

BIO-OPTICS FOR OCEAN COLOR REMOTE SENSING  
OF THE BLACK SEA  
(Black Sea Color)

TN9 Analysis and QA of biological data

<i>Workpackage:</i>	3	<i>Data analysis and QA</i>
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### **CHLOROPHYLL A QC/QA PROCEDURE ( in situ measurements)**

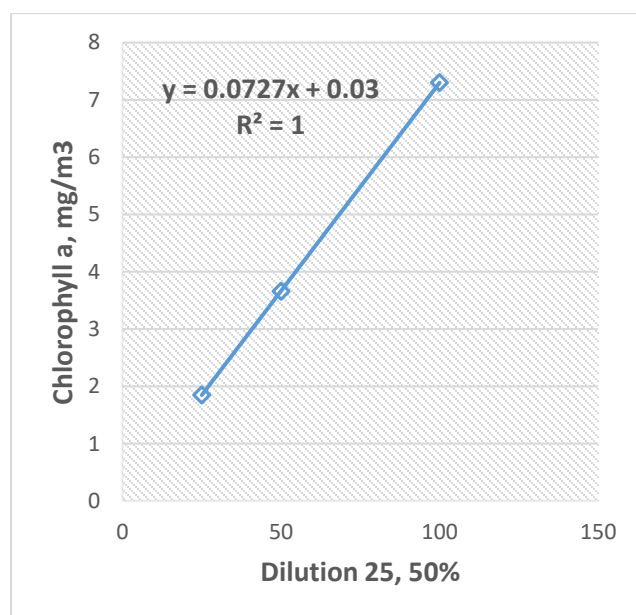
The Protocol for measurement of chlorophyll a and QC/QA procedure followed ISO 10260 (1992E), EN 15204 (2006) and ICES Techniques in Marine Environmental Sciences (Aminot and Rey, 2000).

The QC/QA routines performed were:

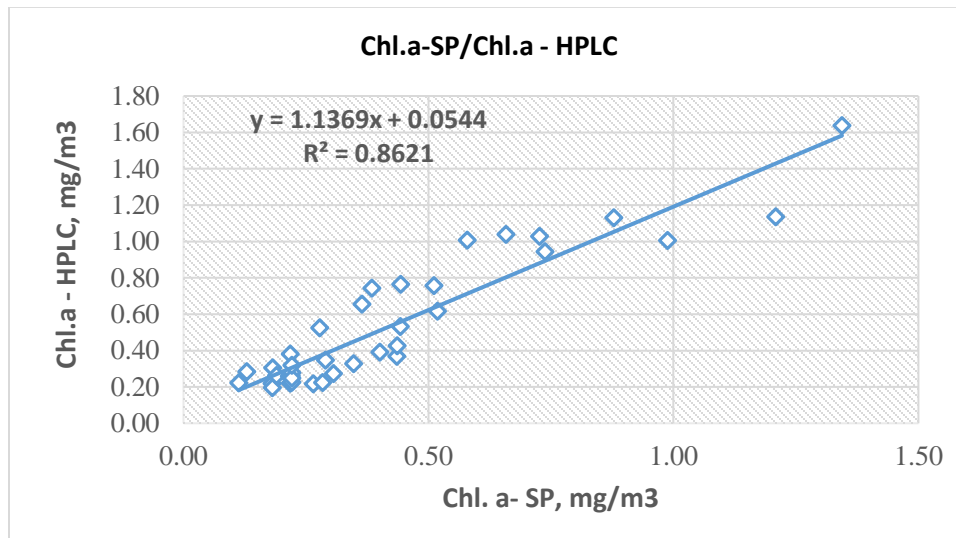
- Prior to the measurements of the sample series the spectrophotometer and the fluorimeter were calibrated against pure chlorophyll a (C6144 – 1 mg CHLOROPHYLL A from *Anacystis nidulans* algae – SIGMA –ALDRICH, UK); preparation of stock standard solution (SSS), Primary dilution standard solution (PDSS), blank, and blank to blank measurements performed for the different cuvettes
- Reproducibility - for checking the reproducibility of the measurements from a natural sample (100%) 2 diluted samples were prepared (25% dilution and 50% dilution) and 12 replicates were measured for each sample concentration. The results are presented on Table 1, Figure 1
- Accuracy - for checking the accuracy of the lab measurements the results were compared with the results produced by HPLC (based on JRC-IO-BAS data for 2016) – Figure 2

**Table 1.** Measurements reproducibility

Replicates	100%	50%	25%
R1	7.22	3.81	1.91
R2	7.12	3.72	1.91
R3	7.45	3.69	1.76
R4	7.11	3.80	1.81
R5	7.26	3.69	1.79
R6	7.28	3.51	1.88
R7	7.56	3.74	1.80
R8	7.34	3.66	1.78
R9	7.32	3.52	1.93
R10	7.55	3.49	1.89
R11	7.24	3.53	1.85
R12	7.19	3.73	1.88
average	<b>7.30</b>	<b>3.66</b>	<b>1.85</b>
stdev	<b>0.15</b>	<b>0.12</b>	<b>0.06</b>
CV%	<b>2.05</b>	<b>3.16</b>	<b>3.17</b>



**Figure 1.** Mean chlorophyll a values (n=12 replicates) of dilution samples (25 and 50 %)



**Figure 2.** Comparison between chlorophyll a data set measured by spectroscopic method (SP) and HPLC ( 90<sup>th</sup> percentile of the data, n= 37; SP av=0.43, stdev=0.29; HPLCav = 0.53, stdev=0.36)

The One – way ANOVA test show an f-ratio value of 2.12628 and the p-value of 0.149141, e.g. there is no statistically significant difference between the two data sets (the result is not significant at  $p < .05$  ).

### ***PHYTOPLANKTON ANALYSIS and QC/QA PROCEDURE***

For phytoplankton analysis and QC/QA procedure followed the Black Sea Manuals: For phytoplankton QC/QA procedure followed the Black Sea Manuals: Moncheva, Parr (2015) and Moncheva (2014) referring to EN 15204 (2006) standard procedure.

As recommended the microscopic counts (done on Nikon Eclipse Ti-U inverted microscope with software for image analysis, LUCIA) and calculation of phytoplankton cell abundance and biomass was performed by a single analyst and the species nomenclature followed strictly the WoRMs taxonomic nomenclature.

Three replicates of a randomly selected sample were analyzed for checking the reproducibility of the method. The results are summarized on Table 2 and Table 3.

**Table 2.** Results and statistical summary of the three replicates of sample K11-002, Phytoplankton abundance (N, cells/l)

TAXON	Replicate, N cells/l			Statistical summary		
	1	2	3	Average	stdev	CV%
Bacillariophyceae	332166	332698	319838	328234	7275.9	2.2
Dinophyceae	95827	110967	94789	100528	9055.8	9.0
Chlorophyceae	0	15327	15030	10119	8764.6	86.6
Conjugatophyceae (Zygnematophyceae, Charophyceae)	521	0	100	207	276.3	133.5
Cryptophyceae	26040	33209	12525	23925	10502.8	43.9
Cyanophyceae	2604	1667	5010	3094	1724.6	55.7
Euglenoidea	260	613	451	441	176.5	40.0
Nephroselmidophyceae	7812	12773	5010	8532	3930.9	46.1
Pyramimonadophyceae	5208	0	2505	2571	2604.6	101.3
Prymnesiophyceae	401016	337194	315630	351280	44401.6	12.6
Microflagellates	59892	89408	70140	73147	14985.7	20.5
<b>Total</b>	<b>931347</b>	<b>933855</b>	<b>841029</b>	<b>902077</b>	<b>52884.0</b>	<b>5.9</b>

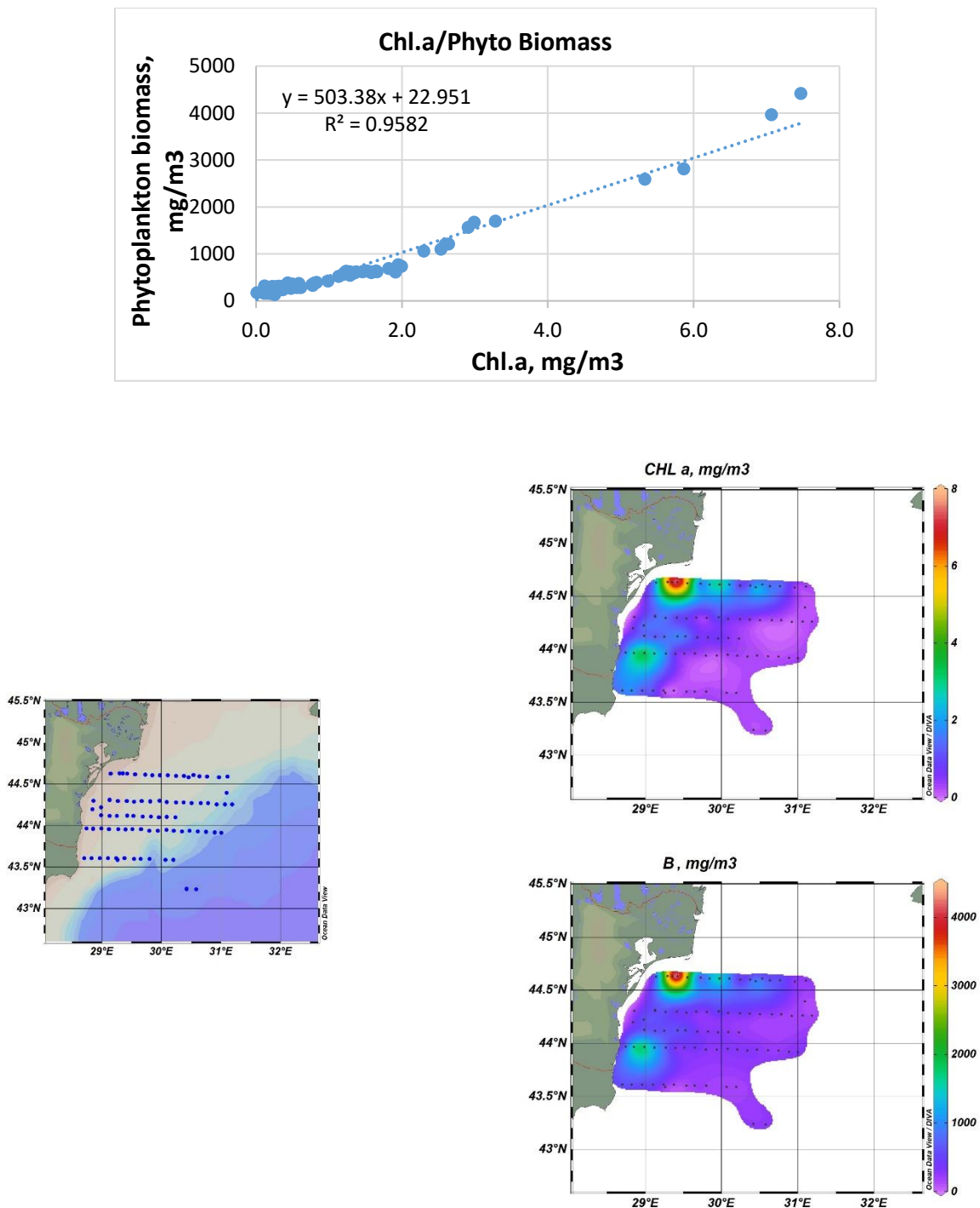
For the very rare and low abundant groups the CV is expected (normally) high. For the Total abundance, the CV is 5.6 (bellow the accepted difference of 20%). The one-way ANOVA results show no statistically significant difference between the replicates (f-ratio value is 0.01294. the p-value is .987153, the result is not significant at  $p < .05$ ).

**Table 3.** Results and statistical summary of the three replicates of sample K11-002, Phytoplankton biomass (B, mg/m<sup>3</sup>)

TAXON	Replicate, B mg/m <sup>3</sup>			Statistical summary		
	1	2	3	Average	stdev	CV%
Bacillariophyceae	373.831	384.453	402.036	386.8	14.2	3.7
Dinophyceae	171.851	224.138	169.389	188.5	30.9	16.4
Chlorophyceae	0.000	2.721	0.575	1.1	1.4	130.5
Conjugatophyceae (Zygnematophyceae, Charophyceae)	0.103	0.000	0.020	0.0	0.1	133.5
Cryptophyceae	7.691	4.941	3.166	5.3	2.3	43.3
Cyanophyceae	0.039	0.256	0.075	0.1	0.1	94.5
Euglenoidea	0.056	1.020	0.679	0.6	0.5	83.6
Nephroselmidophyceae	1.605	2.624	1.029	1.8	0.8	46.1
Pyramimonadophyceae	0.206	0.000	0.099	0.1	0.1	101.3
Prymnesiophyceae	55.784	44.079	39.821	46.6	8.3	17.8
Microflagellates	2.461	3.674	2.882	3.0	0.6	20.5
<b>Total</b>	<b>613.626</b>	<b>667.905</b>	<b>619.770</b>	<b>633.8</b>	<b>29.7</b>	<b>4.7</b>

Similar to the phytoplankton biomass replicates for the Total Biomass, the CV is below the accepted difference of 20% - 4.7%. The one-way ANOVA results show no statistically significant difference between the replicates (f-ratio value is 0.0053, the p-value is .994716, the result is not significant at  $p < .05$ ).

There is a good agreement between the chlorophyll a data and the Total phytoplankton biomass values and pattern of surface spatial distribution – Figure 3.



**Figure 3.** Comparison between chlorophyll a data [mg/m<sup>3</sup>] and the Total phytoplankton biomass values [mg/m<sup>3</sup>] and the pattern of spatial distribution

## References

Aminot A. and F. Rey (2000). Determination of chlorophyll a by spectroscopic methods. ICES Techniques in Marine Environmental Sciences, 17pp., ISSN 0903-2606.

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