Intercalibration Report
Phytoplankton

Black Sea
monitoring harmonization process

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I. SCOPE

The quality of biological data has gained recognition as an essential part of 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 as a BQE (WFD) and key biological component in MSFD has a key role 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.

In line with one of the main objectives of MISIS Project “Carrying out ecological assessment of the Black Sea, taking into consideration the requirements in the WFD and the descriptors of the MSFD the task “Organizing inter-comparison exercises to evaluate the performance of laboratories involved” is considered a critical step in producing harmonized data sets.

The aim of this report is to assess the comparability of phytoplankton data produced by the partners in MISIS Project – IO-BAS (Bulgaria), NIMRD (Romania) and SUFF (Turkey) in order to be able to construct a common data set as a bases for application of unified phytoplankton related indicators for assessment of NW Black Sea environmental status in a harmonized way.

II. SAMPLING DESIGN

Two sampling stations were selected for the intercalibration exercise - an open sea station (13) and a coastal station (18) - Fig.1 (For more details see MISIS Joint Cruise Report, 22-31st Jult 2013).

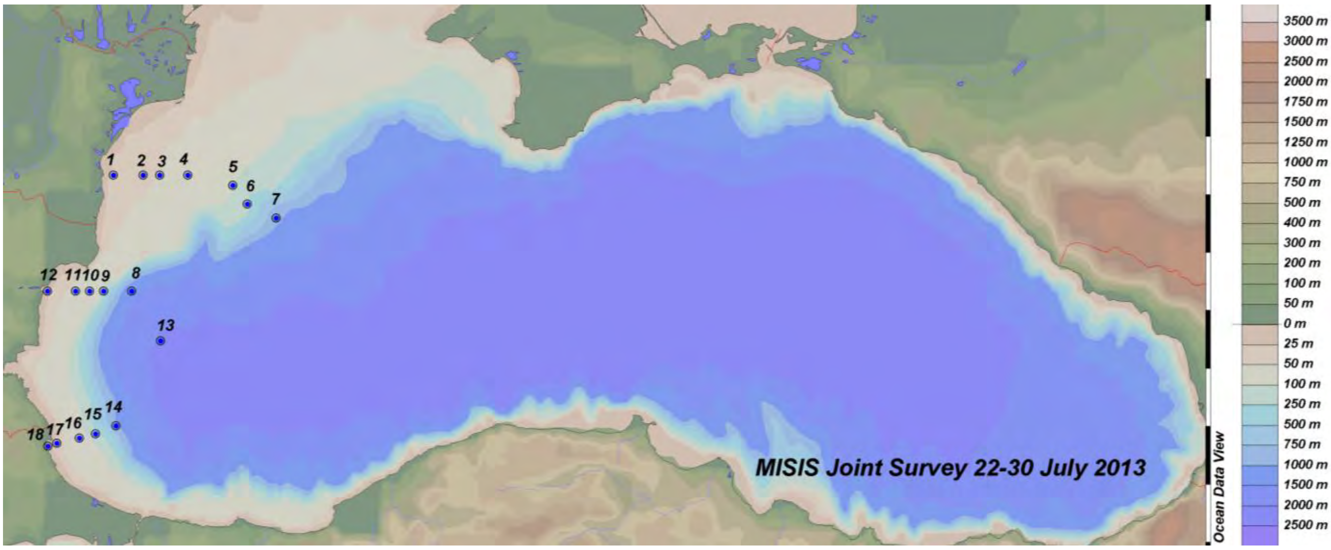


Figure 1. Map of MISIS cruise stations – intercalibration stations: st. 13 (Lat 42.74 N, Long 29.34 E, depth 2015.5m and st. 18 (Lat 41. 84 N Long. 28.30 E, depth 27m)

Samples preparation and lab methods

Samples were collected from the chlorophyll a max depth (43m at st.13 and 15 m at st.18) by 5L Teflon Niskin bottles attached to CTD - SBE 911 - Rosette System equipped with in situ fluorometer (Chelsea Minitraca). 1l seawater samples in three replicates were collected in plastic bottles for each Lab following a scheme assuring a max homogeneity of the samples distributed among partners. The samples were fixed in 4% formaldehyde solution, buffered to pH 8-8.2 with disodiumtetraborate by a single participant. In addition from st. 13 another 3 replicates per partner from were fixed in Lugol following the same sampling scheme. In total 27 samples were used for the intercomparison exercise.

The details of the in-house procedures for phytoplankton lab analysis of the participant laboratories and their codes used in the results are presented on Table1. The individual cell biovolume (V, µm3) was derived by measurements through the approximation of the cell shape of each species to the most similar regular solid, calculated by the respective formulas used routinely in the respective lab. The average of at least 10 measurements per species was agreed to be used for the biovolume calculation. Cell bio-volume was converted to weight (W, ng) following Hatchinson (1967).

Table 1. Inventory of in –house routines of phytoplankton lab analysis

Laboratory Code	Sample concentration	Microscope type	Counting chamber	Volume of subsample	Magnification	Counting area of chamber analyzed
SUFF-TR **Code 1**	Decantation Ütermol	Inverted epiflourescence attachment	Sedgwick Rafter, Ütermol	0.1 ml	20X 40X	Entire chamber
NIMRD-RO **Code 2**	Decantation Ütermol	Olympus Inverted Image analysis	Ütermol	0.1 ml/1ml	20X 40X	Entire chamber
IO-BAS- BG **Code 3**	Decantation Ütermol	Nikon inverted image analysis	Sedgwick Rafter, Ütermol	1ml	40X	At least 400 cells

III. STATISTICAL ANALYSIS

The phytoplankton attributes subject to intercomparison were:

- Phytoplankton total abundance [cells/] and biomass [mg/m^3]
- Phytoplankton abundance [cells/l] and biomass [mg/m^3] by classess
- Phytoplankton total abundance [cells/l] and biomass [mg/m^3] depending on the fixation: Formalin (F) and Lugol (L)
- Species biovolume [µm^3] and the related geometric shapes
- Taxonomic identification (species lists)

Several statistical treatments were applied to the data.

A. Statistical evaluation based on the z-score according to "The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (IUPAC Technical Report) (IUPAC, 2006), ISO 13528 (2005) with a standard uncertainty following the approach applied for phytoplankton proficiency test in the Baltic (Reports of the Finnish Environment Institute 5, 2010).

The z-score is a measure of the performance of the laboratory against established criteria based on fitness for a common purpose while compliance with these criteria is judged on the basis of the deviation of measurement results from "assigned" values. Than the laboratories are assessed by the difference between their result and the assigned value. A performance score is calculated for each laboratory, using the Z-score based on a fitness-for-purpose criterion.

Z scores calculation

For the selected phytoplankton attributes (abundance and biomass), a participant's result X is converted into a Z-score according to the equation

$Z= (X – Xa)/\sigma p$

where Xa is the "assigned" value, and σp is the fitness-for-purpose-based "standard deviation for proficiency assessment", that underline the importance of assigning a range appropriate to a particular purpose (ISO Guide 43; Statistical Guide ISO 13528).

In the equation the term (X – Xa) is the error in the measurement. The parameter σp describes the standard uncertainty that is most appropriate for the application area of the results of the analysis, assumed as "fitness-for-purpose". Measurement uncertainty can be thought of as the sum of the intra-laboratory reproducibility and the trueness. Trueness is difficult to assess as the true value in the case of counting is actually always unknown.

Uncertainty (u) of the assigned values was evaluated as follows: $u = 1.25*srob/\sqrt{n}$, in which srob = robust standard deviation calculated using Algorithm A (ISO 13528) and n = number of results. Robust standard deviation (srob) is calculated as median of absolute deviation of median

(MAD) multiplied by 1.483. or divided by 0.6745. The MAD (Hoaglin et al., 2000) is a robust measure of the spread of the data, and is used as an estimate of the sample standard deviation if scaled by a factor of 1.483, a correction factor to make the estimator consistent with the usual parameter of a normal distribution. If the MAD value is scaled by a factor of 1.483 it becomes comparable with a standard deviation, this is the MADE value. Criterion for the reliability of the assigned values was $u \leq 0.3\ \sigma p$. If $u \leq 0{,}3\sigma$, then the standard uncertainty of the assigned value is negligible and need not be included in the interpretation of the results of the proficiency test. The criterion, srob < 1.2*sp, was also tested and presented.

The uncertainty that is fit for purpose in a measurement result depends on the application. As described in the IUPAC guidelines, the choice of σ is dependent upon the data quality objective of a particular program. The most common approach is to specify the criterion as a relative standard deviation (RSD). Specific σp values are then obtained by multiplying the selected RSD by the assigned value.

Definition of assigned value

According to the IUPAC's technical report, an assigned value is an estimate of the value of the measured that is used for the purpose of calculating scores. From the suggested methods for its determination in the technical report the only applicable for the phytoplankton test is the "consensus value" that is, a value derived directly from reported results. The consensus of the participants is currently the most widely used method for determining the assigned value. The idea of consensus is not that all of the participants agree within bounds determined by the repeatability precision, but that the results produced by the majority are unbiased and their dispersion has a readily identifiable mode.

For the establishment of the assigned consensus value we followed the next steps:

- Visualize the data
- Calculate mean and 90% confidence limit.
- Observations outside the 90% confidence limit were interpreted as outliers.
- Exclude the values outside the 90% confidence limit
- Recalculate the mean which is assumed to be the assigned consensus value
- Test the uncertainty criterion for the assigned consensus value

For this test σp- fitness-for-purpose-based "standard deviation for proficiency assessment" was obtained by multiplying the selected RSD by the assigned consensus value.

Interpretation of the z-scores

According to IUPAC, the interpretation of z-scores uses an assumed model based on the scheme provider's fitness-for-purpose criterion, which is represented by the standard deviation for proficiency assessment σp:

– A score of zero implies a perfect result. This will happen rarely even in the most competent laboratories.
– Z-scores fall between –2 and +2. The sign (i.e., – or +) of the score indicates a negative or positive error respectively. Scores in this range are commonly designated "acceptable" or "satisfactory".
– Scores in the ranges –2 to –3 and 2 to 3 are designated as "questionable".
– A score outside the range from –3 to 3 indicate that the cause of the event should be investigated and remedied. Scores in this class are commonly designated "unacceptable" or "unsatisfactory".

B. MANOVA tests were conducted to investigate the effects of independent variables across dependent variables using IBM SPSS Statistics. In MANOVA, a new dependant variable that maximizes group differences is created from the set of dependant variables. The new dependant variable is a linear combination of measured by dependant variables, combined so as to separate the groups as much as possible (Tabachnick & Fidell, 2007). MANOVA could be used to examine all of the dependant variables at the same time. Additionally, MANOVA controls Type 1 error (the probability of rejecting the null hypothesis when it is true) across all of the dependant variables in the model.

Unlike conducting multiple ANOVAs, MANOVA accounts for the co-variances of the other dependent variables, which might increase statistical power.

The main objective in using MANOVA was to determine if the response variables e.g. phytoplankton abundance & biomass (total and by classes), are altered by the manipulation of the independent variables, e.g. Laboratory/ Replicates and the type of fixation (Formalin /Lugol).

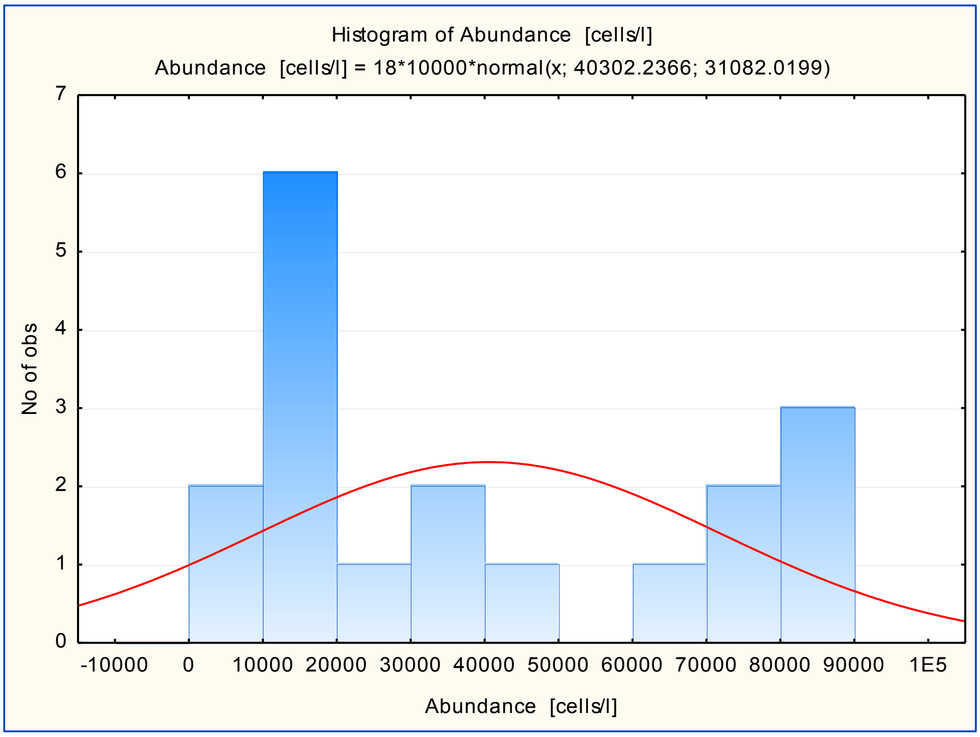
C. Similarity percentage - SIMPER, (PRIMER, 2006). This analysis breaks down the contribution of each species to the observed similarity (or dissimilarity) between samples and allows to identify the species that are most important in creating the observed pattern of similarity. The method uses the Bray-Curtis measure of similarity, comparing in turn, each sample by pair of laboratories (each sample in Lab 1 with each sample in Lab 2). The Bray-Curtis method operates at the species level and therefore the mean similarity between Lab 1 & Lab 2 can be obtained for each species. The analysis was applied for the comparison of the species biovolumes used by the participating laboratories.

IV. RESULTS

The raw data and the results of the scoring (Z-scores) are presented on Figures 2-16 and the related statistical values are given in the corresponding Tables. All classes except Bacillariophyceae and Peridinea are treated as one group - Others.

IV.1 Phytoplankton total abundance and biomass

A)



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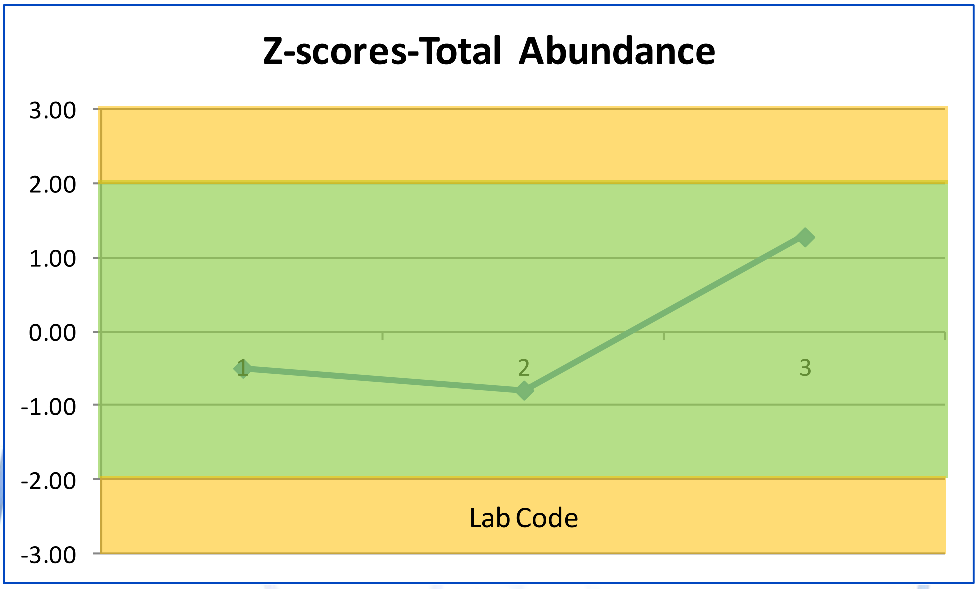
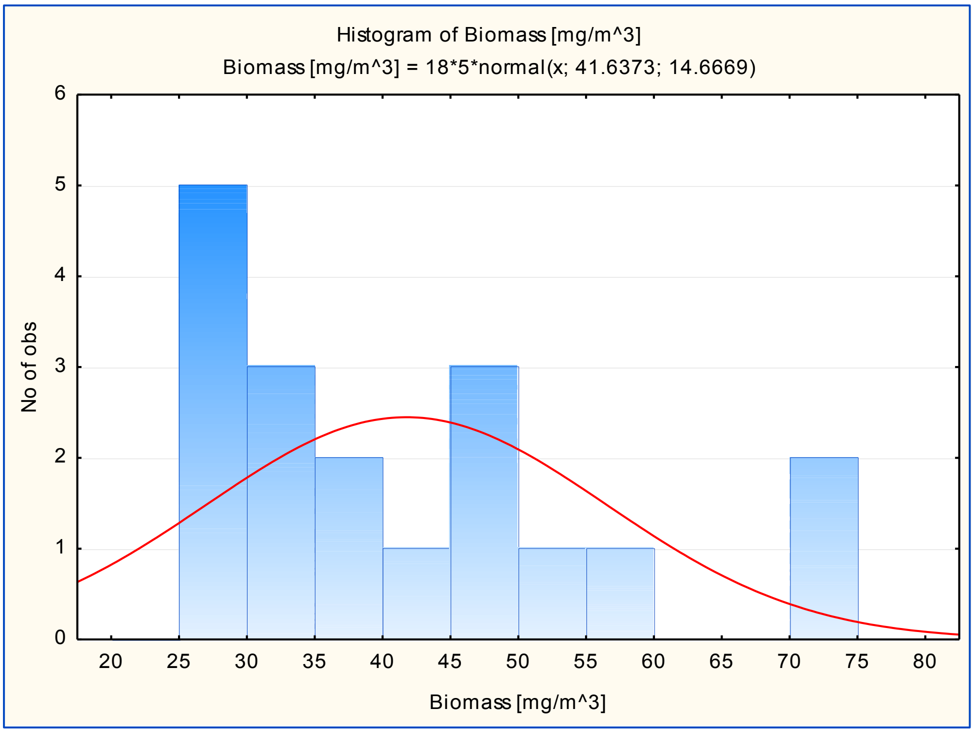


Figure 2. Histogram of raw data (A) and Z scores plot (B) of Total abundance [cells/l], st. 13.

A)



B)

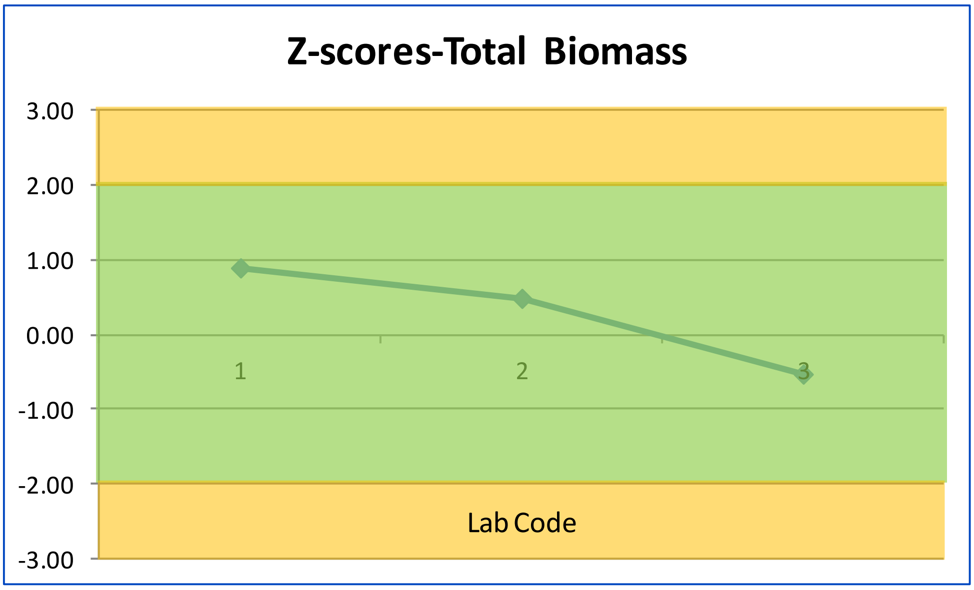
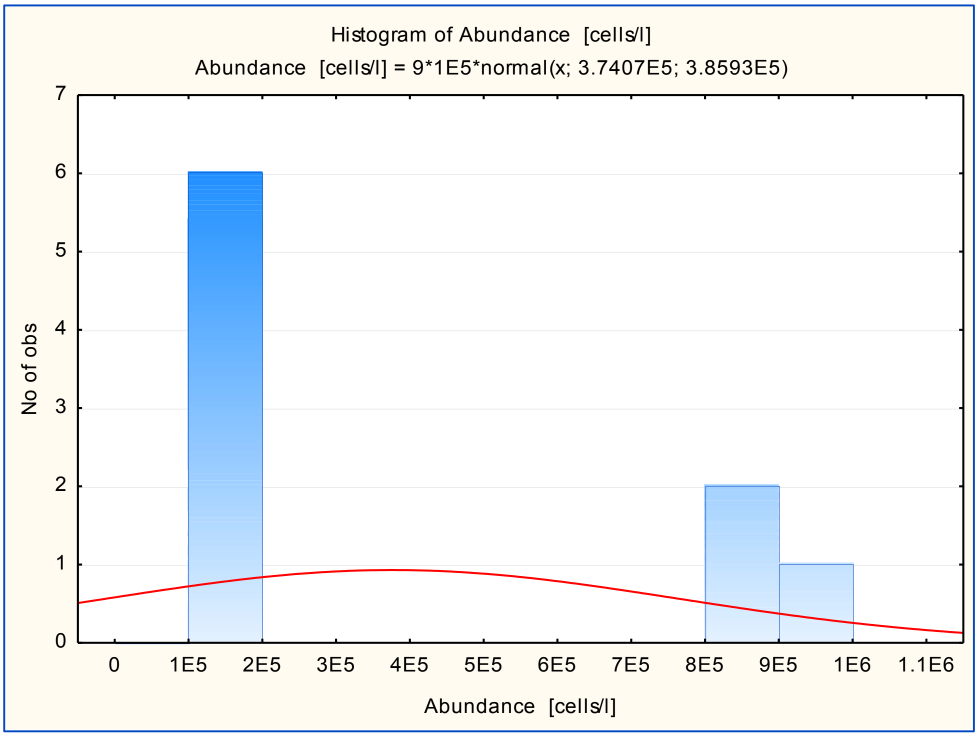


Figure 3. Histogram of raw data (A) and Z scores plot (B) of Total biomass [mg/m³], st. 13.

Station	Lab code	Z-score Abundance [cells/l]	Assigned value	RSD	σ
13	1	-0.49	38625	0.9	36572
	2	-0.79			
	3	1.28			
		Biomass [mg/m^3]			
13	1	0.9	36.6	0.3	9.6
	2	0.49			
	3	-0.53			

A)



B)

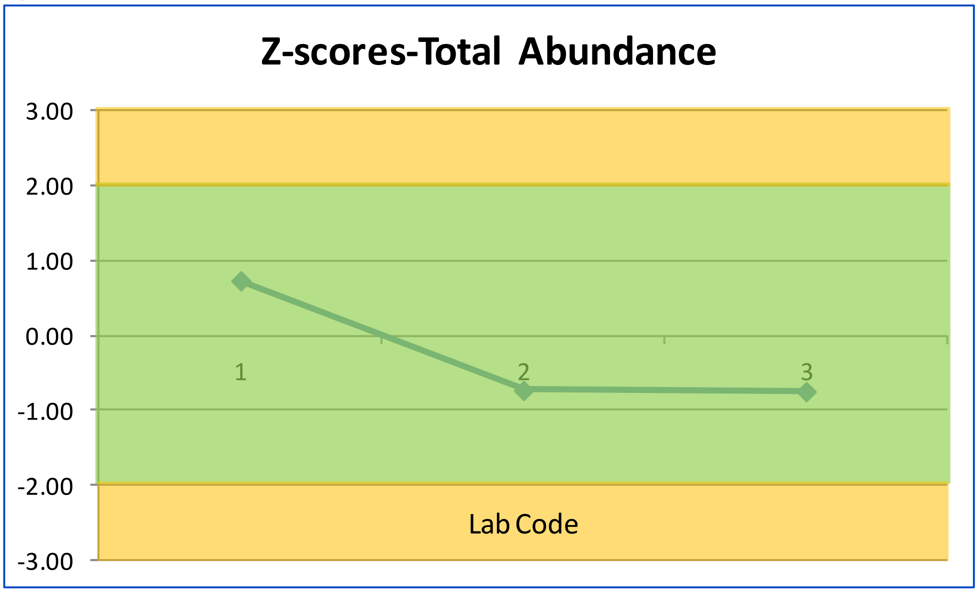
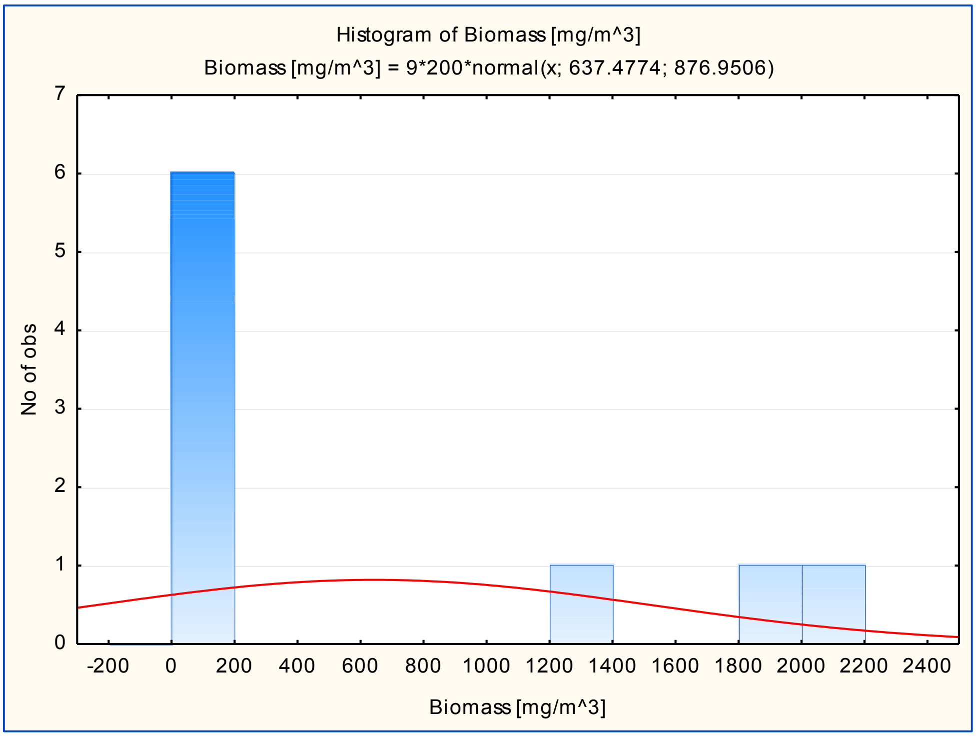


Figure 4. Histogram of raw data (A) and Z scores plot (B) of Total abundance [cells/l], st. 18.

A)



B)

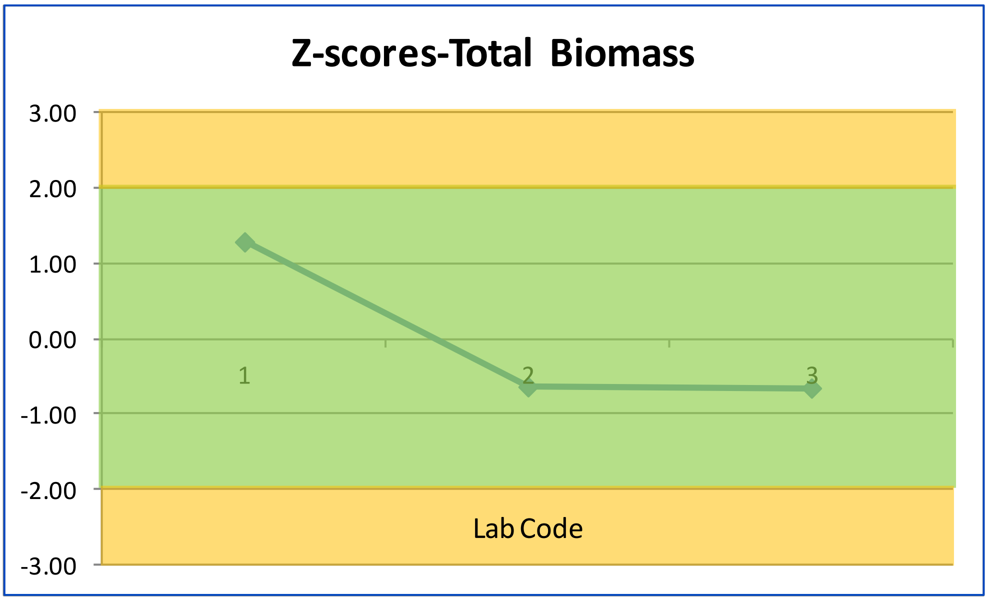
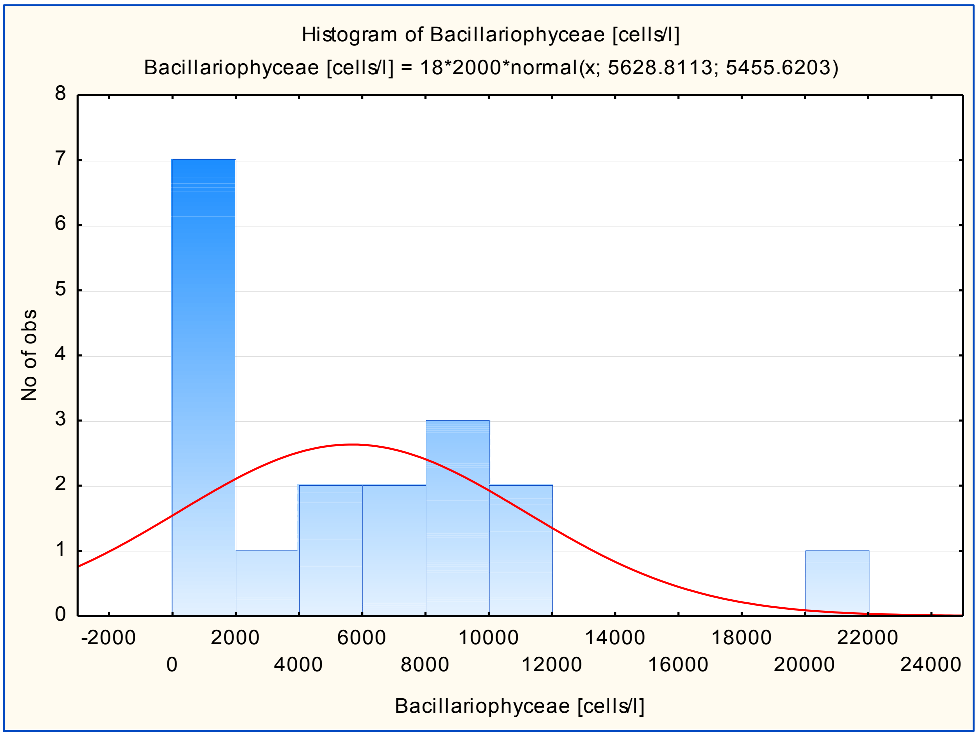


Figure 5. Histogram of raw data (A) and Z scores plot (B) of Total biomass [mg/m³], st. 18.

Station	Lab code	Z-score	Assigned value	RSD	σ
		Abundance [cells/l]			
18	1	0.73	503690	1.03	519663
	2	-0.73			
	3	-0.75			
		Biomass [mg/m^3]			
18	1	0.9	637.5	1.4	877
	2	0.49			
	3	-0.53			

IV.2 Phytoplankton abundance and biomass by taxonomic classes

A)



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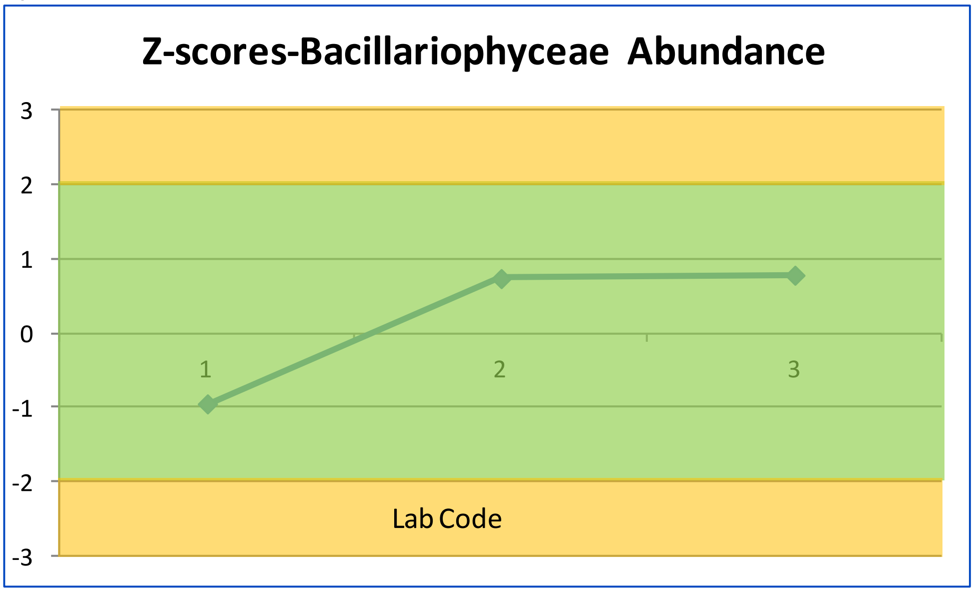
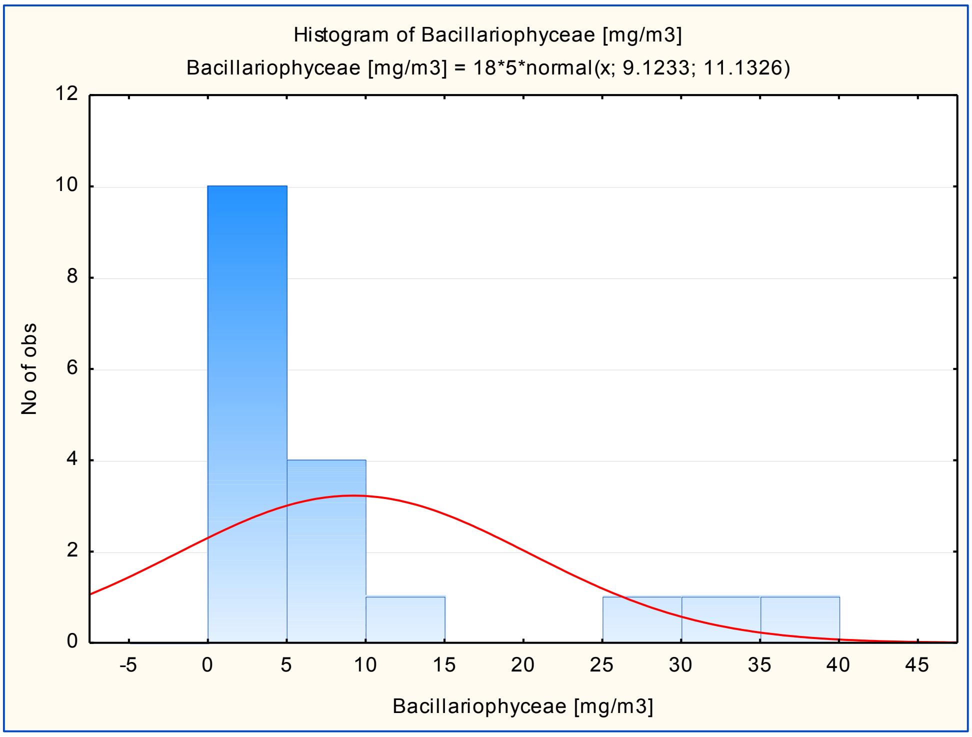


Figure 6. Histogram of raw data (A) and Z scores plot (B) of Bacillariophyceae abundance, st. 13.

A)



B)

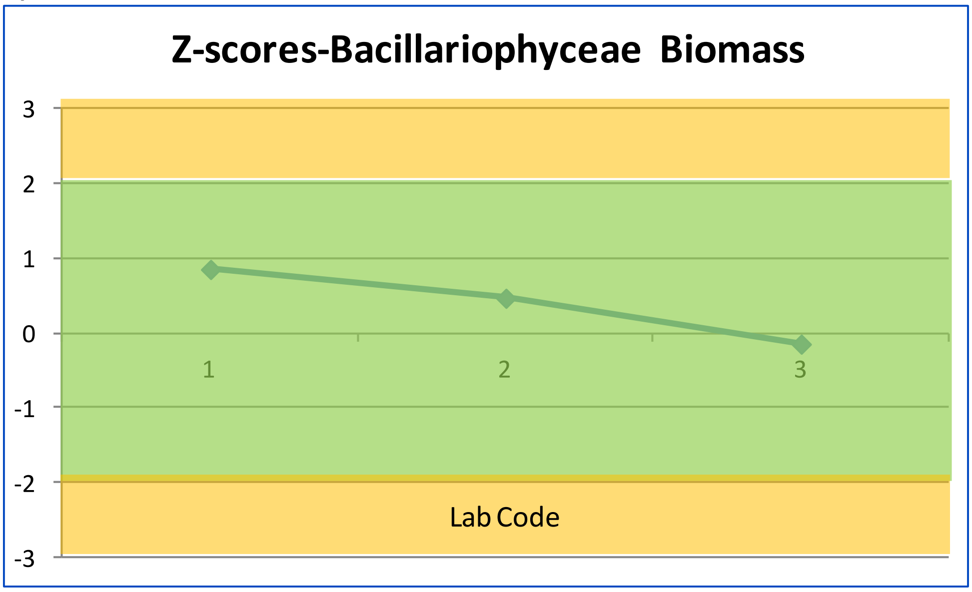
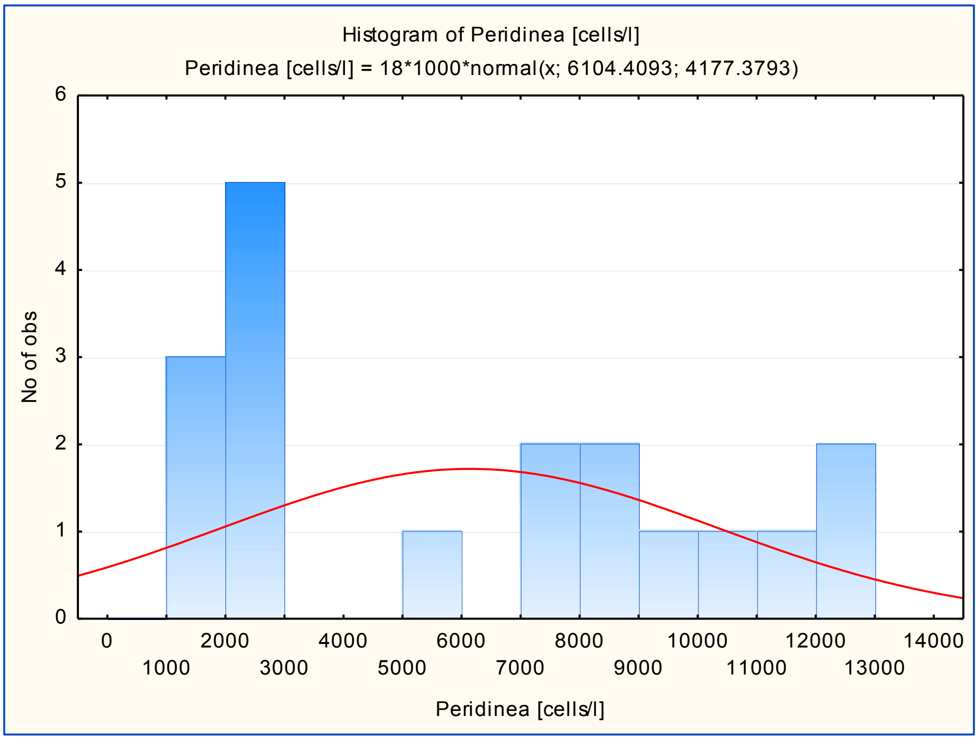


Figure 7. Histogram of raw data (A) and Z scores plot (B) of Bacillariophyceae biomass, st. 13.

Station	Lab code	Z-score Bacillariophyceae [cells/l]	Assigned value	RSD	σ
18	1	-0.95	4759	0.97	4612
	2	0.74			
	3	0.78			
		Bacillariophyceae [mg/m^3]			
18	1	0.85	6.2	1.22	7.5
	2	0.47			
	3	-0.15			

A)



B)

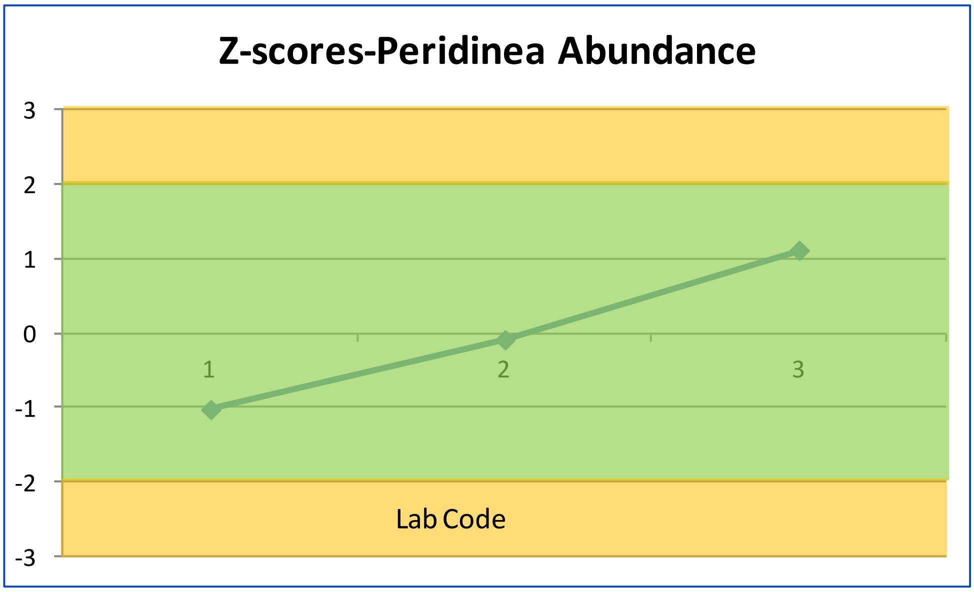
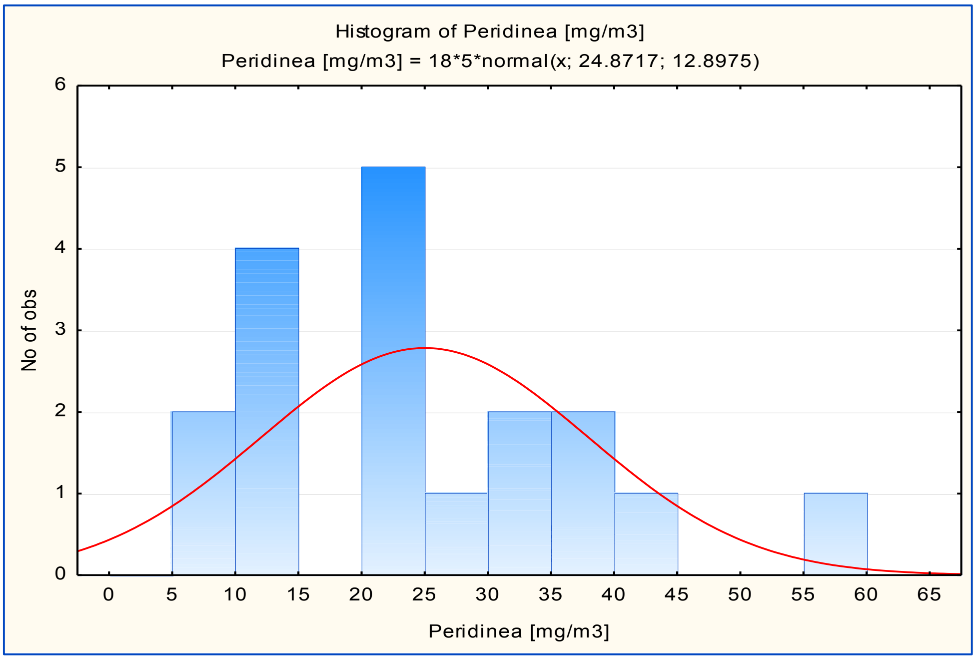


Figure 8. Histogram of raw data (A) and Z scores plot (B) of Peridinea abundance, st. 13.

A)



B)

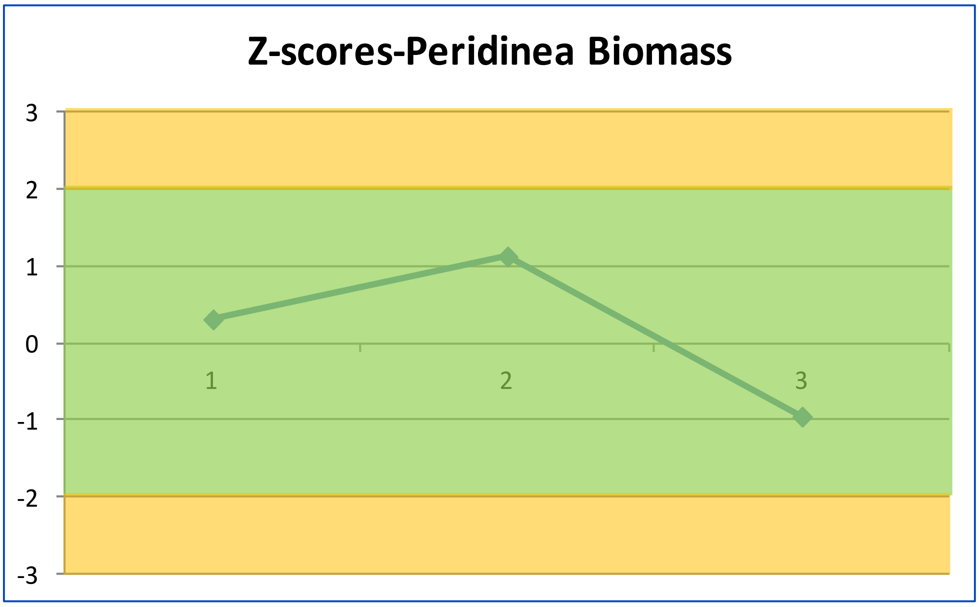
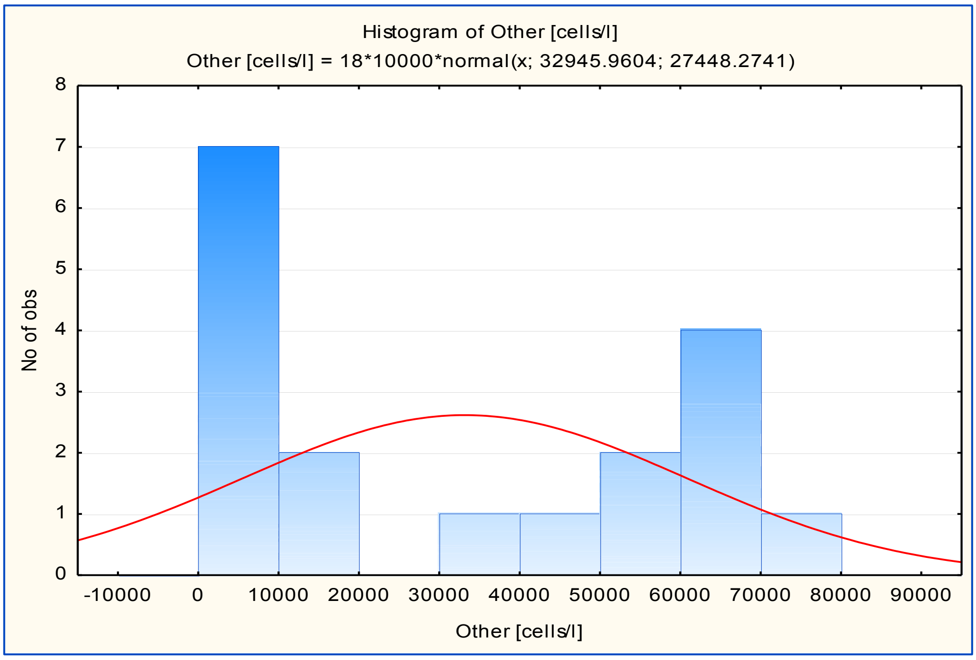


Figure 9. Histogram of raw data (A) and Z scores plot (B) of Peridinea biomass, st. 13.

Station	Lab code	Z-score	Assigned value	RSD	σ
		Peridinea [cells/l]			
18	1	-1.03	6104	0.68	4177
	2	-0.09			
	3	1.12			
		Peridinea [mg/m^3]			
18	1	0.31	22.96	0.52	11.9
	2	1.13			
	3	-0.96			

A)



B)

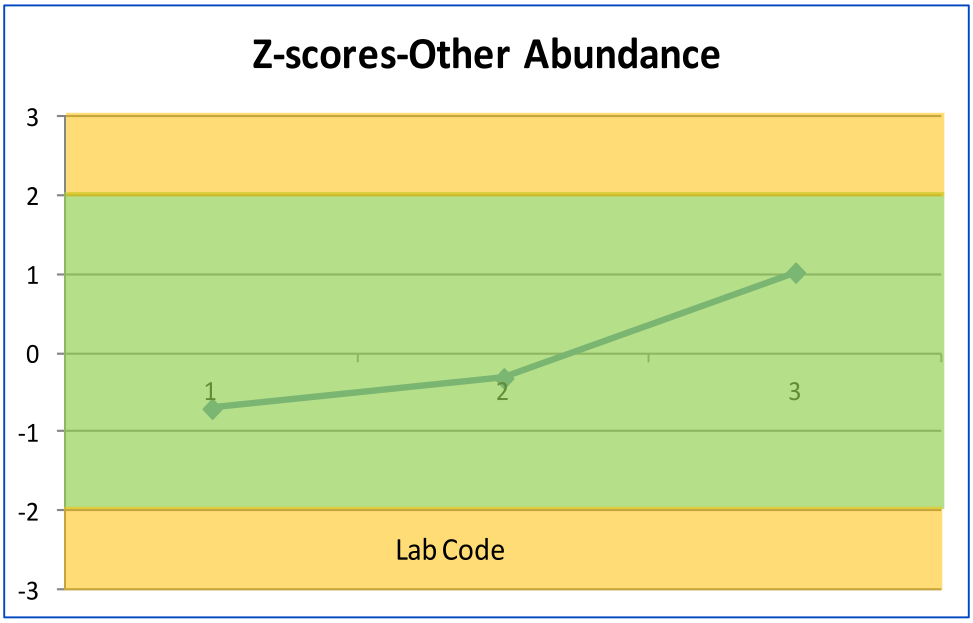
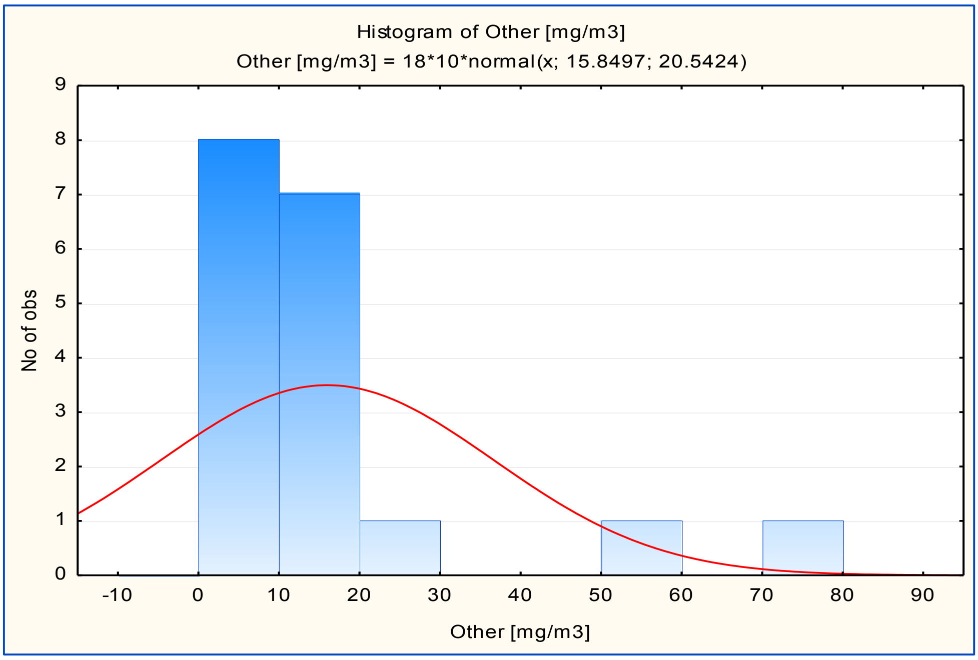


Figure 10. Histogram of raw data (A) and Z scores plot (B) of Others abundance, st. 13.

A)



B)

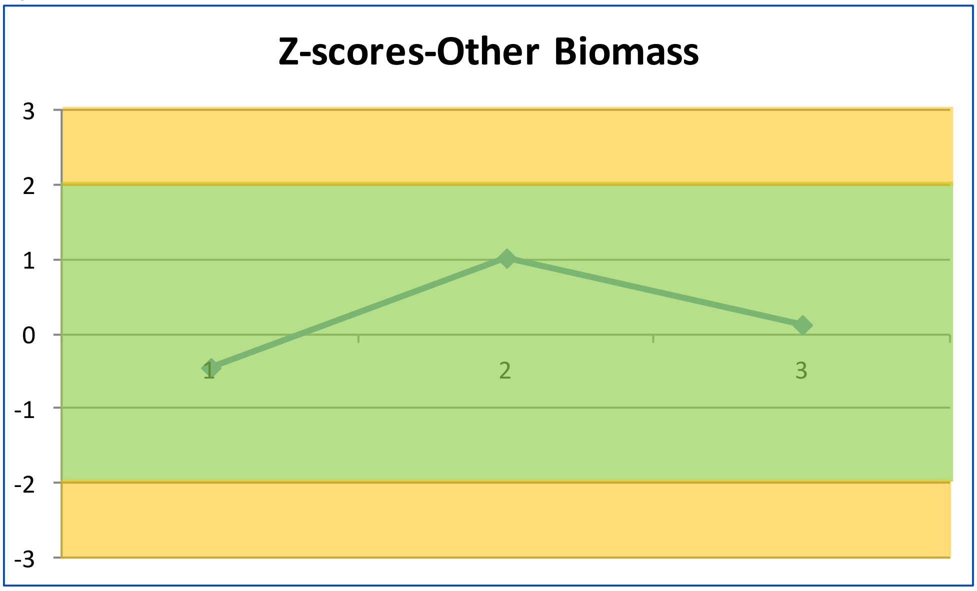
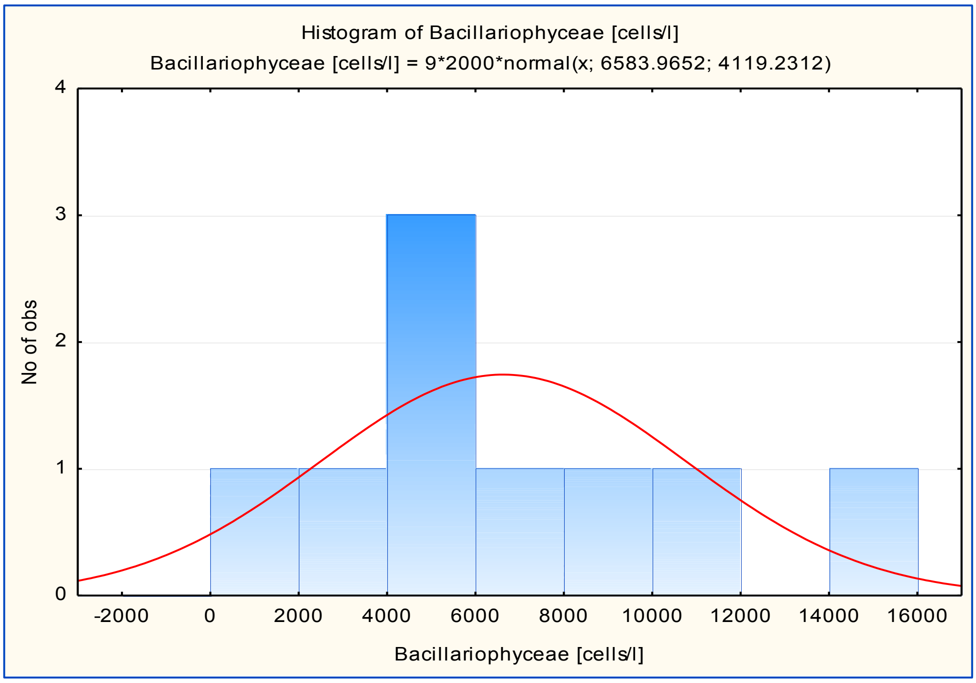


Figure 11. Histogram of raw data (A) and Z scores plot (B) of Others biomass, st. 13.

Station	Lab code	Z-score	Assigned value	RSD	σ
		Others [cells/l]			
18	1	-0.71	32946	0.83	27448
	2	-0.32			
	3	1.03			
		Peridinea [mg/m^3]			
18	1	-0.45	12.17	1.3	15.77
	2	1.02			
	3	0.13			

A)



B)

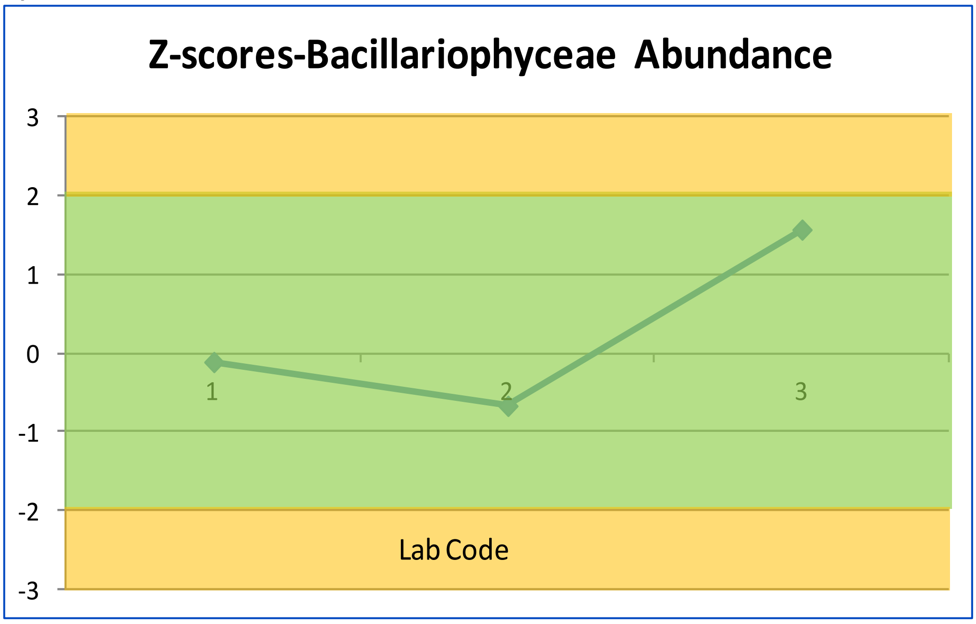
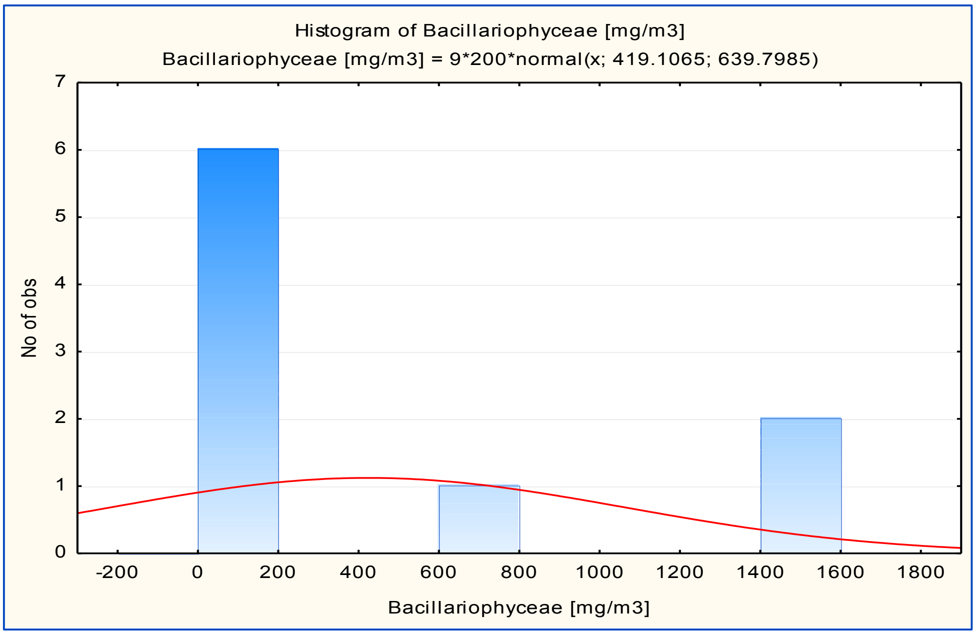


Figure 12. Histogram of raw data (A) and Z scores plot (B) of Bacillariophyceae abundance, st. 18.

A)



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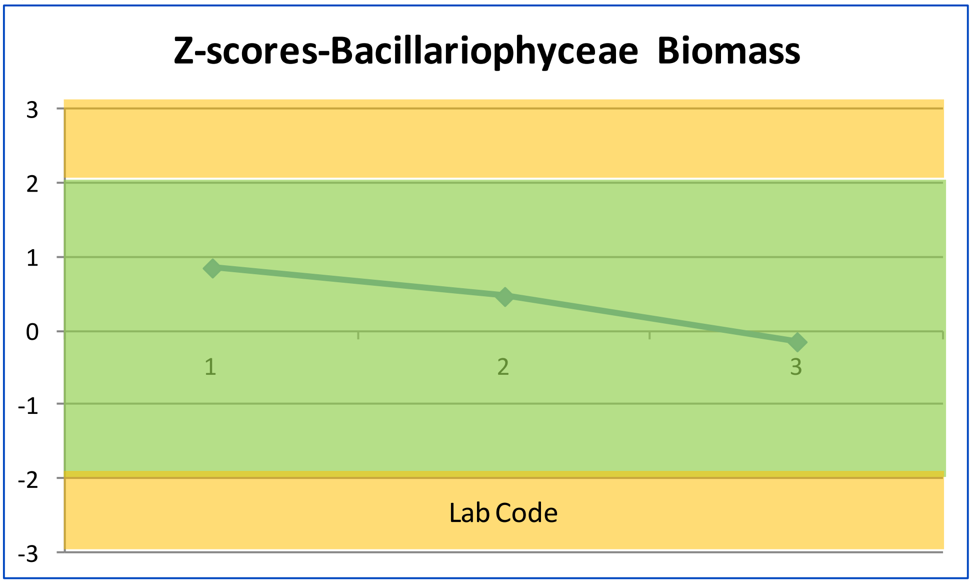
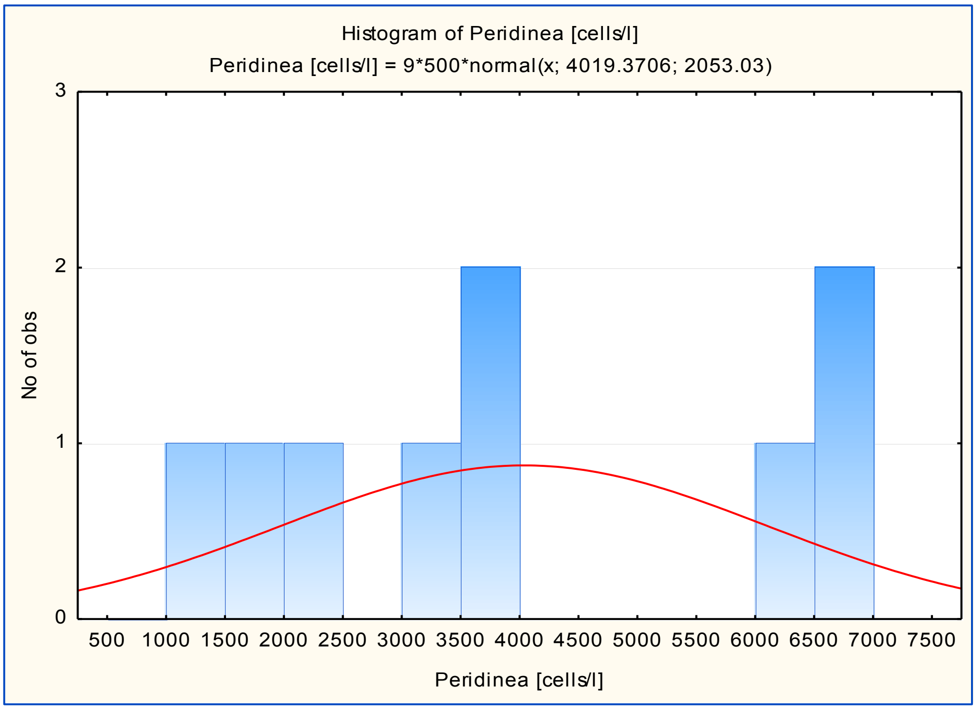


Figure 13. Histogram of raw data (A) and Z scores plot (B) of Bacillariophyceae biomass, st.18.

Station	Lab code	Z-score	Assigned value	RSD	σ
		Bacillariophyceae [cells/l			
18	1	-0.11	5650	0.63	3534
	2	-0.67			
	3	1.57			
		Peridinea [mg/m^3]			
18	1	1.26	419.11	1.53	639.8
	2	-0.63			
	3	-0.63			

A)



B)

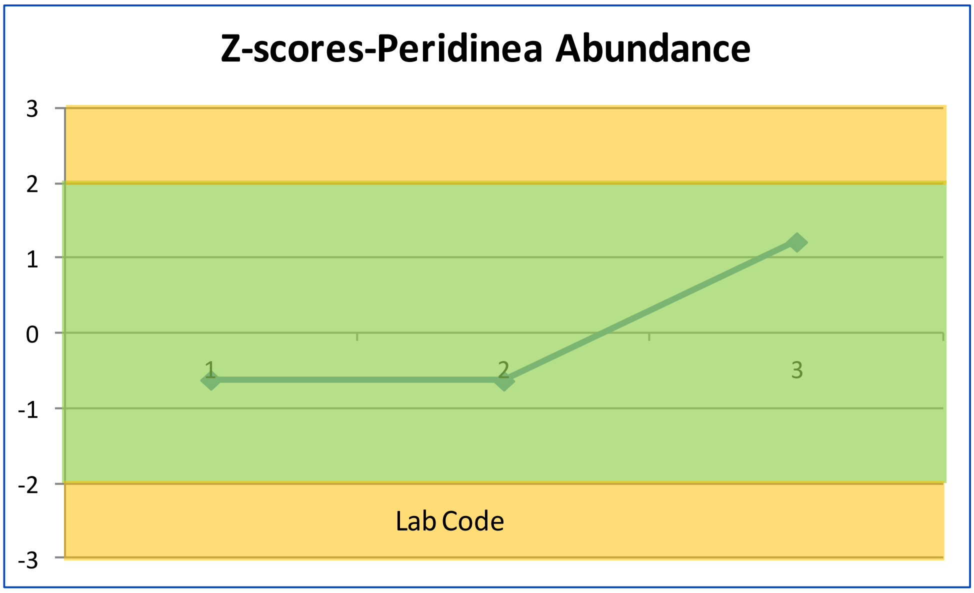
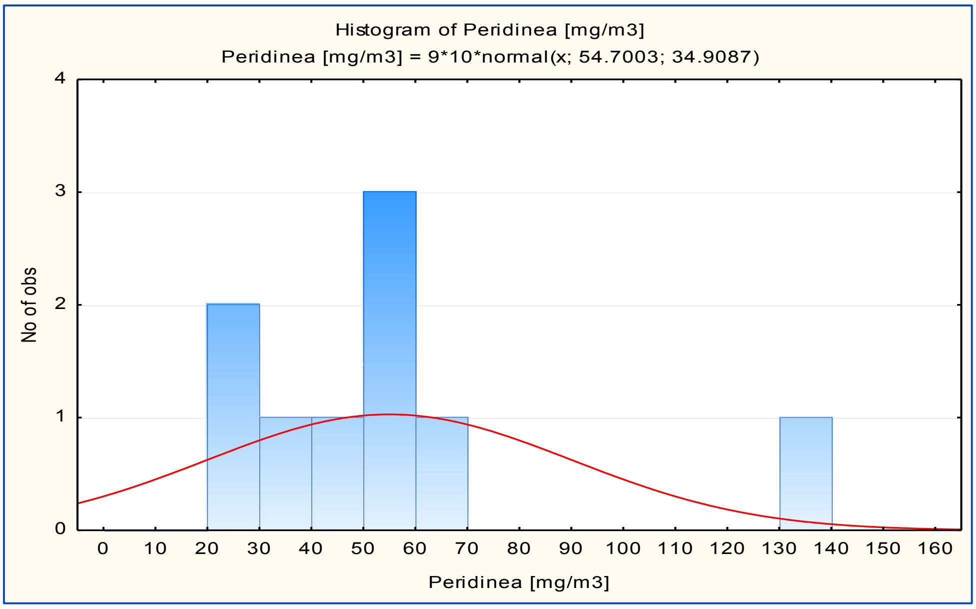


Figure 14. Histogram of raw data (A) and Z scores plot (B) of Peridinea abundance, st 18.

A)



B)

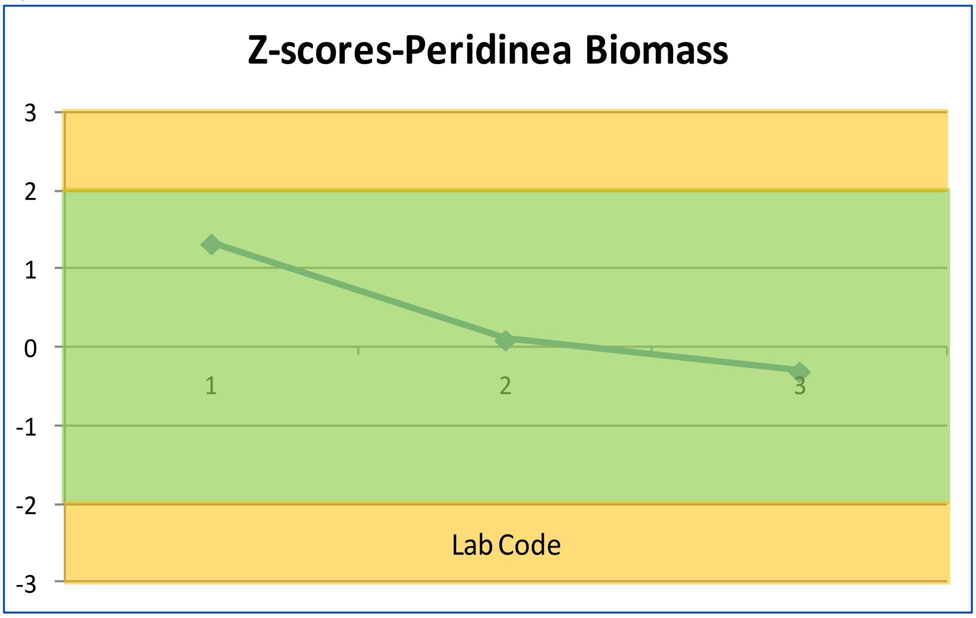
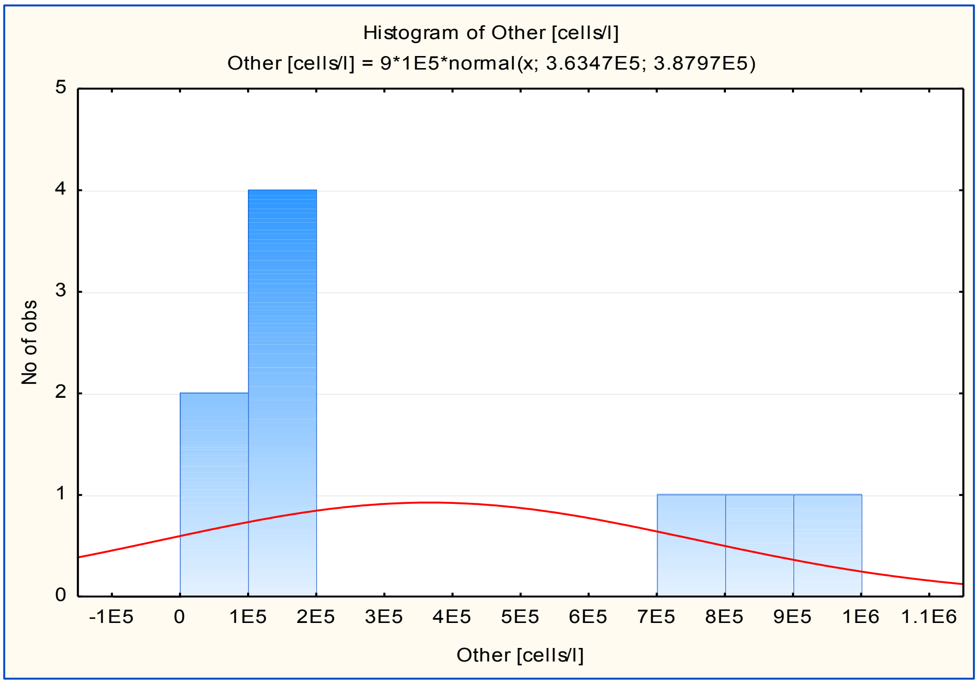


Figure 15. Histogram of raw data (A) and Z scores plot (B) of Peridinea biomass, st.18.

Station	Lab code	Z-score	Assigned value	RSD	σ
		Peridinea [cells/l]			
18	1	-0.6	395346	1.07	422001
	2	-0.6			
	3	1.2			
		Peridinea [mg/m^3]			
18	1	1.3	163.67	1.36	222.93
	2	0.1			
	3	-0.3			

A)



B)

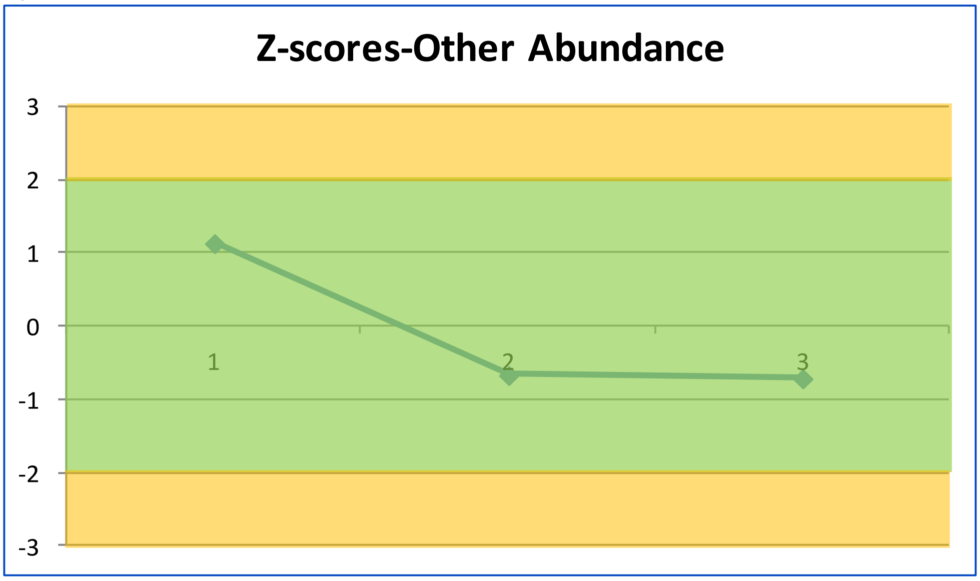
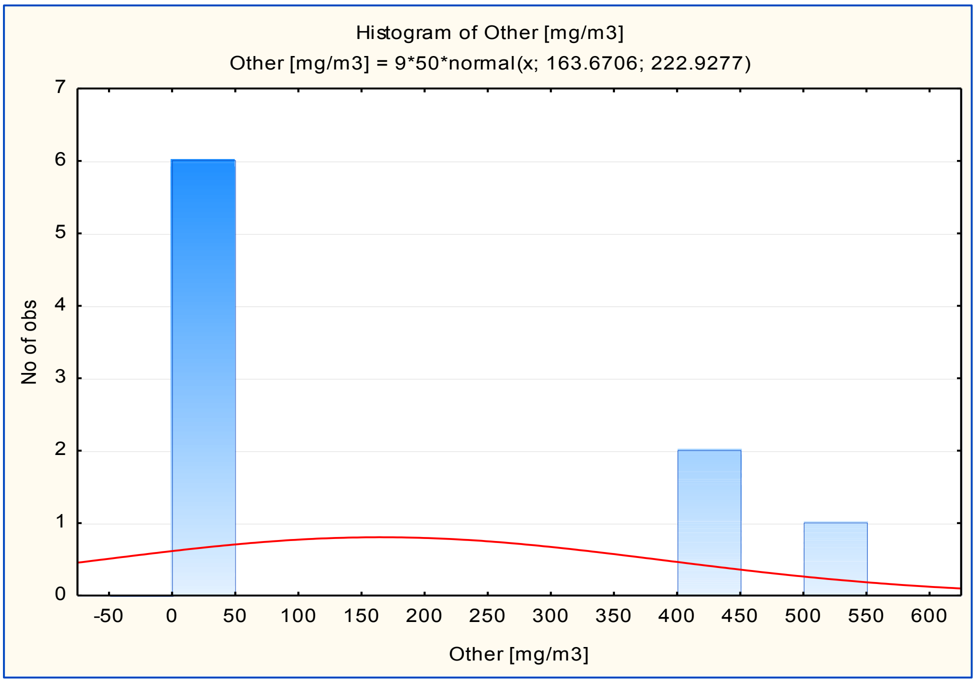


Figure 16. Histogram of raw data (A) and Z scores plot (B) of Others abundance, st.18.

A)



B)

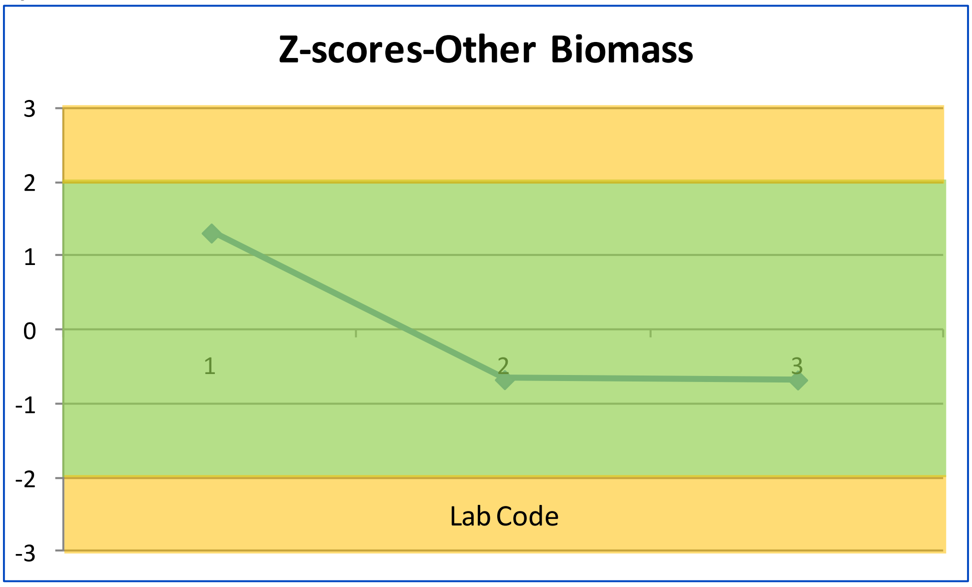


Figure 17. Histogram of raw data (A) and Z scores plot (B) of Others biomass, st.18.

B. MANOVA tests

The results of the MANOVA tests are presented on Tables

Table 2. MANOVA test results Laboratory, Replicates (RLAB) and fixation type (F-formaline, L-lugol) applied on Abundance by classes, st.13; gray shade indicates significant effect of the factor

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
FixationType	Pillai's Trace	,506	2,046[b]	3,000	6,000	,209
	Wilks' Lambda	,494	2,046[b]	3,000	6,000	,209
	Hotelling's Trace	1,023	2,046[b]	3,000	6,000	,209
	Roy's Largest Root	1,023	2,046[b]	3,000	6,000	,209
RLAB	Pillai's Trace	1,727	1,357	24,000	24,000	,230
	Wilks' Lambda	,019	2,207	24,000	18,003	,045
	Hotelling's Trace	14,924	2,902	24,000	14,000	,021
	Roy's Largest Root	12,012	12,012[c]	8,000	8,000	,001

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
FixationType	Bacillariophyceae [cells/l]	3949354	1	3949354	,165	,695
	Peridinea [cells/l]	22763613	1	22763613	5,932	,041
	Other [cells/l]	810454480	1	810454480	1,915	,204
RLAB	Bacillariophyceae [cells/l]	310498094	8	38812261	1,621	,255
	Peridinea [cells/l]	243195437	8	30399429	7,922	,004
	Other [cells/l]	8612200691	8	1076525086	2,544	,104

Table 3. MANOVA test results Laboratory, Replicates (RLAB) and fixation type (F-formaline, L-lugol) applied on Biomass by classes, st.13; gray shade indicates significant effect of the factor.

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
FixationType	Pillai's Trace	,554	2,484[b]	3,000	6,000	,158
	Wilks' Lambda	,446	2,484[b]	3,000	6,000	,158
	Hotelling's Trace	1,242	2,484[b]	3,000	6,000	,158
	Roy's Largest Root	1,242	2,484[b]	3,000	6,000	,158
RLAB	*Pillai's Trace*	*1,597*	*1,139*	*24,000*	*24,000*	*,376*
	Wilks' Lambda	*,020*	*2,150*	*24,000*	*18,003*	*,050*
	Hotelling's Trace	*22,050*	*4,287*	*24,000*	*14,000*	*,003*
	Roy's Largest Root	*20,922*	*20,922[c]*	*8,000*	*8,000*	*,000*

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
FixationType	Bacillariophyceae [mg/m3]	537,799	1	537,799	4,521	,066
	Peridinea [mg/m3]	324,034	1	324,034	8,040	,022
	Other [mg/m3]	1588,223	1	1588,223	3,777	,088
RLAB	Bacillariophyceae [mg/m3]	617,422	8	77,178	,649	,723
	Peridinea [mg/m3]	2181,403	8	272,675	6,765	,007
	Other [mg/m3]	2221,973	8	277,747	,661	,714

Table 4. MANOVA test results Laboratory, Replicates (RLAB) and fixation type (F-formaline, L-lugol) applied on Abundance by classes, st.18; gray shade indicates significant effect of the factor.

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Lab	Pillai's Trace	1,968	62,485	6,000	6,000	,000
	Wilks' Lambda	,000	72,437[b]	6,000	4,000	,000
	Hotelling's Trace	376,811	62,802	6,000	2,000	,016
	Roy's Largest Root	342,840	342,840[c]	3,000	3,000	,000
R	Pillai's Trace	1,128	1,293	6,000	6,000	,382
	Wilks' Lambda	,095	1,499[b]	6,000	4,000	,362
	Hotelling's Trace	7,206	1,201	6,000	2,000	,520
	Roy's Largest Root	6,864	6,864[c]	3,000	3,000	,074

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	
Lab	AbBacilariophiceae	102141628	2	51070814	6,315	,759
	AbPeridinea	28745872	2	14372936	20,531	,911
	AbOther	1186016661823	2	593008330911	188,353	,989
R	AbBacilariophiceae	1252568	2	626284	,077	,037
	AbPeridinea	2173379	2	1086689	1,552	,437
	AbOther	5566736790,549	2	2783368395	,884	,307

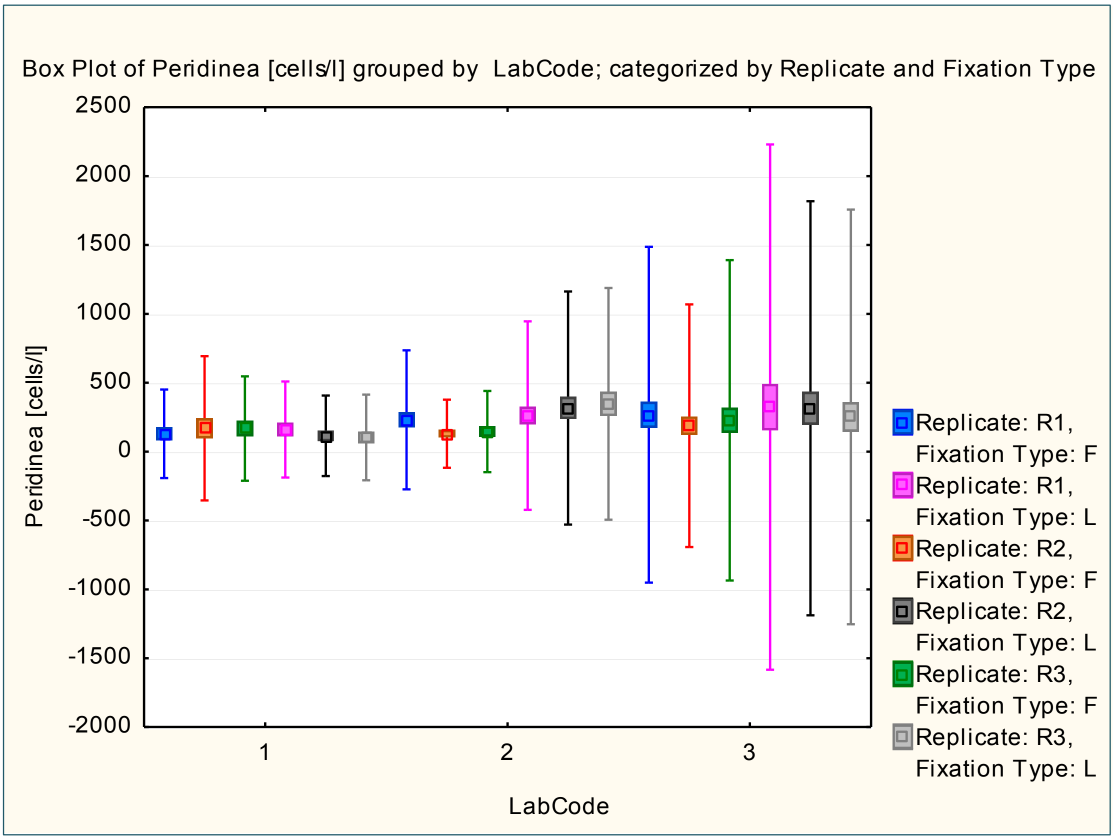
Table 5. MANOVA test results Laboratory, Replicates (RLAB) and fixation type (F-formaline, L-lugol) applied on Abundance by classes, st.18; gray shade indicates significant effect of the factor.

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Lab	Pillai's Trace	1,078	1,170	6,000	6,000	,427
	Wilks' Lambda	,001	20,937b	6,000	4,000	,005
	Hotelling's Trace	965,840	160,973	6,000	2,000	,006
	Roy's Largest Root	965,753	965,753c	3,000	3,000	,000
R	Pillai's Trace	1,197	1,491	6,000	6,000	,320
	Wilks' Lambda	,096	1,482b	6,000	4,000	,366
	Hotelling's Trace	6,343	1,057	6,000	2,000	,561
	Roy's Largest Root	5,819	5,819c	3,000	3,000	,091

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Lab	BMBacilariophiceae	2945794,149	2	1472897,074	27,301	,005
	BMPeridinea	3418,481	2	1709,240	2,943	,164
	BMOther	392630,258	2	196315,129	240,511	,000
R	BMBacilariophiceae	112881,326	2	56440,663	1,046	,431
	BMPeridinea	3981,446	2	1990,723	3,428	,136
	BMOther	1645,150	2	822,575	1,008	,442

The abundance of Bacillariophyceae and Peridinea as major classes in the phytoplankton community structure and the sum of the remaining phytoplankton classes (Other) as dependent variables was analyzed with the factors Fixation Type and combined Replicates and Laboratory (RLAB). According to MANOVA output Fixation Type and RLab have significant effect on both the abundance and biomass of all classes - Peridinea (at st. 13), as illustrated on Figs. 17 &18 and Bacillariophyceae and Others (st.18) e.g. the result from the two station did not show similar trends.



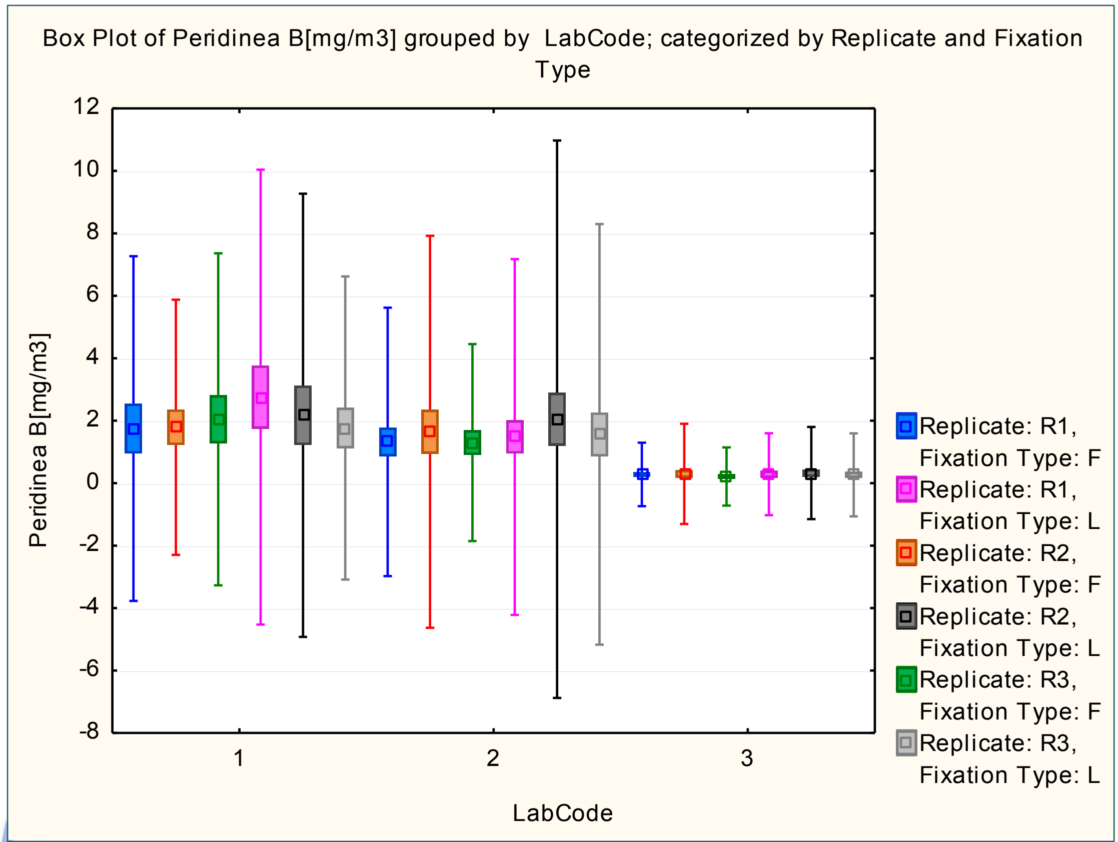


Figure 18. Box plot of Peridinea abundance and biomass by labs replicate and fixation.

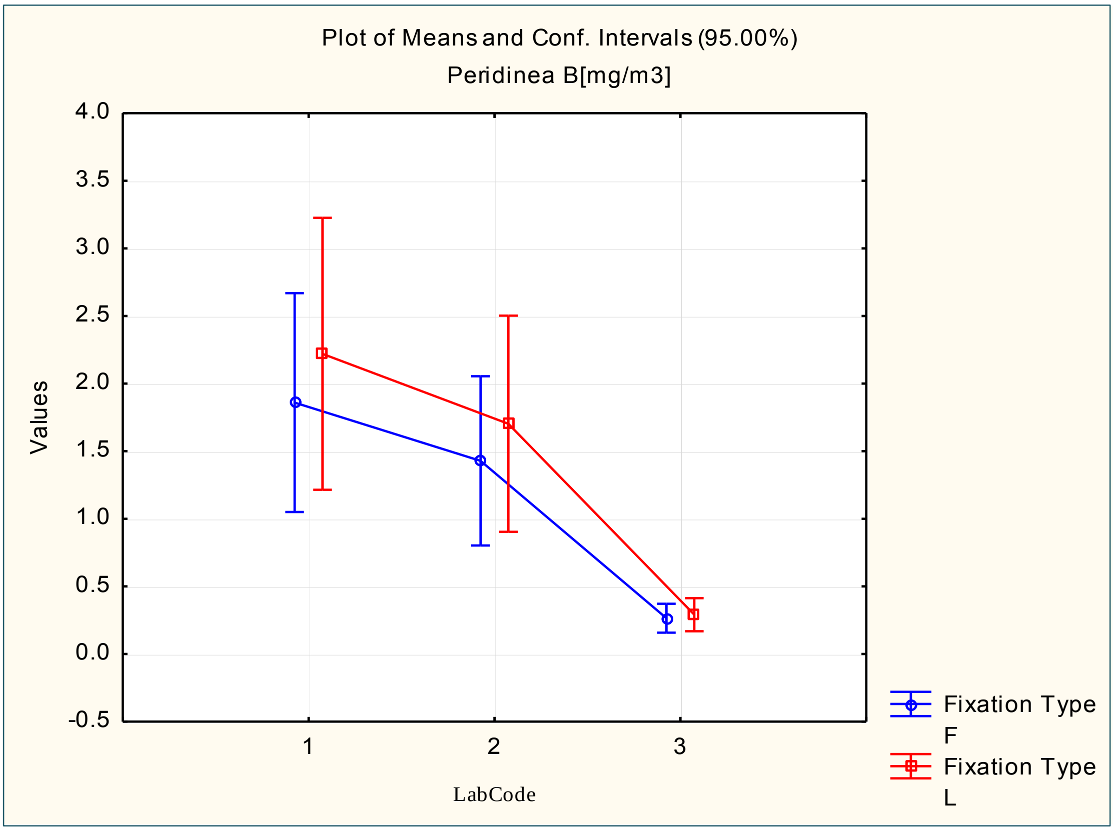


Figure 19. Plot of Peridinea mean biomass by laboratories and fixation type (F-formalin, L-Lugol).

A consistent difference (higher values of biomass) between the samples fixed by Lugol as compared to Formalin fixation is evident only in the overall biomass averages of labs replicates (Fig. 18), while this trend is not consistent between the replicates and laboratories (shown by the MANOVA).

The MANOVA results are in line with the uncertainty test in the Z-score approach. As evident from the Uncertainty Table the results of the Z scores could be considered reliable only for the total biomass and total phytoplankton abundance. At the level of taxonomic classes the uncertainty in the definition of assigned consensus values and z-scores respectively is high (> 0.3*σp) and again there is no consistency between the results of the 2 stations – Table 6.

Table 6. Phytoplankton parameter, uncertainty value (u) and coefficient 0.3*σ.

Station	Parameter	u	0.3*σ	Srob	1.2 σ
13	total Abundance [cells/l]	8629	9325	29289	37298
13	total Biomass [mg/m^3]	4	4	12.35	15.99
18	total Abundance [cells/l]	4169	155899	14150	623596
18	total Biomass[mg/m^3]	17	263	57.72	1052.34
13	Bacillariophyceae [cells/l]	1829	1384	6207	5535
13	Bacillariophyceae [mg/m^3]	1	2	3.34	9.05
13	Peridinea [cells/l]	1780	1253	6041	5013
13	Peridinea [mg/m^3]	5	4	15.8	14.29
13	Other [cells/l]	9598	8234	32575.7	4241.64
13	Other [mg/m^3]	4	5	14	767.76
18	Bacillariophyceae [cells/l]	1758	1060	4220	2464
18	Bacillariophyceae [mg/m^3]	996	616	13.55	33.73
18	Peridinea [cells/l]	11055	126601	2390.21	2463.64
18	Peridinea [mg/m^3]	6	192	22.32	33.73
18	Other [cells/l]	9	8	26531	506402
18	Other [mg/m^3]	1	67	2.99	267.51

As the biomass is a function of counts (cell abundance) and species biovolumes (converted to wet biomass) we test the difference between the specific biovolumes used by the participating labs by SIMPER analysis and by checking the geometric shapes to assess the degree and the source of the differences.

IV.1 Phytoplankton biovolume

C. SIMPER analysis

The analysis was applied for the comparison of the species biovolumes used by the participating laboratories in a pair-wise mode (Lab1-Lab2, Lab 1-Lab3 and LB2-Lab3). The results are assessed based on the dissimilarity coefficient and the species with high contribution to it (big difference between the species specific biovolumes) - Table 7 and Fig. 19.

Table 7. Average dissimilarity between the species specific biovolumes and list of species contributing to >90% cumulative difference (SIMPER test).

Average dissimilarity = 34.54				
Species	**BV-Lab 3**	**BV-Lab 2**	**Av.Diss**	**Cum.%**
Neoceratium tripos	70384	286962	17.35	50.24
Thalassiosira eccentrica	52691	2892	3.99	61.79
Protoperidinium steinii	13936	48530	2.77	69.82
Neoceratium furca	30749	63306	2.61	77.37
Protoperidinium divergens	86740	60852	2.07	83.38
Pseudosolenia calcar-avis	45000	61155	1.29	87.12
Protoperidinium granii	49335	35735	1.09	90.28
Phalacroma rotundatum	18440	28902	0.84	92.7
Prorocentrum compressum	10049	459	0.77	94.93
Neoceratium fusus	49298	42901	0.51	96.41

Average dissimilarity = 46.92				
Species	**BV-Lab 3**	**BV-Lab 1**	**Av.Diss**	**Cum.%**
Pseudosolenia calcar-avis	45000	226980	14.48	30.86
Neoceratium furca	30749	90718	4.77	41.03
Protoperidinium divergens	86740	26884	4.76	51.19
Protoperidinium steinii	13936	69272	4.4	60.57
Thalassiosira eccentrica	52691	8384	3.53	68.08
Neoceratium tripos	70384	26610	3.48	75.51
Proboscia alata	3002	46087	3.43	82.82
Phalacroma rotundatum	18440	58076	3.15	89.54
Neoceratium fusus	49298	12137	2.96	95.84
Prorocentrum compressum	10049	19008	0.71	97.36
Protoperidinium brevipes	4479	12215	0.62	98.67

Average dissimilarity = 45.22				
Species	**BV-Lab 2**	**BV-Lab 1**	**Av.Diss**	**Cum.%**
Neoceratium tripos	286962	26610	17.78	39.32
Pseudosolenia calcar-avis	61155	226980	11.32	64.36
Proboscia alata	6293	46087	2.72	70.37
Protoperidinium divergens	60852	26884	2.32	75.5
Neoceratium fusus	42901	12137	2.1	80.14
Phalacroma rotundatum	28902	58076	1.99	84.55
Neoceratium furca	63306	90718	1.87	88.69
Protoperidinium steinii	48530	69272	1.42	91.82
Prorocentrum compressum	459	19008	1.27	94.62
Protoperidinium granii	35735	48793	0.89	96.6
Protoperidinium brevipes	6125	12215	0.42	97.51
Thalassiosira eccentrica	2892	8384	0.38	98.34
Dinophysis caudata	39365	44401	0.34	99.1
Gonyaulax spinifera	18948	20706	0.12	99.37
Scrippsiella trochoidea (22/17)	1966	3219	0.09	99.56
Pseudo-nitzschia delicatissima	1226	294	0.06	99.7
Skeletonema costatum	194	880	0.05	99.8

The average dissimilarity varies between 35 and 47% and is due mostly to Peridinea species, although species from Bacillariophyceae are also present in the list (gray shaded) - Table 7. For some species the biovolume differs between 5-9 times, which is partly related to the differences in the geometric shapes assigned to the species (geometric formulas) - AnnexVII. 1.

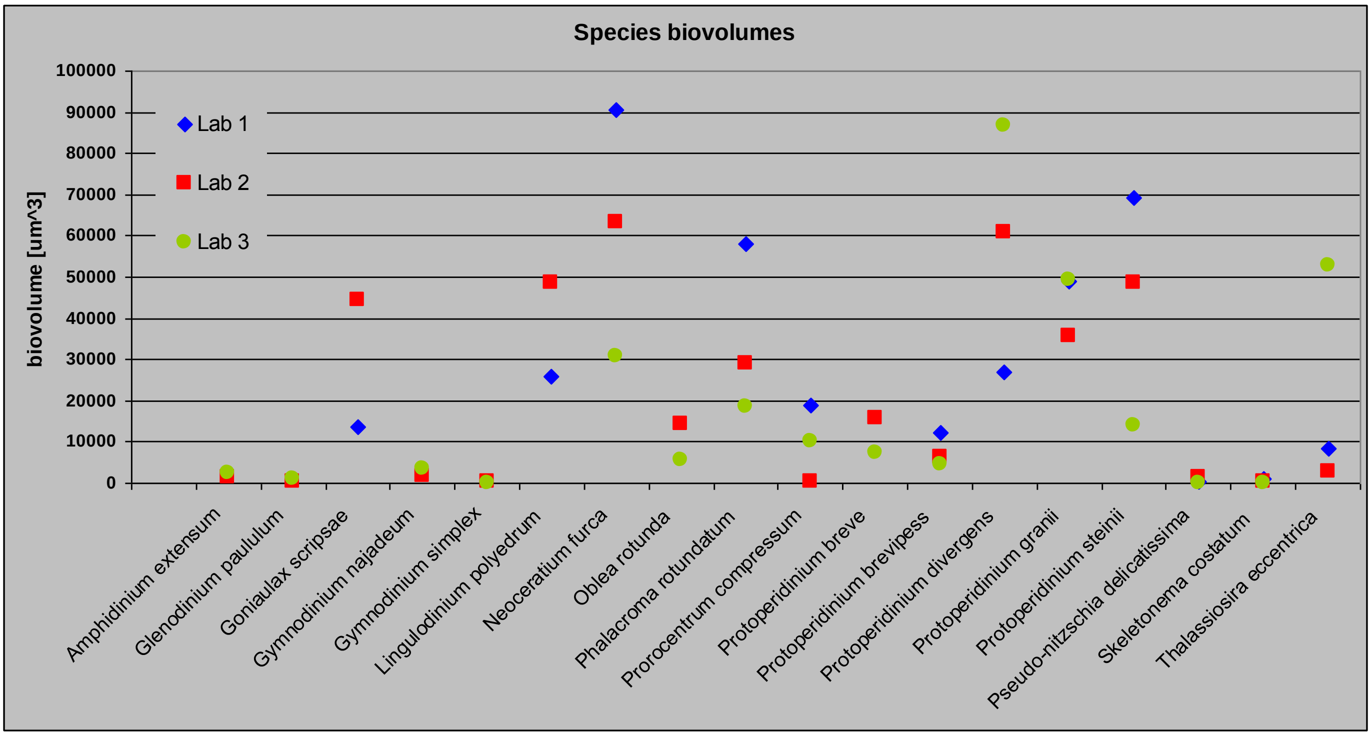


Figure 20. Plot of species specific biovolumes of selected species reported by the participating labs.

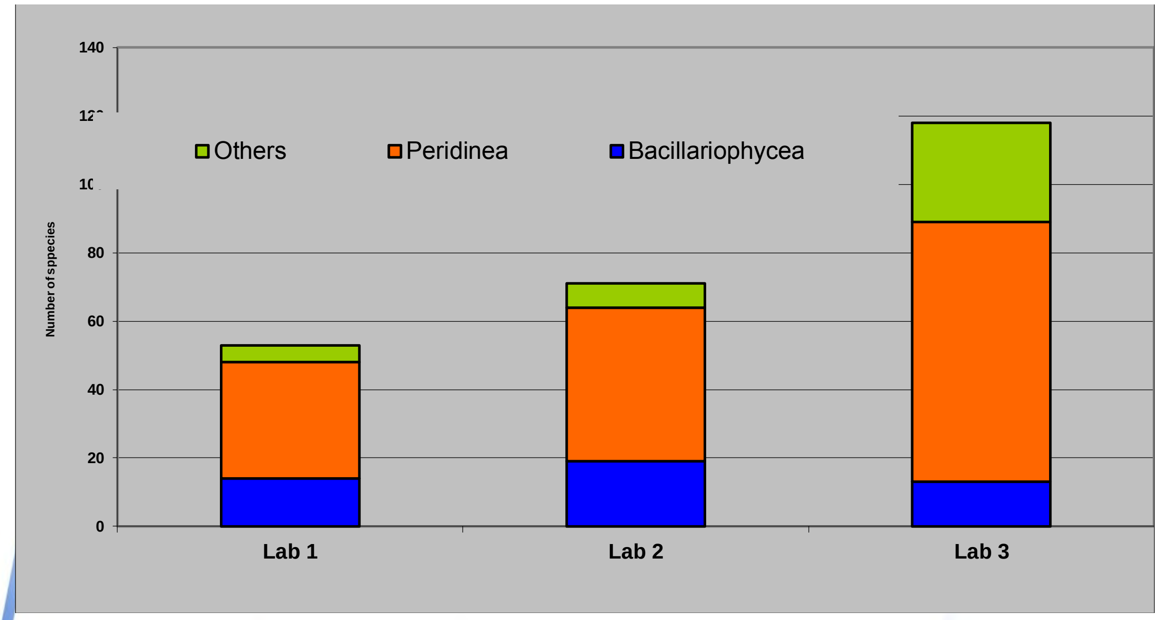


Figure 21. Number of species by Taxonomic classess identified by the participating labs.

The comparison of taxonomic lists of species identified in the samples by the participating labs also differs significantly especially regarding the “other” classes – Fig.21. In total Lab 1 reported 53, Lab 2 - 71 and Lab 3 - 118 species, but notably not all identifications were to species level (reported “sp”). Out of 15 taxonomic classes, only one lab identified species belonging to all of them including microflagellates, one lab reported representatives of 6 classes and one lab representatives of 7 classes (*Annex VII.1.)*.

V. CONCLUSIONS and RECOMMENDATIONS

The result give ground to conclude that by total biomass and abundance the data could be treated as a common data set.

If taxonomically based indicators will be applied the data should be considered with caution, especially regarding classes “other”.

The inetercalibration exercise reveal differences in the taxonomic skills of the participants that call for further training and more frequent intercallibration campaigns.

During a workshop held in Varna (23-25 April, 2014) a follow up actions were taken aimed to reduce the differences. At the level of taxonomic classes they were partly overcome by revision of the specific biovolumes used, especially for the species for which different geometric shapes were used and those for which the differences in the estimated biovolumes were high (Table 7 and Annex VII.1. Table with all species biovolumes). A final list of biovolumes based on agreed shapes was prepared along with correction of some technical errors in the calculations (Annex VII.1-corrected). All protocols were recalculated accordingly, using unified shapes. In addition the NIMRD team prepared a “web phytoplankton identification tool”, where microscopic pictures of some doubtful species were posted and taxonomic consensus reached. Altogether these assured the best possible harmonized common data set which was used for the preparation of the State of the Environmental Report.

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VII. ANNEXES

VII.1 Phytoplankton species biovolumes

STATIONS M13+M18 Species geometric shapes and biovolume

Species	BG-shape	RO-shape	TR-shape	BG-BV	RO-BV	TR-BV
			Bacillariophyceae			
Amphora sp.		*Ellipsoid*			315	
Cerataulina pelagica		Cylinder			3605	
Chaetoceros (cysts)		*Sphere*			1517	
Chaetoceros affinis			Cylinder			20362
Chaetoceros curvisetus		Cylinder	Cylinder		7531	14148
Chaetoceros heterovalvatus		*Eliptic prism + 4 cilinders*			276	
Chaetoceros similis			Cylinder			3700
Coscinodiscus granii		Cylinder	Cylinder		247953	102704
Cyclotella choctawhatcheeana	Cylinder	Cylinder	Cylinder	115	203	111
Cyclotella sp.		*Sphere*			287	
Ditylum brightwellii		*Prism on triangular base*			83320	
Nitzschia sp. (15,4/6,1)	Prism on parallelogramm base			145		
Nitzschia sp.		*Prism on parallelogram base*2*			79	
Cylindrotheca closterium		*2 cones*	2 cones		602	757
Navicula sp.		*Prism on elliptic base*			527	
Nitzschia tenuirostris	Spheroid + 2 cylinders	*Spheroid + 2 cylinders		672	323	
Nitzschia sp. (52,4/6,8)	Prism on parallelogramm base			345		
Pleurosigma elongatum			Half parallelepiped			12240
Proboscia alata	Cylinder	Cylinder	Cylinder	3002	6293	7018
Pseudo-nitzschia delicatissima	Prism on parallelogramm base	Prism on parallelogramm base	Prism on parallelogramm base	134	246	294
Pseudo-nitzschia seriata	Prism on parallelogramm base			1338		
Pseudosolenia calcar-avis	Cylinder	Cylinder	Cylinder	45000	61155	59003
Skeletonema costatum	Cylinder	Cylinder	Cylinder	76	194	123
Thalassionema nitzschioides	Parallelepiped	Parallelipiped	Parallelipiped	641	946	1178
Thalassiosira eccentrica	Cylinder		Cylinder	52691		32600
Thalassiosira sp. (20)	Cylinder	Cylinder		2531	2892	
Thalassiosira parva	Cylinder		Cylinder	303		398
				13	**19**	**14**
			Dinophyceae			
Akashiwo sanguinea		Ellipsoid			34268	
Alexandrium sp. 2 (32/32)	Ellipsoid	Ellipsoid		8247	8928	
Alexandrium sp. 7 (27/22)	Ellipsoid			3359		
Alexandrium sp. 8 (35/36)	Ellipsoid			11797		
Amphidinium acutissimum	Ellipsoid			435		
Amphidinium crassum	Ellipsoid	Ellipsoid		3579	3354	
Amphidinium extensum	Ellipsoid	Ellipsoid		2346	1318	
Amphidinium longum	Ellipsoid			2176		
Amphidinium sp.		Ellipsoid			1463	
Archaeperidinium minutum	Sphere			12750		
Neoceratium furca	Ellipsoid + 2 cones + cylinder	Ellipsoid + 2 cones + cylinder	Ellipsoid + 2 cones + cylinder	63306	38484	61353
Neoceratium fusus	Two cone	2 Cones	2 Cones	49298	42901	43464
Neoceratium tripos	cilinder+3 cones	cilinder+3 cones	cilinder+3 cones	165718	261051	171822
Cochlodinium pupa	Prolate spheroid	Prolate spheroid		18595	13063	
Cochlodinium sp. (31,96/22,21)	Prolate spheroid			8251		
cyst 27	Sphere	Sphere		9850	7616	
cyst (18)	Sphere			3083		
Dinophysis acuta	Ellipsoid			39421		
Dinophysis acuminata	Ellipsoid		Ellipsoid	26267		25656
Dinophysis sacculus		Ellipsoid	Ellipsoid		26286	15559
Dinophysis fortii			Ellipsoid			48967
Dinophysis meunieri			Ellipsoid			20251
Dinophysis caudata	cone + Ellipsoid	Cone+ellipsoid	Cone+Elilipsoid	42682	39365	44401
Ensiculifera carinata		Cone+half sphere			34888	
Glenodiniopsis steinii	Ellipsoid			7125		
Diplopsalis lenticula	Ellipsoid		Ellipsoid	9119		12566
Glenodinium pilula	Ellipsoid			1837		
Glenodinium paululum	Ellipsoid	Ellipsoid		1128	505	
Glenodinium sp. 2 (13,41/11,89)	Ellipsoid			496		
Glenodinium sp. 6 (23,76/17,65)	Ellipsoid		Ellipsoid	1742		1123

Species	BG-shape	RO-shape	TR-shape	BG-BV	RO-BV	TR-BV
Glenodinium sp. 8 (42,13/27,82)	Ellipsoid			8532		
Glenodinium sp. 9 (58/42)	Ellipsoid			26950		
Gonyaulax grindleyi	Sphere	Sphere		18841	21501	
Goniodoma sp.	Sphere			31548		
Goniodoma sphaericum	Sphere			52856		
Gonyaulax digitale	Prolate spheroid			23968		
Gonyaulax spinifera	Cone+half sphere	Cone+half sphere	Cone+half sphere	21709	18948	20706
Gonyaulax polygramma	Prolate spheroid			10829	15102	
Gonyaulax scrippsae		Two cone	Two cone	44312		13720
Gonyaulax monacantha			Cone+half sphere			25862
Gymnodinium helveticum		Ellipsoid			626	
Gymnodinium lacustre	Ellipsoid			754		
Gymnodinium agiliforme		Ellipsoid			349	
Gymnodinium hamulus	Ellipsoid			264		
Gymnodinium lantzschii	Ellipsoid			541		
Gymnodinium nanum	Ellipsoid			41		
Gymnodinium punctatum	Ellipsoid			107		
Gymnodinium rubrum	Ellipsoid			48543		
Gymnodinium sp.2 (h,46/l,42)		Ellipsoid			25673	
Gymnodinium najadeum	Ellipsoid	Ellipsoid		3398	1813	
Gymnodinium sp. 13 (11,63/8,67)	Ellipsoid		Ellipsoid	229		314
Gymnodinium voukii	Ellipsoid			1649		
Gymnodinium wulffii		Ellipsoid			236	
Gymnodinium simplex	Ellipsoid	Ellipsoid		133	322	
Gymnodinium sp.1 (h,20/l,14)		Ellipsoid			1030	
Gyrodinium fusiforme		Ellipsoid			16887	
Gyrodinium nasutum	Ellipsoid			51635		
Gyrodinium sp. 6 (42/18)	Ellipsoid			3211		
Gyrodinium lachryma			Flattended Ellipsoid			152132
Herdmania litoralis		Prolate spheroid				
Heterocapsa rotundata	Ellipsoid			253		
Heterocapsa triquetra	2 Cones	2 Cones		3484	3299	
Katodinium fungiforme	Ellipsoid			215		
Lessardia elongata	Two cone	2 Cones		884	474	
Lingulodinium polyedrum	Prolate spheroid		Prolate spheroid	48585		46923
Oblea rotunda	Sphere	Sphere		5588	14336	
Oxyrrhis marina	Ellipsoid			692		
Peridinium morzinense	Two cone			39306		
Peridinium sp. 2 (69,23/51,21)	Ellipsoid			50241		
Peridinium sp. 3 (17,5/18,5)	Ellipsoid			1567		
Peridinium sp. 6 (42,9/40,4)	Ellipsoid			17119		
Peridinium sp. 7 (44,7/45,2/40,4)	Ellipsoid			23891		
Peridinium sp. 8 (24,52/20,58)	Ellipsoid			2707		
Peridinee (vegetative stages)		Sphere			19168	
Peridiniella danica		Ellipsoid			739	
Peridinium granii f. mite		Ellipsoid			19140	
Peridinium quinquecorne		Ellipsoid			3081	
Phalacroma acutum			Ellipsoid			63355
Phalacroma rotundatum	Ellipsoid	Ellipsoid	Ellipsoid	18440	23799	20665
Polykrikos schwartzii	Ellipsoid			27310		
Preperidinium meunierii		Cone+half sphere			23811	
Prorocentrum compressum	Ellipsoid	Ellipsoid	Ellipsoid	10049	9173	10673
Prorocentrum cordatum	Ellipsoid	Ellipsoid	Ellipsoid	1099	1038	1144
Prorocentrum micans	Prolate spheroid	Prolate spheroid	Prolate spheroid	17214	19537	19030
Protoperidinium bipes		Ellipsoid	Ellipsoid		1125	3272
Protoperidinium breve	Two cone	Two cones		7456	6309	
Protoperidinium brevipes	Two cone	Two cones	Two cones	4479	6125	5747
Protoperidinium claudicans	2 Cones	2 Cones	2 Cones	120211	93668	71838
Protoperidinium globosum	Sphere		Sphere	17800		22449
Protoperidinium granii	Two cone	2 Cones	Two cone	49335	35735	48793
Protoperidinium leonis	Two cone			190392		
Protoperidinium pallidum	Two cone		Two cone	36855		8790
Protoperidinium pellucidum	Two cone		Two cone	13489		6465
Protoperidinium divergens	Two cone	Two cone	Two cone	86740	60852	89204
Protoperidinium steinii	Cone+half sphere	Cone+half sphere	Cone+half sphere	58900	48530	69272
Protoperidinium depressum	Two cone		Two cone	105657		105645
Protoperidinium cerasus						8579
Scrippsiella trochoidea (22/17)	Ellipsoid	Ellipsoid	Ellipsoid	2298	1966	3219
Torodinium robustum	Ellipsoid			3020		
Tyrannodinium edax	Ellipsoid			9190		
				76	**45**	**34**

Species	BG-shape	RO-shape	TR-shape	BG-BV	RO-BV	TR-BV
Chlorophyceae						
Chlamydomonas sp.	Prolate spheroid			999		
filament unit	Cylinder			40		
round cell 4,1	Sphere			37		
				3	**0**	**0**
Cryptophyceae						
Chroomonas sp.	Prolate spheroid			662		
Hemiselmis sp.	Prolate spheroid			103		
Hillea fusiformis	Prolate spheroid	Prolate spheroid	Prolate spheroid	163	356	141
Plagioselmis sp.	Prolate spheroid			282		
Rhodomonas marina	Prolate spheroid			1244		
Teleaulax sp.	Prolate spheroid					
Cryptomonas sp.		Prolate spheroid			1563	
				5	**2**	**1**
Cyanophyceae						
Monoraphidium sp.	Two cone			104		
Romeria sp.	Cylinder			14		
Synechococcus sp.	Cylinder			141		
Phormidium hormoides		Sphere			16	
Anabaena sp.	Cylinder	Sphere		342	318	
				4	**2**	**0**
Dictyochophyceae						
Apedinella radians	Prolate spheroid			386		
Dictyocha speculum			Half sphere			5301
				1	**0**	**1**
Nephroselmidophyceae						
Nephroselmis astigmatica	Sphere			199		
Nephroselmis pyriformis	Prolate spheroid			326		
				2	**0**	**0**
Noctilucales						
Pronoctiluca pelagica	Prolate spheroid		Flattended Ellipsoid	13181		7890
Pronoctiluca spinifera	Prolate spheroid			4648		
				2	**0**	**1**
Prasinophyceae						
Pyramimonas amylifera	Cone			145		
Pyramimonas sp.	Cone			38		
				2	**0**	**0**
Prymnesiophyceae						
Calyptrosphaera oblonga	Prolate spheroid			976		
Chrysochromulina sp.	Prolate spheroid			439		
Coccolithos sp. 1	Sphere			271		
Coccolithos sp. 2	Sphere			1563		
Corymbellus aureus	Prolate spheroid					
Emiliania huxleyi	Sphere	Sphere	Sphere	118	141	382
Pavlova sp.	Prolate spheroid			241		
				6	**1**	**1**
Trebouxiophyceae						
Trochiscia sp.	Sphere			293		
				1	**0**	**0**
Raphidophyceae						
Heterosigma inlandica	Prolate spheroid			2269		
				1	**0**	**0**
Microflagellates						
microflagellates	Sphere			40		
				1	**0**	**0**
Euglenoidea						
Eutreptia lanowii		cilinder + cone			2676	
Lepocinclis acus		*2 Cones*			106	
				0	**2**	**0**
Ebriophyceae						
Ebria tripartita	Sphere		Sphere	13843		8621
				1	**0**	**1**

INTERCALIBRATION

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Intercalibration Exercise

Phytoplankton

MISIS

MSFD Guiding Improvements in the
Black Sea Integrated Monitoring System

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