LifeWatch ERIC Scientific,
Community Meeting
Rome, 27-29 May 2019

Working session: Marine Biodiversity & Ecosystem Functioning
Flash talk

PhytoNumb3rs: An easy-to-use computer toolkit for counting microalgae by the Utermöhl method

ARPA PUGLIA

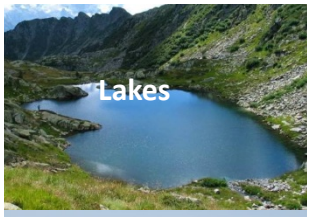
Maria Rosaria Vadrucci, Leonilde Roselli, Tiziana Di Festa, Daniela Donadei, Marisa Florio, Emanuela Longo, Stefania D'Arpa, Flavia Maci, Sergio Ranieri,Mariangela Spinelli, Annamaria Pastorelli, Nicola Ungaro

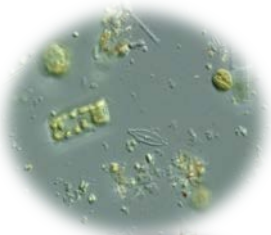
Phytoplankton is recognized as a BQE and a biological descriptor in several European directives and national laws.

The Marine Strategy Framework Directive (MSFD)

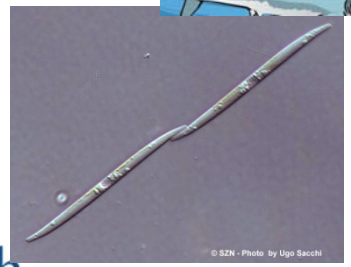
56/2008/EC
D. Lgs 190/2010

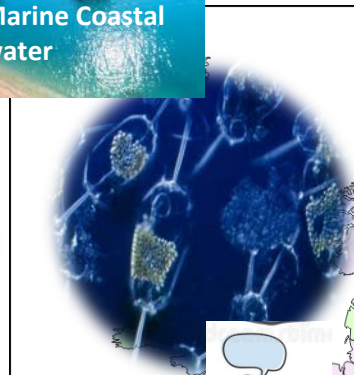
EC/2000/60
D.Lgs 152/2006





Descriptors
1 - Biodiversity
2 - NIS
4 - Food web
5 - Eutrophication/HABs blooms





The heterogeneity of data sources creates a barrier in terms of making connections within and among multiple domains of information

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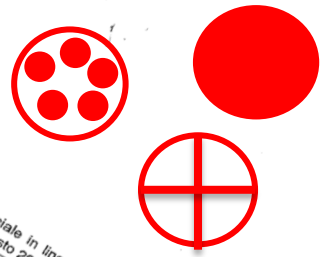
The Utermöhl method (Lund et al., 1958) is the most widely adopted method to determine the abundance of phytoplankton assemblages

The procedures used for phytoplankton analysis vary widely between research and monitoring groups, despite the numerous efforts to standardise phytoplankton data.

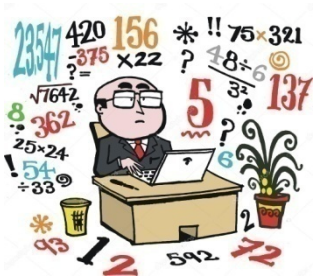
Settling volume

Magnification



Counting strategy

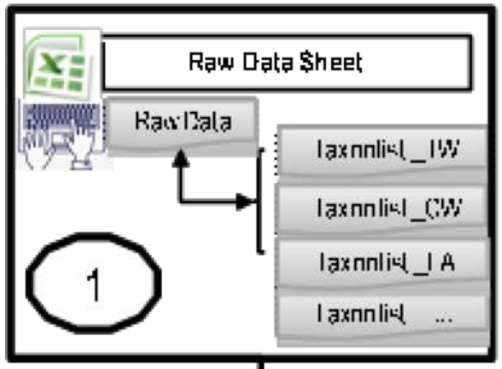
Number of cell to count



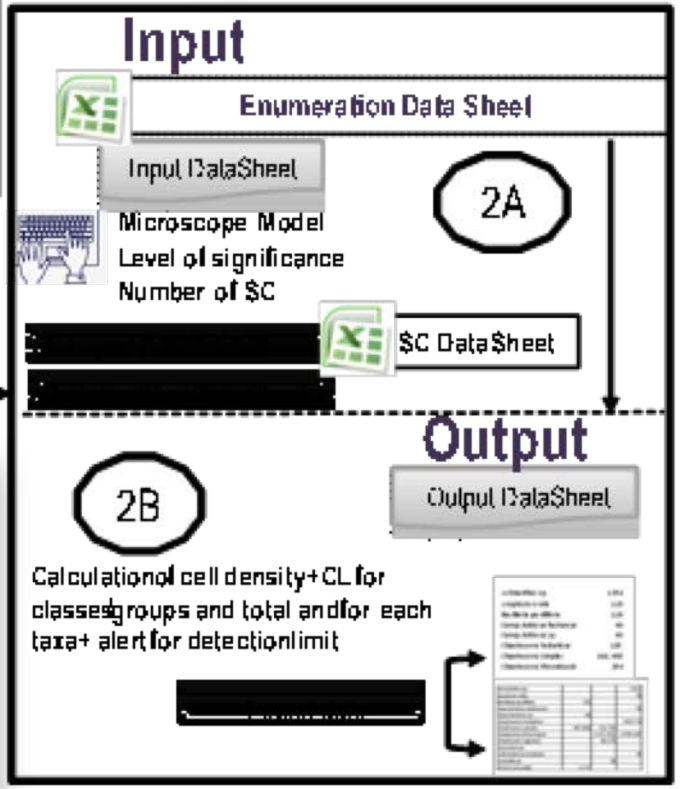
Cell density and confidence limits calculus

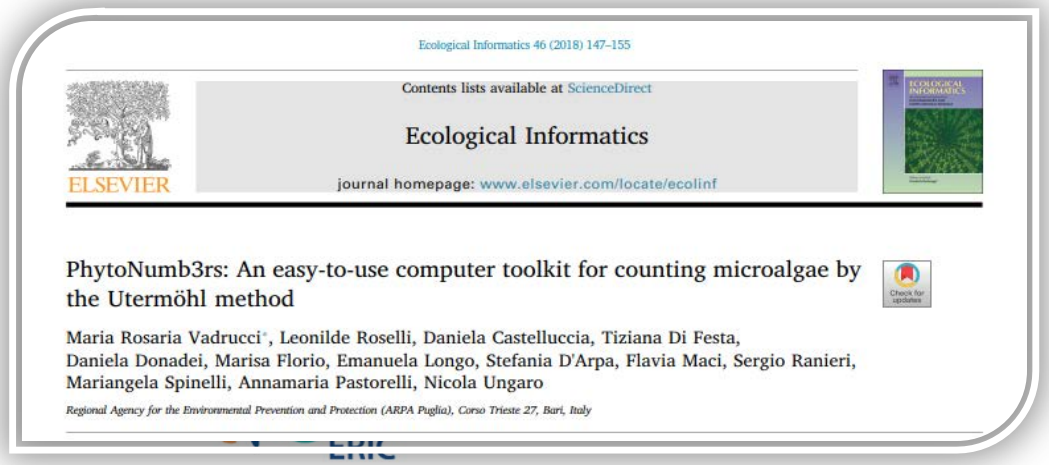
Results are not comparable

PHYTONUMB3RS Tool Kit
A simplified visualisation of PhytoNumb3rs workflow



PHYTONUMB3RS
Counting Phytoplankton





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PHYTONUMB3RS – Raw Data Sheet

PHYTONUMB3RS

RAW DATA SHEET

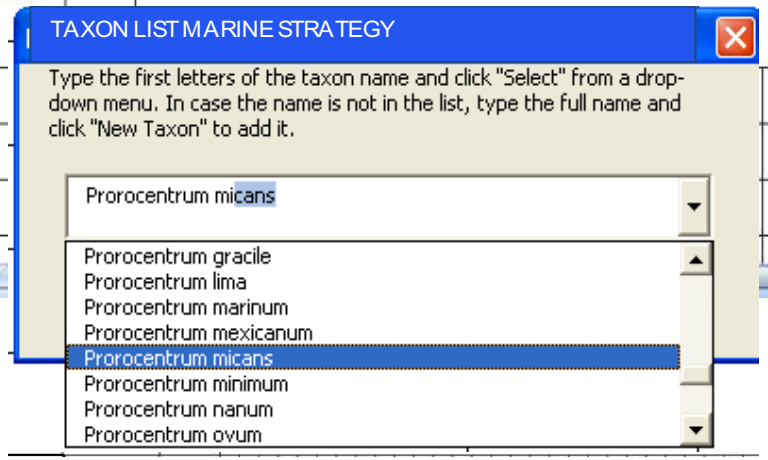
Rev.1 March 2018

Method: UNI EN 15204:2006

N° LIMS_SAMPLE ID	Start of analysis	STATION NAME	ANALYZED BY	VOLUME OF SEDIMENTATION CHAMBER (ml)	N° OF SEDIMENTATION CHAMBER
Sampling Date	End of analysis				

Counting Strategy			TAXA	Maximum Linear Dimension (N/M)	TOTAL	NUMBER OF CELLS/THREADS/COENOBIA/COLONIES
C=Counting Field T=Transect F=Whole Chamber	Magnification	N° of Counting fields/ Transects/ Whole Chamber				

Raw Data | Taxon list_TW | Taxon list_CW | Taxon list_LA | Taxon list_Marine Strategy



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PYTONUMB3RS Counting Phytoplankton

Enumeration Data Sheet –INPUT Data Sheet

Insert the number of chamber

1. Upload SCDS

ENUMERATION DATA SHEET — Ver 1 March 2018 — A

N Lims_SampleID		Microscope :			
Name of sampling station		Chamber n°			
Sampling Time					1. Import SCDS
Operator's name					
Settling volume in liter = v (0,01 - 0,025 - 0,050 - 1 - 2)		Counting strategy			
Magnification		100	200	400	600
Number of counted random fields = c		#DIV/0!	#DIV/0!	#DIV/0!	#DIV
Number of fields in half chamber		#DIV/0!	#DIV/0!	#DIV/0!	#DIV
Number of fields in a transect * n° of transects		#DIV/0!	#DIV/0!	#DIV/0!	#DIV
Number of fields in whole chamber		#DIV/0!	#DIV/0!	#DIV/0!	#DIV
Area of sedimentation chamber (µm2) = A					
Area of counting field = a					
Area of transect					
Number of transects					
Dilution factors = d (nd = 1; 1:2 = 2; 1:4 = 4; 1:10 = 10;...)					
Level of significance (α)					
Quantitative detection limit = -ln(α)* ftotal/(V*fcounted)		#DIV/0!	#DIV/0!	#DIV/0!	
TAXA	N° of total cells (x)	Result CELL/L = X * (A * d) / (a * v) - where "X"is the average of n per field (x/c)			

α

2

2. Upload Raw Data

Input Data Sheet | Output Data Sheet | Microscope parameters | Legends | Va

Sedimentation Chambers Data Sheet — MD - 264 Rev. 1 May 2017

DATE: 16/03/2017 — Analyst: Vadrucci

N° OF SEDIMENTATION CHAMBER	Empty Weight Chamber (P1) (g)	Full Weight Chamber - Distilled Water (P2) (g)	Volume Chamber - (P2-P1) (L)	Diameter Chamber (µm)	Radius Chamber (µm)	Total Area of the Chamber π*(d/2)2 (µm2)
1	29,28	54,43	0,025	25.000	12.500	490.873.852
2	29,19	54,28	0,025	26.000	13.000	530.929.158
3	27,78	53,02	0,025	26.000	13.000	530.929.158
4	29,25	54,43	0,025	25.000	12.500	490.873.852

2-Upload Raw Data Sheet

ENUMERATION DATA SHEET — Ver 1 March 2018 — B

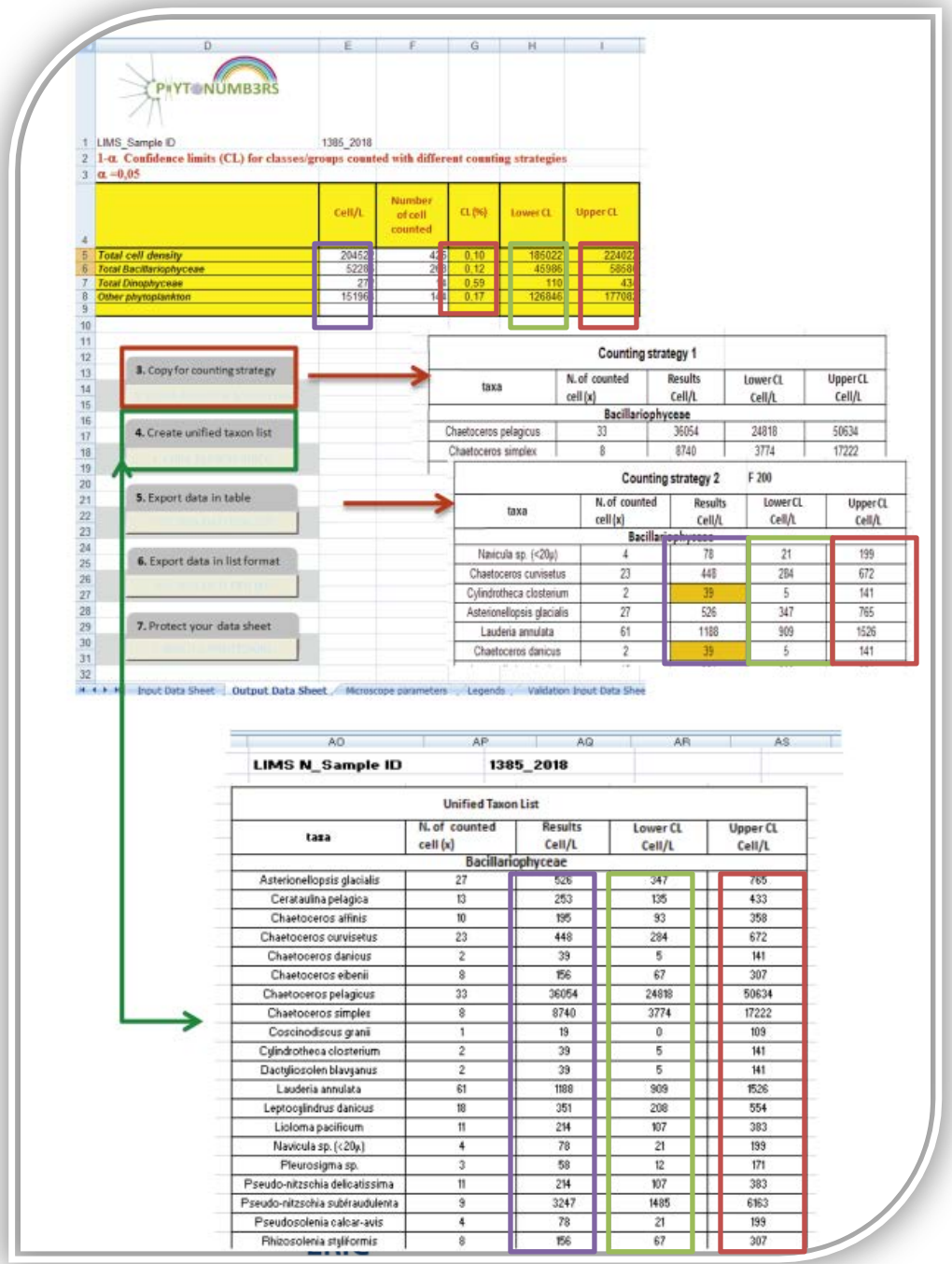
N Lims_Sample ID	1385_2018	Microscope :	Nikon Eclipse Ti -S N. Inv. 686		
Name of sampling station	MC_UG01S	Chamber n°	19		
Sampling Time	26/01/2018				1. Import SCDS
Operator's name	XXX				
Settling volume in liter = v (0,01 - 0,025 - 0,050 - 1 - 2)	0,05205	Counting strategy			T 600
Magnification		100	200	400	600
Number of counted random fields = c		139,3711295	578,720432	2229,939289	92,76486057
Number of fields in half chamber		69,69	289,36	1114,97	2654,09
Number of fields in a transect * n° of transects		0,00	0,00	0,00	92,76
Number of fields in whole chamber		139,37	578,72	2229,94	5308,18
Area of sedimentation chamber (µm2) = A		510.705.156	510.705.156	510.705.156	510.705.156
Area of counting field = a		3.664.354	882.473	229.022	96.211
Area of transect		55.080.000	27.030.000	13.770.000	8.925.000
Number of transects					1
Dilution factor = d (nd = 1; 1:2 = 2; 1:4 = 4; 1:10 = 10;...)					1
Level of significance (α)	0,05				
Quantitative detection limit = -ln(α)* ftotal/(V*fcounted)		58	58	58	3.293
TAXA	N° of total cells (x)	Result CELL/L = X * (A * d) / (a * v) - where "X"is the average of n. of cell per field (x/c)			

2. Upload Raw Data

Counting Strategy: C=Counting Field T=Transect F=Whole Chamber	Magnification	N° of Counting fields/ Transects/ Whole Chamber	TAXA	Maximum Linear Dimension (N/M)	TOTAL	NUMBER OF CELLS/THREADS/COENOBIA/COLONIES
F	20	1	*Prorocentrum micans*	M	13	1 1 1 1 2 3 1 1 2
F	20	1	*Protoperidinium quinquecorne*	M	4	1 1 2
F	20	1	*Guinardia striata*	M	3	3
F	20	1	*Asterionellopsis glacialis*	M	15	5 7 3
F	20	1	*Heterocapsa sp.*	N	5	1 1 1 1 1
			PARTIAL COUNT		40	
			CLASS NF		5	
			CLASS MF		35	
T	60	1	*Nitzschia sp.*	N	28	2 5 6 4 1 1 3 3 1 2
			TOTAL COUNT		68	

Detection limit $= -\ln(\alpha) \cdot f_{total} / (V \cdot f_{counted})$

Enumeration Data Sheet –OUTPUT Data Sheet



3. Copy for counting strategy

4. Create unified taxon list

Parameter	Unit
Cell density	Cell/L
Number of cell counted	
Lower confidence limit for taxon	Cell/L
Upper confidence limit for taxon	Cell/L
Quantitative Detection limit	Cell/L
Upper/Lower confidence limit (%) for total or groups	%

If a taxon has been counted below the detection limit the spreadsheet cell is highlighted in yellow

Main advantages of PHYTONUMB3RS Toolkit

Easy to use

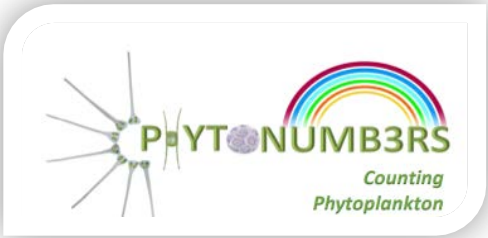
- Completely developed in Excel

Quick counting procedure - Reduce time of analysis

- Automatically counts the number of cells belonging to the same taxon;
- Automatically counts the number of cells counted with the same counting strategy and the total number of cells counted in the sample;

Facilities for data management and storage

- To Carry out quality control by entering the taxon name from a drop-down menu of user-defined taxon lists (reduction of typing errors and formatting errors);
- To Manage a new taxon entry (if the taxon name is not included in the list, the analyst can add it);
- To Calculate cell density uncertainty as for each taxa as for total (for quality assurance procedures);
- To Calculate the LOD;
- To Allert for taxa counted below the LOD
- To Export data in various templates (tables or lists)



Phytonumb3rs represents a first step towards harmonization of data and the promotion of standardized procedures for data management that will save time during database entry and storage. It makes possible to obtain high-quality databases, reducing random errors generated by the operator (typing, wrong names, etc.). The large-scale distribution of **PhytoNumb3rs** is advantageous because can improve the interoperability and integration of phytoplankton data collected by separate research and monitoring programs.

How can we improve PHYTONUMB3RS Toolkit?

Raw Data Sheet

- To Insert alert with advises for suitable counting strategies
- To Create a unified taxon lists of phytoplankton shared among different users

Enumeration Data Sheet – OUTPUT

- To Make the export data sheet function available to a wider range of users
- To Develop data processing workflow for re-using data for long term or spatial data analysis and creation of data reporting

LifeWatch ITALY

Study cases in LifeWatch Italy VREs& MoBiLab –

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