequence of the photosynthetic process over some specified period of time;*
>
> *Net primary C production P_{NP}^C): is gross primary C production minus the losses in carbon due to autotrophic respiration = $P_G^C - R_a^C$ over some specified period of time.*

Practically, whether production is expressed in terms of C (or O_2), or in energy units, is very much dependent on the methodology used to measure production. Therefore, we use the following generally accepted definitions:

- Gross primary production (GPP) = rate of gross photosynthesis (P_G) per volume or m^2 integrated over a specified period of time;
- Net primary production (NPP) = GPP minus autotrophic (algal) respiration (R_a) per volume or m^2 integrated over a specified period of time;
- Net community production (NCP) = GPP- R_a - R_h where R_h is the heterotrophic respiration per volume or m^2 integrated over a specified period of time.

3 Methodologies for measuring phytoplankton primary production

There are several methods available for measuring primary production; generally, these methods are based on the consumption of CO_2, the production of O_2, or on the interpretation of active fluorescence techniques (Fast Repetition Rate fluorometry FRRF, and Pulse Amplitude Modulated PAM). Other methods are promising, but rarely used, such as the photoacoustic method, developed by the group of Zvy Dubinsky (Pinchasov et al. 2007; Pinchasov-Grinblat et al. 2011). This report will only discuss those methods which are relatively easily implemented, although some are more difficult to implement and costlier than others.

A general equation for photosynthesis is:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

This suggest that CO_2 uptake methods are comparable to O_2 production methods and that the photosynthetic quotient PQ = O_2/CO_2 ratio = 1. In reality, the PQ ratio is quite variable and is influenced by the nitrogen source used for growth (Williams and Robertson 1991; Williams 1993a; Williams 1993b):

growth on ammonium: $6CO_2 + 6H_2O + NH_3 \rightarrow C_6H_{12}O_6NH_3 + 6O_2$

growth on nitrate: $6CO_2 + 6H_2O + HNO_3 \rightarrow C_6H_{12}O_6NH_3 + 7.5O_2$

This demonstrates that knowledge of the PQ or of the nitrogen source used for growth by the phytoplankton is important for converting oxygen based estimates of PP to C-based estimates of PP.

3.1 C-based methods

14C-based method

The amount of CO_2 taken up during C-fixation by phytoplankton can be measured by colorimetric determination (Bender et al. 1987; Johnson et al. 1985). However, this method is not / seldom used anymore so this report will concentrate on measurements that determine the uptake of radioactive $^{14}CO_2$ during an incubation.

This technique, originally developed by Steemann Nielsen (1952), is used in different varieties and is the main method used to determine marine phytoplankton primary productivity. It is therefore important to highlight the difficulties with interpretation of PP estimates measured with this technique.

In fact, since Steemann Nielsen (1952) developed this technique, many modifications have been developed; it is still unclear whether this technique measures GPP or NPP. If it is assumed that no recently fixed CO_2 during an incubation is respired, the ^{14}C technique measures GPP. The longer the incubation time, the more likely is that (part of) the newly fixed CO_2 is respired, hence the closer the measurements become to NPP. The problem is further complicated by the fact that (a fraction) of the respired $^{14}CO_2$ can be re-fixed. For this reason, it is assumed that short term (< 2 hours) incubation experiments are generally close to GPP, whereas long incubation times will lead to NPP. Based on a comparison between C-uptake and O_2 fluxes Marra (2009) argues that ^{14}C-uptake approximates NPP. The JGOFS protocol uses 24h incubations and this will indeed produce NPP values. However, such long incubation times can lead to various artefacts (photoacclimation and / or photoinhibition during the incubation, grazer induced artefacts, wall growth, loss of turbulence, damage to cells etc.).

In a series of papers, Halsey (Halsey et al. 2010; Halsey et al. 2011; Halsey et al. 2013; Milligan et al. 2015) investigated the relationship between net and gross photosynthesis, based on C and O_2 evolution. These studies were based on the marine green alga *Dunaliella tertiolecta* and the marine diatom *Thalassiosira weisflogii* grown in nitrogen limited continuous cultures with continuous irradiance. The most striking feature in this work was that short term (20 min) ^{14}C-uptake experiments could measure both gross and net photosynthesis, and that this depended on the growth rate (**Figure 4**). The 20 min ^{14}C-uptake rates were close to NPP when the algal growth rate was low, but moved to GPP when the algae were grown near the maximal growth rate.

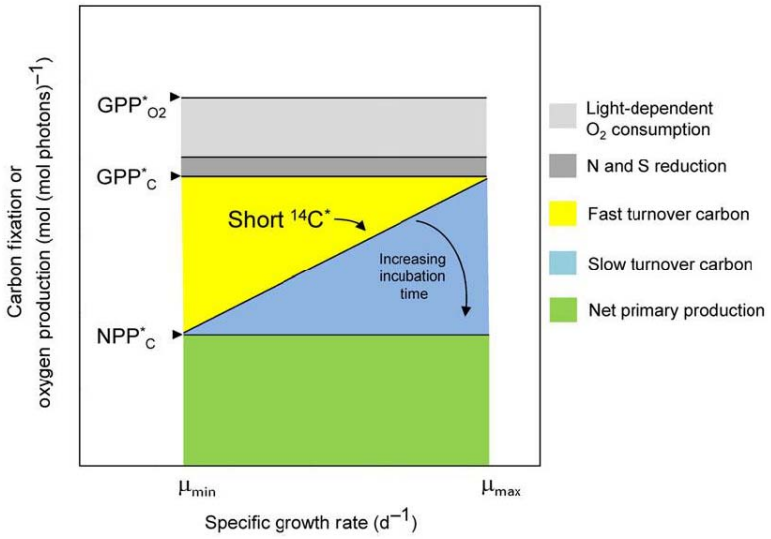

Figure 4: Production rates normalized to absorbed light at different N-limited growth rates. GPP^*_{O2} = O_2-evolution measured with $^{18}O_2$ (using membrane inlet mass spectrometry). NPP^*_C = net primary production (= μ x C_{phyto}), from Milligan et al. (2015). The grey areas are associated with PSII derived electron fluxes which are related to light-dependent rates of O_2 consumption (e.g. Mehler reaction, photorespiration) or with the reduction of NO_3^- to NH_4^+ (and S for a small fraction).

It is thus important to realize that the interpretation of the C^{14}-method is difficult without knowledge of the algal growth rate. This variability might be one of the reasons of noise in comparison between active fluorescence measurements (see Section 3.4) and C-fixation measurements.

The major differences between the variations of the $^{14}CO_2$ uptake measurements can be related to three main aspects:

- **Natural light vs artificial light**: samples can be incubated in bottles placed in an incubator with an artificial light source, or in the field by suspending bottles at different depths. In the field, the colour of the available light changes with depth and hence the absorption efficiency of the pigments will vary with depth. It is important to correct for the chromatic changes in light as this can cause changes in the initial slope of the photosynthesis-light curve (i.e. the curve that describes the rate of photosynthesis as a function of irradiance). Samples incubated in bottles (on deck using different filters for shading or in the field) are often suspended (exposed) for the day light period or for 24 h and the resulting production rates are then integrated over depth to obtain the daily water column primary production (NCP). Gross primary production (GPP) is calculated by adding community respiration (CR) to NCP. If an

incubator is used, the light dependency of the rate of photosynthesis is often normalised to the chla concentration and then fitted using an empirical model (e.g. Eilers and Peeters 1988; Platt and Gallegos 1980; Platt and Jassby 1976; Webb et al. 1974). From the fitted photosynthesis-light (P/E) curve, the maximum rate of photosynthesis P^B_{max} and the initial slope α^B are obtained (**Figure 5**). With these parameters, the daily primary production can be obtained by calculating the rate of C-fixation over the entire water column (which requires knowledge of the light attenuation coefficient and surface irradiance). The P/E parameters are also useful for ecosystem models calculating GPP and NPP.

- **Total or particulate production**. After a ^{14}C incubation, samples can be filtered or acidified to remove the unused (unfixed) inorganic $^{14}CO_2$. When samples are filtered and the radioactivity of the filters is determined, the particulate PP is measured. Any CO_2 fixed and later excreted into the environment (often as glycolate) is missed. After acidification, when the total radioactivity is measured, the total PP is measured. Whether excretion is a significant part of the overall PP depends on the physiological conditions. Phytoplankton from turbid environment, during short term incubations, often show very little excretion, while phytoplankton communities experiencing high light, combined with nutrient limitation, can excrete a substantial amount of the CO_2 fixed (Leboulanger et al. 1997). Incubation or filtration artefacts leading to cell lysis can also be interpreted as excretion.
- **Length of incubation**. Long incubation times generally lead to the measurement of NPP. However, substantial photoacclimation can take place during the incubation, as well as various types of artefacts, leading to results sometimes difficult to understand. Short term incubations however provide results in between NPP and GPP, depending on the length of the incubation, and on the growth rate (see **Figure 4**). Both long and short-term incubation require knowledge of the dissolved inorganic carbon (DIC) concentration.

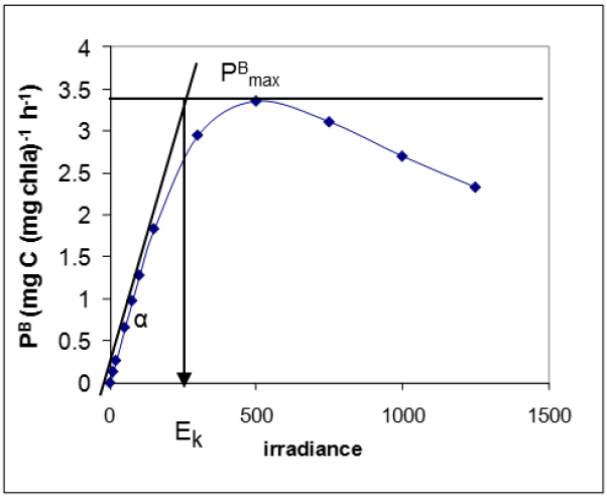

Figure 5: Example of a P/E-curve (data JC Kromkamp). $E_k = P^B_{max}/\alpha^B$ is a measure of the light acclimation status of the phytoplankton. In particular, E_k is the irradiance where light limits photosynthesis (when the ability to harvest light switches to the light saturated photosynthesis, which is determined by the capacity of the Calvin-cycle to fix CO_2).

13C-based method

Due to health and safety issues, it is more difficult to use radioactive labelled $^{14}CO_2$; therefore, $^{14}CO_2$ is now often replaced by the use of $^{13}CO_2$ (i.e. the stable and non-radioactive isotope of C; De Kluijver et al. 2013). In principle, the ^{13}C method does not differ from the ^{14}C-method; however, the analytical procedure is different. The incorporated amount of ^{13}C is measured with an isotope ratio mass spectrometer rather than a liquid scintillation counter (used to determine the rate of radioactivity). Furthermore, an isotope ratio

mass spectrometer is more expensive than a scintillation counter. The sensitivity of the ^{14}C-method is higher than the ^{13}C-method, but very good uptake rates with $^{13}CO_2$ labelled bicarbonate were achieved at low chlorophyll (< 0.5 mg m^{-3}) concentrations in the Atlantic Ocean, at the NOC Porcupine Abyssal Plane (PAP) site (49°0'0"N, 16°30'0" W, Kromkamp and Silsbe, unpublished).

3.2 Oxygen based methods

Other oxygen-based methods to measure phytoplankton primary production are: incubation of light-dark bottles, changes in the ambient pO_2, changes in the O_2/Ar-ratio, and the triple isotope method.

Light-dark bottles

The rate of photosynthesis can be obtained by measuring the oxygen concentration in water samples (transferred in light and dark bottles) at the start and at the end of an incubation; samples can be incubated in the field, at different depth of the water column, or in an incubator. The change in pO_2 in the light bottles is the net community production (NCP), while the change in pO_2 in the dark bottles is the net community respiration (CR). GPP is calculated as the sum of NCP and CR. This method has some drawback; firstly, it is not as sensitivity as the ^{14}C-method, especially when the O_2 concentrations are measured with an oxygen sensor (polarographic sensor or optode). The accuracy of the method is increased when the change in pO_2 is measured with Winkler titrations. Because the O_2 concentrations are measured at the start and end of the incubation it is assumed that the rate of change during the incubation is linear (this also holds for the ^{14}C-method). All the incubation artefacts valid for the ^{14}C methods apply also for this method. In addition, it is assumed that the rate of respiration in the light bottles equals that in the dark bottles; this is doubtful (e.g. see Claquin et al. 2004; Lewitus and Kana 1995) and knowledge of the photosynthetic quotient is necessary for converting oxygen based estimates of PP to C-based estimates of PP.

Natural changes in the ambient pO_2 in the field

Due to photosynthetic activity, oxygen concentration in the water rises during the day (NCP), and decreases during the night, as a result of respiratory processes by both autotrophs and heterotrophs (CR). Hence, daily GPP can be obtained, provided the changes in pO_2 are corrected for water-air exchange. This method was pioneered by Odum (1956) and used by several other scientists (e.g. Staehr et al. 2012), and has the advantage of not sufferring from "bottle effects". Like for the light-dark bottle experiments, it is assumed that CR does not vary during the day. Furthermore, calculating the oxygen-water exchanges is not trivial. Changes in the ambient O_2-concentrations can also be due to advection processes, and if a correction is not applied for this, the resulting GPP can either be over- or underestimated. For this reason, this method is more suitable for measuring production in lakes or lagoons with a long residence time.

In situ oxygen concentration can be measured continuously with optodes, for example, as part of monitoring stations. Hull et al. (2016) investigated uncertainty and sensitivity of net community production estimates at a monitoring site in the Thames estuary (UK) based on optode measurements. The authors demonstrated the difficulties in making accurate daily estimates of net community production (due to short-term variability in oxygen) and the effects of super saturation induced by bubbles (Hull et al. 2016).

Recently a new **fourier based method** has been developed by Cox et al. (2015) to estimate GPP from changes in oxygen time series. The method uses a fourier analysis on the amplitude of the daily harmonics in pO_2 and seems suitable for a range of in air-water O_2-exchange rates and mixing events. This method is also very suitable for systems with tidal movement. Fluctuations of GPP are related to the light-dark cycle but tidal movements have a very different frequency, therefore, effects of tides (advection) on changes in the pO_2 are eliminated by the fourier treatment of the data. Conversion to C-based estimates of GPP requires knowledge of the PQ, therefore, in order to produce reliable estimates, this method requires a good knowledge of the oxygen exchange depth (the mixing depth) and of the photic depth. Furthermore, oxygen measurements need to be averaged over a number of days to obtain good estimates. This approach

is regarded a good method to obtain high resolution time series with weekly estimates of production, however it requires further testing in order to be fully validated.

NPP can also be obtained from **O_2/Ar ratios**. The solubility properties of Argon are nearly the same as those of oxygen. Thus, any change in the O_2/Ar-ratio is due to biological activity, i.e. NCP and CR. This method has been used in the Southern Ocean by Cassar et al. (2011), Gueguen and Tortell (2008), Hamme et al. (2012) and in the Gulf of Eilat and in the Atlantic Ocean near Bermuda (Luz and Barkan 2009a). With a membrane inlet mass spectrometer the change in the O_2/Ar can be followed on-line. NCP can be assessed as follows:

$$NCP = kO_2\left(\frac{\Delta O_2}{Ar}\right)[O_2]_{eq}\rho + h\frac{d\left(\frac{O_2}{Ar}\right)}{dt}[O_2]_{eq}\rho$$

where kO_2 is the non-weighted gas transfer velocity for O_2 (m d^{-1}) and h is the mixed layer depth (m). A requirement for this method is that the change in O_2/Ar-ratio is measured within the same body of water, hence a complex Lagrangian approach is necessary (requiring following the same body of water using a tracer). Hamme et al. (2012) used data segments spanning several days in order to avoid biases caused by diel changes in pO_2. This technique may not be suitable for OSPAR waters considering their complex hydrodynamic conditions. In fact, to the best of our knowledge, this technique has not been used in OSPAR waters, so it will not be considered further in this document.

GPP can also be estimated from the **Triple Oxygen isotope method** developed by Luz and Barkan (2009b), Luz et al. (1999). This method requires analysis of the concentration of the three isotopes of oxygen, determined with isotope ratio mass spectrometry. As for the O_2/Ar-method, the triple oxygen isotope method is sensitive to changes in water masses. Thus, this technique will not be considered further in this report.

Despite these potential problems and pitfalls of the several methods, when applied consistently, PP estimates still give a good indication of the productivity of water bodies and trends in PP can be detected, provided long time series are available.

3.3 Absorption based methods

Most ecosystem models estimate primary production by using a chlorophyll-based photosynthesis model. The big drawback of these models is that they require knowledge of the C/chl*a*-ratio. It is well known that this ratio is under stringent physiological and thus environmental control (Geider and La Roche 2002; Geider et al. 1997), and it is not possible to measure this factor in the field. As photosynthesis is a direct result of the absorption of light, it is perhaps more appropriate to base methods to estimate PP on the absorption of light. In fact, the rate of photosynthesis equals the rate of light absorption (E_{abs}) times the light use efficiency (ϕ):

$P = E_{abs} \times \phi$

Light absorption by phytoplankton can be measured by the filterpad method (Cleveland and Weidemann 1993; Tassan and Ferrari 2002). This method measures the absorption of a filter before and after the extraction of the photosynthetic pigments and requires a path length amplification factor (resulting from scattering processes on the filter). Barnes et al. (2014) investigated the relationship between PP and light absorption, measured at the red chla absorption peak, and obtained good results for samples collected in the English Channel (near Plymouth), and for several stations in the North Sea. The method worked well for samples collected at several depths (**Figure 6**). With the advancement of new absorption techniques, such as the point source integrating cavity (PSICAM) technique, routine measurements of algal absorption (at least at the red chla absorption peak) seem now possible. Furthermore, the PSICAM measures absorption

of a whole water sample thus does not require filtering of the water sample (Roettgers and Gehnke 2012; Röttgers et al. 2007).

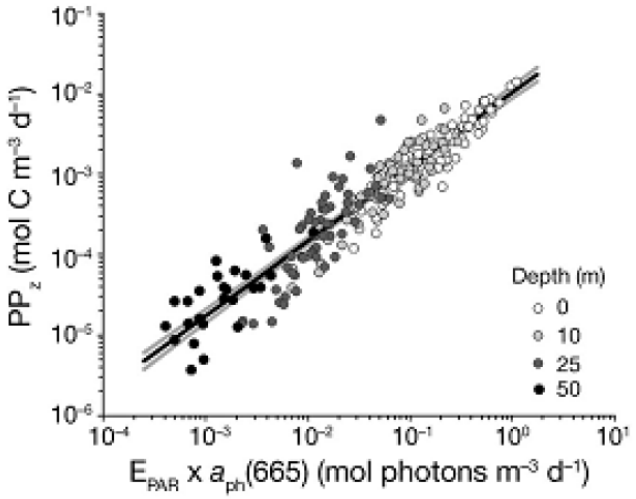

Figure 6: Relationship between algal absorption and 665 nm and primary production for samples obtained at several depths at the L1 monitoring site in the English Channel. From Barnes et al 2014.

An on-line version of the PSICAM was developed as product of the EU program PROTOOL. This gave the possibility to carry out autonomous measurements of algal absorption (Wöllschläger et al. 2014); however, as this method is very sensitive to fouling of the integrating cavity, further testing and development are required before the on-line PSICAM can be used as robust method to estimate PP.

3.4 Fluorescence based methods

This method is particularly emphasized as it is recommended in the proposed measuring strategy of Section 4. It is important to highlight that the technique described in this section measures the rate of photosynthetic electron transport and that this photosynthetic activity is related (but not equal) to C-fixation. Hence this technique requires a calibration, which, when carried out successfully, lead to the automation of this method. If the reader is not interested in the details of the method, the remainder of this section can be skipped.

A small fraction of the light absorbed by algae is re-emitted as red light (fluorescence). This property of algae (and all plants) is used to gain information about the algae (or plants) biomass. However, the intensity of the fluorescence is not only dependent on the algal biomass present, but also on the photosynthetic activity. This can be explained as follows: an absorbed photon can be used for three different processes: photochemistry (with a quantum efficiency factor ϕ_P), heat dissipation (ϕ_H) and fluorescence (ϕ_f). As only photosystem II (PSII) fluoresces at environmental temperatures, the following section is specific for PSII. Heat dissipation is a process in which absorbed light is lost as heat and this is often the result of photoprotection mechanisms, but it can also be caused by redistribution of absorbed light between photosystem II and photosystem I (state transitions), and damage to the photosystems, especially of PSII. The sum of these 3 efficiency factors is equal to 1, so if one process increases, the other(s) have to decrease. In particular, the fluorescence efficiency (ϕ_f) and the efficiency of photosynthesis (ϕ_P) can be calculated as follow:

$\phi_f = \phi_f / (\phi_f + \phi_H + \phi_P)$

$\phi_P = \phi_P / (\phi_f + \phi_H + \phi_P)$.

"Active" fluorometers, such as the Fast Repetition Rate Fluorometer (FRRF) (Kolber and Falkowski 1993; Kolber et al. 1998) or the Pulse Amplitude Modulated (PAM) fluorometer (Schreiber et al. 1986) measure ϕ_P by briefly giving a pulse of very high light which closes all reaction centres of PSII (RCII). When a RCII is in the open state (like in the dark), it quenches fluorescence giving the minimal fluorescence (F_0). When the RCII is closed by the application of the high light pulse, the quenching disappears and the fluorescence rises to a maximum (F_m) because when RCII is in the closed state, ϕ_P=0. From both parameters, the fluorometer calculates the maximum quantum efficiency:

$\phi_{P,0} \approx F_v/F_m = (F_m - F_o)/F_m$

In the light, a fraction of the PSII is in the closed state, depending on the rate of light absorption (thus on the light intensity and the absorption properties of the alga). This results in the "steady state" fluorescence in the light (F_t) > F_o. The effective quantum efficiency equals:

$\phi_P \approx \Delta F/F_m' = F_q'/F_m' = (F_m' - F_t)/F_{m'}$

By multiplying the effective PSII quantum efficiency with the incident irradiance (E) the relative PSII photosynthetic electron transport rate is obtained:

$rETR = \Delta F/F_m' \times E$

PAM and FRR fluorometers differ slightly because the PAM fluorometers normally use a "long" multiple turnover (0.4-1 sec) saturation pulse which does not only reduce the primary electron acceptor of PSII (Q_A), but also the following electrons acceptors Q_B and Plastoquinone (PQ). As the redox state of the PQ-pool is an important environmental sensor, the PAM technique is in general more intrusive than the FRRF-technique; the latter technique uses a single turnover protocol, which only leads to reduction of the Q_A pool. As part of the variable fluorescence is associated with the reduction of the PQ-pool, the F_m measured with a PAM fluorometer is generally slightly higher than F_m measured with a FRRF (for more information see Kromkamp and Forster 2003). Both methods seem very suitable to measure C-fixation (e.g. see Kromkamp et al. 2008).

FRR-fluorometers can measure the functional absorption cross section of PSII ($\sigma_{PSII,}$ nm^2 $photon^{-1}$ $PSII^{-1}$). The functional cross section is related to the optical absorption cross section of PSII (a_{PSII}) by multiplying a_{PSII} by the rate constant of photochemistry, divided by the rate constants of fluorescence, heat dissipation and photochemistry:

$\sigma_{PSII} = a_{PSII} \times (k_P/(k_P + k_f + k_H)$

Thus σ_{PSII} measures the amount of light absorbed by the photosynthetic pigments of PSII used to drive photochemistry. Hence, the absolute rate of ETR (Electron Transport Rate) can be calculated as:

ETR (µmol electrons mol^{-1} PSII s^{-1}) = $\Delta F/F_m' \times E \times \sigma_{PSII}$

So, if the concentration of PSII is known, the absolute rate of PSII electron transport in the water can be measured. A recent development allows to derive this property directly from the FRRF; simply, this is an absorption technique that also measures the photosynthetic efficiency (Oxborough et al. 2012; Silsbe et al. 2015). There are two alternative approaches for estimating the absolute ETR:

The "sigma" algorithm:

ETR (µmol electrons mol^{-1} mg^{-1} chla s^{-1}) = $\Delta F/F_m' \times E \times \sigma_{PSII} \times n_{PSII}$

Here, n_{PSII} is the concentration of PSII centres per mg chla, which equals the RCII concentration per mg chla (n_{PSII}= RCII/chla x 8.259 x 10^5, where the latter factor is a unit conversion from mol to mg chla). The

concentration of RCII can be obtained as: K_R/E_{LED} x F_o/σ_{PSII}. Here K_R is an instrument specific calibration factor and E_{LED} is the intensity of the LED current used to measure F_o, F_m and σ_{PSII}.

The absorption algorithm:

As mentioned above, the functional and optical absorption coefficients are related to each other and Oxborough et al. (2012) demonstrated that the optical absorption cross section of PSII (a_{PSII}) can be derived from FRRF-parameters after proper derivation of the K_R:

$$a_{PSII} = \frac{F_m \times F_o}{F_m - F_o} \times \frac{K_R}{E_{LED}}$$

From this the volumetric rate of ETR can be obtained as follows:

$$ETR_V = \frac{F_m \times F_o}{F_m - F_o} \times \frac{\Delta F}{F_m'} \times \frac{K_R}{E_{LED}} \times E_z$$

where E_z is the irradiance at depth z. To convert ETR to C-fixation, knowledge of the electron yield of C-fixation ($\phi_{e,C}$ mol C/mol electrons) is needed. Theoretically, the minimal value of $\phi_{e,C}$ is 0.25 (i.e. there are 4 electrons necessary to fix one CO_2 molecule). $\phi_{e,C}$ can be obtained from a comparison between C-fixation and ETR measurements. In a meta-analysis, Lawrenz et al. (2013) demonstrated that there can be a substantial variability in this factor, especially in clear waters with low nutrient concentrations. However, the meta-analysis also showed that the variability in coastal waters is limited, with values close to the theoretical maximum. Kromkamp (in prep., see **Figure 7**) demonstrated that GPP can be accurately predicted with both the sigma and the absorption algorithms, assuming a single value for $\phi_{e,C}$. Hence, this approach seems very suitable for estimates of primary production, as it can be measured (semi) automatically, preferably together with Ferrybox measurements such as temperature, salinity and turbidity.

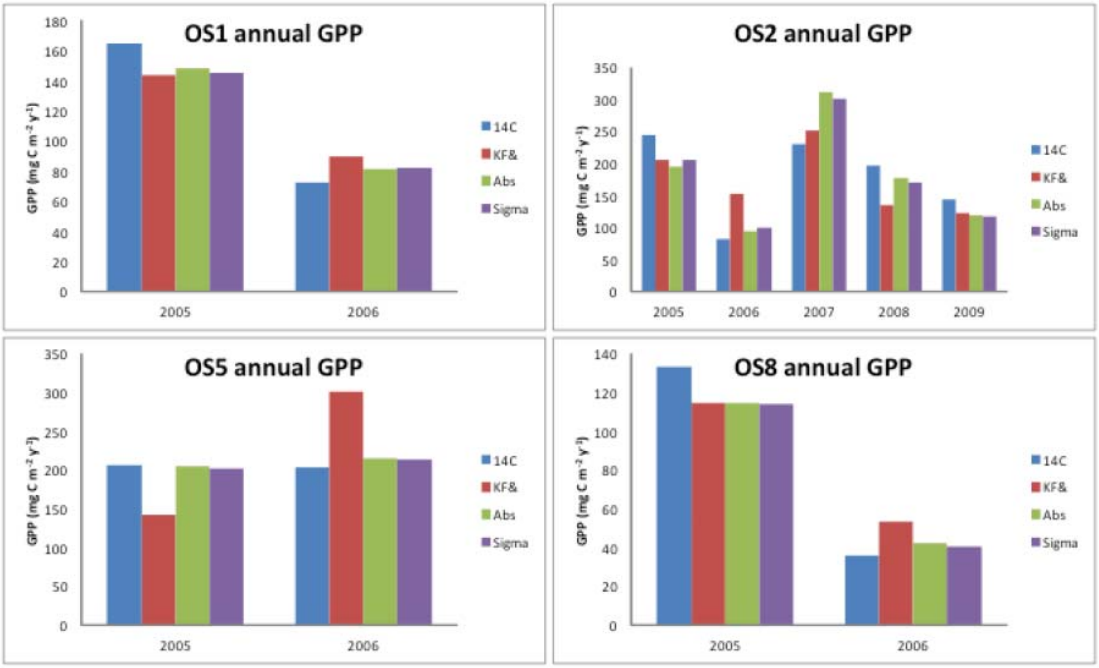

Figure 7: Annual GPP for 4 stations in the Oosterschelde estuary (the Netherlands) calculated using 3 different FRRF protocols: a) the K&F ("Kolber and Falkowski algorithm), b) the Absorption and c) the Sigma algorithm. The K&F algorithm is equal to the Sigma algorithm, with one important difference: it assumes that n_{PSII} = 0.002 (i.e. a RCII contains 500 chla molecules). This assumption was made before it was possible to measure the RCII concentration

with the FRRF. The blue bars are GPP estimates obtained using the classical ^{14}C-technique. As can be seen, GPP can be estimated with ~ 90% accuracy from FRRF-data for this estuary.

Napoléon and Claquin (2012) demonstrated that C-fixation estimates from PAM measurements, made on a ferry crossing the English Channel (from Ouistreham to Portsmouth), can be used to obtain estimates of PP, and that the technique clearly delineated different water masses (**Figure 8**).

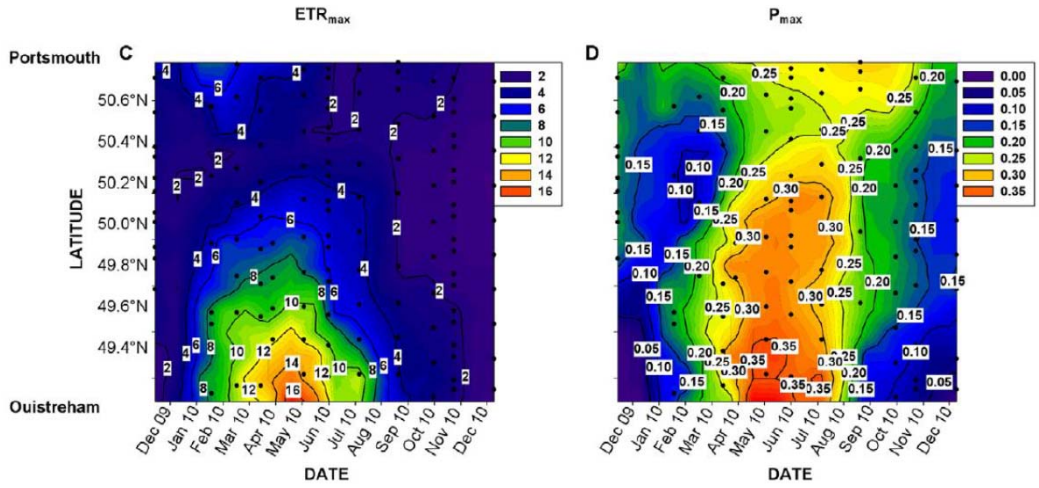

Figure 8: Contour Plot showing seasonal changes in ETR (µmol electrons $L^{-1}h^{-1}$, panel C left) and Pmax (µmol C $L^{-1}h^{-1}$ right panel) on the transect from Ouistreham to Portsmouth.

NIOZ is pioneering an automated measuring system based on FRRF measurements. **Figure 9** shows an example of automated FRRF light curve measurements made on a cruise organized by NOC from Scotland to Iceland and back. The sailing track is colour coded and shows measured chla concentrations estimated from the automated Light Curves (LC). The data show a clear relationship between "shallow" areas and higher chla concentrations (**Figure 9**). The bottom panel shows the maximum rate of rETR (rETR$_{max}$) obtained after fitting the light curves. A clear diel pattern in photosynthetic activity is also evident in the data (**Figure 9**). Similar diel patterns were observed with the setup at the NIOZ jetty in the Wadden Sea.

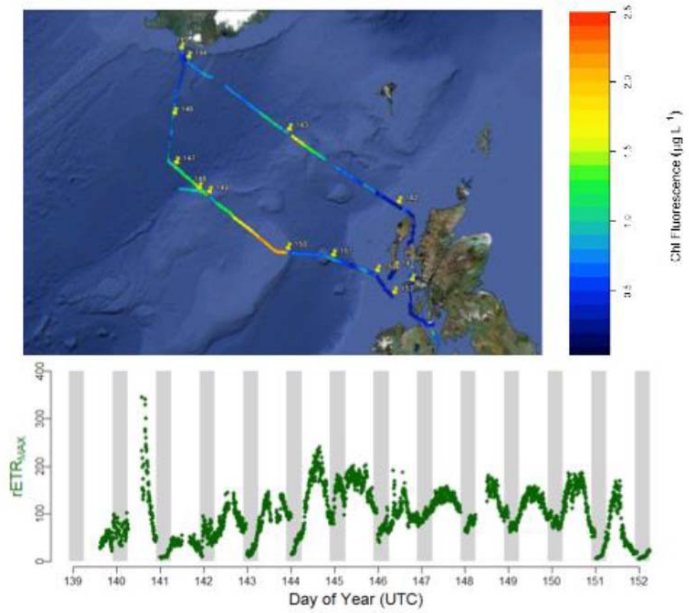

Figure 9: Results of an automated FRRF setup during a survey from Scotland to Iceland and back. The bottom panel shows the maximum rate of rETR ($rETR_{max}$).

3.5 Semi-empirical algorithms based on chlorophyll concentrations, photic depth and surface irradiance

Although it was argued in the introduction that the concentration of chla can be a poor proxy for PP, this does not necessarily mean that there is no correlation between chla concentration and PP. For example, Joint and Pomroy (1993) and Gowen et al. (1996) observed significant relationships between daily estimates of gross primary production (from ^{14}C) and water column integrated chlorophyll concentration, for the North Sea and the Irish Sea respectively (r2 ~ 0.69-0.71).

For San Francisco Bay, Puget sound and New York Bight, Cole and Cloern (1987) developed what they called a semi-empirical algorithm to estimate PP, which relied on the phytoplankton biomass (using chla as proxy), the photic depth (Z_P) and the daily irradiance (E_o) and which did not differ significantly between the estuaries.

$NPZ = a \times [chla] \times Z_P \times E_0 + b$

Where a and b are empirical coefficients obtained after linear regression analysis. The parameter "a" is a measure of the light utilization efficiency, also denoted as ψ (Jassby et al. 2002). As the NPZ relationship was based on 24h incubations, Cole and Cloern (1984) estimated net photic zone production. The standard error in ψ was substantial: 410 mg C m^{-2} d^{-1}. The offset parameter "b" (equivalent to 150 mg C m^{-2} d^{-1}) was not significantly different from zero.

This approach (also called BPI) was applied to phytoplankton production (using 2h incubations) in the Westerschelde (Kromkamp and Peene 2005) and Oosterschelde estuaries (Heip et al. 1995; **Figure 10**) and in shallow estuaries by Brush and Brawley (2009). The limitation of this equation is that the regression parameters can vary with time and space (see Heip et al. 1995). Parker et al. (2012) observed that ψ measured in north San Francisco Bay varied more than 2-fold from year to year and that ψ was substantially lower since the invasion of the clam *Corbula amurensis*, which resulted in generally much lower chla concentrations than observed before the invasion of the clam.

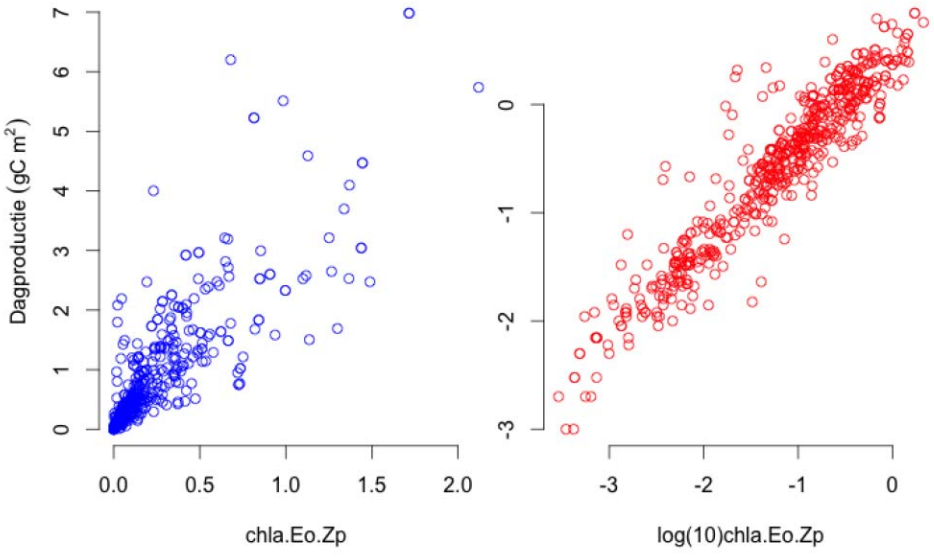

Figure 10: Relationship between the product of the chla concentration, the daily incident irradiance (mol photons m^{-2}) and the photic depth (m) and the measured PP. In the right panel the data are log transformed. Note the noise in the relationship, which has an r^2 of 0.705.

Nevertheless, despite these difficulties, this model can be useful to upscale PP estimates to larger areas, for example with the aid of remote sensing (see also Section 3.6 on Remote sensing of primary production). A possible explanation for the 'noise' observed in **Figure 10** is that the model does not take into consideration differences in photosynthetic physiology, which itself is influenced by changes in environmental conditions. This can be demonstrated by multiplying the term 'chla.E_0.Zp' by P^B_{max} (the chla normalized maximum rate of photosynthesis; **Figure 11**, Kromkamp in preparation). The validity of this approach needs to be tested for other systems.

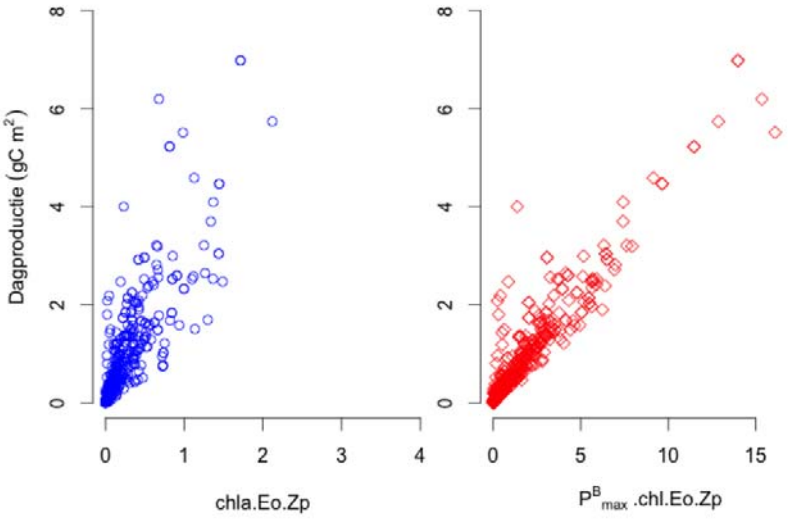

Figure 11: Updated version of the BPI model (given in **Figure 10**), including P^B_{max} (the chla normalized maximum rate of photosynthesis) in the calculations. Data for Oosterschelde (Kromkamp, in preparation).

3.6 Remote sensing of primary production

A potential limitation of the methods described in the previous sections is that, when being carried out during research surveys and/or at fixed locations, the measurements of production may not be adequate to resolve the spatial variability of the water body. The only way of obtaining a synoptic measurement of a water body at a large scale is to measure by satellite (Platt and Sathyendranath 1988). At the same time, measurements from satellite are limited to the surface. It follows that for calculating primary production from satellite remote sensing measurements, two main steps are required: 1. Development of an algorithm that describes water column production, and 2. Extrapolation of the results to a wider spatial scale (Platt and Sathyendranath 1988; Sathyendranath et al. 1995).

As indicated in Section 3.5, primary production can be estimated based on light dependent models requiring measurements of the subsurface light field (E_0), light attenuation (K_d), chlorophyll (through the water column), and the parameters of the P-E curve (P^B_{max}). Satellite data can provide some of these measurements (E_0, K_d, chlorophyll), while details of the P-E parameters or the chlorophyll profile through the water column can be obtained from in situ measurements (Sathyendranath et al. 1995). Therefore, remote sensing is used for extrapolating localized in situ measurements to a wider scale. Examples of empirical and semi-analytical algorithms used for estimating primary production from remote sensing are given in Balch et al. (1989).

More recently Saba et al. (2011) evaluated 21 ocean color models for estimating primary production in coastal and pelagic waters, during a Primary Production Algorithm Round Robin. The models (with various

complexity from simple depth/wavelength integrated to wavelength resolved) were inputted with field of chlorophyll-a, sea surface temperature, PAR and mixed layer depth, latitude/longitude, date and day length, and the resulting estimates of primary production were compared with in situ measurements. The study by Saba et al. (2011) showed that model performance was poor in shallow waters (turbid Case-2 waters), and the ocean colour chlorophyll algorithms performed poorly also in similar water types. Furthermore, the performance of the model was linked to the accuracy of the input data, particularly of chlorophyll. Hence, the BPI model described in Section 3.5 seems a promising addition to the existing remote sensing models, especially for application in coastal Case-2 waters.

Jacox et al. (2015) demonstrated that autonomous underwater gliders can significantly improve estimates of primary production thanks to glider's ability to measure subsurface properties (e.g. chlorophyll) on long-term and long-range deployments. Furthermore, the authors suggested that a combination of measurements from satellite and in situ with glider would improve estimates of primary production.

4 Towards a common measuring approach

Primary production is not measured routinely by most of the OSPAR member states, and when it is estimated, it is normally measured within a specific project and not as part of a monitoring programme. There are several reasons for this:

- The "standard" ^{14}C-method is a complicated procedure, quite costly and time consuming.
- Health and Safety regulations make it more and more difficult to use radioactive materials on board a ship.
- This ^{14}C-method needs a dedicated laboratory with a special license.
- It needs well trained personnel.

However, as it has been highlighted throughout this document, new techniques (i.e. automated FRRF technology) make it possible to measure primary production without the issues encountered by the ^{14}C-method.

The hardware is now available commercially from at least two suppliers and the raw data processing is performed by at Least the FastOcean/Ac2 combination from Chelsea technologies group.

If the FRR-fluorometer is interfaced with a Ferrybox, measurements of PP could be carried out continuously. Alternatively, water samples could be taken for discrete measurements at fixed stations or when required. NIOZ is currently developing a software for use on FRRF data to compute primary production that will become most likely freely available. Furthermore, the R-package '*phytotools*' by Silsbe and Malkin (2015) fits FRRF derived light curves and calculates water column PP, based on simulated incident irradiance and user defined light field.

4.1 Scenario 1: continuation of existing PP monitoring program

The simplest primary production monitoring scenario depends on whether PP is already measured. If so, this will likely be measured at stations, and based on the ^{14}C-technique. This might suffice, but it is recommended to measure PP twice a month during the growing season (e.g. March to October), and monthly outside this period. If only monthly measurements are made, interpolation between measurements might be necessary based on the semi-empirical BPI.P^{B}_{max} model (described in Section 3.5).

4.2 Scenario 2: implementation of the FRRF-technique

If no PP monitoring is implemented, it is advised to install a semi-automated FRRF system on the vessel used for monitoring activities. PP measurements can be carried out at monitoring stations/locations, but they can also be extended to wider areas using the FRRF-system in the flow through (on-line) mode, so to obtain a higher spatial resolution. For both approaches it is necessary to measure the conversion factor ("calibration") which relates photosynthetic electron transport to C-fixation ($\phi_{e,C}$). It is recommended that this is done at least once during each season, and twice during the spring bloom at locations which are representative of the physico-chemical and biological characteristics of the water body. When more insight is obtained in the variability of $\phi_{e,C}$, the number of calibration measurements can be reduced. Calibration

should be performed at 2-3 irradiances (at $0.5E_k$, E_k and $2E_k$, see **Figure 5**) by measuring the rate of ^{13}C-labeled $NaH^{13}CO_2$. This approach for measuring PP is very cost effective: The purchase of the equipment is a one-off event, but the running of the FRRF is free of costs. Data treatment could be performed by a dedicated partner, by trained personnel or it could be outsourced to a potential interested commercial partner.

Annual primary production at monitoring stations can be obtained by linear interpolation of chla values, P/E-parameters, daily incident irradiances, and measured light attenuation coefficient (K_d), between the sampling dates.

4.3 Scenario 3: upscaling of PP to the total EEZ

This PP monitoring scenario is the combination of Scenario 2, application of a model (which can be a simple semi-empirical algorithm as the BPI model, or a full scale coupled hydrodynamical and ecosystem model) and remote sensing. We will only briefly describe a simple case here, avoiding application of a complex ecosystem/hydrodynamic model, as outside the scope of this framework document.

Biogeochemical provinces within the Exclusive Economic Zone of a OSPAR member state can be delineated, using remote sensed observation, in-situ measurements or data from a hydrodynamic model. For each of these provinces phytoplankton biomass and light attenuation coefficients can be obtained from remote sensing. Photosynthetic parameters can be obtained from FRRF measurements, and ideally, at least one station should be sampled within each biogeochemical provinces. PP can then be calculated for each station in each of these provinces. Upscaling from the point measurement to the complete province, and to the total EEZ, can then be done by using the P^B_{max} values obtained from the FRRF measurements and the BPI.P^B_{max} model (where B (chla) and P (=$4.6/K_d$) and I (the incident irradiance) are obtained from continuous in situ measurements or remote sensed measurements). Annual primary production for the total EEZ can then be calculated by linear interpolation between the dates of measurements, adopting higher frequency data (e.g. from remote sensing).

A further advance in the interpolation can be achieved when it becomes possible to model P^B_{max} as a function of temperature and nutrient availability, using an empirical algorithm.

5 Glossary

Term	Definition	Source
Descriptor	Qualitative features which are used to assess GES. This report addresses the descriptor D4, Foodweb, particularly FW2.	EU 2008.
Indicator	Distinct features that help quantify descriptors outlined within the MSFD. More precise: a measure, index, or model used to estimate the current state and future trends in physical, chemical, biological, or socioeconomic	EU. 2008 resp. Rees et al 2008 – ICES Journal of Marine Science, 65: 1381–1386. doi:10.1093/icesjms/fsn153

	conditions of the environment, along with thresholds for management action to achieve desired ecosystem goals. This reports is about indicator FW2: Production of phytoplankton	
Production of phytoplankton or primary production	Operational definition of the amount of organic C that phytoplankton produces by fixation of CO_2 during the photosynthetic process.	this document
Gross primary production	Organic carbon produced by the fixation of CO_2 as a result of the photosynthetic process over a specified period of time (with no losses due to autotrophic respiration)	Williams, 1993
Net primary production	Gross primary production (GPP) minus the losses in C due to autotrophic respiration (= respiration by algae)	Williams, 1993
Net community production	GPP minus respiration by both autotrophs as well as heterotrophs organisms (CR): NCP=GPP-CR	
Community Respiration	Respiration by all living organism in the system studied	
Carrying capacity	Maximum population size of a species that a specific habitat can support. This depends on the resources available. The primary production sets the upper limit as this determines the amount of organic C entering the foodweb.	Wikipedia

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