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Black Sea Phytoplankton Data Quality – Problems and Progress

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Abstract

The quality of biological data has gained recognition as an essential part of international monitoring programmes, in response to the demand for strategic environmental evaluations such as the EU WFD, the MSFD and informed decisions for environmental sound management. The paper presents the results of an intercalibration exercise among four Black Sea phytoplankton laboratories (NIMRD-RO, IBSS-UKR, IO-RAS – RUS and IO-BAS - BLG) conducted under SESAME FP6 Project with the objectives: 1) to assess the degree of comparability of phytoplankton and chlorophyll a data produced by routine in-house methods; 2) to formulate recommendations for progress towards harmonization of the research methodology in the Black Sea. The statistical treatment of the results reveal that at the level of total phytoplankton abundance and biomass as well as chlorophyll a the data were in a good agreement, while for some taxonomic classes (Prymnesiophyceae and small flagellates) the differences were significant. The counted sample volume proves essential for detection of species diversity and the methods of species specific biovolume measurements - for the total biomass. As a follow up Guidelines for QC/QA of phytoplankton data and check-list with suggested shapes for biovolume calculation were produced under UP-Grade Black Sea SCENE FP7 Project that offer key options for progress.

Keywords: phytoplankton data comparability, intercalibration, cell count, biovolume, manual.

Introduction

The quality of biological data has gained a recognition as an essential part of international monitoring programmes, in response to the demand for strategic environmental evaluations such as the EU WFD, the MSFD and informed decisions for environmental sound management.

Phytoplankton is an essential part in the process of understanding and predicting changes in the marine environment. Community structural characteristics bear valuable information about the evolution of phytoplankton assembly and the trajectories of shifts under multiple environmental factors, including anthropogenic and global climatic impacts. Details of phytoplankton analytical procedures are essential to compare data produced by different analysts either during long-term monitoring programs in one area or between different areas in order to evaluate statistically significant long-term trends or spatial differences. Carbon biomass of plankton organisms is a fundamental parameter in ecosystem models and biogeochemical carbon budgets. Temporal and spatial variability in total and export primary production can be quantified and predicted only if the carbon content of the major plankton organisms is known. Estimates of carbon biomass of plankton organisms are usually made by converting microscopic size measurements to cell volumes, which are then converted to carbon biomass using empirically or theoretically derived carbon to volume ratios. Irrespective of the available manual for phytoplankton sampling and analysis in the Black Sea (Moncheva and Par, 2005) based on agreed procedures among laboratories from the 6 Black Sea countries the latter are not fully followed, or labs are working according to there own routines. Standards such as the ISO 9000 series and ISO 17025 provide a general framework for quality assurance but so far criteria for determining the acceptability of data from surveys of biological communities to meet specified information needs at international level are still under development, and should be given high priority.

Hence the importance of comparability of data when regional data bases are composed and further used in various regional studies. In the present paper the results of an intercalibration exercise conducted among four Black Sea phytoplankton laboratories

(NIMRD-RO, IBSS-UKR, IO-RAS – RUS and IO-BAS - BLG) are presented.

The objective of the intercalibartion exercise was to compare the results of phytoplankton and chlorophyll *a* measurements following in-house routine methodology in order to:

1) assess the degree of comparability/differences in phytoplankton and chlorophyll *a* data collected during the SESAME field campaigns;

2) where possible to make recommendations and catalyze actions for further improvement and harmonization of research methodology in the Black Sea.

We focus on the abundance and biovolume analysis, where the differences may impact significantly the final results. This exercise was expected to produce valuable results for assisting the modeling stage of SESAME Project and contribute to phytoplankton data management in the Black Sea.

Materials and Methods

Sampling Logistics

Prior to the cruise a one day workshop was organized at IO-BAS to design the sampling strategy and refine the approach of the intercalibration exercise. 2 sampling stations were selected along the SESAME sampling polygon – one in the coastal area and one in the open sea (SESAME intercalibration & sediment trap station), where data have been already collected during previous campaigns by several SESAME partners.

Phytoplankton and Chlorophyll a Sample Preparation

Water samples were collected from a depth 1m bellow the surface by CTD Rosette System to which 5l sampling bottles were attached. In order to minimize possible sampling difference the water from the CTD bottles was homogenized in a large vessel (15 L) prior to collecting 1l water samples distributed in three replicates to each partner. The samples were fixed in 4% buffered to pH 8-8.2 with disodiumtetraborate (borax-$Na_2B_40_3$ • 10 H_20) formaldehyde solution and stored in plastic containers for further lab analysis. For IO-RAS the samples were fixed in 2% buffered formaldehyde to comply with the routine practice.

Similar homogenizing procedure was applied for chlorophyll samples. The samples were filtered through 47 mm GF/F, Whatman MILLIPORE filters at 0.2 atm vacuum (Vacuum pump MILLIPORE). The filters were stored in liquid N and delivered to the partners lab.

Basic information of the in- house routines for phytoplankton and chlorophyll *a* lab analysis among the different participants labs is summarized in Table 1 and Table 2.

Statistical Analysis

The data were checked for statistical differences among the 4 laboratories (Bulgaria, Russia, Romania and Ukraine) on the following parameters:

• Phytoplankton Total Abundance [cells/L]

• Phytoplankton common taxonomic classes (Bacillariophyceae, Dinophyceae, Prymnesiophyceae, Cryptophyceae, Small flagellates and Chrysophyceae) Total Abundance [cells/L]

• Phytoplankton Total Biomass (wet weight) – [mg/m^3]

• Chlorophyll *a* measurements [μg/L]

In this comparison test we applied the following statistic treatement:

1) Robust statistics-ANOVA test and the Tukey's test at a confidence of 0.95, on log - transformed data in order to normalize the skewed distribution and to stabilize the variance and Lavene

Table 1. Inventory of in-house methods of phytoplankton analysis

Participant	Sample concentration	Counting chamber	Type of microscope	Volume of subsample
BLG	Decantation Utermol	Segwick Rafter Utermol	Nicon inverted+ immage analysis	1.0 ml
RO	Decantation Utermol	Utermol	Inverted	0.1 ml
RUS	Decantation/inverse filtration	Nogott's - 0.1 ml Nauman's chamber 1 or 5 ml	Light compound	1.0 ml
UKR	Decantation	Nauman's chamber 1 ml - 0.05 ml	Light compound	0.1 ml

Table 2. Inventory of in-house methods of chlorophyll *a* analysis

Partner	Extraction Solvent/duration	Sample preparation	Instrument	Equations reference
BLG	90% acetone 24 hours	7000 rpm cuvette 1cm L	Spectrophotometer	Jeffrey and Humphrey (1975)
RO	90% acetone 24 hours	4000 rpm cuvette 1cm L	Spectrophotometer	SCOR UNESCO (1968)
RUS	90% acetone		Fluorometer	

statistic for checking the homogeneity of the variance between the groups; Bray-Curtis similarity among samples on square root transformed data of taxon species specific abundance and biomass. These methods were applied to BLG, UKR and the RUS data (no replicates by the RO partner).

2) Common statistics employed during phytoplankton ring tests - average±standard deviation (SD) and CV <20% (Rott *et al*., 2007; Lacouture, 2001; HELCOM, 2003)

Results and Discussion

Comparison of Phytoplankton Numerical Abundance

The statistical summary of the results for the total abundance and biomass and major taxonomic classes by the different labs are presented on Table 3.

The ANOVA statistics for total abundance show significant differences between the Ukrainian-Bulgarian results, and between the Ukrainian-Russian results, while the difference between Bulgarian and Russian data were not significant (Figure 1 and Table 4). The same stands for the comparison between the abundance of the taxonomic classes Dinophyceae, Small flagellates and Prymnesiophyceae, where the deviations were much higher.

For the remaining taxonomic groups (Bacillariophyceae, Cryptophyceae and Chrysophyceae) as well as for the biomass the ANOVA results could not be considered reliable, since the F statistic value was higher than 0.05 (F>0.05) (results not shown).

Hierarchical clustering based on species specific and total abundance and biomass of the replicates showed high similarity - >85% between Bulgarian and Russian data and between Ukrainian-Bulgarian and Ukrainian-Russian >75% respectively (Figure 2).

The reproducibility of the in-house analysis (CV <20%) for the total numerical abundance and biomass yield very close results both between the replicates and between the different labs (Table 5).

At the level of taxonomic classes the differences among the participating labs were significant, especially critical for Prymnesiophyceae and small flagellates, where also the in-house results show inconsistencies for all partners. Albeit the good agreement between the data among some of the labs this was not systematic for all the taxonomic groups that add further complexity to the comparability of the result.

As apparent from the comparative analysis of the common species biovolume used by the participating labs out of 18 species that compose the bulk of the phytoplankton assembly only for 2 species the specific biovolume was similar (CV<20%). Thus for example for the dominant species such as *Pseudo-nitzschia delicatissima* and *Emiliania huxleyi* the biovolume varied more than twice (202-409 μm^3 and 145-268 μm^3 respectively) for other species the differences exceeded 3 fold – Table 6.

Chlorophyll *a*

The results of chlorophyll *a* measurements reveal good in-house reproducibility for BLG and RO and higher than 10% difference for RUS lab – Table 7. The difference between the BLG and RO data was within the average ± stdev, the higher deviation observed in the values lower than 1 µg/L, most likely related to the different methodology (spectrophotometry and fluorimetry). Among the higher values as expected the difference was not significant (Table 7).

There are various approaches used during ring

Table 3. Statistical summary of phytoplankton abundance (cells/L) and biomass (mg/m^3)

LAB	BLG		RUS		UKR	
	Phytoplankton abundance (cells/L)					
Taxon	Mean	SD	Mean	SD	Mean	SD
Bacillariophyceae	2942260	26535	2318659	261721	3787862	294578
Dinophyceae	9913	2342	7803	1172	54495	9179
Prymnesiophyceae	72798	1155	62422	12837	227412	36967
Cryptophyceae	147526	2040	125343	24977	456157	72049
Small flagellates	347712	142143	753600	421620	65790	55824
Chrysophyceae	108	0.2	180	282	28316	40044
Total Abundance	3520319	167824	3268008	178087	4620032	160602
	Phytoplankton biomass (mg/m^3)					
Taxon	Mean	SD	Mean	SD	Mean	SD
Bacillariophyceae	2007.95	150.30	2614.26	86.00	1620.71	420.33
Dinophyceae	88.63	12.36	297.02	95.00	111.80	5.02
Small flagellates	93.12	38.07	4.26	3.61	58.87	21.84
Prymnesiophyceae	11.77	0.03	52.26	14.51	9.10	2.55
Cryptophyceae	0.36	0.05	0.70	0.99	0.09	0.11
Chrysophyceae	0.47	0.00	5.45	7.71	0.39	0.34
Total Biomass	2284.55	103.02	2974.87	4.91	1935.74	372.04

SD – standard deviation

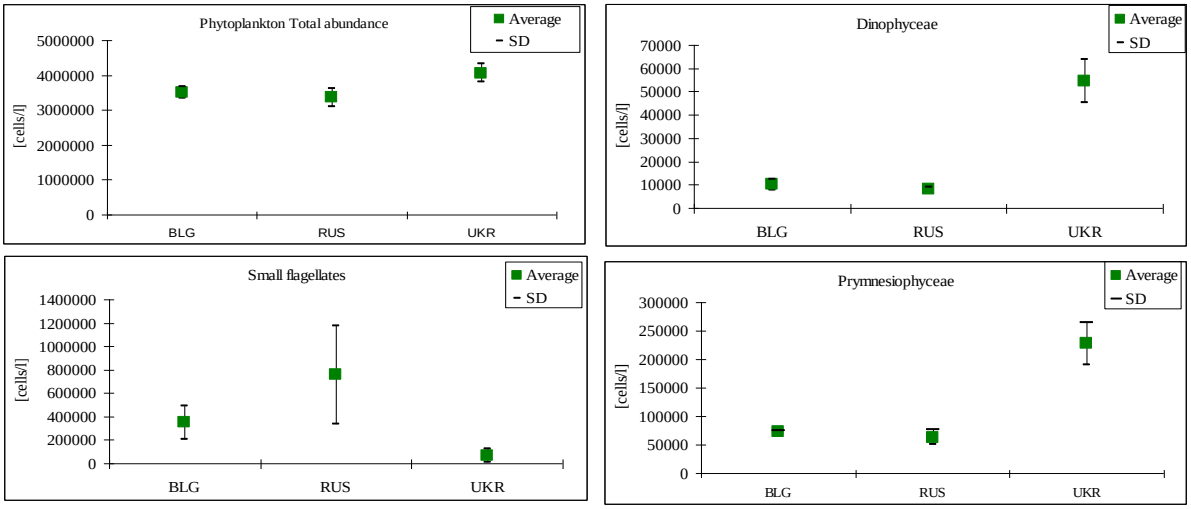

Figure 1. Stock plot of total phytoplankton and selected taxonomic classes abundance [cells/L - average and standard deviation (SD)]; BLG - Bulgaria, RUS – Russia, UKR – Ukraine.

Table 4. Statistical summary of ANOVA and Tukey's test

Analysis of variance: Phytoplankton total abundance (cells/L)					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	0.028	0.014	31.233	0.004
Error	4	0.002	0.000		
Corr- total	6	0.030			
Tukey (HSD) at confidence 95%					
Countries	Difference	Standardized difference	Critical value	Pr > Diff	Significant
UKR / RUS	0.151	7.748	3.564	0.003	Yes
UKR / BLG	0.118	5.548	3.564	0.011	Yes
BLG / RUS	0.032	1.671	3.564	0.321	No
Tukey's d critical value: total abundance			5.04		
UKR /RUS	46692.000	10.636	3.564	0.001	Yes
UKR /BLG	44581.865	9.271	3.564	0.002	Yes
BLG /RUS	2110.135	0.481	3.564	0.884	No
Tukey's d critical value: Dinophyceae			5.040		
BLG /UKR	1.417	5.530	3.564	0.011	Yes
BLG /RUS	0.199	0.852	3.564	0.695	No
RUS /UKR	1.217	5.206	3.564	0.014	Yes
Tukey's d critical value: microflagellates			5.04		
UKR / RUS	0.762	7.987	3.564	0.003	Yes
UKR /BLG	0.639	6.113	3.564	0.008	Yes
BLG / RUS	0.123	1.291	3.564	0.471	No
Tukey's d critical value: Prymnesiophyceae			5.04		

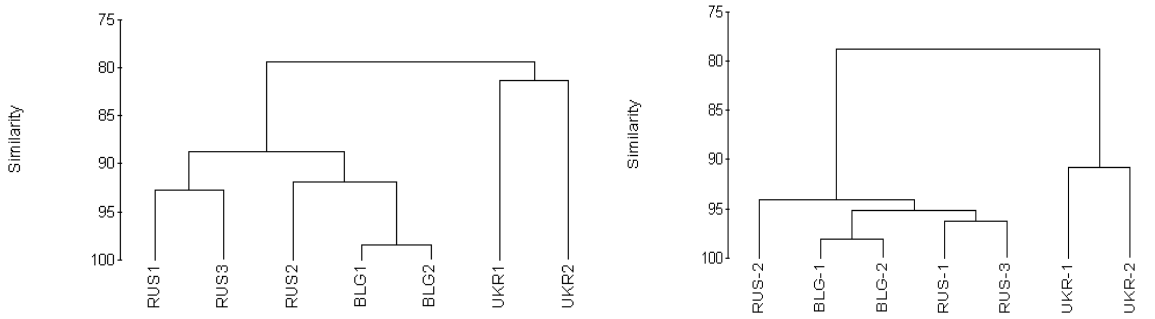

Figure 2. Similarity cluster matrix of total phytoplankton abundance (cells/L) and biomass (mg/m^3) (square root transformed data); 1, 2, 3- sample replicates

Table 5. Average taxonomic classes abundance (cells/L) and biomass (mg/m^3) and CV (%) by partners

	Phytoplankton Abundance (cells/L)– CV%					Phytoplankton Biomass (mg/m^3) – CV%				
LAB	Total N	Bac	Din	Prymn	microfl	Total B	Bac	Din	Prymn	microfl
BLG	4.8	7	23.6	11.0	40.9	19.2	7.5	13.9	8.5	40.9
RUS	5.4	11.3	15.0	22.6	55.9	4.5	25.9	4.5	28.0	37.1
UKR	3.5	7.8	16.8	15.7	84.9	0.2	3.3	32.0	27.8	84.7
All	17.7	20.0	93.1	45.5	117.4	19.5	22.0	54.2	101.6	114.7

Table 6. Common phytoplankton species biovolume (μm^3) used by the different participants

Taxon/species	RUS	BLG	RO	UKR	Average	SD	CV%
Bacillariophyceae							
Cerataulina pelagica	25000	6138	6496	7691	11331	9137	81
Chaetoceros socialis		76	500	115	230	234	102
Chaetoceros curvisetus	2600	2800	5000	2478	3220	1194	37
Nitzschia tenuirostris		205	101	63	123	74	60
Proboscia alata (RH.alata)	35000	52000	12000	10351	27338	19923	73
Pseudonitzschia delicatissima	280	220	202	409	278	94	34
Skeletonema costatum		76	300	247	208	117	56
Thalassionema nitzschioides	60	320	572	294	312	209	67
Dinophyceae							
Ceratium fusus	101690	23770	77000		67487	39822	59
Gyrodinium fusiforme	1600	17479	14474	62393	23986	26514	111
Heterocapsa triquetra	5000	4658	3040	7432	5032	1814	36
Prorocentrum compressum	15300	10689		19008	14999	4168	28
Prorocentrum micans	13091	13500	8570	12600	11940	2277	19
Protoperidinium bipes	4200	2110	6000	2304	3654	1826	50
Protoperidinium granii	22450	35000	30000	17760	26303	7684	29
Scrippsiella trochoidea	5237	5859			5548	440	8
Prymnesiophyceae							
Emiliania huxleyi	180	200	145	268	198	52	26
Small flagellates	134	260	310	65	192	113	59
Total No of species counted in the sample	57	59	39	31			

Table 7. Chlorophyll *a* (μg/L) data statistical summary

Station	BLG	RO	RUS
1	7.53	8.08	7.53
1	7.13	7.85	6.11
1	7.97	10.19	7.92
Average	7.54	8.70	7.19
SD	0.42	1.29	0.95
CV%	5.6	14.8	13.3
2	0.61	0.68	0.46
2	0.61	0.72	0.59
2	0.61	0.68	0.38
Average	0.61	0.69	0.48
SD	0.00	0.02	0.15
CV%	0.00	3.06	30.41
3	6.33	6.24	4.18
3	6.32	6.29	3.82
3	6.86	6.84	4.71
Average	6.50	6.46	4.24
SD	0.31	0.33	0.45
CV%	4.75	5.16	10.50

tests and intercalibration exercises to measure comparability/uncertainty of data. Uncertainty of a final result encompasses the uncertainties of the whole measurement process (sampling, sub-sampling, homogeneity, identification, quantification etc.). In biological methods it should be taken into consideration that uncertainties are sometimes qualitative in nature (misidentification) and difficult to combine with other uncertainties into a final one. Often the absolute statistical limits are difficult to

assess, particularly when no standards or other reference methods exist. Obviously sophisticated statistical methods are not applicable to phytoplankton data, mainly due to the lower precision of microscopic analysis (HELCOM, 2003). Yet statistically valid targets for cell counts are still a major subject of standardization (European Commission, 2006). A 20% difference between the replicates analyzed by one and the same counter is considered acceptable measure of intra - laboratory reproducibility fitting also the null hypothesis for the differences among the participating labs (Rott *et al*., 2007), in other cases a confidence level within the average ± stdev, or a CV <20% was recommended (Lacouture, R., 2001) or observations that were outside the 90% confidence limit, were interpreted as outliers (Vuorio *et al*., 2010). In any case as suggested by Vuorio *et al*. (2007) the mean value obtained in inter-laboratory studies organized among proficient laboratories could be adopted as practical limits.

Conclusions

The results reported here reveal that for the total phytoplankton abundance the results between Bulgaria, Russia and Romania could be considered comparable at CV <20% while with Ukrainian lab at CV between 25-30%. For the total phytoplankton biomass there is a good agreement between Romania and Ukraine, about 20% difference between Bulgaria and all other labs and a 30% difference between Russia, Romania and Ukraine that should be taken into consideration if the data should be combined in a single data set. At the level of taxonomic classes the differences were substantial especially for Prymnesiophyceae and Small flagellates, e.g. these data should be treated with caution.

The result underline the importance of counting chamber and sub-sample volume accounting for the degree of species detection and the unification of species specific biovolume estimation for achieving comparable biomass results.

Furthermore a good knowledge of phytoplankton taxonomy is essential in order to correctly identify species; therefore in addition to the technical performance of phytoplankton enumeration ring-test for species identification would also prove necessary.

As a follow-up under FP7 Project UP-Grade Black Sea SCENE a taxonomically up-dated Black Sea phytoplankton species check - list with agreed geometric formulas has been developed and an automated system for biovolume calculation and phytoplankton data-base is in progress http://phyto.bss.ibss.org.ua/wiki/List_checked. A QC/QA guidelines were produced (Moncheva, 2010) that altogether offer opportunities for progress towards increasing the accuracy of phytoplankton measurements in the Black Sea region.

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