



Intercalibration Report Zooplankton

Black Sea monitoring harmonization process

Group II

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Table 1. Inventory of in -house routines of mesozooplankton lab analysis

I. SCOPE

In order to carry out a comparison between different Black Sea regions regarding the mesozooplankton community current status, an intercomparison/intercalibration exercise between different laboratories/institutions was considered necessary. For this purpose, 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 " was achieved through organisation of the Joint Survey in the summer of 2013.

Main objective of this report is to evaluate the comparability of mesozooplankton data produced by the partners in MISIS Project – IO-BAS (Bulgaria), NIMRD "Grigore Antipa" (Romania) and SNUFF (Turkey) in order to be able to construct a common data set as a bases for application of unified indicators for assessment of Western Black Sea environmental status in a harmonised way.



II. SAMPLING DESIGN

An open sea station was selected for the intercalibration exercise - (station marked 13 o the map) - Fig.1. First step taken to obtain homogeneous samples was the performance of integrated water column sampling (from the lower limit of the oxic layer to the surface) in order to avoid zooplankton vertical migration.



Figure 1. Map of MISIS cruise mesozooplankton intercalibration station 13 (Lat 42.74 N, Long 29.34 E)

Samples preparation and lab methods

Samples were collected with the help of the Juday net (36 cm opening with 150 micron mesh) in a vertical tow from 120 meters depth to surface. For each laboratory were collected three replicates. The second step taken to assure a higher homogeneity of samples was sampling in three rounds and in each of the rounds one sample was collected for each laboratory. Samples have been preserved on board in 11 bottles with 4% formaldehyde (buffered to pH 8-8.2 with disodiumtetraborate).

Laboratory sample processing was done according to the "Manual for mesozooplankton sampling and analysis in the Black Sea monitoring" compiled by Alexander Korshenko and Boris Alexandrov.



Laboratory	Sample	Microscope type	Counting	Volume of	Magnification	Counting
	concentration		chamber	subsample		
IO-BAS BG	Decantation	Olympus stereomicroscope SZ30	Bogorov	2 ml	40X	First three dominant species up to 100 individuals
NIMRD RO	Decantation	Olympus stereomicroscope (SZ61)	Bogorov	2 ml	45X	First three dominant species up to 100 individuals
SUFF TR	Decantation	Novex RZB-SF stereomicroscope	Bogorov	1 ml		Subsampling

Table 1. Inventory of in -house routines of mesozooplankton lab analysis



III. STATISTICAL ANALYSIS

The mesozooplankton components subject to inercomparison for the attributes (abundance and biomass) were:

- mesozooplankton total abundance [ind/m³] and biomass [mg/m³]
- Copepoda total abundance [ind/m³] and biomass [mg/m³]
- Cladocera total abundance [ind/m³] and biomass [mg/m³]
- Meroplankton total abundance [ind/m³] and biomass [mg/m³]
- Oikopleura dioica abundance [ind/m³] and biomass [mg/m³]
- Parasagitta setosa abundance [ind/m³] and biomass [mg/m³]
- Noctiluca scintillans abundance [ind/m³] and biomass [mg/m³]
- Individual biomass
- Taxonomic identification (species lists)

A. Statistical evaluation was based on the z-score according to "The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (IUPAC Technical Report) (IUPAC, 2006) and ISO 13528:2005 (Statistical methods for use in proficiency testing by interlaboratory comparisons)

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 mesozooplankton components attributes a participant's result X is converted into a Z-score according to the equation

Z= (X – Xa)/σp

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where Xa is the "assigned" value, and op 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 13528:2005).

In the equation the term (X - Xa) is the error in the measurement. The parameter op describes the standard uncertainty that is most appropriate for the application area of the results of the analysis, assumed as "fitnessfor-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 and n = number of results. Criterion for the reliability of the assigned values was $u/\sigma p \le 0.3$. The fulfillment of this criterion indicates that the z scores are reliable.

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 op values are then obtained by multiplying the selected RSD by the assigned value. The standard deviation (σ p) for the proficiency assessment is commonly set at 20%.

Definition of assigned value

According to the IUPAC's technical report, an assigned value is an estimate of the value of the measurand 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 mesozooplankton 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 that were outside the 90% confidence limit were interpreted as outliers.
- Exclude the values which are not included in the 90% confidence limit
- Recalculate the mean which is assumed to be the assigned consensus value
- Recalculate the standard deviation which is assumed as robust

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".



IV. RESULTS

The raw data and the results of the scoring (Z-scores) are presented on Figures 2 to 15 and the related statistical values are given in the corresponding Tables. The group "Others" was treated as separate species (*Oikopleura dioica* and *Parasagitta setosa*), the non-fodder zooplankton was constituted only by *Noctiluca scintillans*, other non-fodder species (gelatinous plankton) were sampled with different equipment and it were not a part of this report.

1 Mesozooplankton total abundance and biomass









Figure 3. Histogram of raw data (up) and Z scores plot (down) of total mesozooplankton biomass [mg/m³]

		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	0.17			
	RO	-0.02			
13	TR	-0.02	484.47	3.27	1620.91
		Biomass [mg/m^3]			
	BG	0.18			
	RO	0.10			
13	TR	-0.07	28.89	2.92	84.51

2 Mesozooplankton abundance and biomass by components





Figure 4. Histogram of raw data (up) and Z scores plot (down) of Copepoda abundance [ind/m³]







Figure 5. Histogram of raw data (up) and Z scores plot (down) of Copepoda biomass [mg/m³]

		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	0.17			
	RO	-0.02			
13	TR	-0.01	409.47	3.32	1362.87
		Biomass [mg/m^3]			
	BG	0.16			
	RO	0.10			
13	TR	-0.11	26.53	2.78	73.86





Figure 6. Histogram of raw data (up) and Z scores plot (down) of Cladocera abundance [ind/m³]







Figure 7. Histogram of raw data (up) and Z scores plot (down) of Cladocera biomass [mg/m³]

		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	0.22			
	RO	-0.28			
13	TR	-0.27	34.44	2.58	89.02
		Biomass [mg/m^3]			
	BG	0.27	· · · · ·		
	RO	-0.08			
13	TR	-0.01	0.87	2.29	2.01















		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	0.11			
	RO	-0.03			
13	TR	0.14	10.14	2.54	25.80
		Biomass [mg/m^3]			
	BG	0.10		/	
	RO	0.02		(
13	TR	0.12	0.32	3.01	0.95
	17		XX		





Figure 10. Histogram of raw data (up) and Z scores plot (down) of *Oikopleura dioica* abundance [ind/m³]









		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	1.09			
	RO	-0.19			
13	TR	-0.13	17.56	1.11	19.64
		Biomass [mg/m^3]			
	BG	0.67			
	RO	3.72			
13	TR	-0.29	0.13	1.05	0.13
	19	k	XX		





Figure 12. Histogram of raw data (up) and Z scores plot (down) of *Parasagitta setosa* abundance [ind/m³]









		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	0.06			
	RO	0.39			
13	TR	-0.19	17.05	2.09	35.69
		Biomass [mg/m^3]			
	BG	0.60		/	
	RO	-0.10			
13	TR	0.28	1.96	1.54	3.02
	21		XYT		





Figure 14. Histogram of raw data (up) and Z scores plot (down) of *Noctiluca scintillans* abundance [ind/m³]









		Z-score			
Station	Lab code	Abundance [ind/m ³]	Assigned value	RSD	σ
	BG	-0.31			
	RO	1.39			
13	TR	-0.10	1.54	1.07	1.64
		Biomass [mg/m^3]			
	BG	-0.31			
	RO	1.39			
13	TR	-0.10	0.14	1.07	0.14
	23		AL		
	X				

V. CONCLUSIONS and RECOMMENDATIONS

 According to z-score agreements, satisfactory (z-score ≤ 2) results were obtained for the most measurements (98 %), whereas unsatisfactory (zscore ≥ 3) results in 2 % of cases (table below).

	Abundance [ind/m ³]			Biomass [mg/m ³]		
Participant	BG	RO	TR	BG	RO	TR
Total Mesozooplankton	0.17	-0.02	-0.02	0.18	0.10	-0.07
Total Copepoda	0.17	-0.02	-0.01	0.16	0.10	-0.11
Total Cladocera	0.22	-0.28	-0.27	0.27	-0.08	-0.01
Total Meroplankton	0.11	-0.03	0.14	0.10	0.02	0.12
Oikopleura dioica	1.09	-0.19	-0.13	0.67	3.72	-0.29
Parasagitta setosa	0.06	0.39	-0.19	0.60	-0.10	0.28
Noctiluca scintillans	-0.31	1.39	-0.10	-0.31	1.39	-0.10

- The sole unsatisfactory result was related to *Oikopleura dioica* biomass which is calculated in a different way by NIMRD laboratory.
- Further analysis of biomass data revealed differences related to the calculation of various species. This situation results in a small variation of Z-score for biomass (but still in the satisfactory limit).
- The sampling design assured sufficient homogeneity of the samples;
- As a general conclusion we can consider that all laboratories have obtained good results and the data could be treated as a common data set.
- Due to the differences in the lists of species reported by the participating laboratories in the exercise (*Acartia clausi* and *Acartia tonsa*, *Oithona similis* and *Oithona davisae*) it was revealed the necessity of organizing common training/exercises to harmonize taxonomic identification.
- A common list of equations for the determination of the Black Sea zooplankton biomass and caloricity for all the species should be agreed between all Black Sea zooplankton specialists for the "Manual for mesozooplankton sampling and analysis in the Black Sea monitoring".
- Future intercalibration/intercomparison should be considered and exercises should follow and combine other international sampling and processing guidelines.

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