

Kristin I.M. Andreasson  
Johan Wikner  
Berndt Abrahamsson  
Chris Melrose  
Svante Nyberg

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## **Primary production measurements** -an intercalibration during a cruise in the Kattegat and the Baltic Sea



Cover picture.  
The bow of U/F Argos on a sunny day.

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## **Primary production measurements**

-an intercalibration during a cruise in the Kattegat and the Baltic Sea

Kristin I.M. Andreasson, Swedish Meteorological and Hydrological Institute (SMHI)

Johan Wikner, Umeå Marine Sciences Centre (UMSC)

Berndt Abrahamsson, Department of Systems Ecology, Stockholm University (SISU)

Chris Melrose, National Oceanic and Atmospheric Administration (NOAA)

Svante Nyberg, Department of Systems Ecology, Stockholm University (SISU)



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## **SUMMARY**

In an effort to compare the primary production (PP) measurements in the Baltic Sea, four institutes got together in an intercalibration exercise with the aim to obtain similar values and a common method protocol. The strategy was to compare different methods on the same water sample and to identify sources to any differences.

The four methods showed different results and the differences were systematic. This was due to that the methods measured different things and to that there were differences in the manuals followed as well as to differences in the measurements. The manuals gave also possibilities to choose different variations of the method.

We have now managed to list all the differences and have a plan to investigate each step further with the aim to agree on a common method. This needs however to be tested to find the best method. The first step is to carefully measure the spectral composition of our incubators. We will see to that we get proper spectra and enough light. Other differences to be tested are the incubation time, the quality of  $^{14}\text{C}$  added and the end addition of hydrochloric acid, HCl.

We think that the measurement of primary production is important and want to do it in the best possible way. To do this we need to have intercalibrations on a regular basis. We also need to test all the steps to find the most suitable method. The discussions will continue until a common manual is agreed upon. We also need to invite institutes from around the Baltic Sea to agree on a change in the common manuals.

## INTRODUCTION

The primary production is the base for the production on earth. A big and measurable part of it is carried out by planktonic microalgae in the photic zone, i.e. the illuminated part of the ocean. A good estimate of the rate of the primary production (PP) is important if we want to estimate possible production in higher trophic levels. An estimation of the ecosystems capacity to assimilate and permanently store CO<sub>2</sub> from the atmosphere is also important.

A common way to measure primary production is to let the assembly of algae incubate *in situ* or in an incubator with <sup>14</sup>C labelled H<sup>14</sup>CO<sub>3</sub><sup>-</sup>; this method was described by Steeman Nielsen in 1952 and has been commonly used since then. Coming as a useful tool is the fluorescence method FRRF where the fluorescence from chlorophyll a is measured *in situ*. The <sup>14</sup>C method has the advantage of being used for many years and it has a proper detection limit. The FRRF method has the advantage of being clean, it works *in situ* with little stress on the algae and there is no laboratory work to be done afterwards.

Steeman Nielsen wrote already in 1975 that the PP measurements had an error of 30% so some variation was to be expected. This uncertainty is composed of natural variation as well as of analytical errors in the technique, from sample collection to the final calculation.

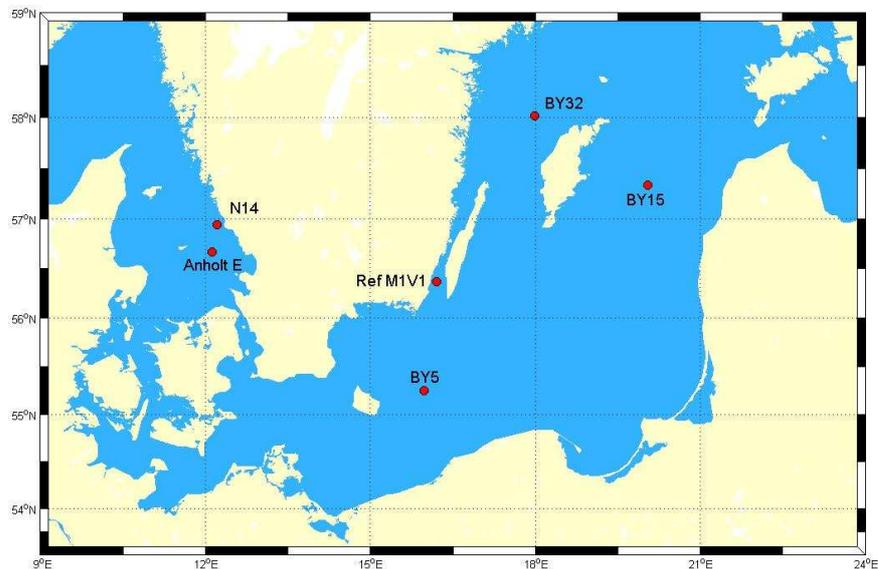
In an earlier comparison exercise 24 laboratories from 15 countries compared their scintillation counting and their calculations (Richardson 1991). The four institutes in this comparison also participated in the earlier exercise. The old results showed that there was much variation in the results even if it was filters to be counted in a scintillation counter or results to be calculated. There was also a comparison of field measurements where differences in results were shown.

Our aim was to again estimate inter-laboratory differences when applying the HELCOM guidelines using our in-house method protocols with all our regular equipment. As a further comparison the FRRF method was used at the same time.

## METHODS

### Location

The intercalibration took place onboard U/F Argos during the week May 11-16, 2009. Samples were taken at seven stations: N14 Falkenberg, Anholt E, BY 5, BY 15, BY 32, RefM1V1 and finally Anholt E again (Fig. 1). Three stations were sampled in the Kattegat and four in the Baltic Sea. Most of the stations were sampled during daytime except BY 32, which was sampled in the middle of the night. The stations used are part of the Swedish monitoring programme under the Swedish Environmental Protection Agency (SEPA), and are sampled once a month by SMHI. In all stations except BY 32 the PP is measured regularly.



*Figure 1. The sampled stations. The station Anholt E was sampled twice with four days between the occasions.*

### The participants and their methods

Participating were the three institutes responsible for the Swedish monitoring of PP in the Baltic Sea: Department of Systems Ecology, Stockholm University – (SISU), Umeå Marine Sciences Centre, Umeå University – (UMSC) and Swedish Meteorological and Hydrological Institute – (SMHI). In addition National Oceanographic and Atmospheric Administration – (NOAA), USA, participated. The Swedish institutes worked with incubation techniques with artificial light and the addition of  $\text{H}^{14}\text{CO}_3$ , while NOAA participated with a fluorescence method, Fast Repetition Rate Fluorescence (FRRF), carried out in natural light.

## **Sampling**

For the incubations two samples were taken with a hose 10 m long and 25 mm wide. The two samples were mixed in a dark bucket. After mixing subsamples were distributed to the participants. From the bucket were also taken samples for pH, alkalinity, salinity and chlorophyll a measurements. The water was gently stirred during the subsampling to avoid sedimentation in the bucket. Meanwhile the incubations took place the FRRF was run in the water column.

## **Light measurements**

The spectra of the incubator light sources were compared using a spectroradiometer, Ocean optics USB 2000 with an optical fibre P-400-2-UV/VIS and a CC3-UV cosine corrector sensor. It was calibrated using a set of lamps also from Ocean optics, DH-2000 Deuterium Tungsten Halogen Light Source. The photosynthetic active radiation (PAR) in the flasks was measured with a submersible light meter from Biospherical instruments QSL 2100 with a spherical sensor small enough to fit in the incubation flasks. The PAR in the water column was measured during the CTD measurements, with a Biospherical instruments QSP-2300 with a spherical sensor attached to the CTD. The FRRF had an additional light meter built in. The data for irradiance in air was taken from the SMHI STRÅNG database.

## **Incubations**

The incubations were made according to the HELCOM Combine and the SEPA manuals with modifications. Two different incubator models were used: two of the model from Hydrobios and one from Danish Hydrological Institute (DHI). The Hydrobios incubator works with 12 individually shaded 50 ml bottles. The rotation is propelled by the cooling medium. The DHI incubator works with 11 40 ml clear bottles that are placed in a row to shade each other with the help of a black mesh, tulle. The rotation is propelled by a motor. The cooling medium for SMHI and SISU was seawater pumped from 4m depth and for UMSC a temperature controlled cooling bath with water with polyethylene glycol ( $\pm 0.5$  °C). The incubation time varied between 2 (SMHI) and 3 hours (the others). The incubations were terminated and the samples were either filtered as a whole onto a GF/F filter or a 5 or 10ml subsample was taken out and the whole water was measured in a scintillation counter after addition of scintillation fluids.

## **FRRF**

Fast Repetition Rate Fluorescence, FRRF, measurements were performed at 1 m intervals. The FRRF values were linearly interpolated between neighbouring points to obtain values at depths corresponding to the light readings. Productivity was integrated to the deepest light reading (approx 31 m). FRRF measurements were only performed to 21

m or less depth. It was assumed that all FRRF productivity model parameters except light (chlorophyll, absorption cross section and photosynthetic efficiency) did not change below 21 m and the 21 m values were used in the productivity model for all deeper calculations. During daylight hours, the FRRF could not sample above approx 3 m due to contamination of the fluorescence signal by ambient red light so the FRRF values from the shallowest usable reading were used near surface.

Productivity estimates were made using an FRRF based productivity model developed by Chris Melrose and tuned using C14 data from Narragansett Bay. It uses only dark chamber data unlike traditional FRRF models that require light and dark measurements. Traditional FRRF models (Kolber and Falkowski 1993 or Smyth et al 2004) require a series of measurements throughout the day to estimate daily production. This type of data was not collected. For the comparison values from 0-10 m were calculated.

*Table 1 Oceanographical data for the stations.*

	Lat	Long	Temp °C	Salinity psu	pH	Alkalinity mmol/l	Calculated Inorganic carbon mg /l	Chlorophyll mg/l
<b>N 14</b>	56.94	12.21	10.59	21.16	8.32	2.12	21.03	1.4
<b>Anholt E</b>	56.67	12.12	11.01	18.99	8.30	1.96	20.58	1.2
<b>BY 5</b>	55.25	15.98	7.52	7.50	8.40	1.66	17.47	5.1
<b>BY 15</b>	57.33	20.05	6.92	7.17	8.63	1.67	17.09	1.4
<b>BY 32</b>	58.02	17.98	7.32	6.69	8.51	1.58	16.16	1.6
<b>RefM1V1</b>	56.37	16.20	9.14	7.07	8.26	1.63	17.35	1.0
<b>Anholt E</b>	56.67	12.12	11.27	18.70	8.31	1.95	20.36	0.8

### **Additional measurements**

Together with the PP other oceanographic parameters were measured at the stations. The nutrients were measured using colorimetric methods with an ALPKEM auto analyzer except for ammonium which was measured manually with a spectrophotometer Hitachi U-1800. The chlorophyll was measured with extraction in ethanol and measured with a fluorometer Hitachi F-2500, pH was measured with pH electrode Orion Ross 8102BNUWP, alkalinity with Gran titration using a Metrohm 665 Dosimat and salinity with Mini Sal lab salinometer. All parameters were measured using accredited (SWEDAC) methods.

Table 2 Differences in the regular incubator methods. Differences also occurring during the workshop marked with an \*. UMSC and SMHI also made 1 and 3 comparisons with filtered and whole water samples.

	SISU	UMSC	SMHI
<b>Sampling</b>	19mm hose 0-10m for incubator. for in-situ - water from incubation depth	Hose 0-10 m	25mm hose 0-10 m
<b>Incubation time</b>	Always in daytime	Always in daytime, though dark during winter	Anytime; depends on when the ship comes to station
<b>Incubator</b>	Hydrobios*	Hydrobios*	DHI*
<b>Light source</b>	Light tubes Philips TL8W/33*	Light tubes (Aura. T5. 8 W. 840)* <sup>a</sup>	Phillips HPI-T Plus lamp*
<b><sup>14</sup>C distributor</b>	Amersham*	<sup>14</sup> C- central. DHI*	<sup>14</sup> C- central. DHI*
<b><sup>14</sup>C specific activity (μCi/ml)</b>	5*	50*	10*
<b><sup>14</sup>C solvent</b>	Water and Borax*	Water*	Water*
<b><sup>14</sup>C volume added (μl)</b>	500 in 59ml*	64 in 59ml*	200 in 38 ml*
<b>Incubation time (h)</b>	3 incubator* -4 in-situ	3 *	2 *
<b>End sample volume (ml)</b>	10ml whole water*	5ml whole water*	38ml on a Whatman GF/F filter*
<b>HCl added to remove the excess inorganic <sup>14</sup>C</b>	500 μl 10 % to 10 ml sample*	300 μl 5 M to 5ml sample*	200 μl 0.1 M on the filter*
<b>Mean light (μE m<sup>-2</sup> s<sup>-1</sup>)</b>	470*	415*	808*
<b>SD light (μE m<sup>-2</sup> s<sup>-1</sup>)</b>	80*	123*	481*
<b>Light variation</b>	Individually shaded bottles*	Individually shaded bottles*	Stacked clear bottles shaded with tulle*
<b>Rotation</b>	With the cooling medium*	With the cooling medium*	Motor*
<b>Rotation rate (rpm)</b>	~10*	10-12*	10*
<b>Cooling medium</b>	Water from 4m depth*	Water bath with 30 % polypropyleneglycol*	Water from 4m depth*
<b>Flask wash prior to sample</b>	One flask sample water*	One flask sample water*	One flask sample water*
<b>Flask wash after incubation</b>	3 flasks deionised water*	2 flasks tap water. One flask 1 M HCl. one flask Milli-Q*	New flask each incubation*
<b>Flask wash after expedition</b>	10% HCl now and then	2 flasks tap water. One flask 1 M HCl. one flask Milli-Q	HCl, 3 flasks deionised water
<b>Scintillation</b>	TriCarb 1600 TR*	*	DHI accredited by DANAC*
<b>Scintillation liquid</b>	LumaGel Safe*	OptiPhase High Safe 3*	OptiPhase High Safe 2*
<b>Calculations</b>	Respiration 6% and temperature in the equation  Uses Öström 1975 for inorganic carbon	Respiration 6% and temperature in the equation  Uses Gargas 1975 for inorganic carbon	Uses Zeebe and Wolf-Gladrow 2005 for inorganic carbon

<sup>a</sup> Prior to 2009-01-01 Light tubes Philips TL8W/33 were used.

## Variations

Light irradiance spectra varied markedly between the different light sources. The Aure light tubes used by UMSC in this comparison had undetectable light at some wavelengths. In addition the DHI incubator used by SMHI had  $808 \mu\text{E m}^{-2} \text{ s}^{-1}$  as average light irradiance, while both SISU and UMSC were about half of this value.

The amount of HCl added to remove inorganic  $^{14}\text{CO}_3^-$  from incubated samples also varied markedly. SISU used a final concentration of 50 mM while SMHI and UMSC used final concentrations 2 and 6 times higher, respectively.

Isotope final concentrations were fairly similar, but the pre-handling varied between SISU and the other laboratories. SISU dilute their stock solution in borax buffered distilled water, while the other laboratories withdraw the aliquots directly from the stock vial. In the latter case the volume is chosen to last for one incubation.

We found differences in the HELCOM Combine manual and in the SEPA manual. In the SEPA manual there is a temperature correction for differences in temperature between in-situ and incubator. This correction is not in the HELCOM Combine. There is a respiration factor of 1.06 in SEPA but not in HELCOM.

There are also some different ways to calculate the amount of inorganic carbon available for the algae. SISU and UMSC use the tables from Buch 1945, in SISUs case with modifications by Öström 1975. SMHI uses equations from Zeebe and Wolf-Gladrow 2005. The equations use measurements of pH, salinity and temperature in the calculations and in Zeebe and Wolf-Gladrow 2005 measured alkalinity is used as well.

The differences between the incubator handling can be seen in Table 2. The differences that occur during the cruise are marked with an asterisk.

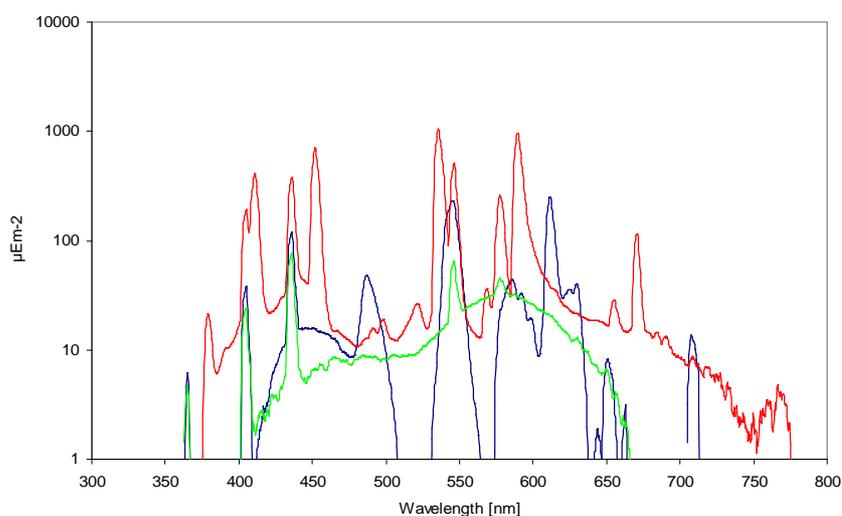
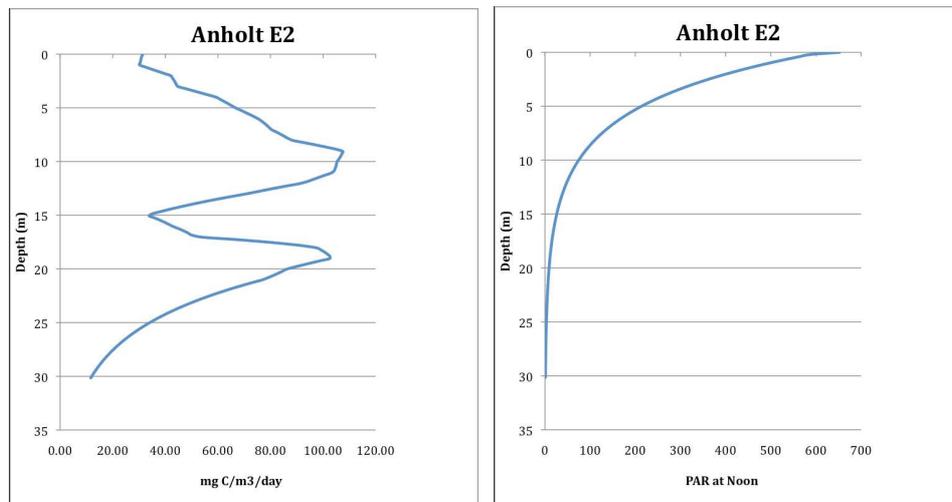


Figure 2 The irradiance from the light sources. Measurements in air. UMSC – blue. SMHI – Red and SISU – Green.

## RESULTS AND DISCUSSION

In this study we covered stations that varied clearly in terms of phytoplankton composition and nutrient composition (Figs. 6 and 7). The station BY 5 had the highest amount of nutrients, chlorophyll a and number of algal cells. There was also a difference in the algal composition between the Kattegat and the Baltic Sea, with Kattegat (N 14 and Anholt E) containing more heterotrophs than the Baltic stations. These environmental factors have a potential impact on the PP measurements.

The results from the FRRF and  $^{14}\text{C}$  assimilation differed in this study (Fig. 5). Four stations were much higher than the  $^{14}\text{C}$  measurements, while two of the stations were in the same range and one of the stations was not measured. A reason can be that the FRRF measured higher productivity with lower light as opposite to the incubators where the opposite was seen lower productivity with lower light (Fig. 3). This depends on the higher chlorophyll a content at these depths. The high production in almost no light is still to be explained. The values in Fig. 5 are calculated from 0-10 m. If we have high production in the water deeper than 10m, we will miss it with our sampling method.



*Figure 3 An example of the productivity pattern measured with the FRRF, together with the accompanying light measurement. This is the last Anholt E station and here the results differ a lot. NB. The incubators measure only down to 10 m.*

Within the incubator experiments there were also differences. The first result we could see was that the light spectra varied a lot between the different incubators (Fig 2). The SMHI lamp had the most complete spectrum but the peaks were very high. The SISU spectrum was mostly in the green part and lacked wavelengths shorter than 445 (except for a peak at 430) and longer than 645. The UMSC spectrum showed gaps here and there. The light intensity was highest in the SMHI incubator (Table 2). The spectra inside the incubators showed signs to be even more different but they have to be measured again to make any certain conclusions. How much the spectrum affects the result is unknown, but it is

potentially a substantial source of differences. It is the most likely candidate to explain the main part of the measurement variation between the incubators seen in this exercise.

For the comparison all the incubator results were calculated in the same ways. Still there were differences (Table 3 and Figs 4 and 5). The SISU results are always the highest and UMSC approximately 50 % of SISU and SMHI 70 % of SISU (Fig. 4). The differences between SISU and SMHI are quite constant while UMSC have results that are lower than 50 % in the Baltic Sea and higher in the first Kattegat stations. This can be speculated to be due to a combination between different algal composition and the spectrum in the incubator, but for now it is only speculations.

The extra samples taken by SMHI and UMSC showed for SMHI higher results when the whole water was counted than when the sample was filtered. For UMSC single sample this was not found, the results were almost identical. Theoretically the result for whole water should be somewhat higher because the algae exude organic compounds to some extent. These compounds are not captured on the filter.

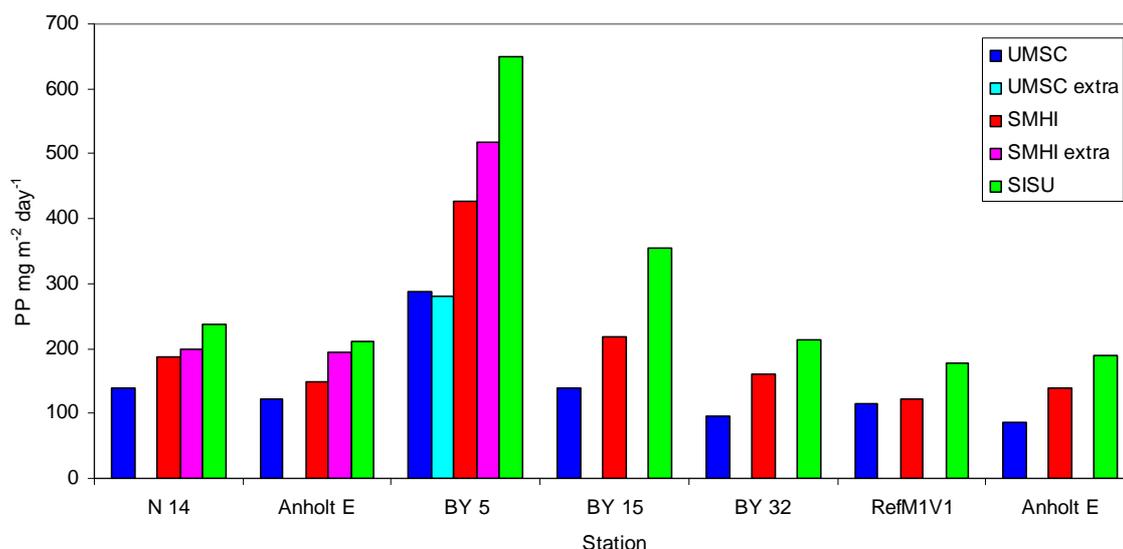


Figure 4 The daily production values measured with the incubators calculated. UMSC - blue and the extra measurement light blue. SMHI - red and the extra measurement pink and SISU – green.

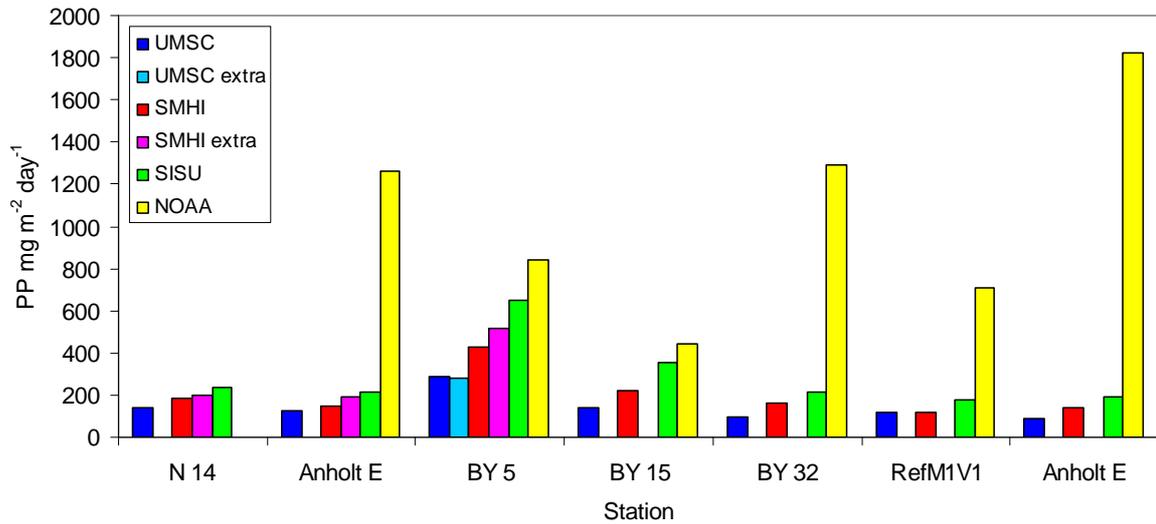


Figure 5 The same plot as Fig. 4 but with the values from FRRF added. The NOAA - yellow.

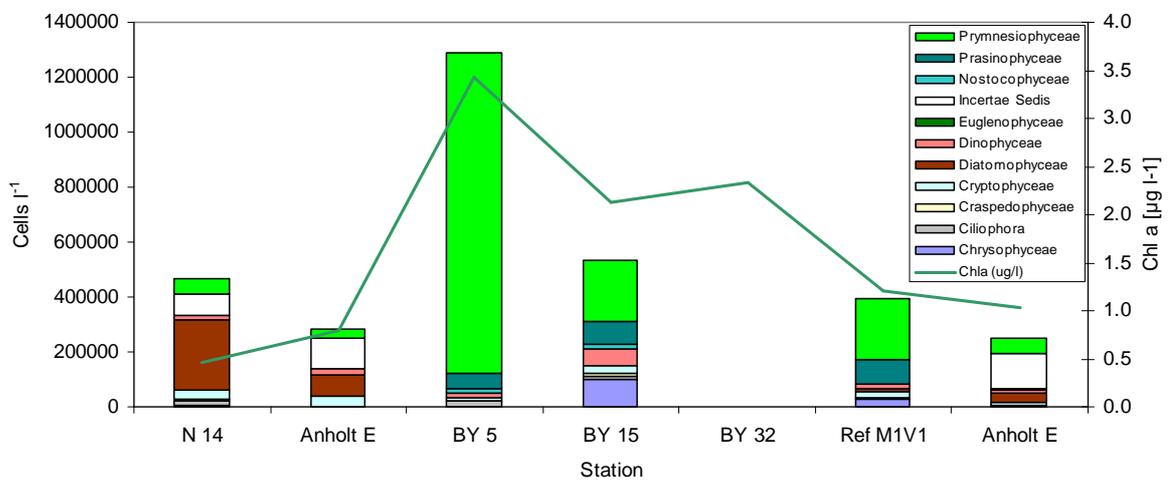


Figure 6 The composition of algal groups in the water. Incertae Sedis consists of heterotrophs. In BY 5 the most common species was the *Chrysocromulina polylepis*. The green line is the chlorophyll concentration in the water.

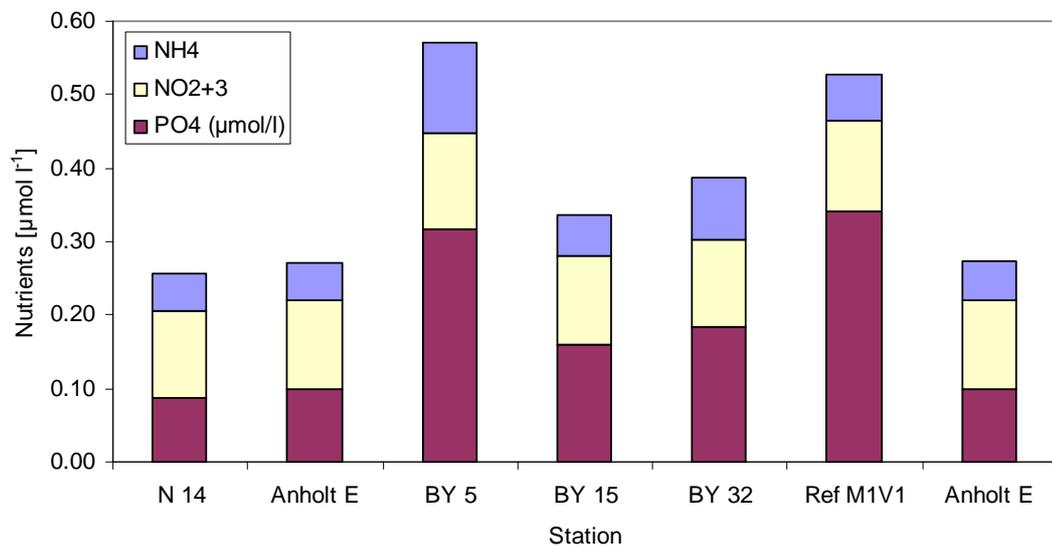


Figure 7 Concentrations of ammonium, nitrate + nitrite and phosphate in the water.

Table 3 The  $P_{max}$  and the  $\alpha$  values. The extra samples are for UMSC one filter and for SMHI tree whole water samples.

	Pmax [ $\mu\text{gCl}^{-1}\text{h}^{-1}$ ]			$\alpha$ [ $\mu\text{gCl}^{-1}\text{h}^{-1}$ ]		
	SISU	UMSC	SMHI	SISU	UMSC	SMHI
<b>N 14</b>	2.04	1.127	1.466	0.011	0.006	0.008
<b>N 14 Extra</b>			1.754			0.007
<b>Anholt E</b>	1.88	1.054	1.345	0.009	0.004	0.005
<b>Anholt E extra</b>			1.557			0.008
<b>BY 5</b>	5.81	2.234	3.901	0.028	0.013	0.015
<b>BY 5 extra</b>		2.161	4.509		0.013	0.018
<b>BY 15</b>	3.10	1.247	2.080	0.018	0.005	0.008
<b>BY 32</b>	1.89	0.717	1.181	0.010	0.005	0.010
<b>RefM1V1</b>	1.45	1.040	0.963	0.009	0.004	0.005
<b>Anholt E</b>	1.93	0.810	1.221	0.010	0.004	0.008

## CONCLUSIONS AND RECOMMENDATIONS

We found differences in the result from our incubation measurements. The FRRF technique suggested significant CO<sub>2</sub> fixation rates also at depths where light irradiance was low or undetectable. It further showed 3 times higher values than the <sup>14</sup>C-based techniques. Also the <sup>14</sup>C-based techniques differed by a factor of 2. We found it necessary to investigate why we got so different results. Some variation is of course expected but it should not be systematic.

The manuals from HELCOM Combine and from the SEPA allow marked deviations in practice (Table 2). The manuals need to be updated and where there are ambiguities the best procedure should be decided through tests. The aim should be a better harmonized method protocol.

We could frame the source of the differences between the <sup>14</sup>C-based techniques to some quality factor of the incubators, light quality, HCl treatment, isotope, or flask washing. This was because the differences were occurring already as dpm uptake in the incubation flasks.

The quality of light spectra could also influence the dpm differences found. The gaps in the UMSC spectra compared to SISU may explain the lower PP values observed in the latter case. However, the spectra measured outside the DHI incubator by SMHI does not fall into the same explaining pattern. The possibility of a compromised spectrum inside the DHI incubator has to be confirmed by further measurements.

Also the isotope quality could influence the <sup>14</sup>CO<sub>2</sub> uptake in the incubation flasks. The borax buffered and diluted isotope of SISU could introduce some stimulating nutrient. Alternatively, the manufacturer's stock solution could contain some hampering substance explaining the lower PP values of SMHI. If the current isotope qualities have no effect on the measurements, direct use of the stock solution from the manufacturer provides the least laborious protocol for routine work.

These hypotheses need to be experimentally tested and the best practices for measurement quality and least laborious procedures adopted for a future standard operating procedure.

We think that the PP measurement is important for the understanding of the dynamics in the sea and we want to measure it in the best possible way. This means that more intercalibration is necessary. We argue also that the method should be possible to do during monitoring cruises, which means that the possible way is perhaps not the optimal way but good enough. The use of the FRRF could, if we can get it to work, be a valuable complement to incubations. It needs to be studied more though. The FRRF can if it is

calibrated easily give us more data points. The only draw back will in that case be that it uses more station time during the measurements.

**How we continue:**

1. Measure the light spectrum in the incubators. See to that it is as natural as possible.
2. Determine the optimal concentration of HCl to remove the extra  $^{14}\text{CO}_2$ .
3. Compare the effect of diluted and undiluted isotope.
4. Test the incubation time to get an optimal time span.
5. Test if we should use filter samples or whole water samples.
6. Discuss with experts in the area how to estimate inorganic carbon in the water.
7. We should remove the respiration factor from the SEPA manual (Marra 2009).
8. The FRRF needs to be investigated further.

This intercalibration has started a necessary discussion and comparison among the institutes that do the PP monitoring for SEPA. The work will continue at each ones lab, but the workshop participants found it important to do common exercises as well and wish to do them on a regular basis. Our recommendation for the future is to do intercalibration and ring tests preferably on land for efficiency and in the process we need to invite scientists from other institutes to participate. The *in situ* measurements should also be a part of the measurement.

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## APPENDIX

### Calculations

The base for the calculations was the equation

$$PP = (\text{Tot CO}_2 * \text{DPM measured} * 1.05 * 60) / (\text{DPM added} * \text{incubation time in minutes})$$

The amount of total CO<sub>2</sub> was calculated according to the equations by Öström 1975. DPM is the concentration of the added <sup>14</sup>C tracer, 1.05 accounts for <sup>14</sup>C lower uptake rate than <sup>12</sup>C. 60 is for converting the incubation time to hours. Additional calculations for sub sampling were used when needed. This PP is valid for the light intensity for the actual bottle.

An equation is fitted to the CO<sub>2</sub> uptake for each light intensity to get the values of the maximum possible production rate (P<sub>max</sub>) and the efficiency of the algae to utilise the light (α). The equation used is a tangential curve fit proposed by Jassby and Platt 1976:

$$PP = P_{\max} * (\text{TANH} (\alpha * E_{\text{PAR}}/P_{\max})).$$

E<sub>PAR</sub> is the actual light in the bottle.

This equation was used to calculate the production in the water column together with the light measured and /or calculated.

The light in the water column was calculated. The reflection on the water surface needs to be estimated. For this exercise we decided to use 7 % reflection. The attenuation coefficient in the water column was calculated from light measurements from the CTD profile. The upper meter was not accounted for because it mainly consisted of disturbances from the ship. From 1 m to the end of the illuminated zone the attenuation coefficient was estimated as

$$K_d = -\ln (I_1-I_0)/(D_1-D_0)$$

I is the irradiance at the depth. D is the depth.

The average K<sub>d</sub> for all the data sets was used. The light at each meter from 0 to 10 meters was calculated.

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Sveriges meteorologiska och hydrologiska institut  
601 76 NORRKÖPING  
Tel 011-495 80 00 Fax 011-495 80 01

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