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Development or Improvement of Standards in Support of the Water Framework Directive - WP 7: Guidance on the estimation of algal biovolume

European interlaboratory comparison for determination of phytoplankton biovolume 2014

Final report

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1 Introduction

The European Standard Document for the estimation of the biovolume of phytoplankton shall be a guideline for harmonisation of the used methods. It shall not serve to unify the national monitoring concepts. The aim of the Standard Document is to harmonise these assignments and prevent unnecessary source of errors or imprecision.

A laboratory intercomparison exercise was performed to test the applicability, usability and manageability of the Standard Document. Main objective of this exercise was to check the compliance of the participants with the proposed document and whether the methods had been described in an appropriate and understandable form. For that reason, the test results have been analysed for reproducibility and repeatability, and statistical comparisons have been carried out.

2 Material and Methods

2.1 Preparation of samples

Prior to the exercise, it had been decided to take freshwater and marine samples from different sites that included algal taxa with different forms and sizes. Then samples were taken by the Alfred-Wegener-Institute, AWI at Helgoland (North Sea sample), the LUNG (State Agency for Environment, Nature Conservation and Geology) at Stralsund (Baltic Sea sample), the BTU (Brandenburg University of Technology) at Cottbus (Scharmützelsee sample) and AquaEcology at Oldenburg (Oldenburg sample). The samples were taken at different sites during summer and fall to get taxa that represented diverse geometrical forms. All samples were analysed with an inverted microscope in Utermöhl chambers to check for quality, taxa and abundance, and suitable mixtures were made. This will be described in detail below.

For the preparation of the North Sea sample, net haul samples and natural water samples were taken at Helgoland Roads in the beginning of August 2013. Originally, these samples were fixed with non-acidified Lugol's iodine solution, which led to a non-optimal fixation, especially for weakly silicified diatoms showing shrinkage and deformation. Nevertheless, it was decided that these samples should be included in the test. The samples were stored and transported in 2.5 L brown glass bottles to the laboratory of AquaEology in Oldenburg. At the beginning of September, the samples were carefully mixed in a 20-L bottle ensuring equal distribution of the organisms, and then were immediately filled into 2.5-L brown glass bottles which were stored cool and dark. In the second half of October, additional autumn net haul samples were taken at Helgoland Roads and treated in the same way as already described. Summer and autumn samples were re-fixed with acidified Lugol's iodine solution mixed on 20th November in a 20-L bottle by taking 4 times 2.5 L of the summer mixture and 2 times 2.5 L of the autumn sample. After carefully shaking the samples for ensuring equal distribution of the organisms, 100-mL subsamples were filled in clear glass bottles which were stored cool and dark until use.

At 11th September, samples were taken at the Baltic sampling site of Stralsund by the LUNG institute. They were fixed with acidified Lugol's iodine solution and filled in 2.5-L brown glass bottles which were transported to Oldenburg. On 20th November 2013, after a visual inspection at the microscope, 6 times 2.5 L of sample were mixed in a 20-L bottle. Then the sample was divided into 100-mL subsamples and stored cool and dark.

The BTU Cottbus took samples at the Scharmützelsee on the 2nd of October. They were fixed with acidified Lugol's solution, filled in 2.5-L brown glass bottles and shipped to AquaEcology. On 22nd November, 6 times 2.5 L were mixed and divided into subsamples of 100 mL each in clear glass bottles.

AquaEcology took samples in several lakes around Oldenburg and fixed them with acidified Lugol's iodine solution on 1st of October. On 22nd November 2013, visual inspection of the samples was carried out with the microscope. 9 L of the not enriched water sample from the pond ''Kennedyteich' was mixed with 1 L of a net haul sample of the 'lake 'Kleiner Bornhorster See'. Additionally 1 L tap water mixed with 10 mL acidified Lugol's iodine solution was added to enhance the fixation of the sample, which had a high biomass. The sample was mixed well and divided into subsamples of 100 mL in clear glass bottles, which were stored cool and dark.

All 100-mL glass bottles got numbers assigned from 1 to 100 for each series. In December, after inspection of the colour of the samples and a microscopical check of the cells, acidified Lugol's solution was added to all bottles to prevent a decrease of the quality.

In order to inform possible participants on the intercalibration exercise, a European-wide call was sent out, using different distribution lists. The period for registration lasted from mid of January until end of February 2014. Potential participants could register online. With the registering procedure, the participants created a username and a password for the online counting software programme 'OrgaCount', which was recommended to be used for the test.

On 30th February 2014, all samples were prepared for shipping. With the samples, each participant received a laboratory number in the range from 11 to 66, which was assigned randomly. The respective sample bottles were safely packed into parcels that were shipped to the participants by a trustworthy parcel service. The laboratory code, the Standard Document, the instructions and a guide for the software OrgaCount was sent to each participant by e-mail. Handling time for the participants lasted from the date of shipping (4th of March) to 11th of April 2014.

2.2 Reference values and performance of interlaboratory comparison

For reference measurements, seven samples from each series were randomly selected and analysed. For testing the stability of the samples, three additional samples were measured during the period the test was running for the participants. For all taxa, the dimensions of at least 30 cells were measured in order to calculate the biovolume. The maximum number of cells being measured was 50. All measurements were done with a magnification of 400 times. Cells were picked randomly and distributed over the whole

sedimentation chamber or in a stripe or counting fields, depending on size and abundance. For chain-forming species only one cell per chain was measured.

OrgaCount is an online software programme for counting taxa and calculating biovolume data for phytoplankton. Prior to the analysis, the programme had been modified for the special needs of the interlaboratory comparison test (see also the OrgaCount User Guide for the intercomparison test in Annex C). Participants had to enter some general information as the laboratory code, their environmental working field and the method used for measurements, which was either eyepiece micrometre or image analysis. The species had to be picked from of a list and the geometry had to be assigned. A correction factor could be assigned as well if necessary. First, the measurements had to be entered, then the programme calculated average, standard deviation, standard error and standard error in percent. A green field indicated when statistical needs were fulfilled and measurements could be stopped. Fields for comments were also implemented in the programme. Participants could additionally calculate deviating biovolumes with different geometries or methods than these to be found in the Standard Document (see also Instruction, OrgaCount User Guide). Data could be downloaded by participants and by AquaEcology. In addition to the User Guide, e-mail and phone support was provided for the handling of the programme. For participants that were not able or did not want to use the programme an excel file for entering the data was prepared and provided.

2.3 General analysis

The data of the participants were downloaded, checked and transferred to a special excel sheet for further analysis. For each species, an inventory of information on general features as measurement method, geometrical form selected and diverse specialties and errors that occurred was set up. In the course of the analysis, it turned out that at for almost all species at least one participant had confused the dimensions. In OrgaCount, the sequence and the equation was predefined and dimensions had to be applied correctly with the help of drawings of the geometrical bodies being offered for each species. This confusion could cause large errors in the determination of the biovolume for certain species. In these cases and when the confusion was obvious, the data were revised, the dimensions changed accordingly and the biovolumes recalculated to make them comparable. Recalculated data got a new labcode. For other cases also new labcodes were given to enable identification and comparison. When two methods of measurement were used, the eyepiece micrometer method got the annex ‘,1’ and the image analysis the annex ‘,2’, for example, the labcode number 99 thus would become 99,1 and 99,2 respectively. For other methods in general, other additional geometrical forms, or differing geometrical forms than requested in the Standard Document the annex ‘,3’ was added. The recalculated data of confused dimensions got the annex ‘,5’. For each species, the biovolume results were presented in a graphic where the reference data were shown as well.

2.4 Statistical analysis

All statistical analysis with regard to the assessment of the ring trial results have been carried out to according to the German standard DIN 38402-45, which has been available in the updated version from June 2014. The statistical analysis of homogeneity and stability of the test samples was based on ISO 13528.

2.4.1 Assessment of ring test results

Before applying any profound statistical analysis, all biovolume results had been grouped for the single species and graphically plotted against arithmetic and median mean values of all biovolume results for that species as well as against the respective reference value. By this, apparent total outliers, mostly caused by the participant confusing the dimensions or the assigned geometric body, became visible and could be eliminated in a first step.

In the next step, all statistical values such as *reproducibility standard deviation* s_R between the results of all laboratories as well as the *repeatability standard deviations* s_r within the results of each single laboratory, have been calculated applying the Q method. Based on the *reproducibility standard deviation* s_R, the HAMPEL estimation method has been used to calculate the *robust mean value* X_{HS} of the participants' results for each analysed species. Within this ring test exercise, this robust mean has not been used as the reference value. Instead the arithmetic mean of the reference samples (analysis of 7 parallel subsamples for each species, also being used for the homogeneity test) has been set as the *assigned reference value* $X_{assigned}$. From the *robust mean value* X_{HS}, the *relative reproducibility standard deviation* CV_R was calculated.

Based on this reference value and on the *reproducibility standard deviation* s_R as the standard target deviation, the corrected (or weighted) z_u score has been calculated for every laboratory and for every species. All laboratories with $|z_u| > 3$ have then been excluded from further analysis of the respective biovolume results. Afterwards, the results of the remaining laboratories have been used for the next calculation round. This process has been carried out repeatedly until no more values of $|z_u| > 3$ occurred in the dataset for each species. The data from this dataset has then been used for the calculation of the standard characteristics for each species in the ring trial.

2.4.2 Analysis of homogeneity of the test samples

In order to check the homogeneity of the biovolume results for each species, a test series of 7 reference subsamples had been taken from the original sample volume. Between 30 and 50 biovolume measurements have been performed on each sample. The standard deviation between these subsamples has been calculated as s_{Ref} according to the standard ISO 13528. From this, CV_{Ref} has been derived as the *relative standard deviation* between the homogeneity samples.

In a next step, the results have been checked against the homogeneity criterion of ISO 13528:

$CV_{Ref} / CV_{Target} \leq 0.3$

where the *relative target standard deviation* CV_{Target} was set as the *relative reproducibility standard deviation* from the ring test results: $CV_{Target} = CV_R$.

Only if this homogeneity criterion was fulfilled for the biovolume test samples for a species, the samples could be considered being homogeneous and an assessment of the participants' performance by determining the z_u scores should be statistically sound.

2.4.3 Analysis of the stability of the test samples

In order to check the stability of the biovolume samples for each species, a series of 3 subsamples had been taken from the original sample volume. These samples have been analysed at three different times: (i) before the ring test had started, (ii) sometimes in the middle of the ring test period, and (iii) in the end. Between 30 and 50 biovolume measurements have been performed on each sample. From the results, the *overall mean value* $mean_{Stab}$ was calculated.

This mean value has then been checked against the *reference mean value* $mean_{Ref}$ from the homogeneity analysis, following the criterion of ISO 13528:

$$\left| mean_{Ref} - mean_{Stab} \right| / s_R \leq 0.3$$

The *reproducibility standard deviation* s_R has been also used as the target standard deviation in this case.

Only if this stability criterion was fulfilled for the biovolume test samples for a species, the samples could be considered having been stable over the ring trial period and an assessment of the participants' performance by determining the z_u scores should be statistically sound.

2.4.4 Analysis of measurement method and expertise

In order to check the comparability of measurements based on different methods, i.e. eyepiece micrometre method vs. image analysis, as well as on different expertise of the participants in the respective environmental field, i.e. marine vs. freshwater expertise, t-tests have been carried out for the respective data groups for each species. For these analysis, a p-value < 0.05 indicated a significant statistical difference between the mean values of the respective data groups.

3 Results and Discussion

3.1 Participants

For the described intercomparison test, AquaEcology had received 56 registrations from 13 different countries: Denmark (5), Finland (4), France (1), Germany (20), Ireland (2), Italy (3), Lithuania (3), Norway (1), Poland (2), Portugal (3), Spain (2), Sweden (4), and United Kingdom (6). There had been 51 returns of results with various numbers of analysed samples and/or species. 28 participants delivered complete datasets for all samples and species. 14 participants omitted some samples due to lack of expertise either in the freshwater or in the marine environment. 9 participants omitted some species. The complete list of the participants can be found in Annex A.

3.2 Homogeneity tests

Table 1 lists the summarised results of the homogeneity tests. For these assessments, 7 randomly selected samples have been analysed for the biovolumes of the contained species. For every species, between 30 and 50 measurements of the required dimensions have been carried out.

The assessment results show that the criterion of ISO 13528 – requiring that the ratio of the standard deviation of the reference samples and the reproducibility standard deviation shall not exceed 0.3 – has been fulfilled in most cases. There have been two exceptions: *Ditylum brightwellii* had a ratio of 0.32 and thus, has been very much nearby the limit. *Ditylum* was the worst fixed species and very variable in size. The second species failing to meet the homogeneity criterion has been *Aulacoseira granulata*. Possibly two different species of *Aulacoseira* (a slightly larger and a slightly smaller one) which are difficult to distinguish were present in the sample. Some participants may have discriminate between both species.

Table 1: Assessment of homogeneity for 7 reference biovolume samples; according to ISO 13528 the homogeneity for each species was calculated as the ratio of the relative standard deviation of the reference samples and the relative reproducibility standard deviation. Values which exceeded the maximum criterion for homogeneity of 0.3 have been marked in red. Description of statistical parameter see chapter 2.4.2.

	Reference samples			Homogeneity
Species	**mean $_{Ref}$**	**s_{Ref}**	**CV_{Ref} %**	**CV_{Ref}/CV_R**
Dactyliosolen fragilissimus	6,824	415	6.08%	0.17
Dinophysis acuminata	16,718	1,143	6.84%	0.17
Ceratium tripos	109,160	6,553	6.00%	0.15
Pseudo-nitzschia cf.pungens	1,355	185	13.65%	0.29
Thalassionema nitzschioides	490	52	10.65%	0.26
Rhizosolenia imbricata	13,980	1,619	11.58%	0.25
Ditylum brightwellii	24,055	3,298	13.71%	0.32
Stephanopyxis turris	99,642	3,162	3.17%	0.07
Odontella sinensis	1,785,462	74,069	4.15%	0.08
Chaetoceros debilis	1,022	68	6.64%	0.13
Fragilaria crotonensis	688	64	9.29%	0.17
Tabellaria fenestrata	2,111	253	11.97%	0.24
Aulacoseira granulata	680	112	16.46%	0.46
Cryptomonas erosa	1,914	74	3.88%	0.11
Rhodomonas lacustris	102	5	4.60%	0.14
Planktothrix agardhii	866	19	2.17%	0.06
Monoraphidium arcuatum	64	4	6.82%	0.14
Woronichinia naegeliana	24	2	7.88%	0.20
Cosmarium ocellatum	5,820	230	3.96%	0.12
Trachelomonas hispida	6,410	381	5.94%	0.21

3.3 Stability tests

For the stability test, 3 samples have been analysed for the biovolume values of all species at different times: The first sample was analysed at the beginning of the counting phase, the second one amidst that phase, and the third one at the end of the ring trial. The results have been assessed according to the requirements of ISO 13528. Table 2 summarises the assessment result. It becomes obvious that for all samples and analysed species the stability criterion – the difference of mean values of reference samples and stability samples divided by the respective reproducibility standard deviation shall not exceed the value of 0.3 – has been met in all cases.

Table 2: Assessment of stability for 3 stability biovolume samples; according to ISO 13528 the stability for each species was calculated as the difference of the mean value of the reference sample and the mean value of the stability sample divided by the reproducibility standard deviation. There were no values exceeding the maximum criterion of 0.3. Description of statistical parameter see chapters 2.4.2 and 2.4.3.

	Reference samples			Stability	
Species	**mean $_{Ref}$**	**s_{Ref}**	**CV_{Ref} %**	**$mean_{Stab}$**	**$(mean_{Ref}-mean_{Stab})/s_R$**
Dactyliosolen fragilissimus	6,824	415	6.08%	6,224	0.23
Dinophysis acuminata	16,718	1,143	6.84%	18,040	0.19
Ceratium tripos	109,160	6,553	6.00%	106,497	0.08
Pseudo-nitzschia cf.pungens	1,355	185	13.65%	1,232	0.24
Thalassionema nitzschioides	490	52	10.65%	549	0.27
Rhizosolenia imbricata	13,980	1,619	11.58%	13,278	0.09
Ditylum brightwellii	24,055	3,298	13.71%	22,277	0.21
Stephanopyxis turris	99,642	3,162	3.17%	102,540	0.08
Odontella sinensis	1,785,462	74,069	4.15%	1,701,181	0.11
Chaetoceros debilis	1,022	68	6.64%	886	0.29
Fragilaria crotonensis	688	64	9.29%	682	0.02
Tabellaria fenestrata	2,111	253	11.97%	2,176	0.07
Aulacoseira granulata	680	112	16.46%	603	0.28
Cryptomonas erosa	1,914	74	3.88%	1,813	0.15
Rhodomonas lacustris ….	102	5	4.60%	104	0.04
Planktothrix agardhii	866	19	2.17%	896	0.09
Monoraphidium arcuatum	64	4	6.82%	68	0.19
Woronichinia naegeliana	24	2	7.88%	23	0.09
Cosmarium ocellatum	5,820	230	3.96%	5,710	0.07
Trachelomonas hispida	6,410	381	5.94%	6,776	0.21

3.4 Species statistics

In the following the results for each species are presented and discussed. For each species an inventory of the main results is summarised in a table (see e.g. Table 3). In the first part general aspects about the participants and their measurement method is

shown. In the second part information about geometry and dimensions with conspicuities are given. The third part shows general statistical aspects as the fulfilment of the minimum requirements for the measurements concerning the Standard Document.

Furthermore, the results and some statistical aspects are visualised graphically for the biovolume (see e.g. Figure 2) and single dimensions. The rhombic symbols for the mean values will appear in blue when the measurements had been done by eyepiece micrometre, and in pink when done by image analysis. The values are shown for each lab code with standard error bars and the standard error in percent given as a number (red coloured if it exceeds 10 %). The expertise for the respective environmental field is marked by a square around the symbol of the mean value. When values were out of range, this is marked by an arrow pointing to the direction of the outlier and the mean value is given. The robust mean of all participants is indicated by a blue line. The reference and stability values are given by red and green solid lines respectively. The standard error for reference and stability data is shown by dashed lines in red and green. z_u scores are represented by orange lines. In some cases, additional information is included and described in the text but also shown in the legend of the figure.

We used a German programme for creating the graphics. Consequently, all figures in this chapter show a full stop as a thousand separator in the y-axis label and if values are marked as outliers for biovolume. A comma is used as a decimal mark for the standard error values in percent and for the z_u scores.

The z_u scores are shown for each lab code in a separate figure (see e.g. Figure 5). The participants' z_u scores are represented by blue (when negative) and yellow (when positive) bars with the values displayed at the bar as well. Green lines indicate a z_u score (absolute value) of 1 and red lines a z_u score (absolute value) of 2, which is the limit for successful passing of the test. All laboratories having a score of $|z_u| > 3$ have been excluded from further analysis for the respective species.

3.4.1 *Dactyliosolen fragilissimus*

Dactyliosolen fragilissimus is a cylindrical species forming chains (Figure 1). All participants that measured this species have correctly assigned the cylinder as geometrical shape (Table 3).

Three participants have apparently confused diameter and height of the cylinder. In the equation for a cylinder, it will be important to apply the dimensions correctly because the diameter goes into the equation exponentially. Thus, confusions mixing-up dimensions will have a huge effect on the biovolume value and cause errors. It might be that the confusions occurred only in OrgaCount, where the calculations had been done automatically. By manually filling in the equations, the users might have applied the dimensions correctly. Nevertheless, OrgaCount provided a figure for each geometrical shape. The coloured dimensions in this three-dimensional shape form corresponded directly with the respective coloured equation terms and thus, enable easy identification. Only the transfer from the orientation of the cell in the microscope to the geometry in OrgaCount had to be done by the user. Indeed, some participants have confirmed that the sequences for the dimensions in OrgaCount had been different from these in their

routine sheets or programmes. Additionally, deviant names for the dimensions had been used in some cases. However, routine procedures have to be corrected or changed when not being in accordance with standard procedures any longer, and this might be necessary when strictly applying the Standard Document. With regard to confusion of dimensions, confused data have been revised and recalculated, whenever obvious and possible.

Table 3: Inventory of the main results (except special statistics) for *Dactyliosolen fragilissimus*.

General			
44	participants (labcode) have measured this species: 12 13 14 15 16 17 18 20 21 23 24 25 26 27 29 30 31 33 34 36 37 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
28	measurements with an eyepiece micrometre		
19	measurements with image analysis software		
Geometry and Dimensions			
45	measurements/calculations with a 'cylinder' assigned as geometrical shape according to the Standard Document		
45	calculations with no correction factor used according to the Standard Document		
3	calculations with 'diameter' and 'height' being confused		
Statistics			
20-53	units have been measured		
	0	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	20	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		0	measurement with the biovolume standard error being ≥ 10 %
	0	total measurements with the biovolume standard error being ≥ 10 %	

Figure 1: Microphotographic picture of the chain forming *Dactyliosolen fragilissimus* in the Stralsund sample.

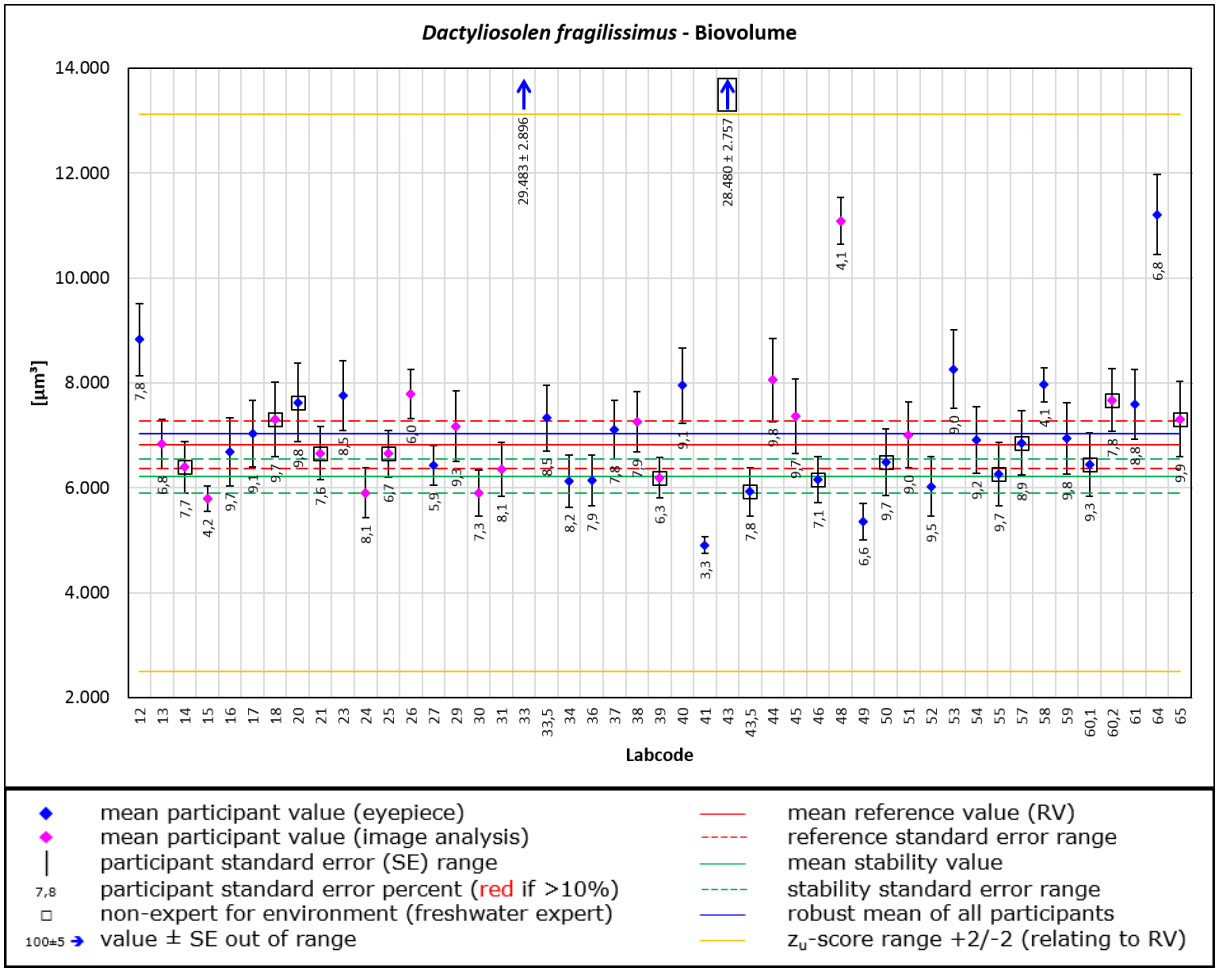

Figure 2: Results and some statistical characteristics for biovolume of *Dactyliosolen fragilissimus*. Please note: Comma used as decimal mark, full stop used as thousands separator.

The values of the participants ranged from 5,000 to 11,000 µm³. The mean of the participants was close to the mean of reference data. The cells are variable in size; however, in all of the measurements the standard error in % was lower than 10 % (Figure 2). Single dimensions showed a similar pattern than biovolumes (Figure 3, Figure 4), but it becomes obvious that the variance, especially for the outliers, was mainly produced by the diameter. Almost all of the participants' results for the biovolume were within the requested z_u range from -2 to +2. There have been 2 outliers due to confusion of dimensions. The same holds principally for the results for the different dimensions.

All participants passed the test for this species (Figure 5). Most of the participants, which successfully have passed the repeated z_u assessment procedures, lay within a z_u range from -1 to +1. This reflects a very good performance for the biovolume analysis of this species.

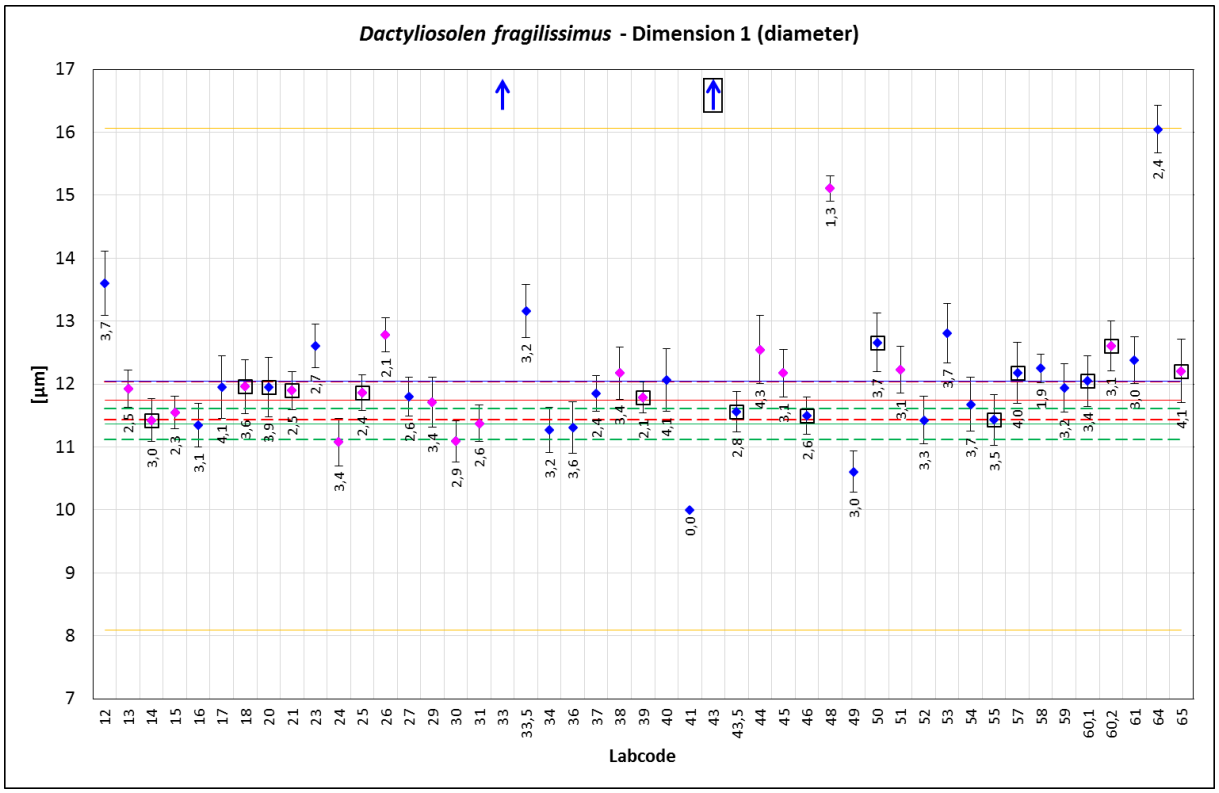

Figure 3: Results and some statistical characteristics for dimension 1 (diameter) of *Dactyliosolen fragilissimus*. Legend see biovolume chart. Please note: Comma used as decimal mark.

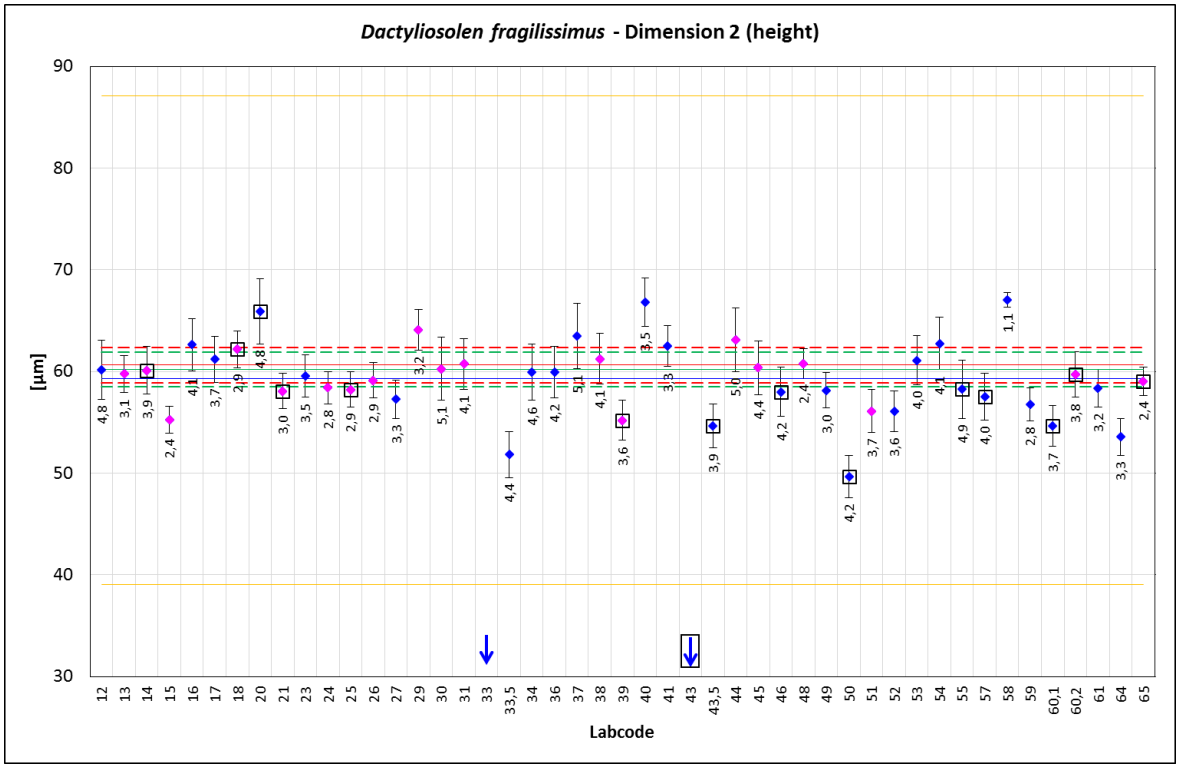

Figure 4: Results and some statistical characteristics for dimension 2 (height) of *Dactyliosolen fragilissimus*. Legend see biovolume chart. Please note: Comma used as decimal mark.

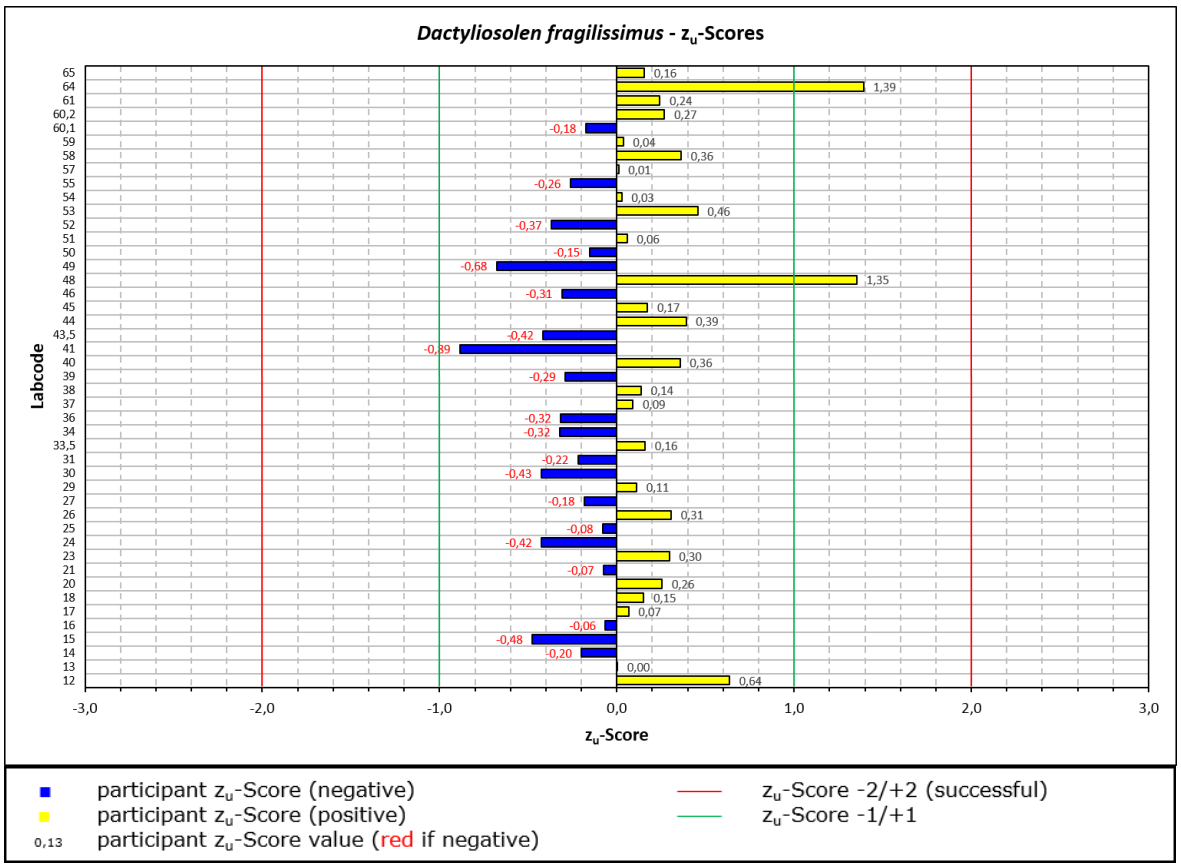

Figure 5: z_u scores of participants for *Dactyliosolen fragilissimus*. Please note: Comma used as decimal mark.

3.4.2 *Dinophysis acuminata*

Dinophysis acuminata is a dinoflagellate with an epitheca and a sulcal wing (Figure 6). All participants that measured this species have correctly used the 'ellipsoid' as geometrical shape according to the Standard Document (Table 4). One participant has used the same value for both diameters but by applying a correction factor to the formula the biovolume values have been calculated correctly in the end. However, for this species no correction factor should have been applied according to the Standard Document. The correction factors in the Standard Document are factors that shall adjust the real form of the species to the best fitting geometrical shape in case it deviates. The factor used by the participant obviously has been a correction factor for the hidden dimension as used in HELCOM. Both factors, the one for the geometry and the other for the hidden dimension shall not be mixed. In the revised taxa list for the standard document both factors have been implemented and are described. That should avoid confusion.

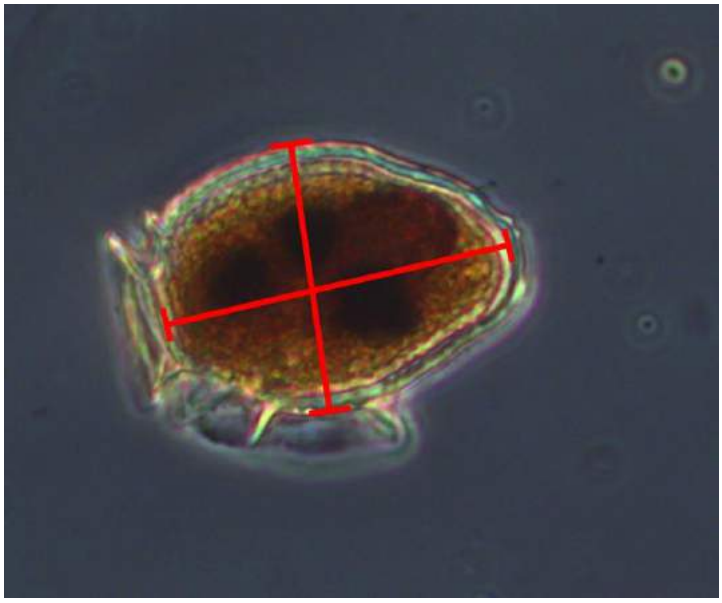

Figure 6: Microphotographic picture of *Dinophysis acuminata* in the Stralsund sample. Red lines display from where to where the dimensions are to measure.

Nevertheless, the hidden dimension is an important topic. The participants should have applied the method they normally use for the hidden dimension (HD). There are several methods for this. Instead of factors that relate the hidden dimension to another dimension, constants can be used, especially referring to literature data. They can be measured either by focussing or by measuring other cells where it is possible to see the respective dimension. It turned out that all of these methods have been applied. However, the data of the participants have been used to calculate a hidden dimension factor for each participant to make it comparable to reference data. For *Dinophysis* the hidden dimension is represented by the 'small diameter' (Figure 9) of the 'ellipsoid' and has been calculated for reference data by applying a factor of 0.6 to the 'large diameter'. The range of the participants calculated the same way lay between 0.3 and 0.98, but the mean was 0.61 (Table 3).

Table 4: Inventory of the main results (except special statistics) for *Dinophysis acuminata.*

General			
43	participants (labcode) have measured this species: 12 13 14 15 16 17 18 20 21 23 24 25 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
25	measurements with an eyepiece micrometre		
19	measurements with image analysis software		
Geometry and Dimensions			
44	measurements/calculations with a 'ellipsoid' assigned as geometrical shape according to the Standard Document		
	1	measurement/calculation with a 'ellipsoid' assigned as geometrical shape according to the Standard Document but using the same values for 'large diameter' as for 'small diameter' which effectively is a 'prolate spheroid'	
43	calculations with no correction factor used according to the Standard Document		
1	calculation with a correction factor of 0.67 used notwithstanding to the Standard Document		
3	calculations with 'small diameter' or 'large diameter' and 'height' being confused		
Statistics			
20-50	units have been measured		
	0	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	31	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		1	measurement with the biovolume standard error being ≥ 10 %
	1	total measurements with the biovolume standard error being ≥ 10 %	

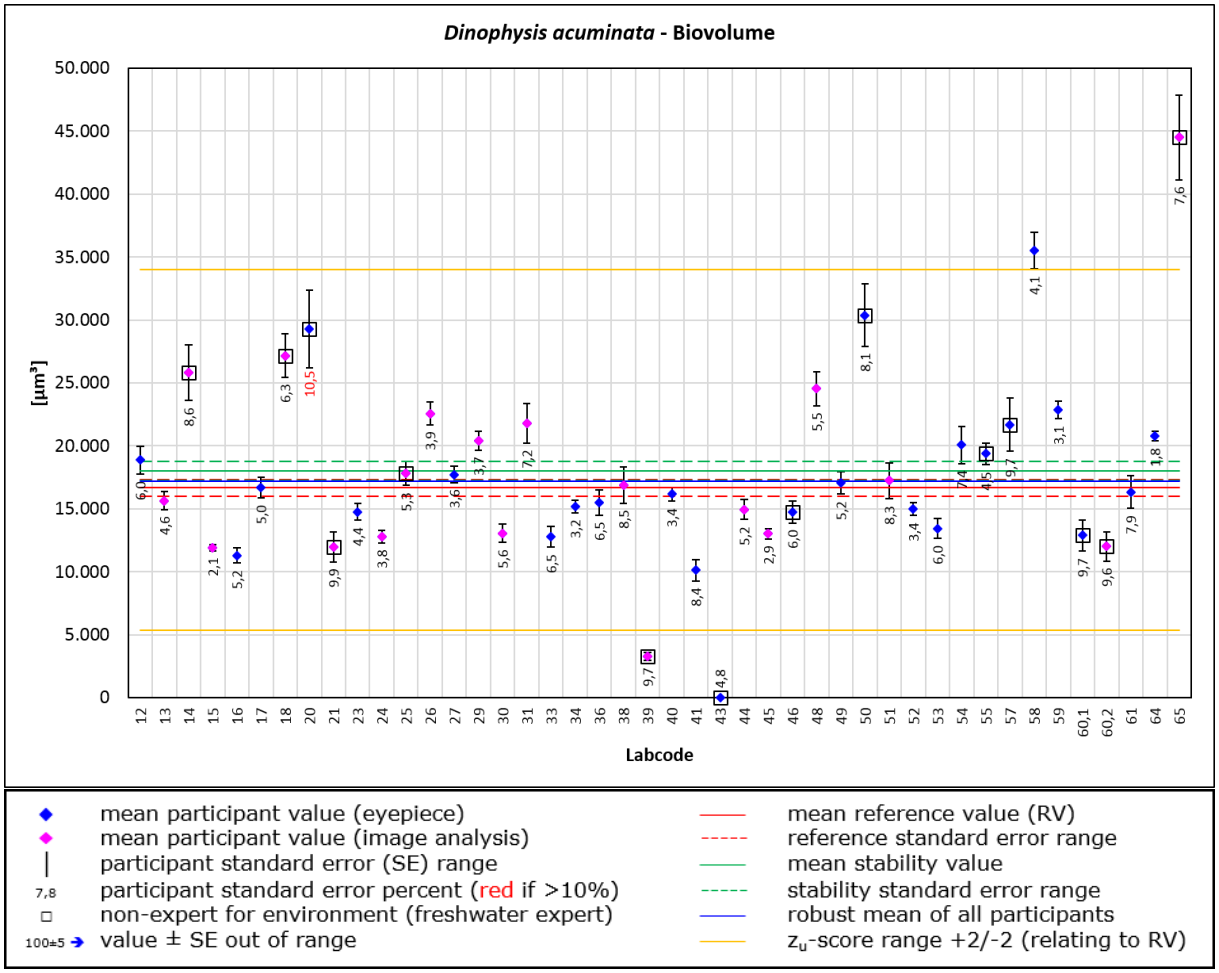

Figure 7: Results and some statistical characteristics for biovolume of *Dinophysis acuminata*. Please note: Comma used as decimal mark, full stop used as thousands separator.

The biovolume values calculated by the participants grouped around the reference biovolume data and ranged from 10,000 to 30,000 µm³ with some outliers, especially for higher biovolumes (Figure 7). This species is an example that sometimes it is necessary to define very clearly, from where to where a dimension has to be measured. In this case, the epitheca and the sulcal wing shall not be included in the measurement (Figure 6). Outliers to higher values may reflect the fact that some participants have included at least parts of the epitheca and the sulcal wing. Checks on the values for the single dimensions have supported that at least for the epitheca (Figure 8, Figure 10), but largely the outliers seemed to have been caused by the hidden dimension. Almost all of the participants' results for the biovolume were within the requested z_u range from -2 to +2. There have been 2 outliers lying above and 2 outliers lying below that range.

The z_u score values showed a high variability for this species (Figure 11). One outlier with a z_u score out of the range from -3 to 3 had been excluded before further analysis (Table 26). Eight of the laboratories which successfully have passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. Three of these laboratories even missed the z_u range from -2 to +2, did not pass the test and thus, produced questionable results for this species. This reflected a good to moderate performance for the biovolume analysis of this species.

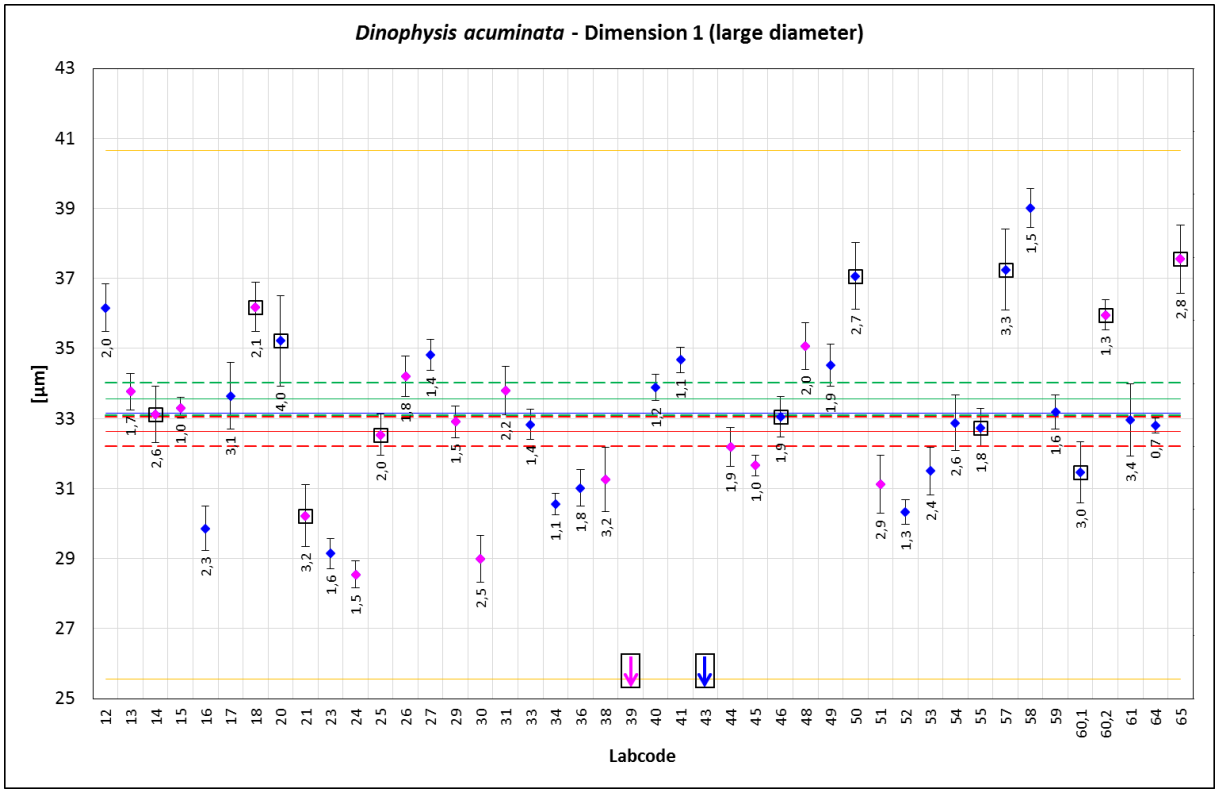

Figure 8: Results and some statistical characteristics for dimension 1 (large diameter) of *Dinophysis acuminata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

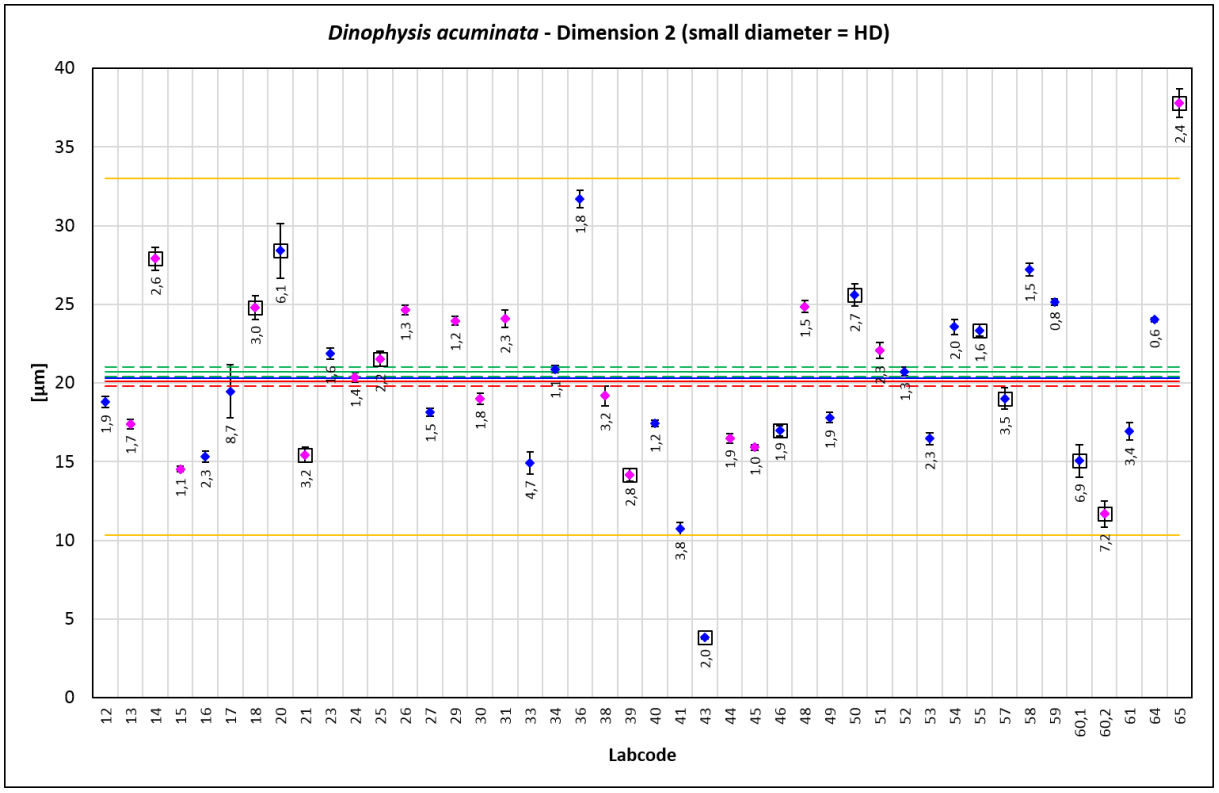

Figure 9: Results and some statistical characteristics for dimension 2 (small diameter = HD) of *Dinophysis acuminata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

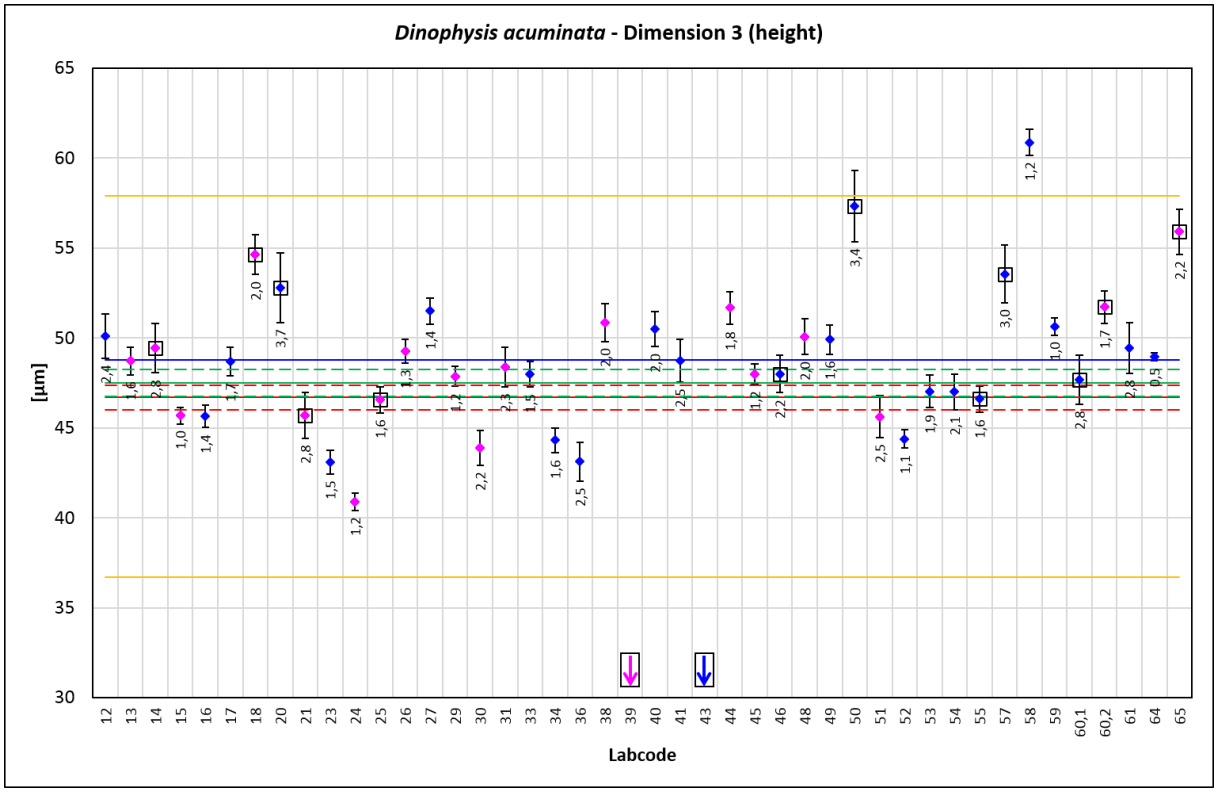

Figure 10: Results and some statistical characteristics for dimension 3 (height) of *Dinophysis acuminata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

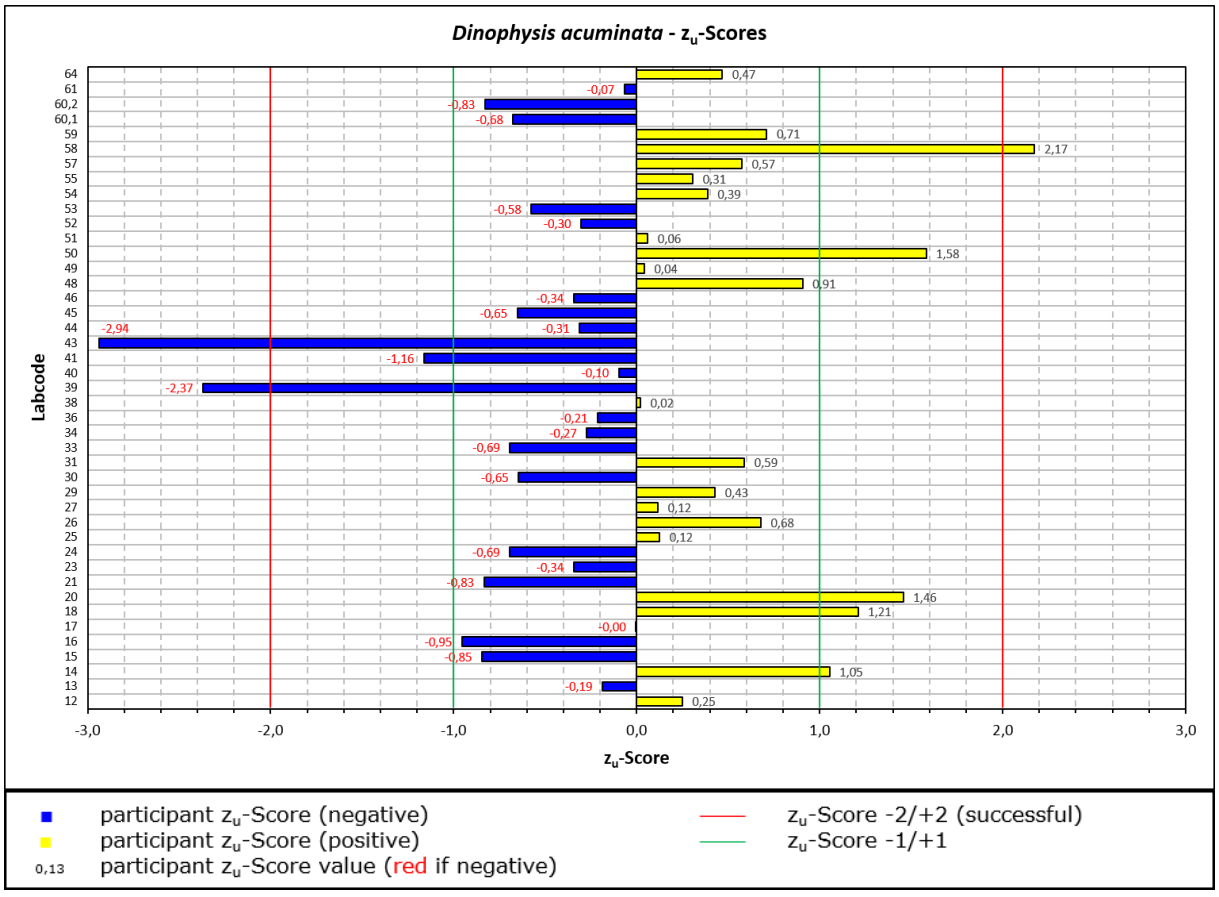

Figure 11: z_u scores of participants for *Dinophysis acuminata*. Please note: Comma used as decimal mark.

3.4.3 *Ceratium tripos*

Ceratium tripos is a larger dinoflagellate with a complicated shape because of its horns and flattened body. It is still under discussion which geometrical form shall be assigned to this organism. So far it had been decided to use the 'elliptic cone with half ellipsoid' (Figure 12), which almost all participants have correctly assigned (Table 5). Two participants had assigned the 'girdle diameter' from HELCOM, which is another approach. It had been possible to compare both calculations within this intercomparison test. Further inspection of data had shown that the 'girdle diameter' fit well in with the data of the 'elliptic cone with half ellipsoid'. Reference data for both calculations did support that as well (Figure 13). Differences in measurements (Figure 14, Figure 15 and Figure 16) and biovolume seem to have been caused mainly by one dimension, the height of the elliptic cone and the question at what position the measurement at the upper horn shall stop (Figure 16, Figure 12). Species comparable to this definitely need a precise description (and decision) where measurements for dimensions shall start and end. Alternatively, in case of *Ceratium tripos*, the 'girdle diameter' can be used, which seems to fit in well and will eliminate this problem. This is implemented in the Standard Document now. However, this approach exists for some but not for all similar species and the question how to measure needs to be answered for several more species.

Figure 12: Microphotographic picture of *Ceratium tripos* in the Stralsund sample. In red the 'elliptic cone with half ellipsoid' is placed on the cell and the extremes of this geometrical shape are shown, depending on how the height is measured.

Three participants had used a correction factor. Probably, this had been meant to serve as a factor for the hidden dimension. In contrast, the correction factor described in the Standard Document is used for adjusting the biovolume of the real shape of the cell to the biovolume of the assigned geometrical shape when no better geometry is available. Both proceedings shall not be mixed. For reference data, the hidden dimension represented by the small diameter had been calculated by applying a factor of 0.65 to the large diameter. The calculated factors of the participants reached from 0.2 to 1.83 with 0.6 as mean value (Table 25).

Table 5: Inventory of the main results (except special statistics) for *Ceratium tripos.*

General			
44	participants (labcode) have measured this species: 12 13 14 15 16 17 18 20 21 23 24 25 26 27 29 30 31 33 34 36 37 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
26	measurements with an eyepiece micrometre		
19	measurements with image analysis software		
Geometry and Dimensions			
43	measurements/calculations with an 'elliptic cone with half ellipsoid' assigned as geometrical shape according to the Standard Document		
2	measurements/calculations with a 'girdle diameter' assigned as geometrical shape notwithstanding to the Standard Document		
42	calculations with no correction factor used according to the Standard Document		
3	calculations with a correction factor of 0.8 used notwithstanding to the Standard Document		
1	calculation with 'height of half ellipsoid' and 'height of cone' being confused		
Statistics			
9-50	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	28	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		0	measurements with the biovolume standard error being ≥ 10 %
	0	total measurements with the biovolume standard error being ≥ 10 %	

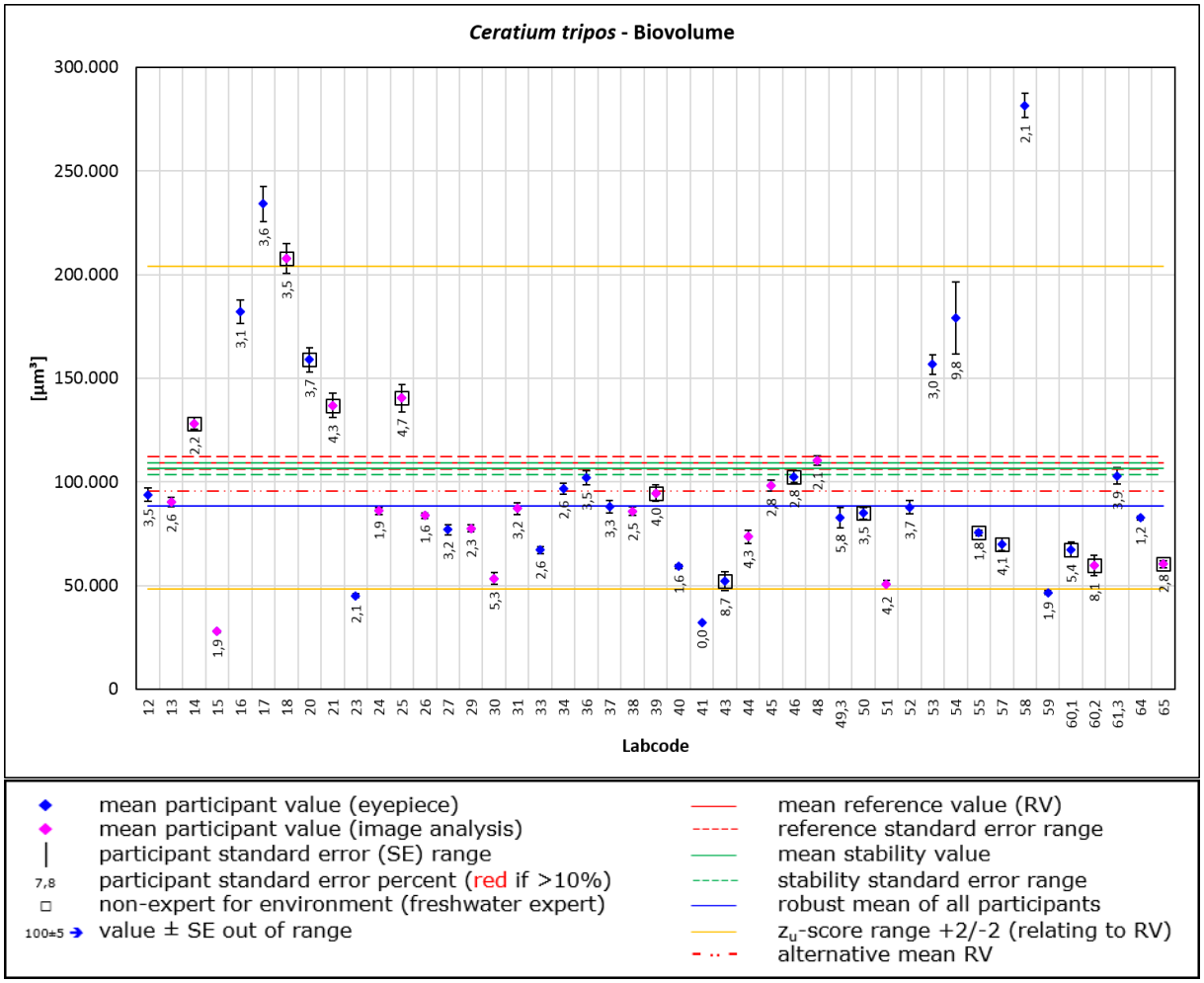

Figure 13: Results and some statistical characteristics for biovolume of *Ceratium tripos*. Please note: Comma used as decimal mark, full stop used as thousands separator.

Biovolume data of the participants had a wide range from 30,000 to 250,000 µm³ (Figure 13). Most of the participants' results for the biovolume were within the requested z_u range from -2 to +2. Furthermore, most result lay below the reference value. There have been 4 outliers lying above and 2 outliers lying below that range.

The z_u score values were mainly negative (Figure 17). 21, i.e. 50 % of the laboratories which successfully have passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. Six of these laboratories even missed the z_u range from -2 to +2, did not pass the test and thus, produced questionable results for this species. Additionally, one participant missed the z_u range from -3 to +3. The z_u scores depend on the reference data. Therefore the difference between reference and participants' data described above was a reason for this result. However, reference measurements have been done in a very careful way and thoughtful method to have a reliable base for comparison. Thus, these results reflect a moderate performance for the biovolume analysis of this species.

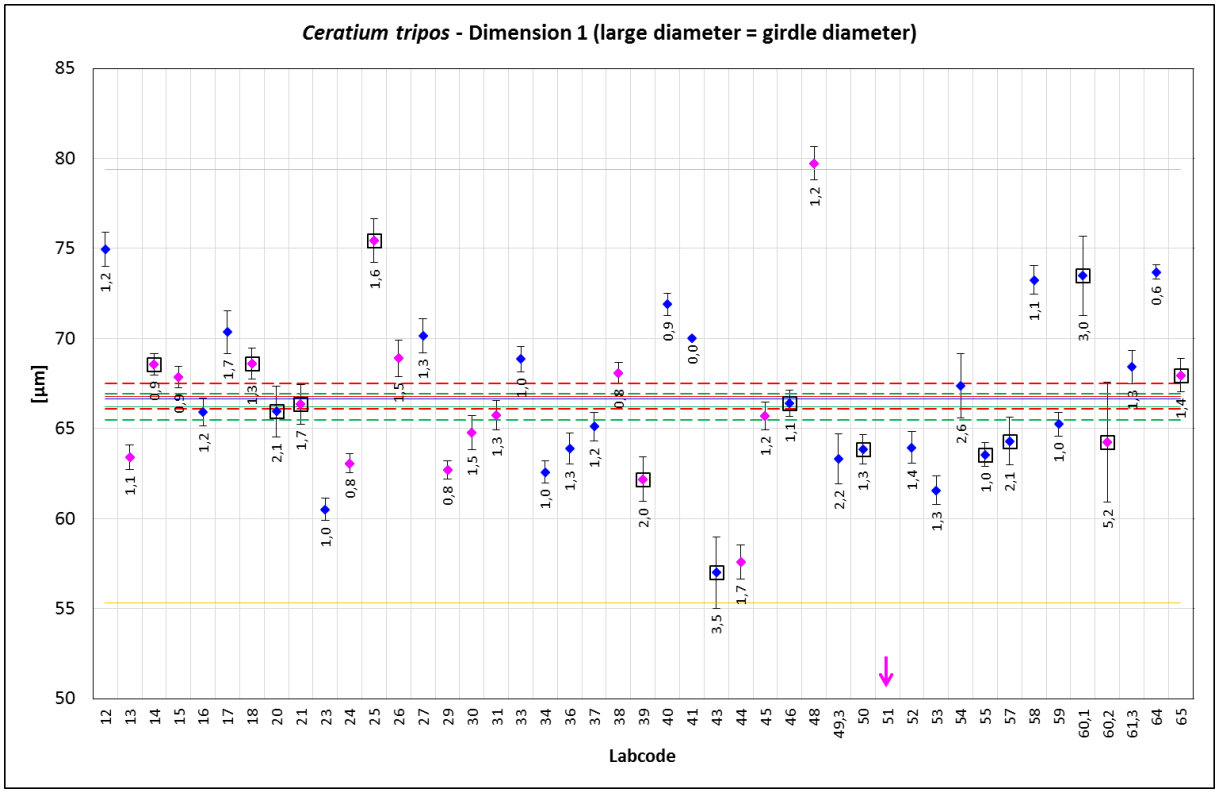

Figure 14: Results and some statistical characteristics for dimension 1 (large diameter = girdle diameter) of *Ceratium tripos*. Legend see biovolume chart. Please note: Comma used as decimal mark.

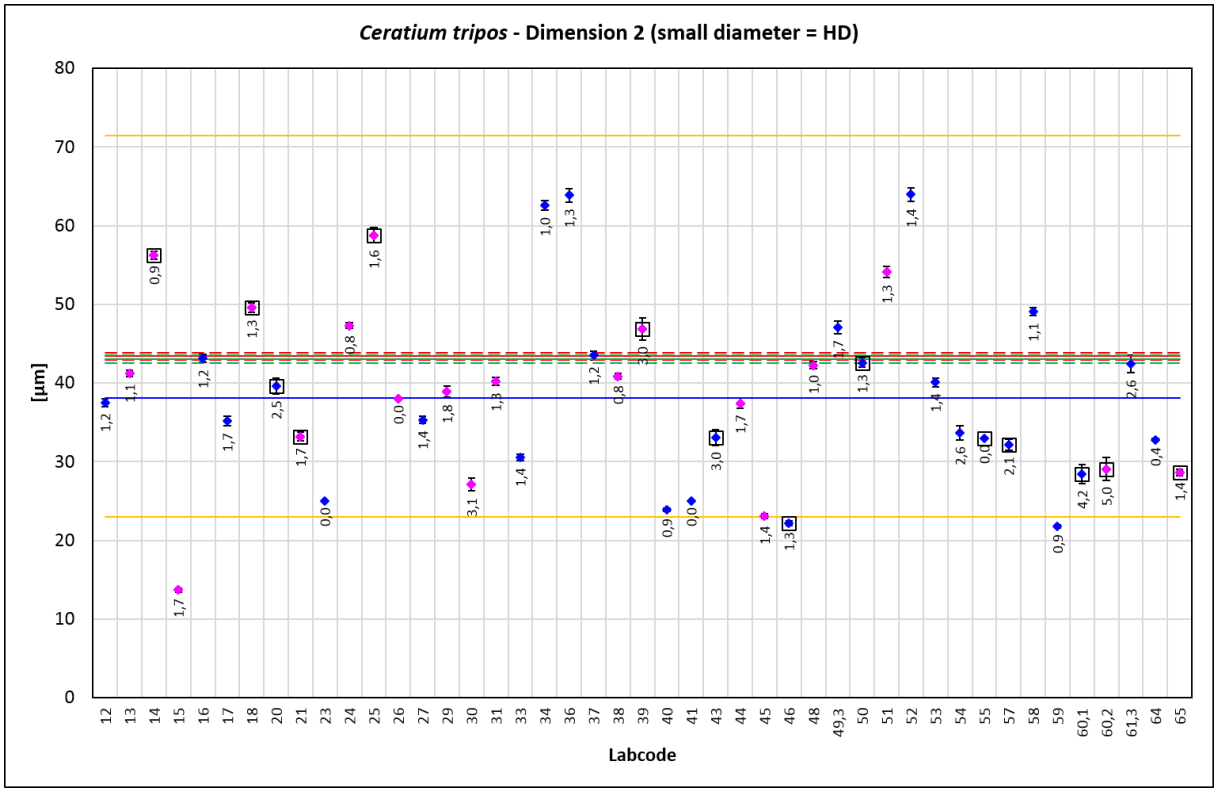

Figure 15: Results and some statistical characteristics for dimension 2 (small diameter = HD) of *Ceratium tripos*. Legend see biovolume chart. Please note: Comma used as decimal mark.

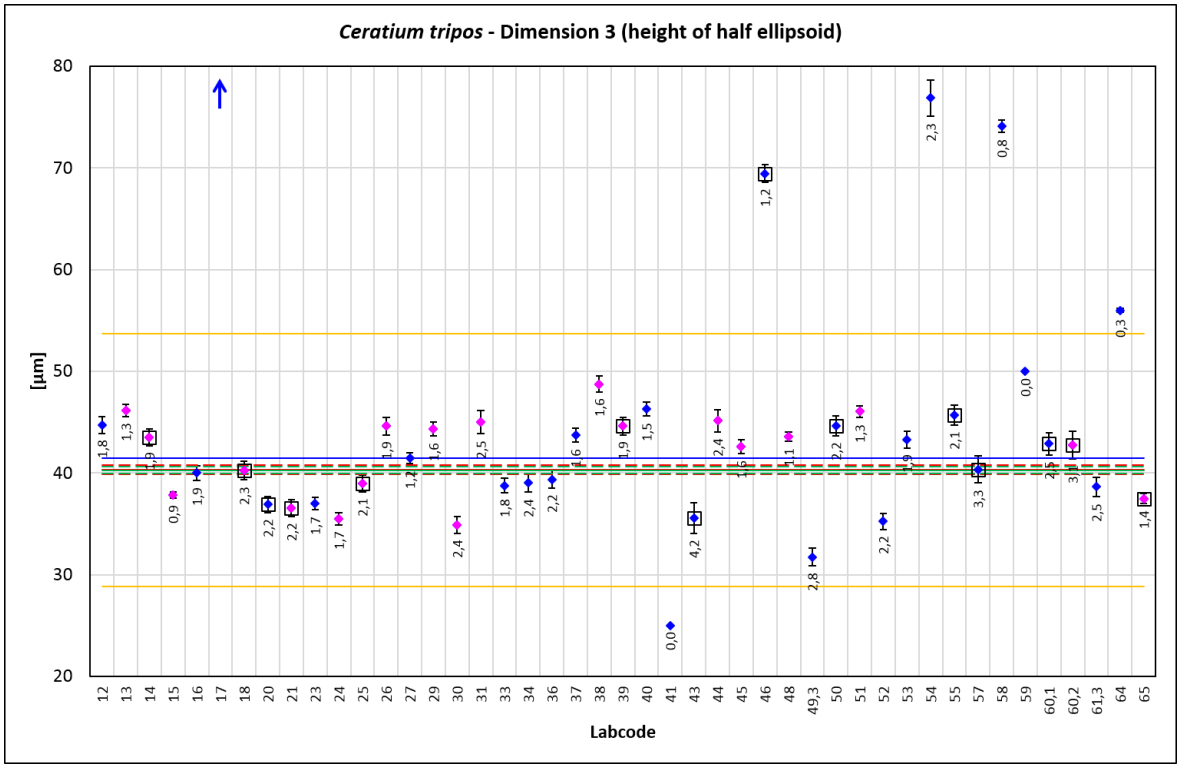

Figure 16: Results and some statistical characteristics for dimension 3 (height of half ellipsoid) of *Ceratium tripos*. Legend see biovolume chart. Please note: Comma used as decimal mark.

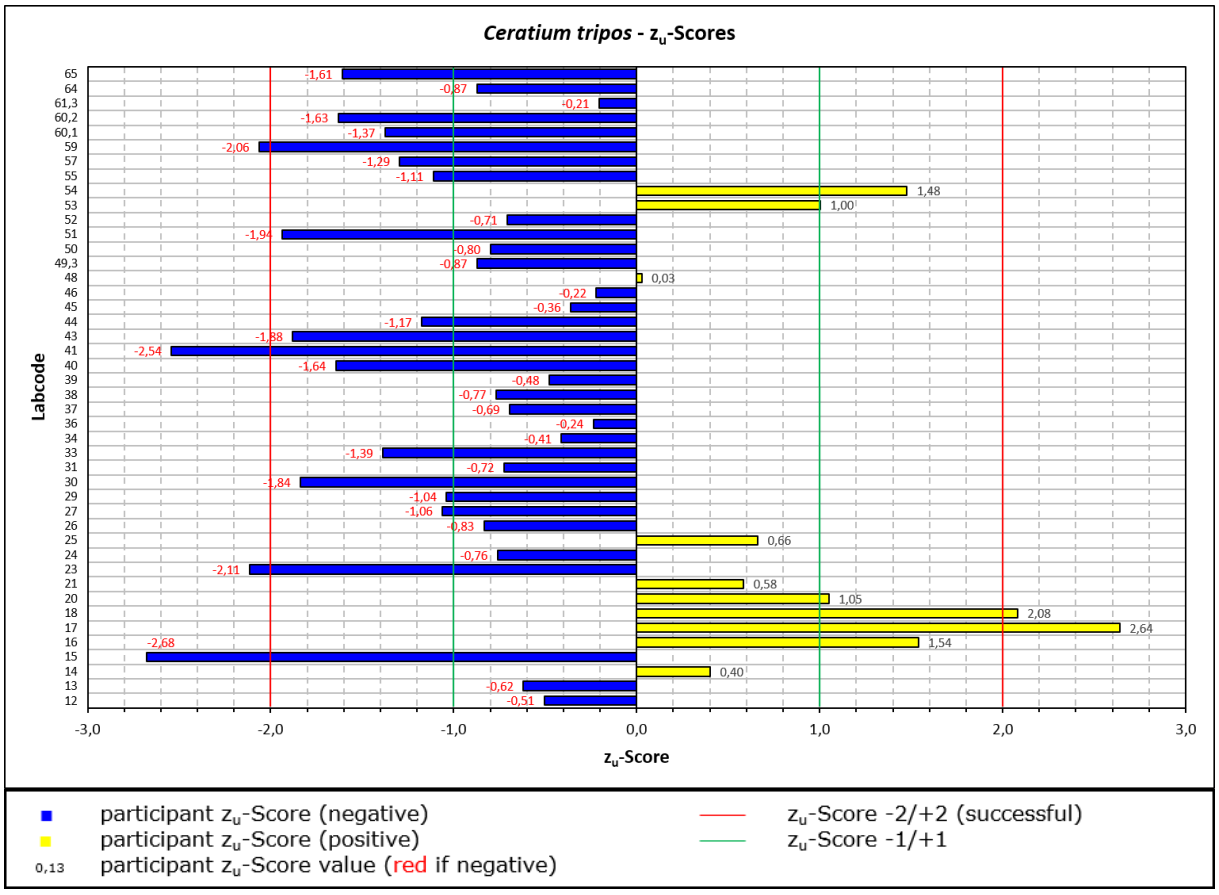

Figure 17: z_u scores of participants for *Ceratium tripos*. Please note: Comma used as decimal mark.

3.4.4 *Pseudo-nitzschia* cf. *pungens*

Pseudo-nitzschia cf. pungens is a marine chain-forming diatom (Figure 18). The chains are built with overlapping ends of the cells. The orientation of single cells cannot be identified, because the ends are pointed at all sides. For that reason, a correction factor should be applied for this species, which is implemented in the Standard Document now. Almost all participants that have measured this species have correctly assigned the 'rhombic prism' as geometrical shape using no correction factor (Table 6). One participant applied the non-correct but similar 'elliptic cylinder' and two participants compared with the 'parallelepiped' form of HELCOM. The comparison has shown the deviation of the geometry recommended by the Standard Document to the special HELCOM geometry which represents a cuboid. The calculations with the 'elliptic cylinder' have generated lower values compared to the average. The biovolume values have been variable anyway. They ranged from 500 to more than 2,500 µm^3 (Figure 19). A reason for this may have been the bad quality of the cells. Due to an alternative preservation for this sample, the cells had been bending, an artefact that has possibly hampered the measurements, but may occur in real samples as well. Additionally, the species has been in dividing stage. This and possible different selection criteria of the participants may have led to the high variability in the results.

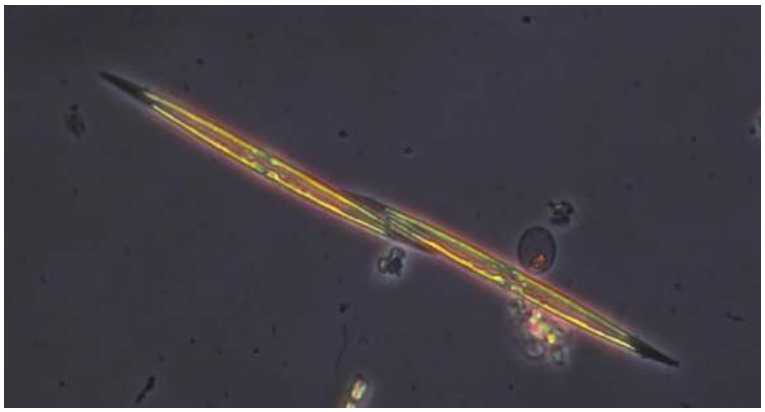

Figure 18: Microphotographic picture of the chain forming *Pseudo-nitzschia* cf. *pungens* in girdle view in the Helgoland sample.

If all dimensions are correctly assigned, the hidden dimension for this species will be the 'small diagonal' of the prism. This will be ensured only if several selection criteria are used. For single cells, the orientation cannot be detected in the light microscope using magnifications of the routine monitoring, because the cells look very similar in girdle and valve view. Therefore, only cells in colonies that lie flat should be selected for the measurement. Because overlapping parts may hinder the identification of the tip of the cell, colonies in girdle view are best for correct measurements. For reference data, the hidden dimension is the 'small diagonal', which has been set to have the same dimension as the 'height'. Calculating the relation for the data of the participants the values range from 0.59 to 2 with a mean of 1.25 (Table 25). However, it is not clear, which dimension was the hidden one in the measurements of the participants. The 'small diameter' and the 'height' are both potential hidden dimensions (Figure 21, Figure 22).

Table 6: Inventory of the main results (except special statistics) for *Pseudo-nitzschia* cf. *pungens.*

General		
42	participants (labcode) have measured this species: 12 13 14 15 16 17 18 20 21 23 24 25 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 65	
25	measurements with an eyepiece micrometre	
19	measurements with image analysis software	
Geometry and Dimensions		
41	measurements/calculations with a 'rhombic prism' assigned as geometrical shape according to the Standard Document	
	2	measurements/calculations with a 'parallelepiped' assigned as geometrical shape notwithstanding to the Standard Document (according to HELCOM PEG-list) for comparison
1	measurement/calculation with a 'elliptic cylinder' assigned as geometrical shape notwithstanding to the Standard Document	
44	calculations with no correction factor used according to the Standard Document	
4	calculations with' large diagonal' and 'height' being confused	
Statistics		
20-50	units have been measured	
	0	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)
	26	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)
	2	measurement with the biovolume standard error being ≥ 10 %
	4	total measurements with the biovolume standard error being ≥ 10 %

The data of the participants grouped well around reference data for biovolume (Figure 19) and all dimensions (Figure 20, Figure 21 Figure 22). However, it depends on the selection criteria and assignment of dimensions by the participants if data for single dimensions are comparable. Most of the participants' results for the biovolume were

within the requested z_u range from -2 to +2. There have been one outlier lying above and one outlier lying below that range.

The z_u scores had mainly negative values but showed a high variability. 22, i.e. 50 % of the laboratories which successfully had passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. Two participants did not pass the test for this species (Figure 23), they even missed the z_u range from -2 to +2. This reflects a moderate performance for the biovolume analysis of this species.

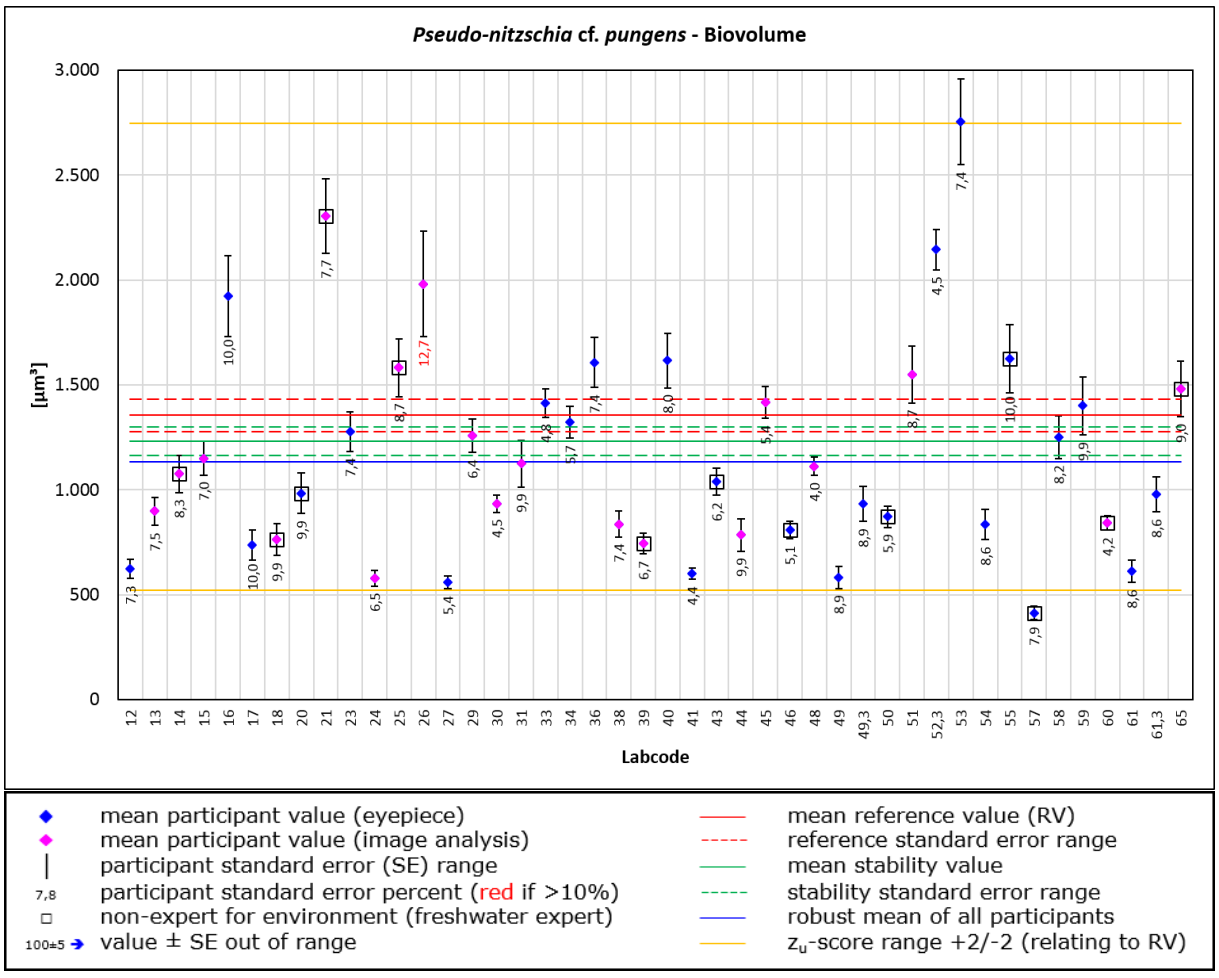

Figure 19: Results and some statistical characteristics for biovolume of *Pseudo-nitzschia* cf. *pungens*. Please note: Comma used as decimal mark, full stop used as thousands separator.

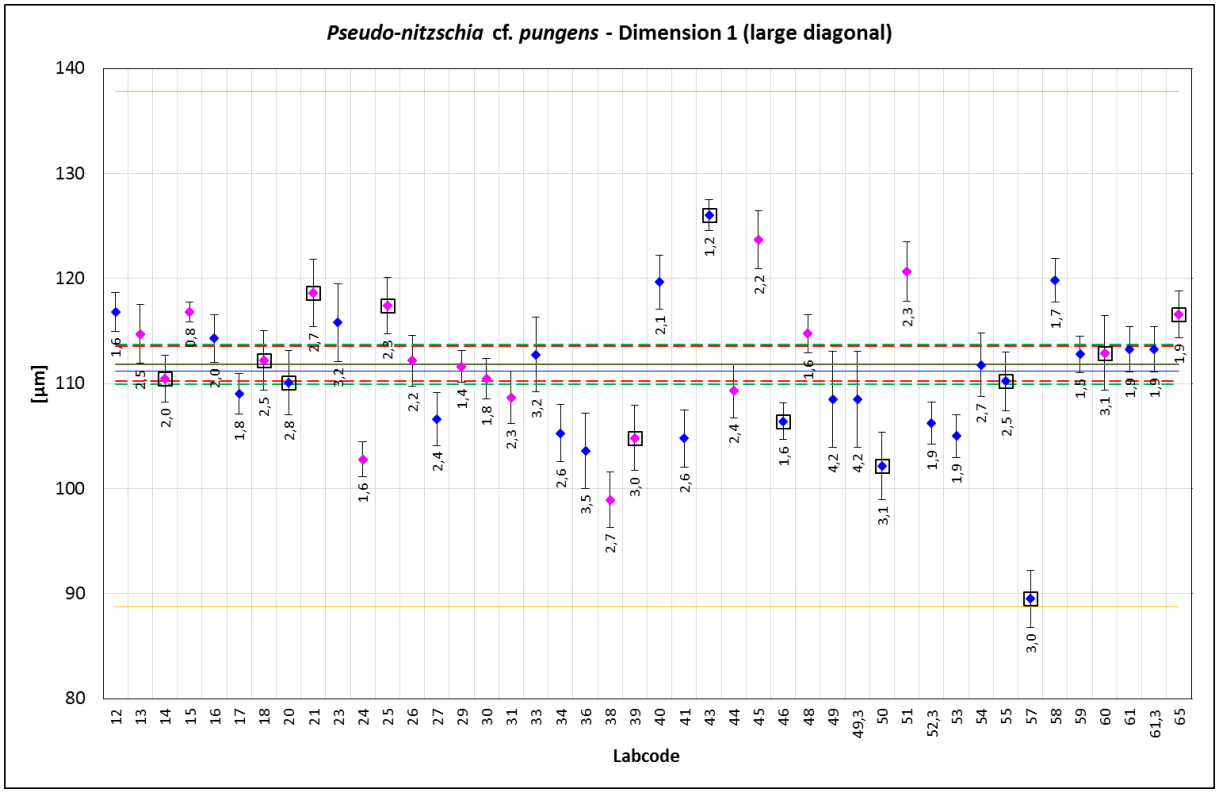

Figure 20: Results and some statistical characteristics for dimension 1 (large diagonal) of *Pseudo-nitzschia* cf. *pungens*. Legend see biovolume chart. Please note: Comma used as decimal mark.

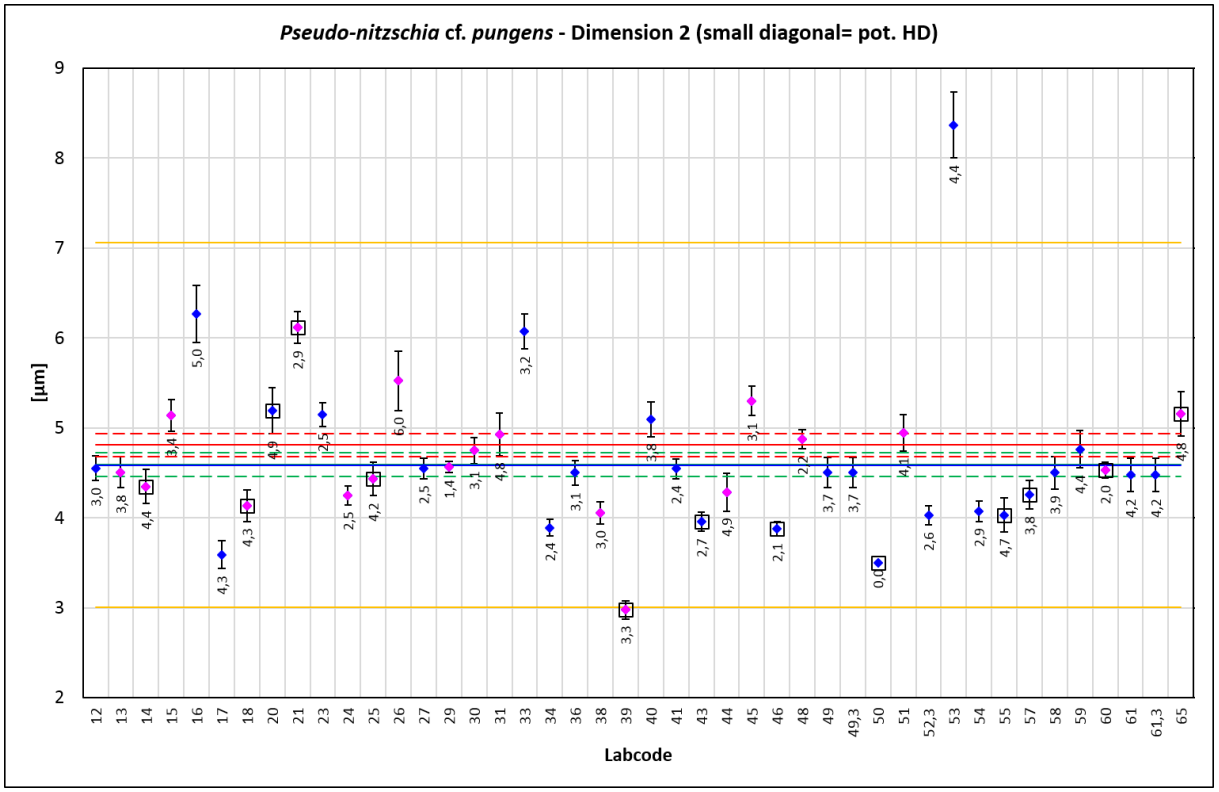

Figure 21: Results and some statistical characteristics for dimension 2 (small diagonal = potential HD) of *Pseudo-nitzschia* cf. *pungens*. Legend see biovolume chart. Please note: Comma used as decimal mark.

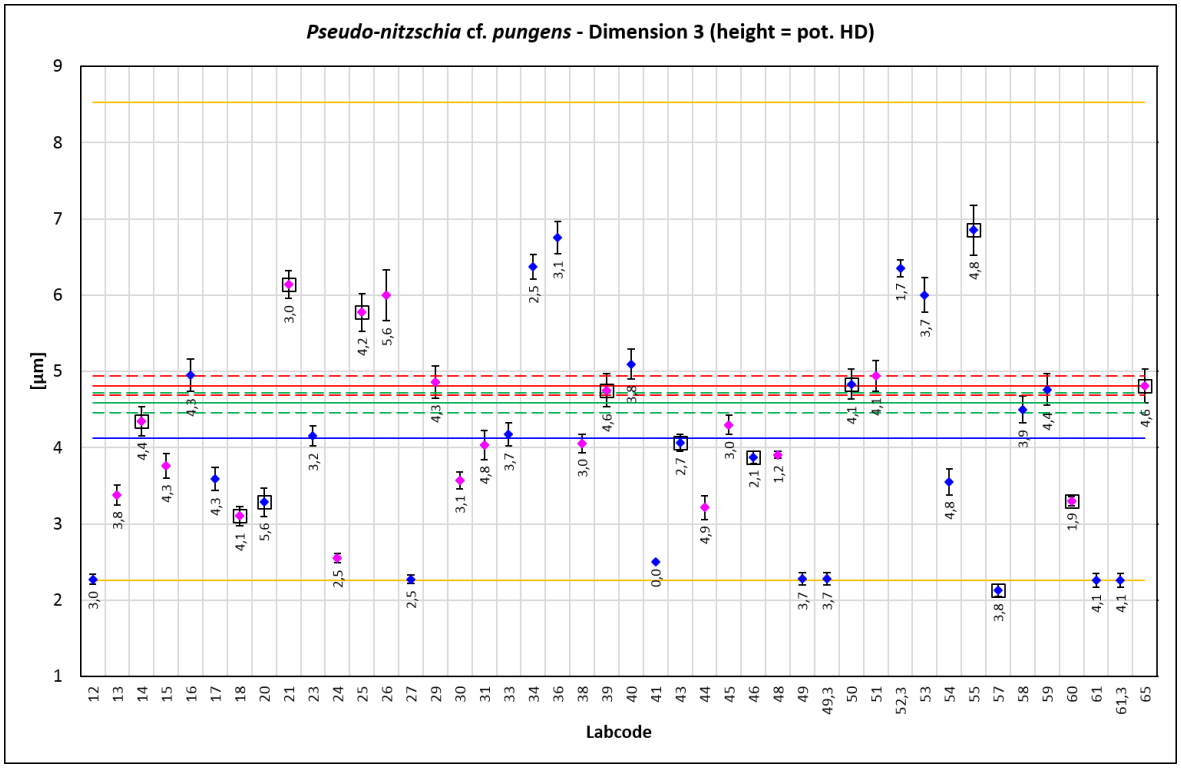

Figure 22: Results and some statistical characteristics for dimension 3 (height = potential HD) of *Pseudo-nitzschia* cf. *pungens*. Legend see biovolume chart. Please note: Comma used as decimal mark.

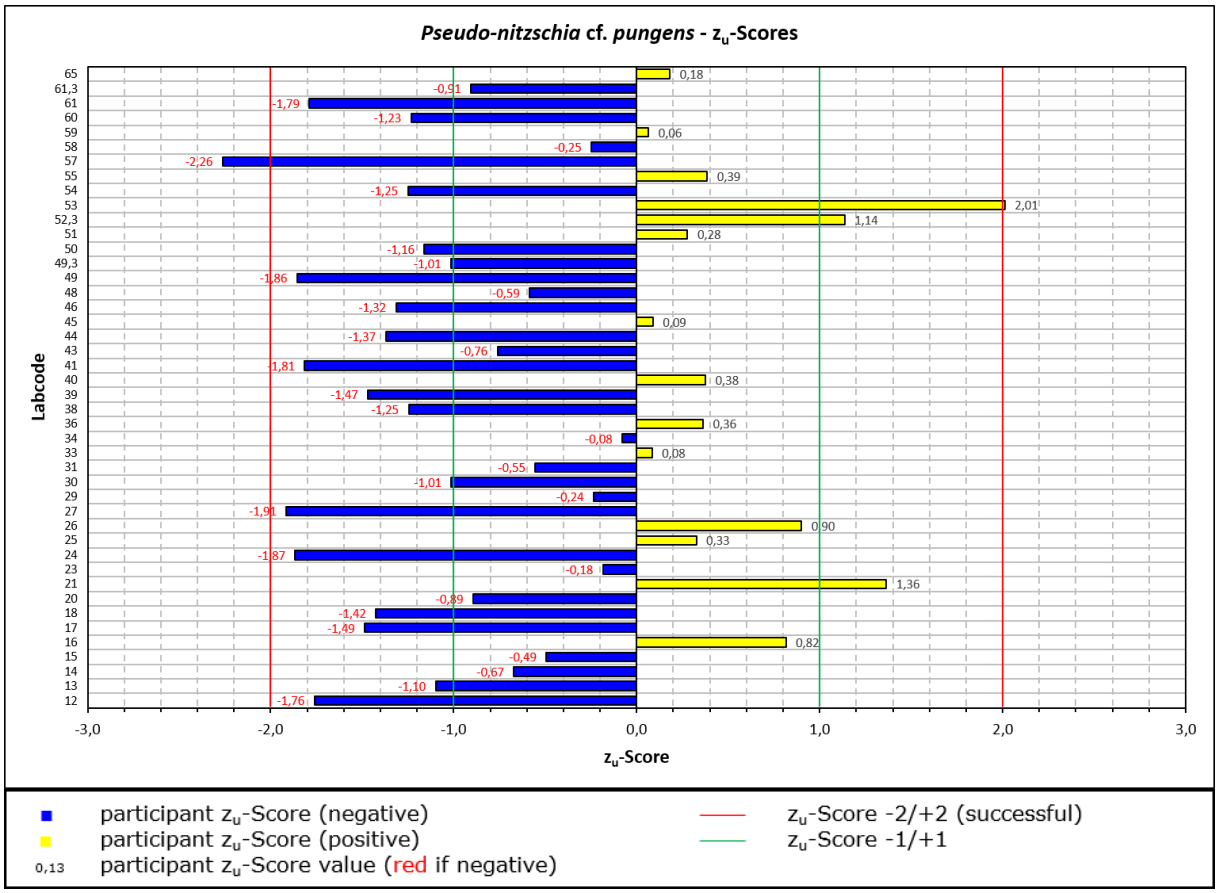

Figure 23: z_u scores of participants for *Pseudo-nitzschia* cf. *pungens*. Please note: Comma used as decimal mark.

3.4.5 *Thalassionema nitzschioides*

Thalassionema nitzschioides is a small diatom that builds colonies in the form of a star (Figure 24). Single cells can be found as well. All participants that measured this species have correctly assigned the 'elliptic cylinder' as geometrical shape (Table 7). Additionally, a special form used by HELCOM, the 'parallelepiped' shape reflecting a cuboid has been used for comparison. The HELCOM special form was comparable.

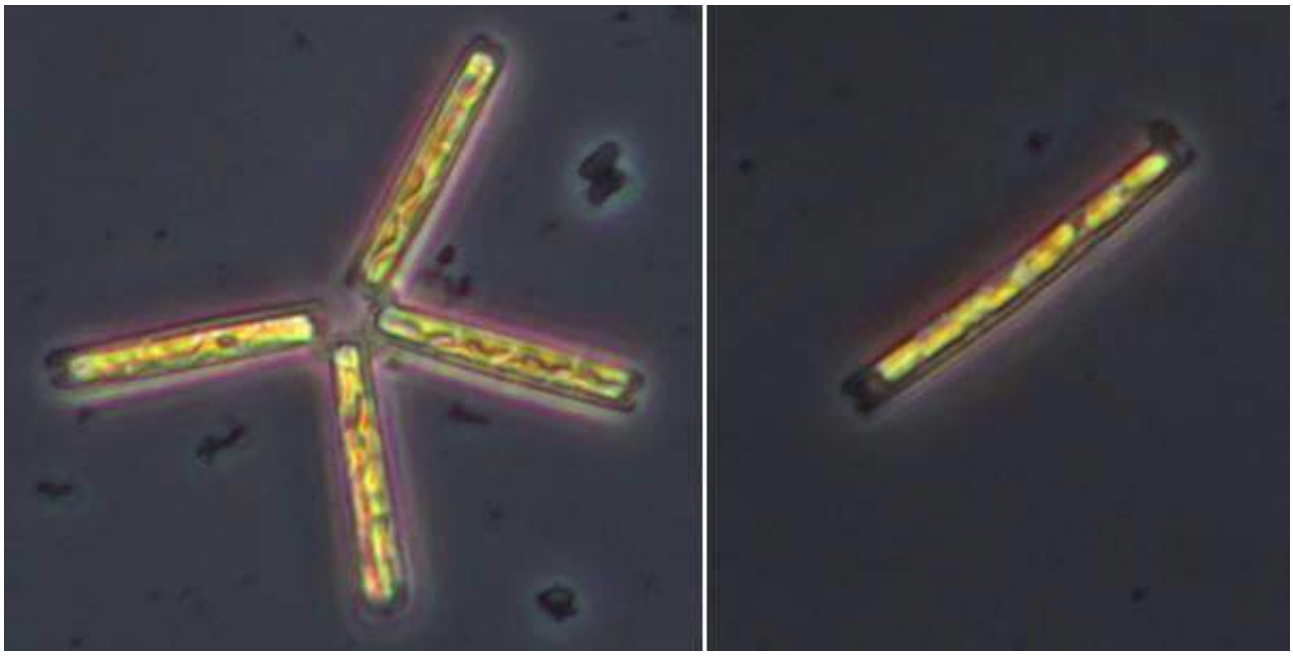

Figure 24: Microphotographic picture of the colony forming *Thalassionema nitzschioides* in the Helgoland sample.

Confusion of the dimensions is not having an effect on biovolume due to the equation. However, it has an effect on the comparability of single dimensions (Figure 26, Figure 27 Figure 28). Thus, statements on single dimensions such as the hidden dimension can be made only with care. Potentially both the 'small diameter' and the 'height' may be hidden. For reference values, the 'small diameter', the transapical axis, represented the hidden dimension. It has been set to the same values as the 'height', the pervalvar axis. The calculation of the relation between transapical and pervalvar axis in the data of the participants revealed that many may have used the factor 1 as well, if they determined the hidden dimension the same way. The values of all participants ranged from 0.5 to 1.49 with a mean of 0.91 (Table 25).

Table 7: Inventory of the main results (except special statistics) for *Thalassionema nitzschioides.*

General			
40	participants (labcode) have measured this species: 12 13 15 16 17 18 20 21 23 24 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 65		
25	measurements with an eyepiece micrometre		
17	measurements with image analysis software		
Geometry and Dimensions			
40	measurements/calculations with a 'elliptic cylinder' assigned as geometrical shape according to the Standard Document		
	2	additional calculations with a 'parallelepiped' assigned as geometrical shape notwithstanding to the Standard Document (according to HELCOM PEG-list) for comparison	
42	calculations with no correction factor used according to the Standard Document		
4	calculations with' large diameter' and 'height' being confused		
Statistics			
4-65	units have been measured		
	2	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	26	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		0	measurements with the biovolume standard error being ≥ 10 %
	0	total measurements with the biovolume standard error being ≥ 10 %	

All biovolume values ranged from 300 to 900 µm^3 and lay around the reference values (Figure 25). Almost all of the participants' results for the biovolume were within the requested z_u range from -2 to +2. There has been one outlier lying above this range.

Most z_u score values lay between -1 and 1 and all participants had passed the test for this species (Figure 29). One dataset of this species has been excluded as outlier with a z_u score outside the range from -3 to +3 (Table 26). Only four laboratories missed the z_u range from -1 to +1. This reflects a very good performance for the biovolume analysis of this species.

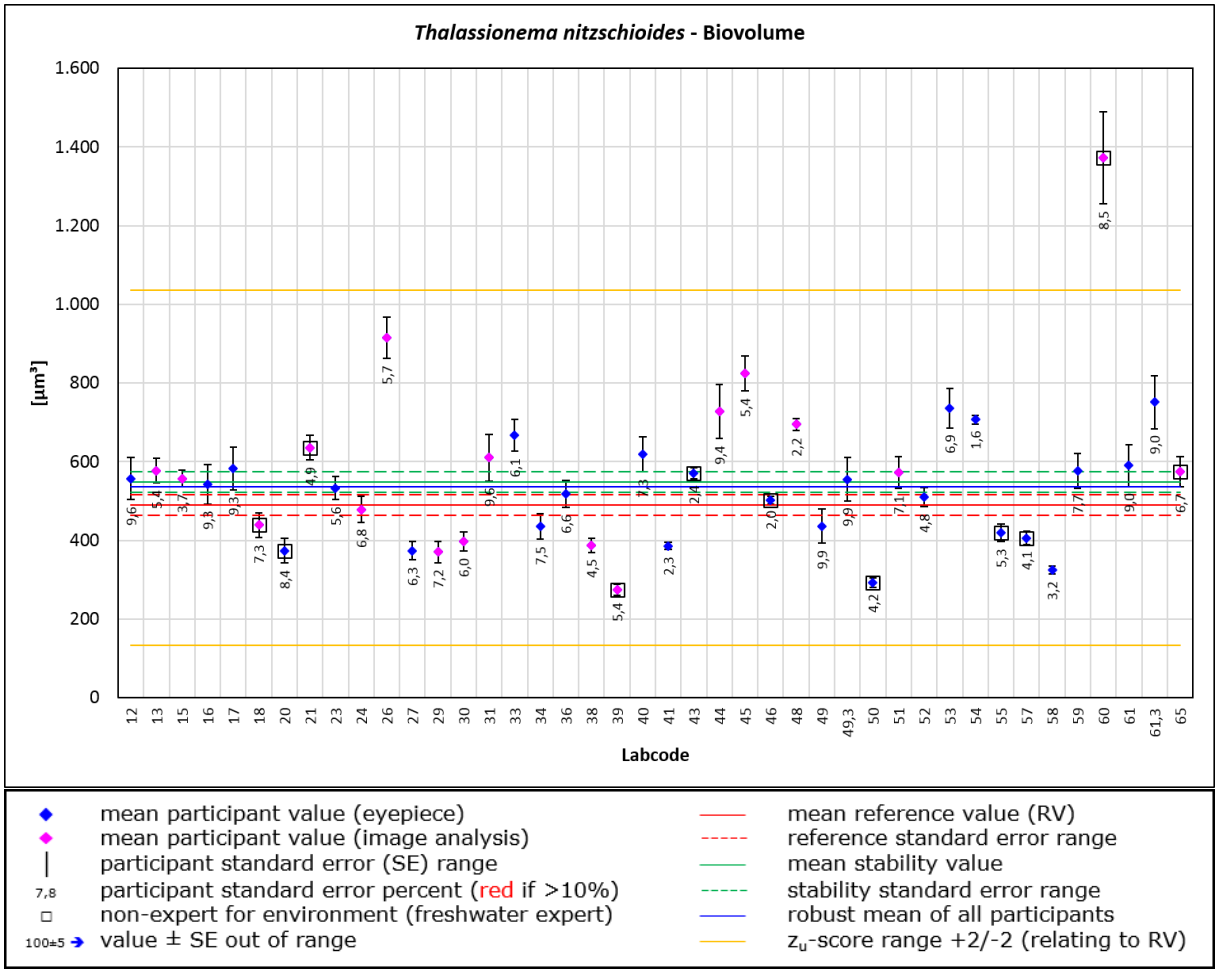

Figure 25: Results and some statistical characteristics for biovolume of *Thalassionema nitzschioides*. Please note: Comma used as decimal mark, full stop used as thousands separator.

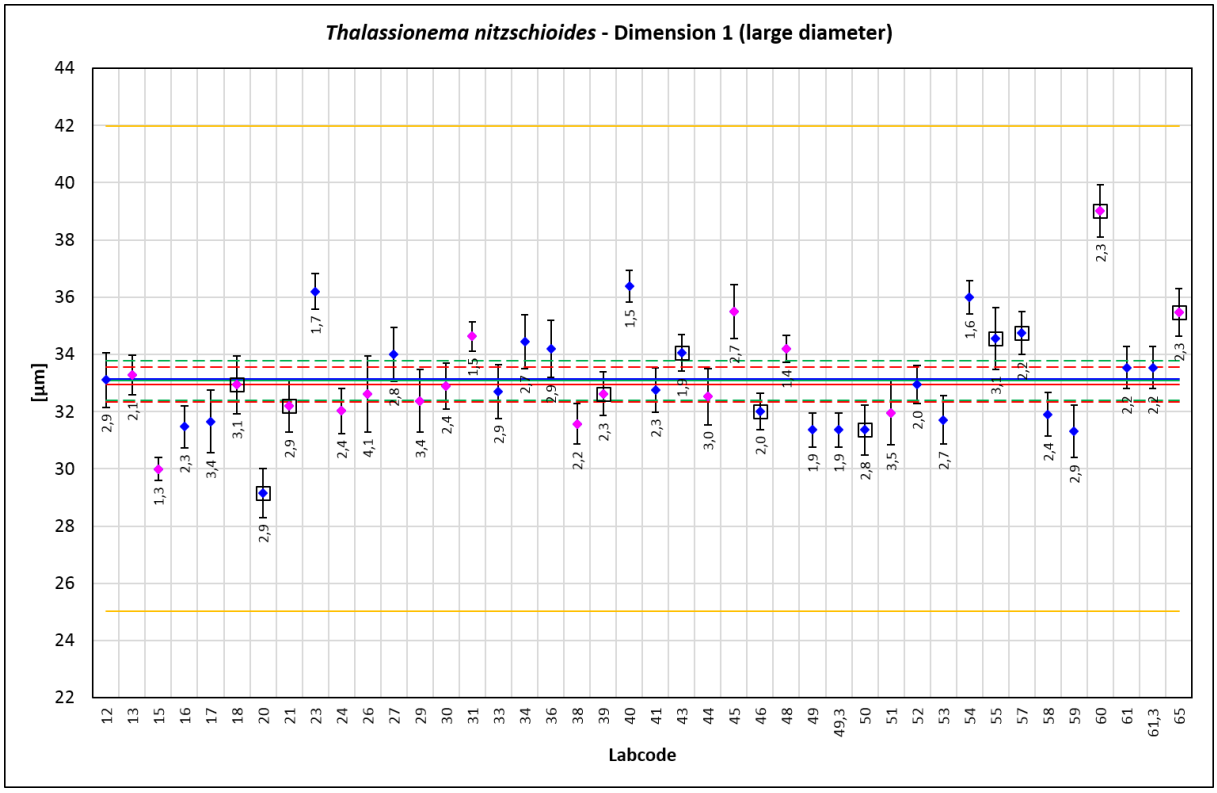

Figure 26: Results and some statistical characteristics for dimension 1 (large diameter) of *Thalassionema nitzschioides*. Legend see biovolume chart. Please note: Comma used as decimal mark.

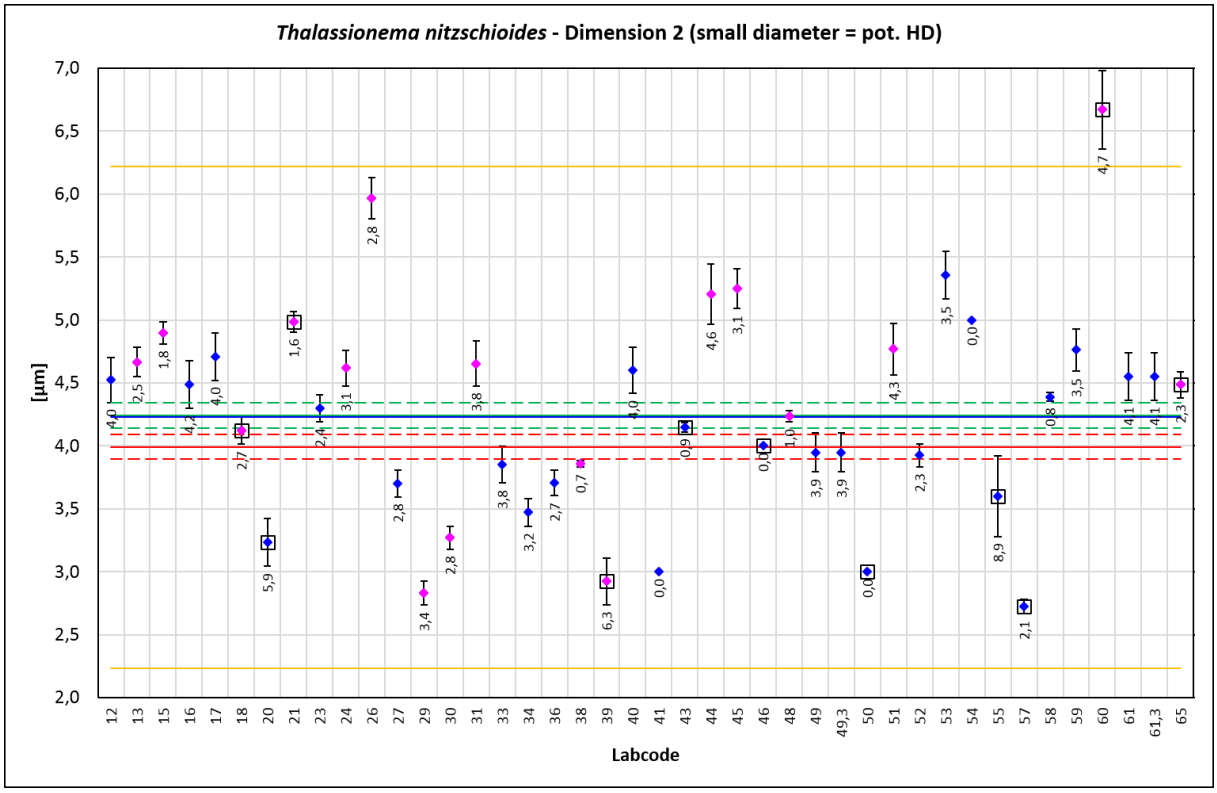

Figure 27: Results and some statistical characteristics for dimension 2 (small diameter = potential HD) of *Thalassionema nitzschioides*. Legend see biovolume chart. Please note: Comma used as decimal mark.

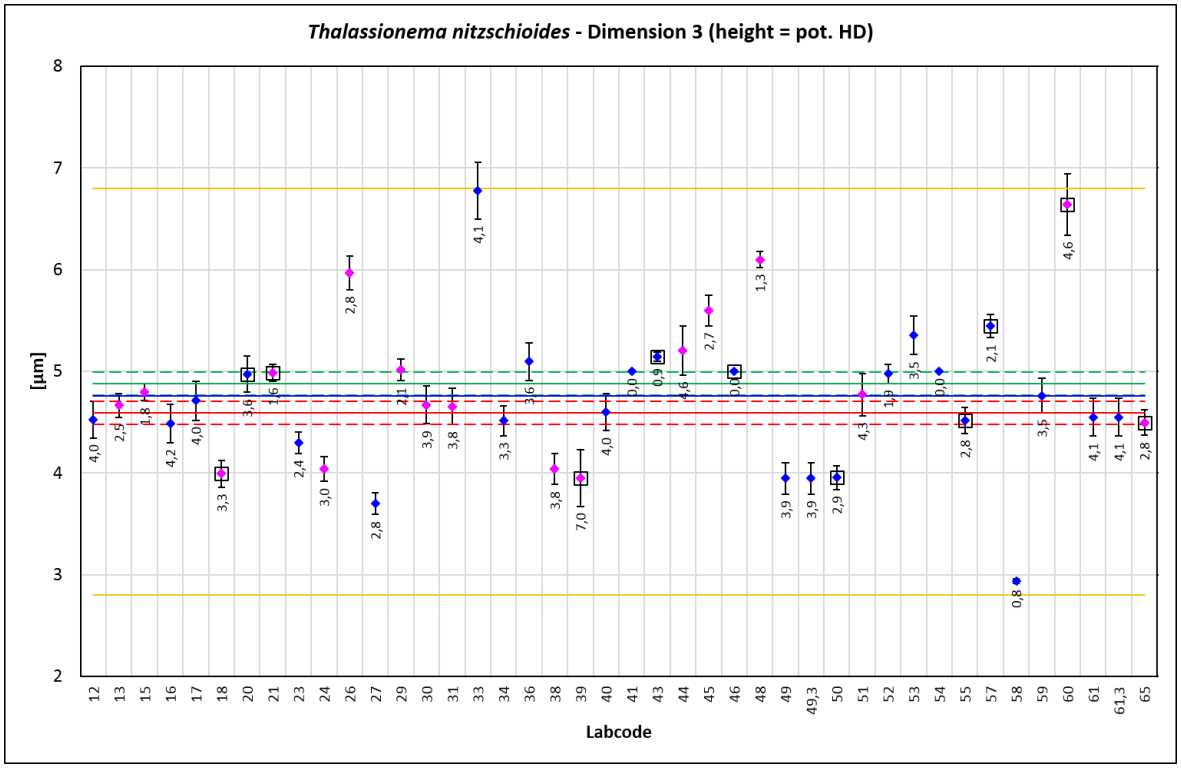

Figure 28: Results and some statistical characteristics for dimension 3 (height = potential HD) of *Thalassionema nitzschioides*. Legend see biovolume chart. Please note: Comma used as decimal mark.

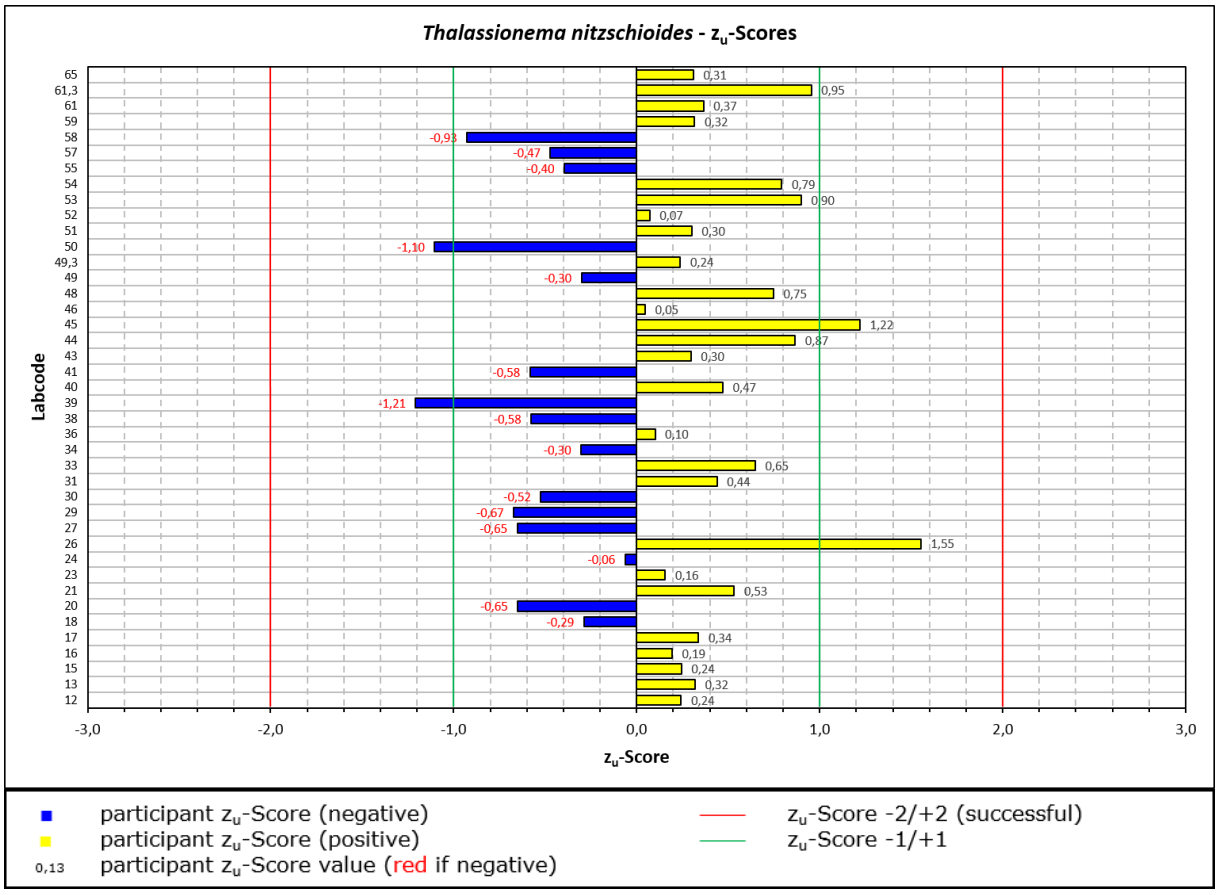

Figure 29: z_u scores of participants for *Thalassionema nitzschioides*. Please note: Comma used as decimal mark.

3.4.6 *Rhizosolenia imbricata*

Rhizosolenia imbricata is a cylindrical species with conical ends (Figure 30). All participants have correctly assigned the cylinder according to the Standard Document (Table 8). Four participants have had problems to relate the measurements to the correct dimensions (Figure 32, Figure 33). That has had a strong impact when applying the geometrical equation and has led to errors with regard to the calculated biovolume.

Table 8: Inventory of the main results (except special statistics) for *Rhizosolenia imbricata.*

General		
42	participants (labcode) have measured this species: 11 12 13 14 15 16 18 20 21 23 24 25 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 65	
23	measurements with an eyepiece micrometre	
19	measurements with image analysis software	
Geometry and Dimensions		
42	measurements/calculations with a 'cylinder' assigned as geometrical shape according to the Standard Document	
16	calculations with a correction factor of 0.9 used according to the Standard Document	
26	calculations with no correction factor used notwithstanding to the Standard Document	
4	calculations with 'diameter' and 'height' being confused	
Statistics		
15-54	units have been measured	
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)
	17	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)
		5 measurement with the biovolume standard error being ≥ 10 %
	10	total measurements with the biovolume standard error being ≥ 10 %

The species is highly variable in size and this may lead to standard errors larger than 10 %. However, the Standard Document requires measuring more than 20 items in that

case, which has not been observed by 5 out of 10 participants, producing a higher standard error. One participant measured only 15 items instead of 20 as requested as minimum by the Standard Document. Only if all individuals of a species are very similar in size, the number of measurements may be reduced.

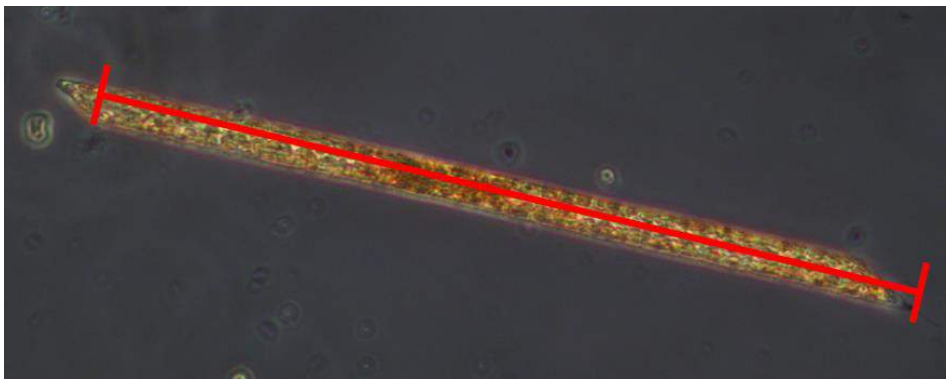

Figure 30: Microphotographic picture of *Rhizosolenia imbricata* in the Helgoland sample. The red line displays from where to where the height is to measure.

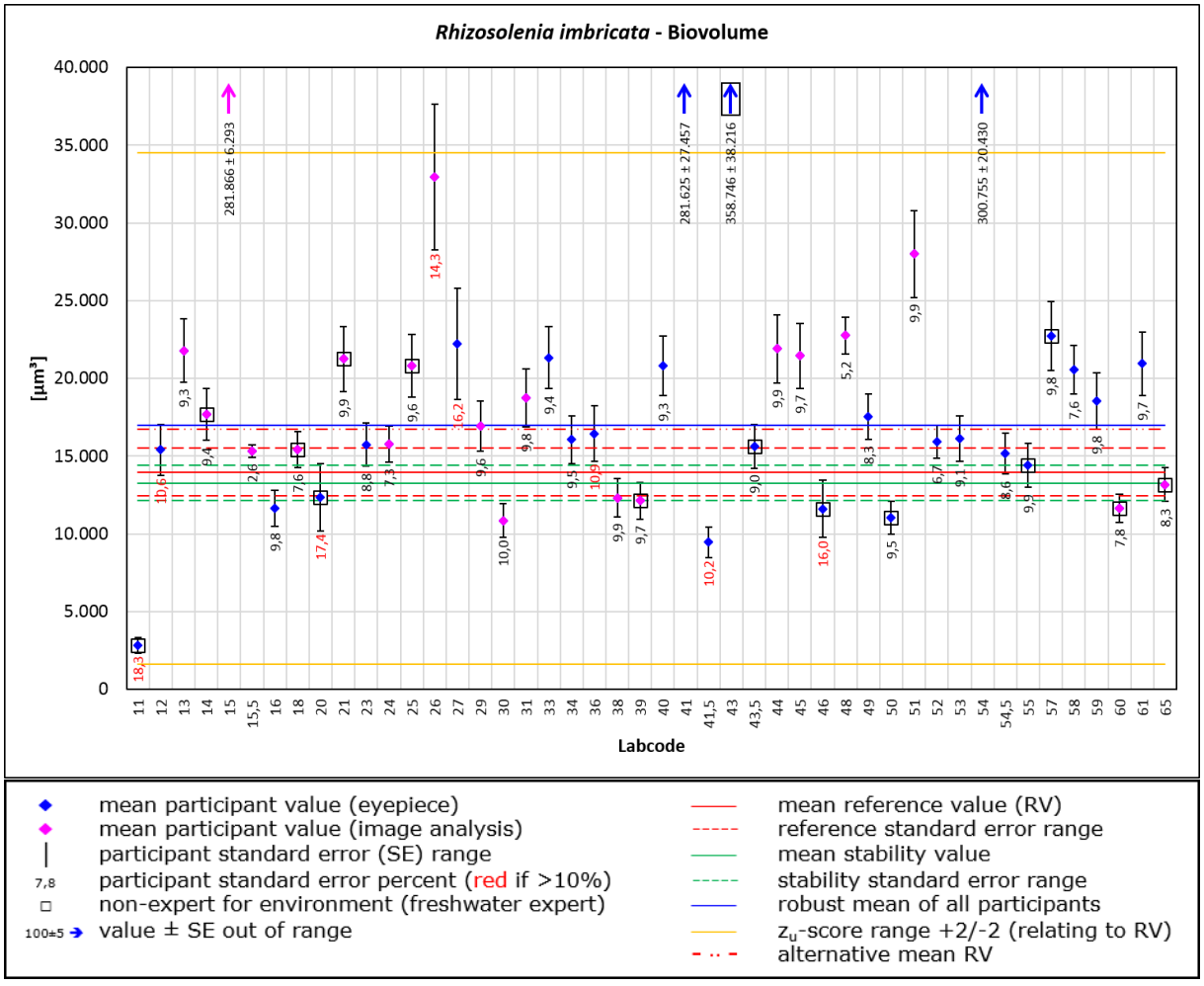

Figure 31: Results and some statistical characteristics for biovolume of *Rhizosolenia imbricata*. Please note: Comma used as decimal mark, full stop used as thousands separator.

The cylinder is slightly flattened; therefore, the Standard Document assigns a correction factor of 0.9 to this species. More than half of the participants have not applied this correction factor and thus, overestimated the biovolume. This is visible in the data. In addition to the correction factor being not applied, it may have been unclear for some participants from where to where the height has to be measured. Because of the two conical ends of the cell, the measurement shall start at the tip of one cone but end at the base of the second cone and thus, not include the second tip (Figure 30). This way a cylinder is measured. If the whole length is measured, the biovolume will be overestimated. By adding an average size for the second cone to the reference data and then calculating the biovolume without correction factor, the reference data fit in much better with the range of the participants (Figure 31, alternative mean RV). All biovolume values ranged from 3,000 to 33,000 µm^3 and lay within the requested z_u range from -2 to +2. The only outliers have been due to confusion of the dimensions.

The z_u scores in general had low values. Most of the laboratories, which successfully had passed the repeated z_u assessment procedures, lay within a z_u range from -1 to +1. Just three lay not within this range, but nevertheless between -2 and +2. All participants passed the test for this species (Figure 34). This reflects a good performance for the biovolume analysis of this species.

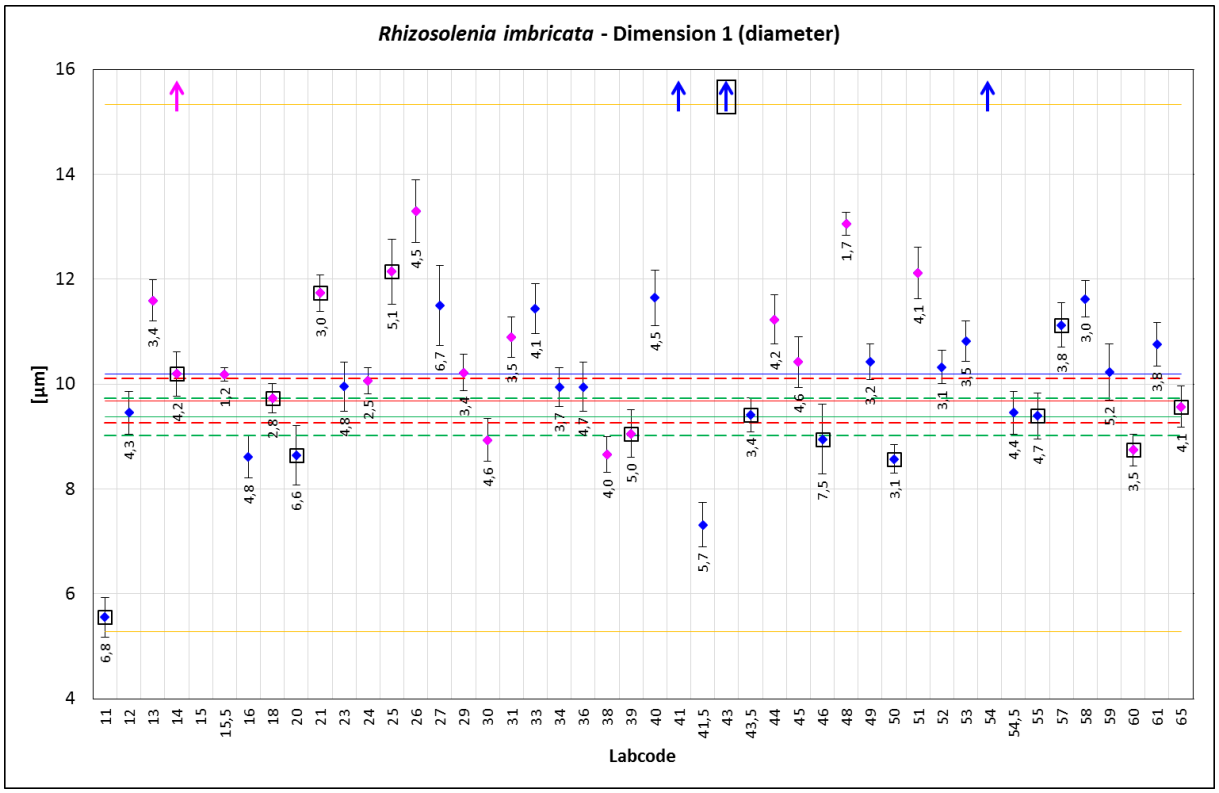

Figure 32: Results and some statistical characteristics for dimension 1 (diameter) of *Rhizosolenia imbricata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

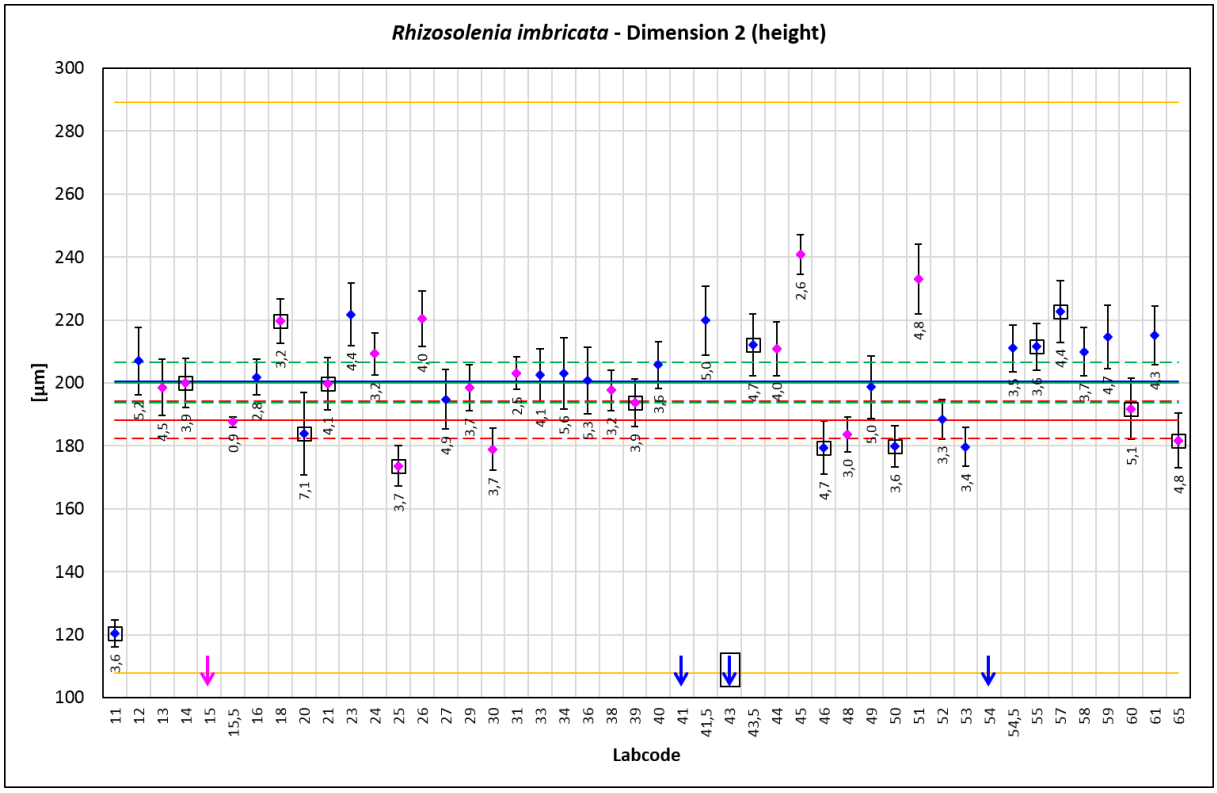

Figure 33: Results and some statistical characteristics for dimension 2 (height) of *Rhizosolenia imbricata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

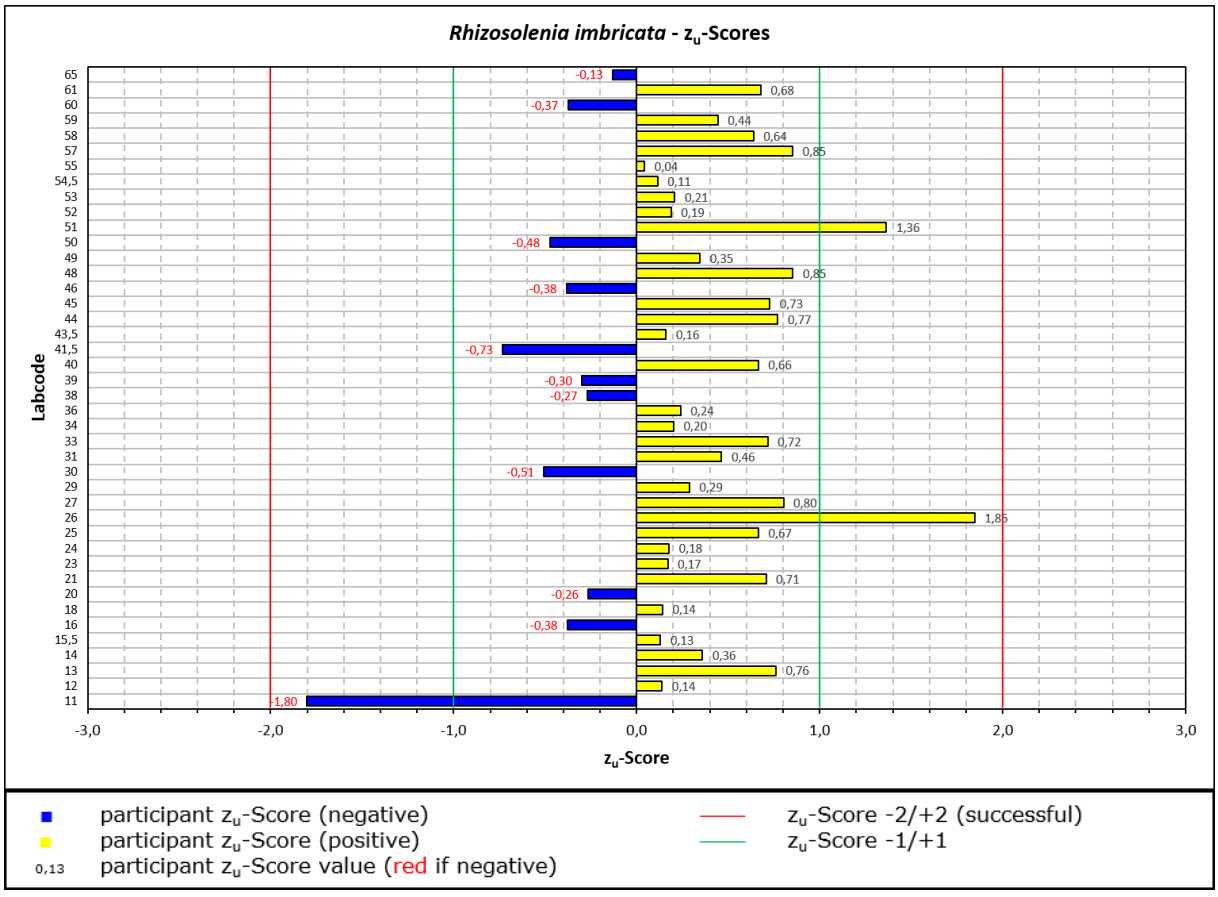

Figure 34: z_u scores of participants for *Rhizosolenia imbricata*. Please note: Comma used as decimal mark.

3.4.7 *Ditylum brightwellii*

Ditylum brightwellii is a centric diatom with a triangular base (Figure 35). All participants that have measured this species have correctly assigned the 'triangular prism' as geometrical shape (Table 9). The hidden dimension can be calculated by a trigonometric function since the base is an almost equal sided triangle. Some participants indeed seem to have calculated that. This trigonometric function gives the factor for the hidden dimension in general, which is exactly 0.8660254. Multiplied by the 'length of basic triangle side' this factor has been used for reference data. This relation calculated for the data of the participants gave values from 0.55 to 1.74, the mean was 0.98 (Table 25).

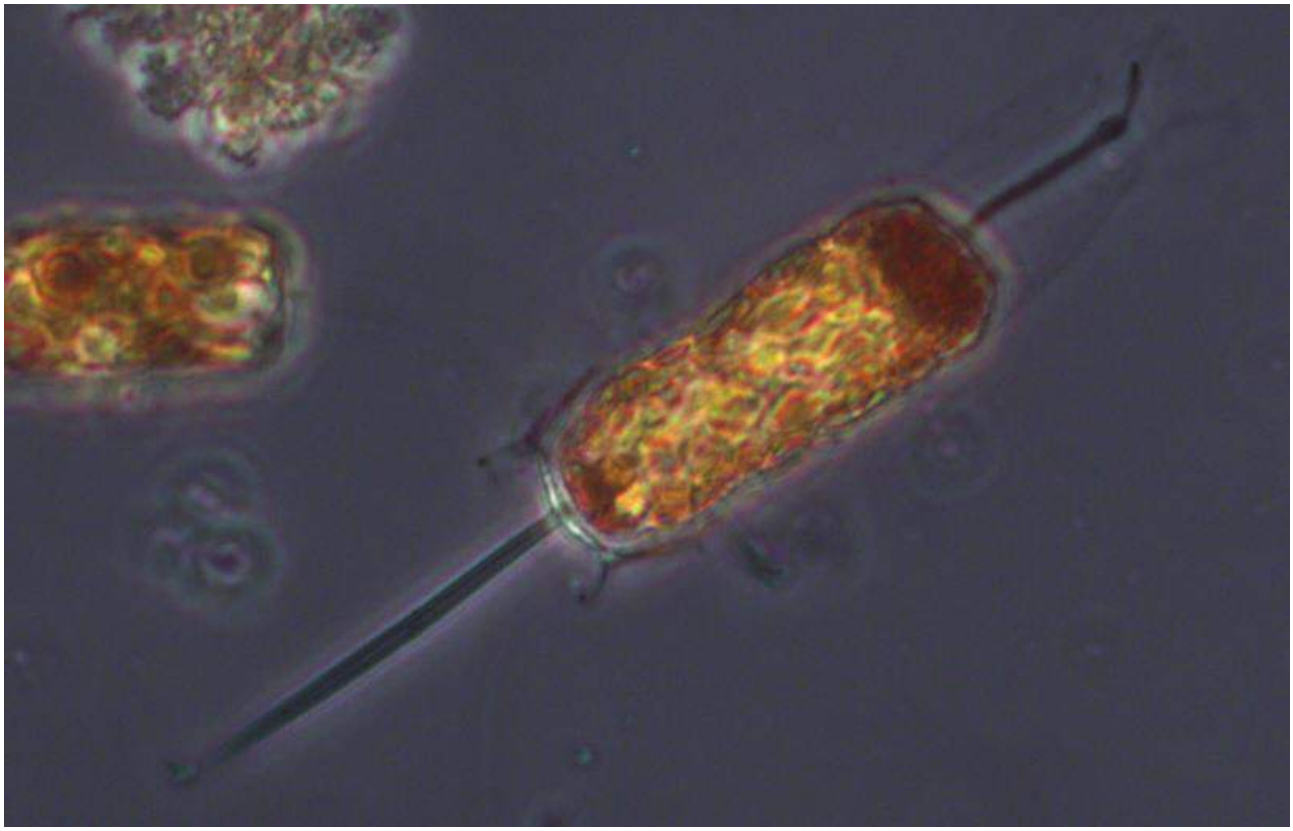

Figure 35: Microphotographic picture of *Ditylum brightwellii* in the Helgoland sample.

Ditylum brightwellii is a quite variable species in size; therefore it is not surprising that for nine calculations the standard error in % has been higher than 10 %, even when higher numbers of cells were measured. The Standard Document requires to measure up to 50 cells if the standard error keeps higher or equal than 10 %. This procedure has not been followed by eight participants, although at least two of them had measured 40 or more cells. Only one of these participants had measured more than 50 cells and nevertheless, the error had been still too high. In other cases, it became obvious that participants had added measurements, until the error became lower than the limit. Often, 20 cells had already been sufficient.

Table 9: Inventory of the main results (except special statistics) for *Ditylum brightwellii.*

General			
40	participants (labcode) have measured this species: 12 13 14 16 17 18 20 21 23 24 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 65		
23	measurements with an eyepiece micrometre		
17	measurements with image analysis software		
Geometry and Dimensions			
40	measurements/calculations with a 'triangular prism' assigned as geometrical shape according to the Standard Document		
40	calculations with no correction factor used according to the Standard Document		
1	calculation with 'height of triangle' and 'height of prism' being confused		
Statistics			
17-69	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	18	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		4	measurements with the biovolume standard error being ≥ 10 %
	9	total measurements with the biovolume standard error being ≥ 10 %	

The variability was reflected in the average biovolumes which ranged from 10,000 to 30,000 µm^3 (Figure 36). Reference values have been high compared to the average. This seems to be caused mainly by the length of the basic triangle side, which has a high weight due to its exponentiation in the equation (Figure 37). The problem may have been the bad quality of the cell, which had been shrunken in the middle and lost its original form over time. For most cells, the measurement for the triangle length have been best at the ends of the cells, where the cell walls have been rigid still, and not in the middle. The shrunken cells may generally have been the cause for underestimating the dimension. Reference data and measurements by participants fit better for the other two dimensions (Figure 38, Figure 39). However, except one all biovolume values lay within the requested z_u range from -2 to +2.

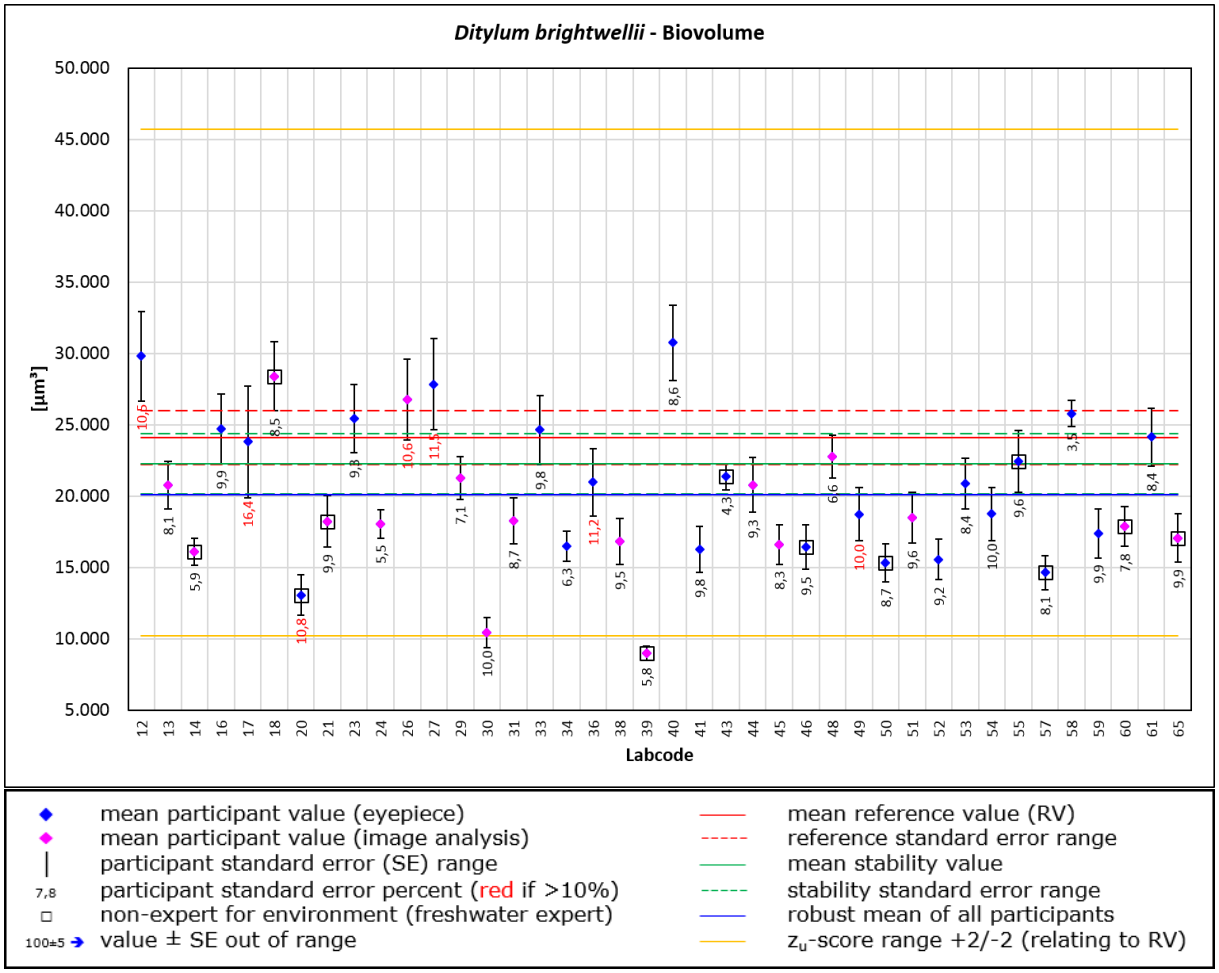

Figure 36: Results and some statistical characteristics for biovolume of *Ditylum brightwellii*. Please note: Comma used as decimal mark, full stop used as thousands separator.

Most of the z_u score values were negative, but only one participant missed the z_u range of -2 to +2 and did not pass the test for this species (Figure 40). 12 z_u score values lay out of the range from -1 to +1. This reflects a moderate performance for the biovolume analysis of this species.

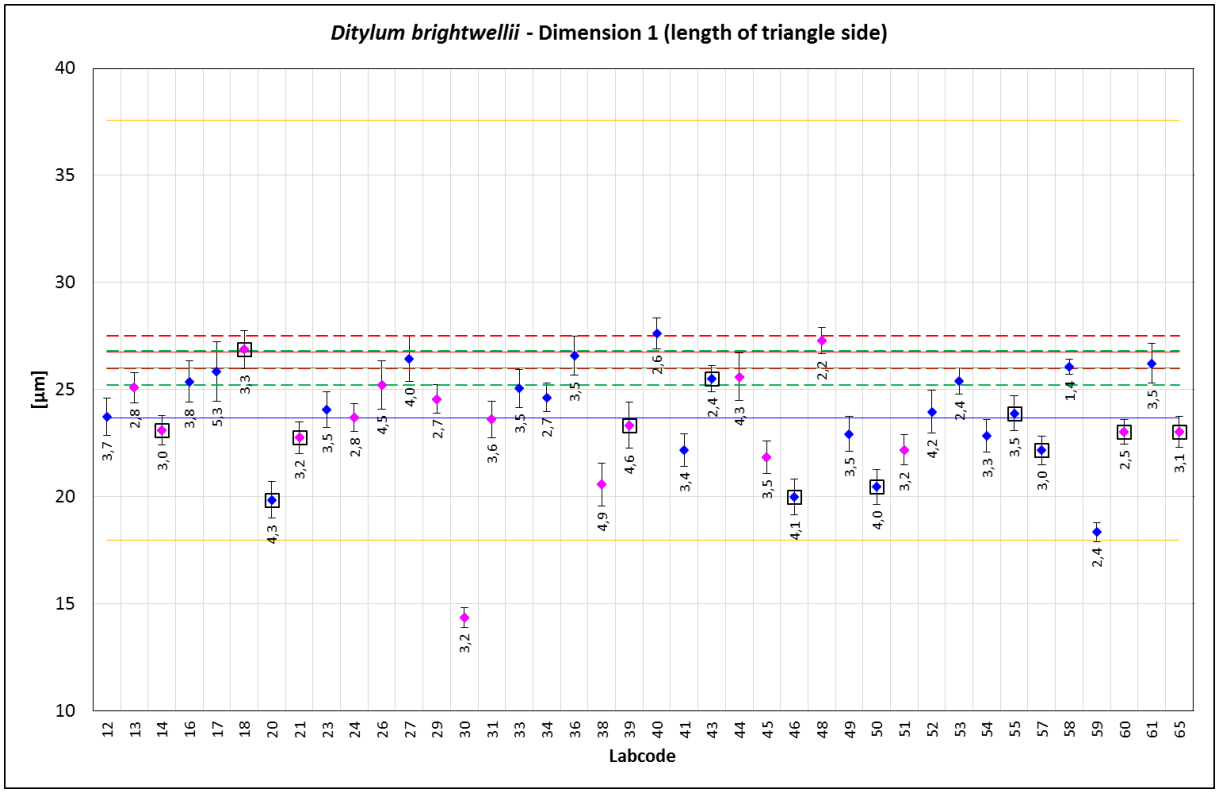

Figure 37: Results and some statistical characteristics for dimension 1 (length of triangle side) of *Ditylum brightwellii*. Legend see biovolume chart. Please note: Comma used as decimal mark.

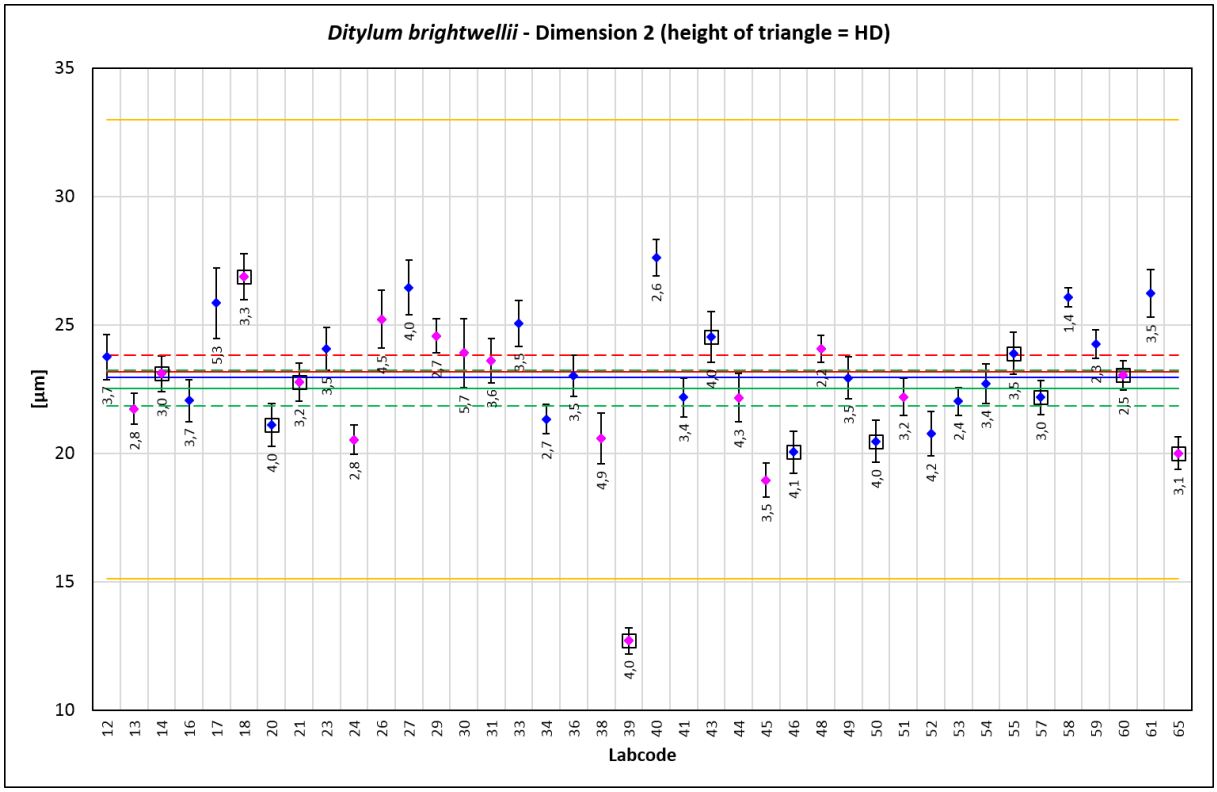

Figure 38: Results and some statistical characteristics for dimension 2 (height of triangle = HD) of *Ditylum brightwellii*. Legend see biovolume chart. Please note: Comma used as decimal mark.

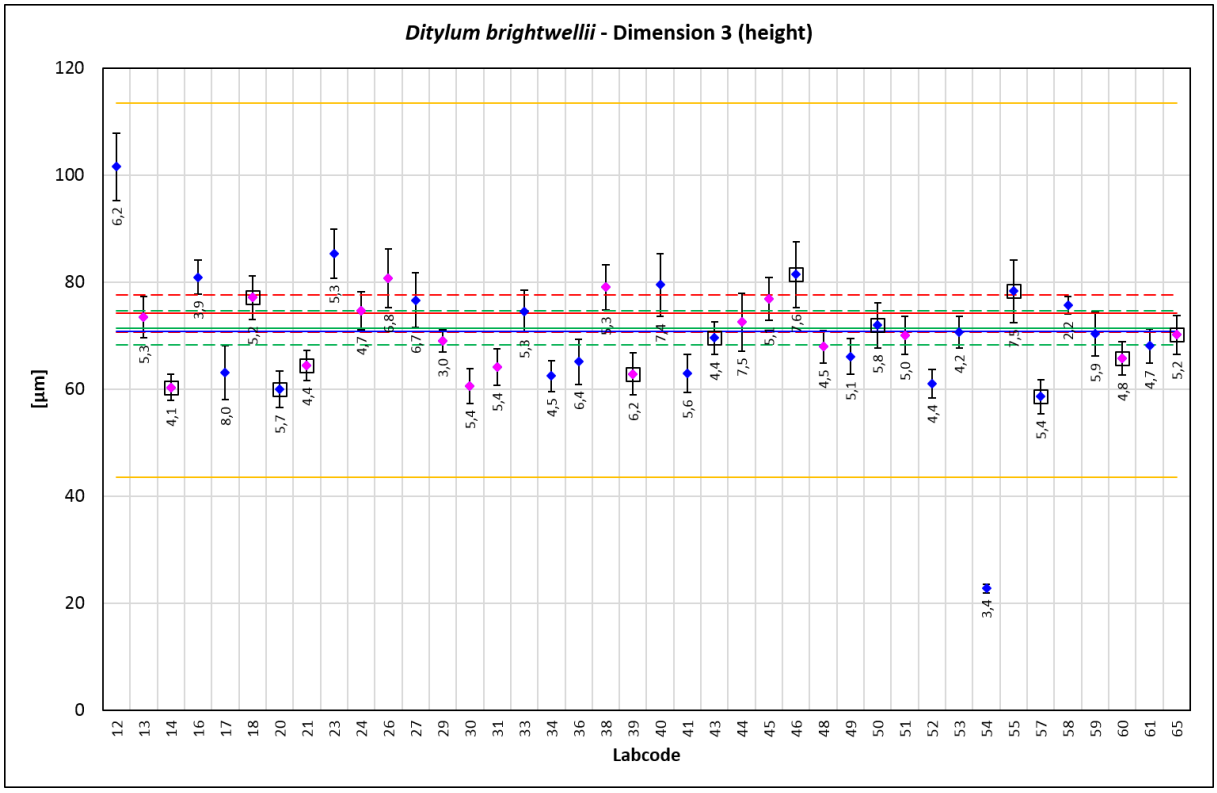

Figure 39: Results and some statistical characteristics for dimension 3 (height) of *Ditylum brightwellii*. Legend see biovolume chart. Please note: Comma used as decimal mark.

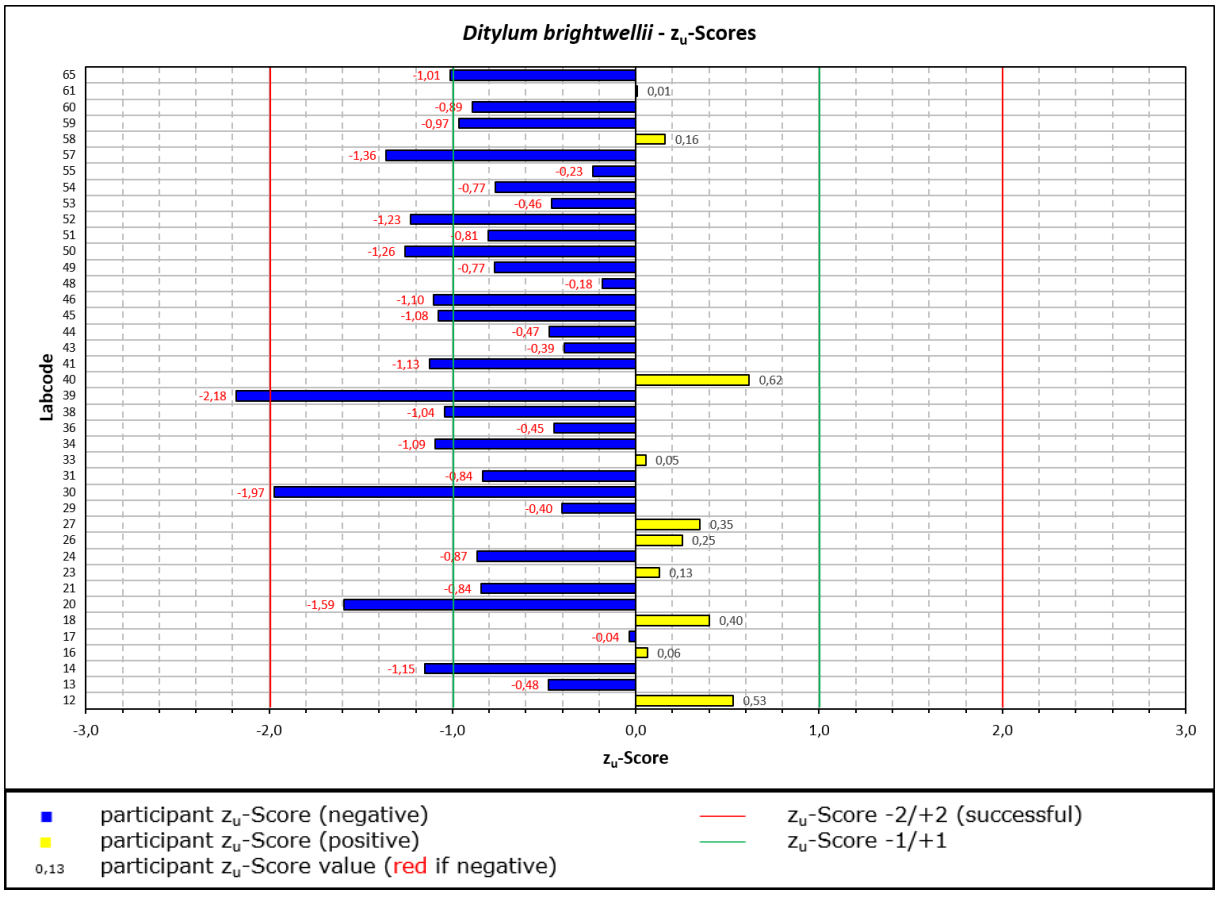

Figure 40: z_u scores of participants for *Ditylum brightwellii*. Please note: Comma used as decimal mark.

3.4.8 *Stephanopyxis turris*

Stephanopyxis turris is a marine diatom forming chains (Figure 41). All participants that have measured this species have correctly assigned the cylinder as geometrical shape, according to the Standard Document (Table 10). However, this shape may be discussed. An alternative could be the 'cylinder with two half prolate spheroids'. But this shape is not included any more in the reduced list of geometric shapes.

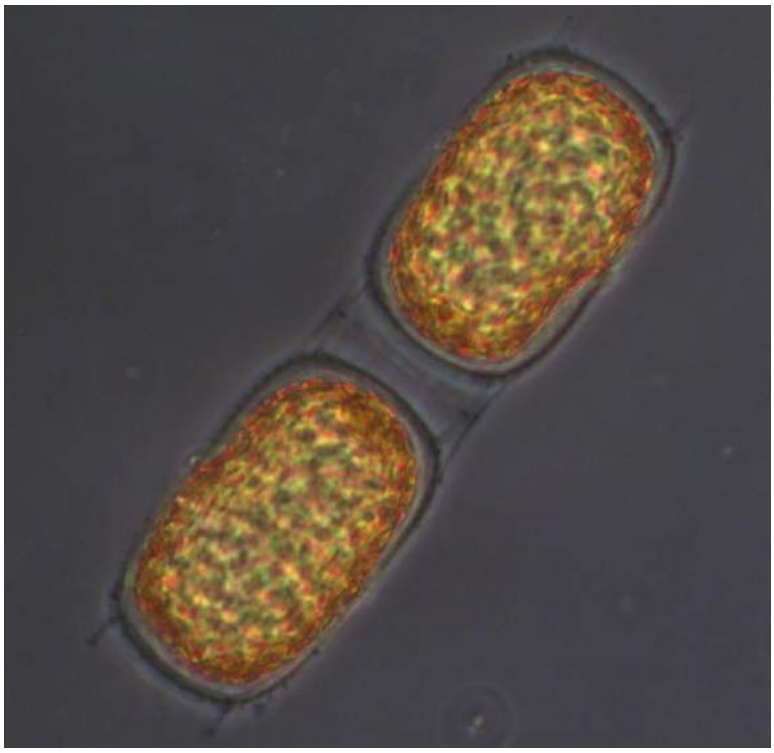

Figure 41: Microphotographic picture of chain forming *Stephanopyxis turris* in the Helgoland sample.

Most biovolume values ranged between 60,000 and 100,000 µm^3, which has been lower than the reference value (Figure 42). Four of the participants' results for the biovolume were not within the requested z_u range from -2 to +2. One possible reason for this was that the participants had not measured the complete height of the cell but only the height of the 'cylinder' taking into account the concavity of the cells and excluding this part. For reference data, the complete length of the cell has been measured. However, both methods need a correction factor, since the cylinder either overestimates or underestimates the true biovolume. The correction factor is now implemented in the Standard Document regarding the complete height. The underestimation compared to reference data has been visible in both dimensions anyway (Figure 43, Figure 44). Another reason for low values of the participants compared to reference values could have lain in the selection of cells for measuring. *Stephanopyxis turris* is variable in size and it might have been the case that the analysts had treated the various cells in a different way. This might hold true especially because a significant difference between the results of the experts and non-experts regarding the marine field has been found (Table 24).

Almost all z_u score values were negative, but just 2 participants did not pass the test for this species (Figure 45). Additionally, two participants missed the z_u range from -3 to +3 and had been excluded as outliers (Table 26). 11 of the participants missed the z_u range from -1 to +1. Thus, the performance of biovolume analysis for this species had been poor.

Table 10: Inventory of the main results (except special statistics) for *Stephanopyxis turris.*

General			
39	participants (labcode) have measured this species: 13 14 16 17 18 20 21 23 24 25 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 57 58 59 60 61 65		
21	measurements with an eyepiece micrometre		
18	measurements with image analysis software		
Geometry and Dimensions			
39	measurements/calculations with a 'cylinder' assigned as geometrical shape according to the Standard Document		
39	calculations with no correction factor used according to the Standard Document		
1	calculation with 'diameter' and 'height' being confused		
Statistics			
6-79	units have been measured		
	3	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	22	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		2	measurement with the biovolume standard error being ≥ 10 %
	4	total measurements with the biovolume standard error being ≥ 10 %	

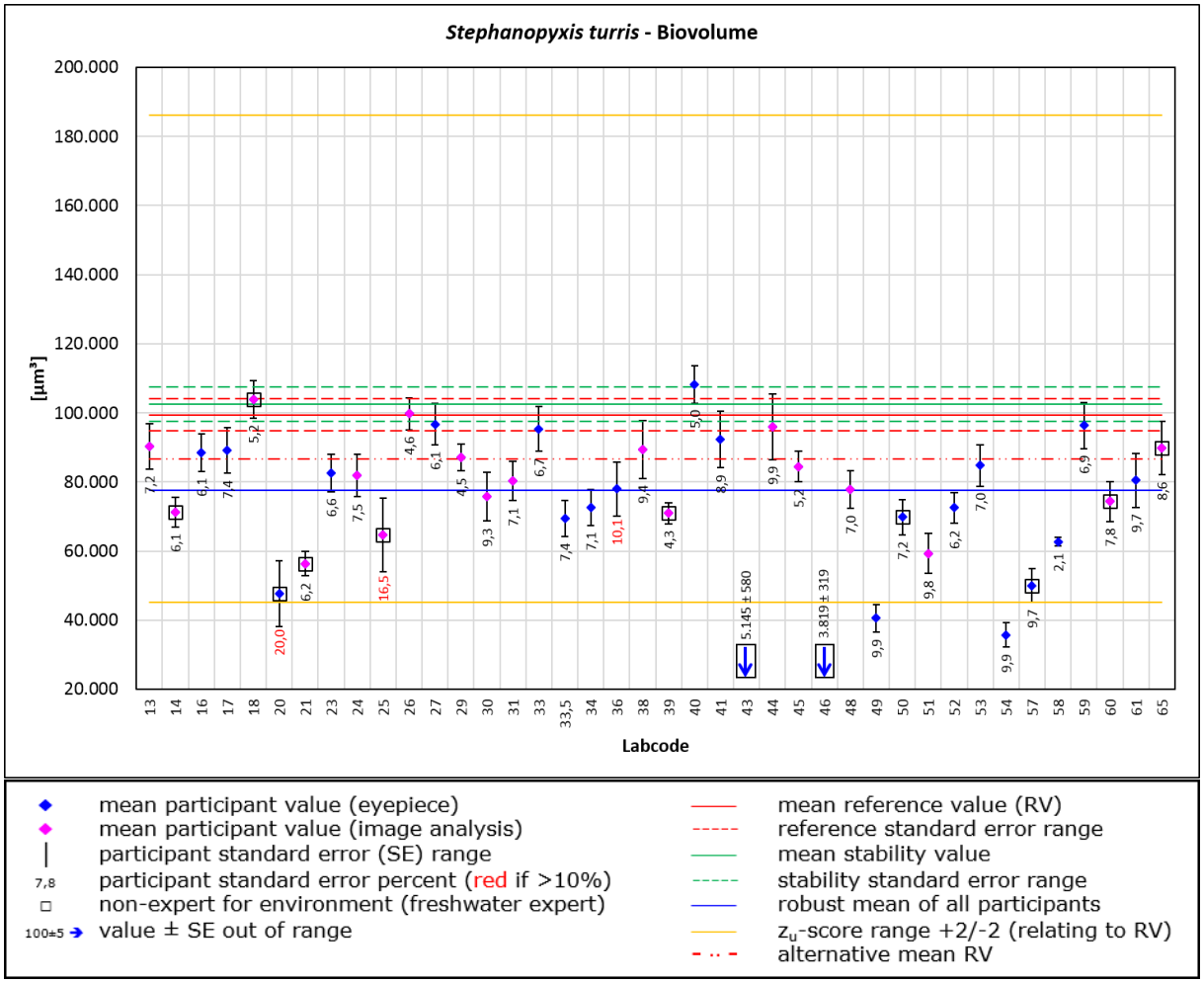

Figure 42: Results and some statistical characteristics for biovolume of *Stephanopyxis turris*. Please note: Comma used as decimal mark, full stop used as thousands separator.

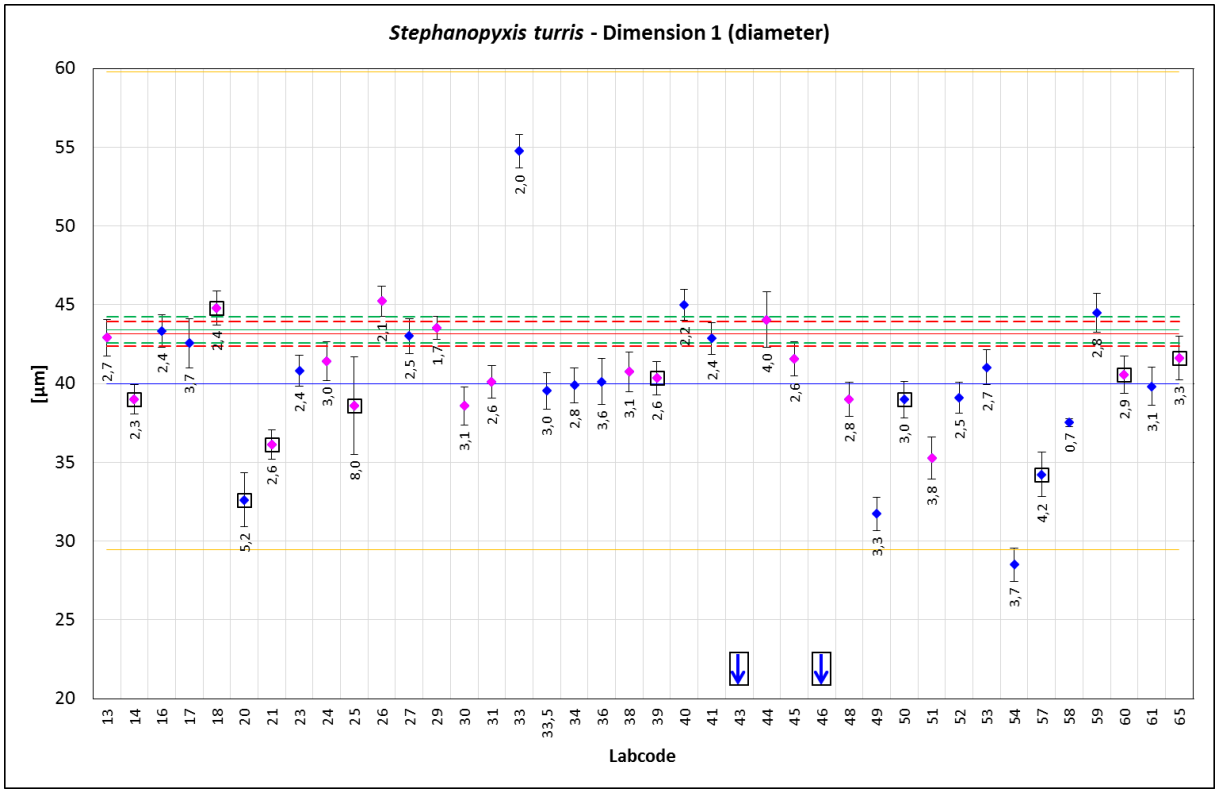

Figure 43: Results and some statistical characteristics for dimension 1 (diameter) of *Stephanopyxis turris*. Legend see biovolume chart. Please note: Comma used as decimal mark.

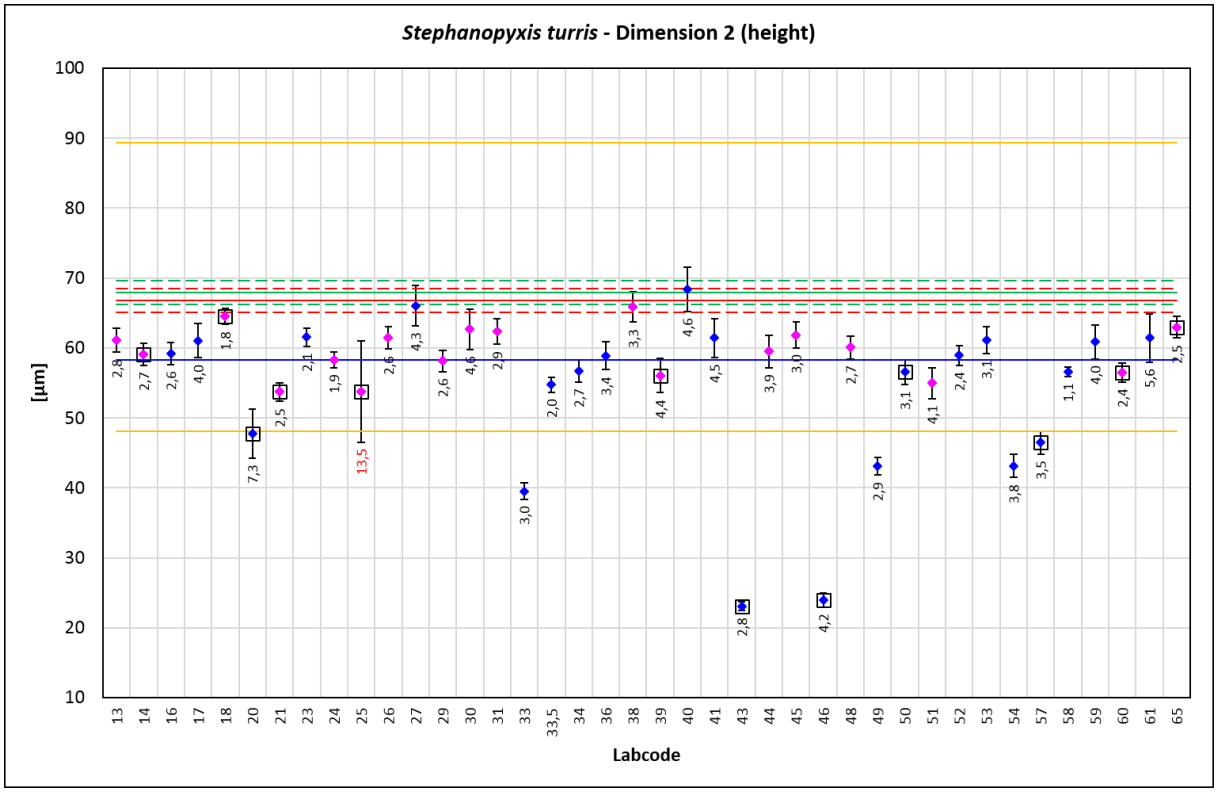

Figure 44: Results and some statistical characteristics for dimension 2 (height) of *Stephanopyxis turris*. Legend see biovolume chart. Please note: Comma used as decimal mark.

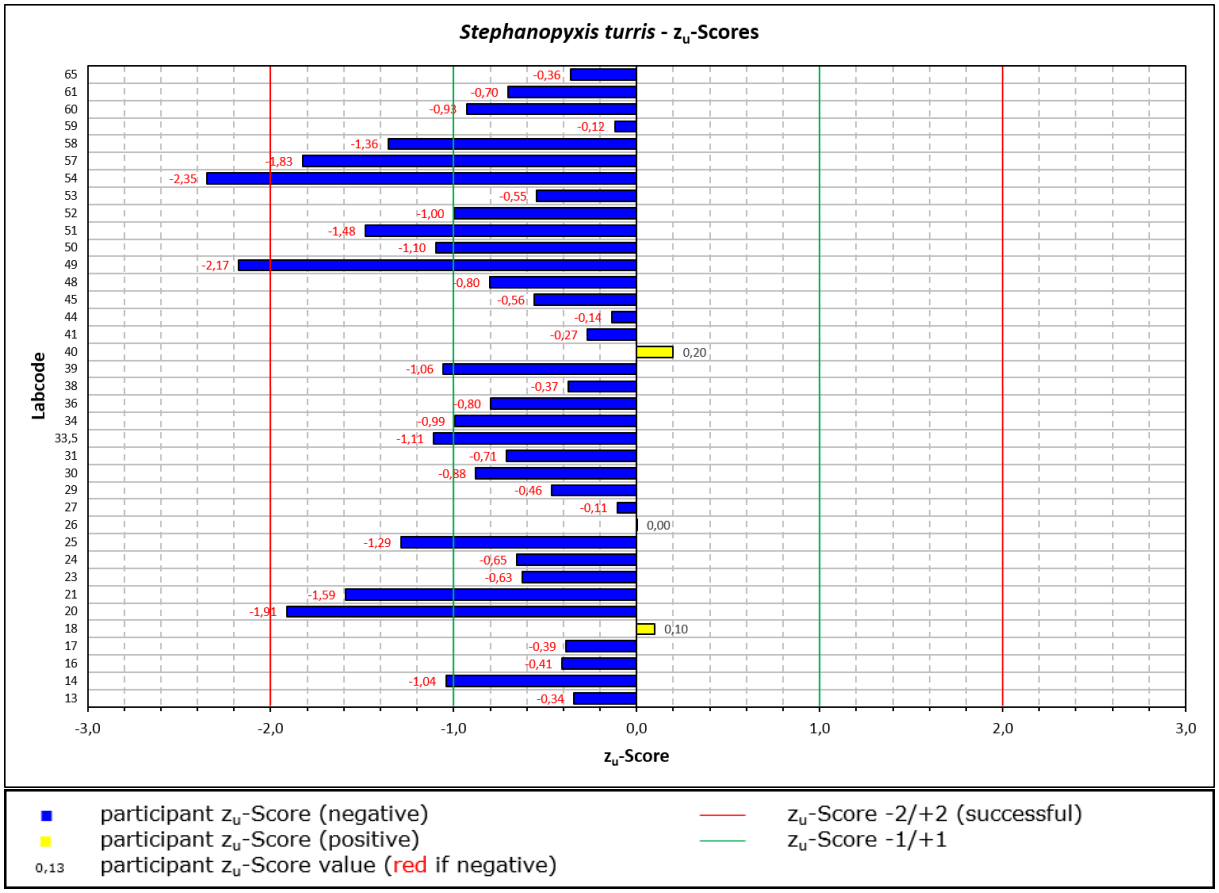

Figure 45: z_u scores of participants for *Stephanopyxis turris*. Please note: Comma used as decimal mark.

3.4.9 *Odontella sinensis*

Odontella sinensis is a large marine diatom forming short chains (Figure 46). With one exception, all participants that have measured this species have correctly assigned the shape 'elliptic cylinder', according to the Standard Document (Table 11). The species is highly variable in size and thus, it will not surprise that in eight cases the standard error has been higher than 10 %. However, the Standard Document is instructing the analyst that as long as the error is not lower than 10 %, more units (up to 50 cells) will have to be measured. It is also stated that for species with very similar sizes less than 20 cells (the general minimum) may be measured if the standard error is low enough. This has been the case for one participant, who has measured only 11 items. On the other hand, *Odontella sinensis* is in fact no species with low variability in size and thus, this rule cannot be applied here.

Generally, the mean of participants and the mean of reference data had been quite similar for all dimensions (Figure 48, Figure 49 and Figure 50), the height had been diverging most. Most average biovolumes felt into the range of 1,000,000 to 2,500,000 μm^3 (Figure 47). Almost all of the participants' results for the biovolume were within the requested z_u range from -2 to +2. There had been one outlier lying above and one outlier lying below that range.

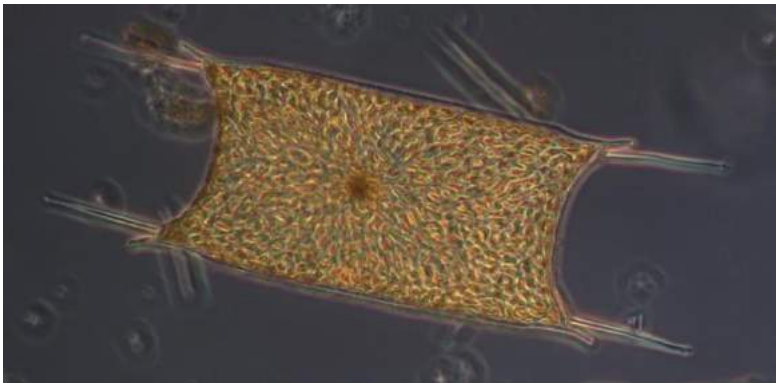

Figure 46: Microphotographic picture of *Odontella sinensis* in the Helgoland sample.

Table 11: Inventory of the main results (except special statistics) for *Odontella sinensis.*

General			
40	participants (labcode) have measured this species: 13 14 15 16 17 18 20 21 23 24 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 65		
22	measurements with an eyepiece micrometre		
18	measurements with image analysis software		
Geometry and Dimensions			
39	measurements/calculations with a 'elliptic cylinder' assigned as geometrical shape according to the Standard Document		
1	measurement/calculation with a 'cuboid' assigned as geometrical shape notwithstanding to the Standard Document		
40	calculations with no correction factor used according to the Standard Document		
9	calculations with 'small diameter' and 'height' being confused		
Statistics			
11-56	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	15	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		4	measurements with the biovolume standard error being ≥ 10 %
	8	total measurements with the biovolume standard error being ≥ 10 %	

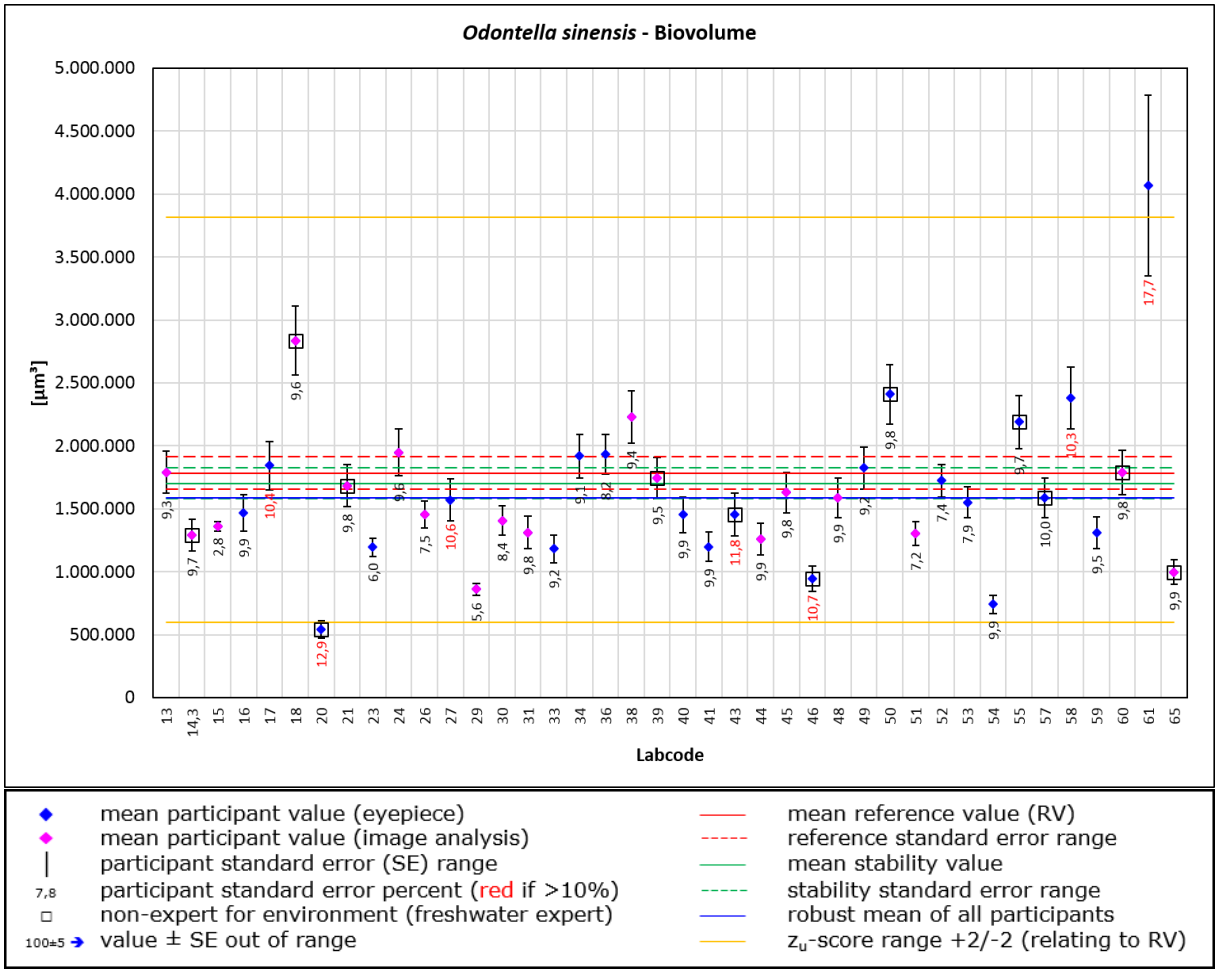

Figure 47: Results and some statistical characteristics for biovolume of *Odontella sinensis*. Please note: Comma used as decimal mark, full stop used as thousands separator.

The 'small diameter' of the 'elliptic cylinder' that represents the transapical axis of the diatom should be the hidden dimension in most cases. For reference values, the hidden dimension has been calculated as 0.4 multiplied by the 'large diameter' value. Results of the participants lay between 0.12 and 0.9; the mean was 0.39 which fit well to the one used for reference data (Table 25).

The z_u score values showed a high variability with a tendency to negative values. Nine of the participants which successfully had passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. Two of these participants even missed the z_u range from -2 to +2 and did not pass the test for this species (Figure 51). This reflects a good to moderate performance for the biovolume analysis of this species.

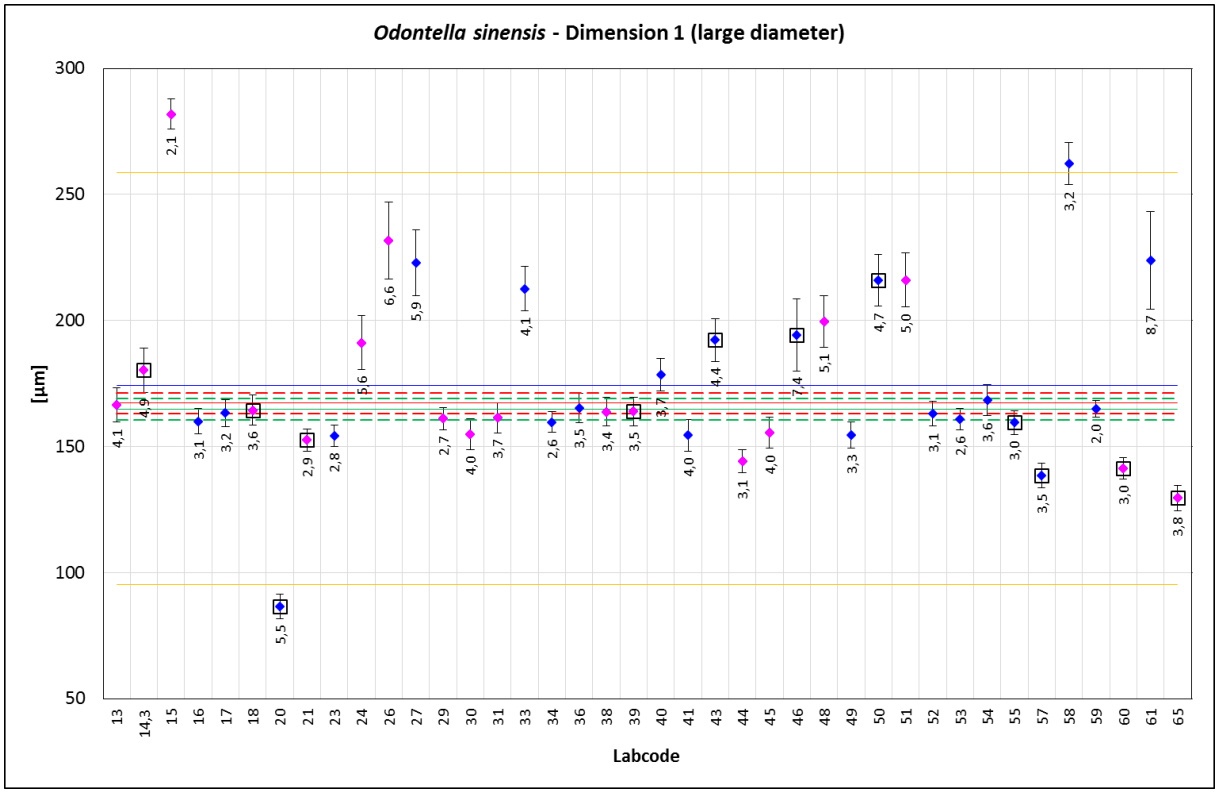

Figure 48: Results and some statistical characteristics for diameter 1 (large diameter) of *Odontella sinensis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

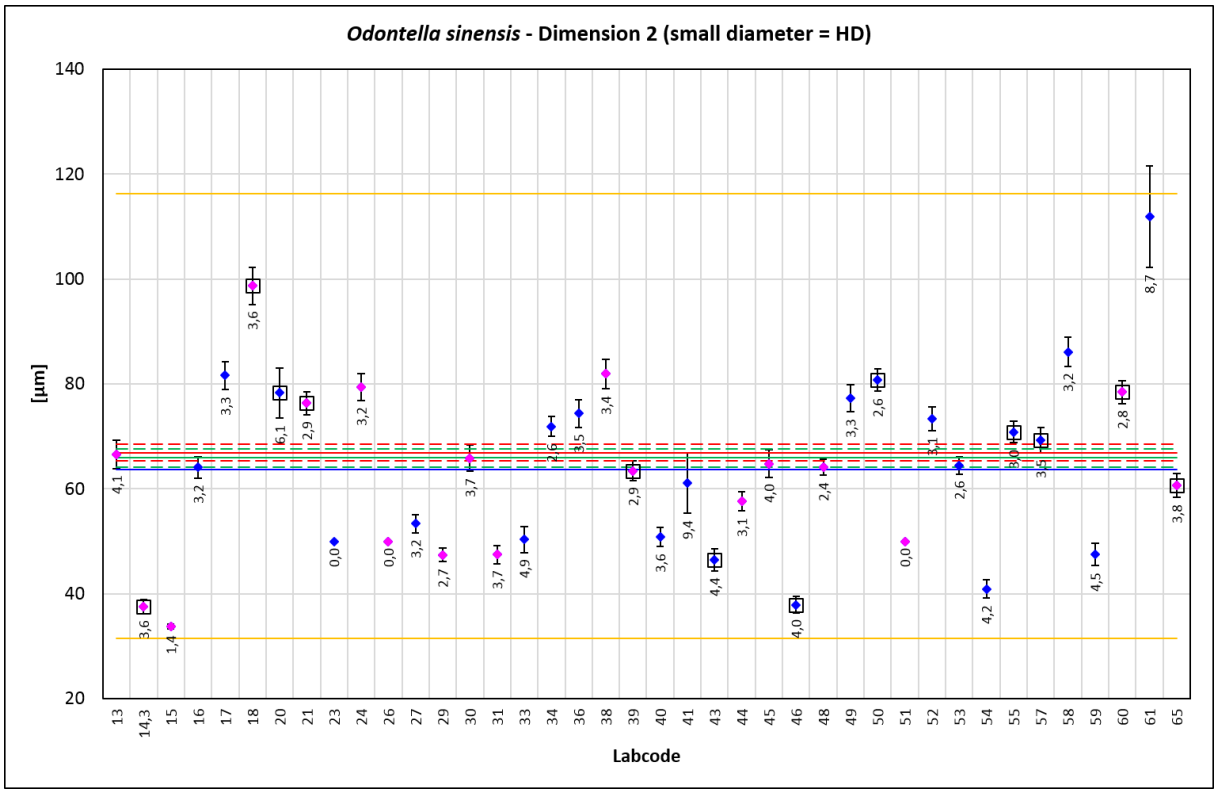

Figure 49: Results and some statistical characteristics for diameter 2 (small diameter = HD) of *Odontella sinensis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

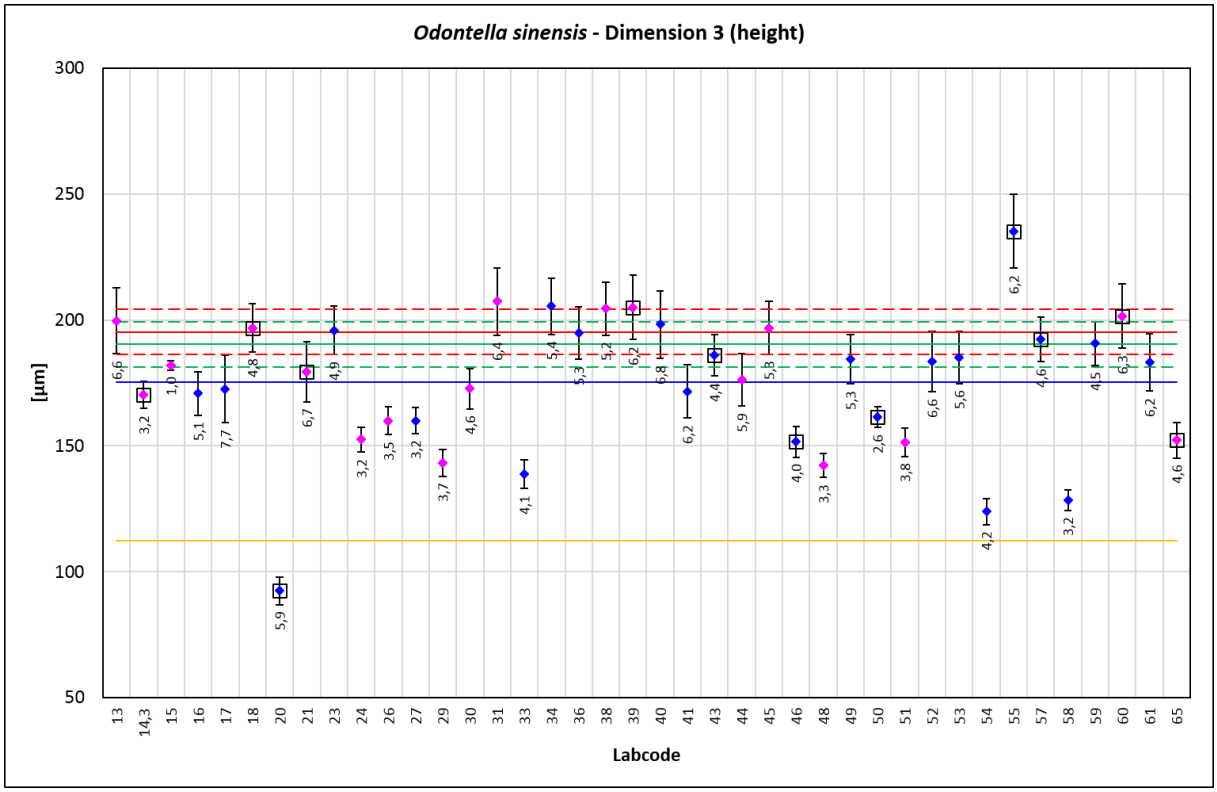

Figure 50: Results and some statistical characteristics for diameter 3 (height) of *Odontella sinensis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

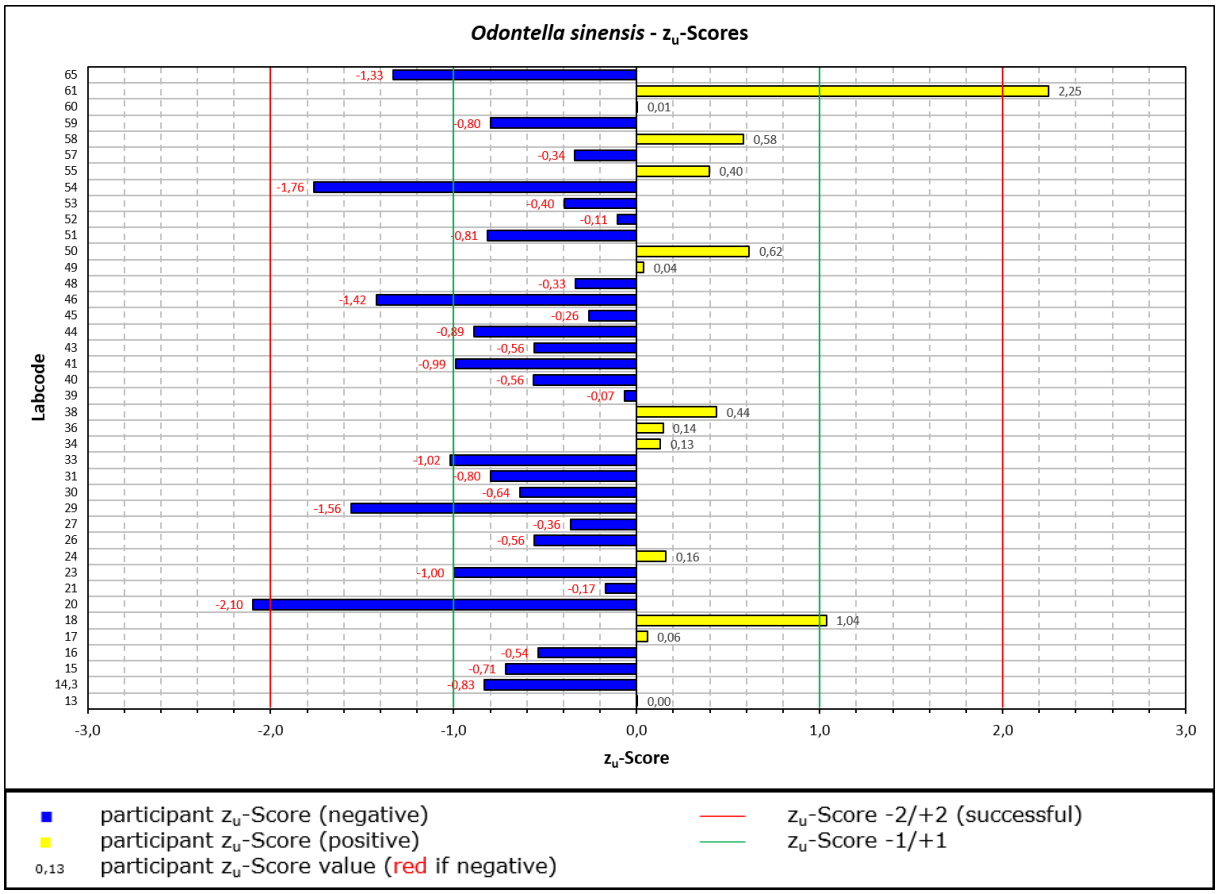

Figure 51: z_u scores of participants for *Odontella sinensis*. Please note: Comma used as decimal mark.

3.4.10 *Chaetoceros debilis*

Chaetoceros debilis is a diatom forming chains (Figure 52). It is a small species of this genus and the cells in this sample did not have an optimal quality. The cell represents an 'elliptic cylinder' which all participants besides one have correctly assigned (Table 12).

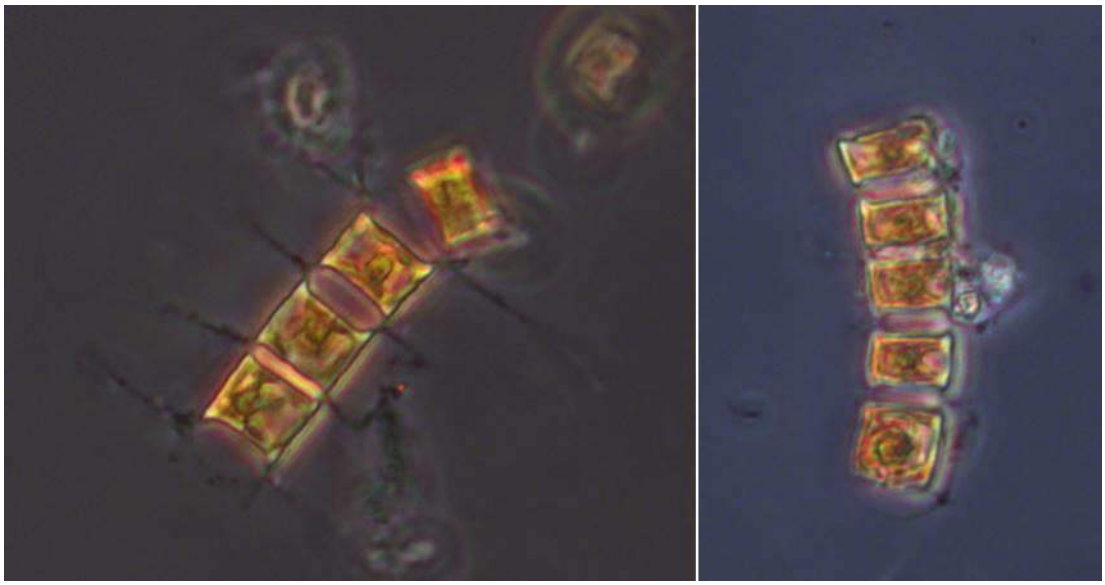

Figure 52: Microphotographic picture of chain forming *Chaetoceros debilis* in the Helgoland sample.

The dimensions are quite variable, thus, the high range from 200 to 1,700 µm^3 is not much surprising (Figure 53). The variability was also visible in the high standard errors and the variability in the measurements of all dimensions (Figure 54, Figure 55 and Figure 56). However, reference values lay well in the middle of this range and only one participant missed the requested z_u range from -2 to +2.

The variability in all dimensions and the fact that participants have confused dimensions in the analysis of other species supports the assumption that confusions may have occurred here as well. Since all dimensions have similar sizes, it is difficult to correctly identify all these confusions. For the same reason, it is a problem to identify the hidden dimension. For reference data, the selection process assured for the 'small diameter' being the hidden dimension. It has been calculated as a value of 0.75 multiplied by the 'large diameter'. Comparing with the calculated data of the participants as if the participants had done the selection and calculation the same way, the calculated hidden dimension ranged between 0.24 and 1.82 and the mean was 0.72 (Table 25).

The z_u scores showed a high variability with a tendency to negative values. Nine of the participants which successfully had passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. One of these participants even missed the z_u range from -2 to +2 and did not pass the test for this species (Figure 57). This reflects a moderate performance for the biovolume analysis of this species.

Table 12: Inventory of the main results (except special statistics) for *Chaetoceros debilis.*

General			
39	participants (labcode) have measured this species: 13 14 16 17 18 20 21 23 24 26 27 29 30 31 33 34 36 38 39 40 41 43 44 45 46 48 49 50 51 52 53 54 55 57 58 59 60 61 65		
22	measurements with an eyepiece micrometre		
17	measurements with image analysis software		
Geometry and Dimensions			
38	measurements/calculations with an 'elliptic cylinder' assigned as geometrical shape according to the Standard Document		
1	measurement/calculation with a 'cylinder' assigned as geometrical shape notwithstanding to the Standard Document		
39	calculations with no correction factor used according to the Standard Document		
Statistics			
7-56	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	18	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		2	measurements with the biovolume standard error being ≥ 10 %
	5	total measurements with the biovolume standard error being ≥ 10 %	

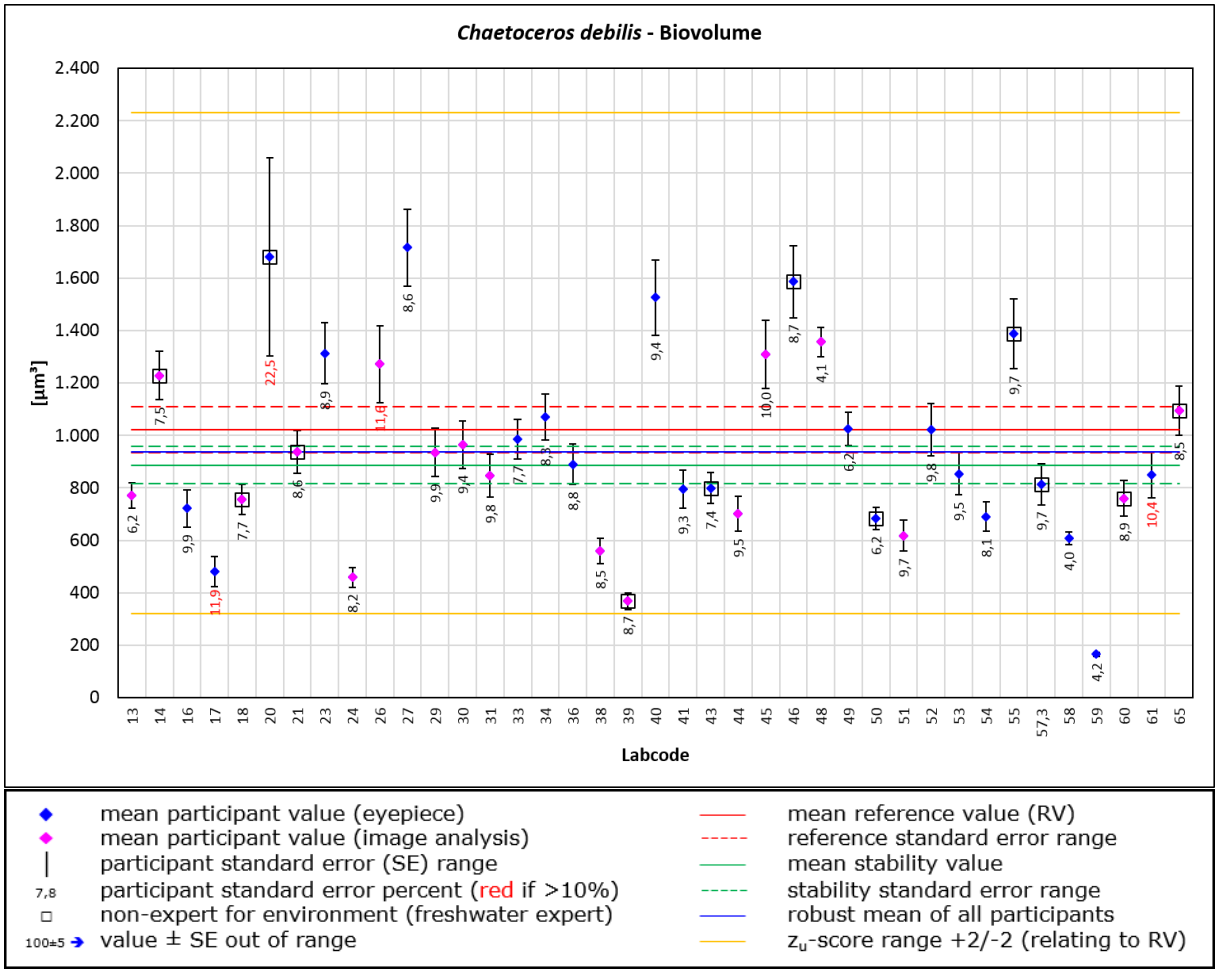

Figure 53: Results and some statistical characteristics for biovolume of *Chaetoceros debilis*. Please note: Comma used as decimal mark, full stop used as thousands separator.

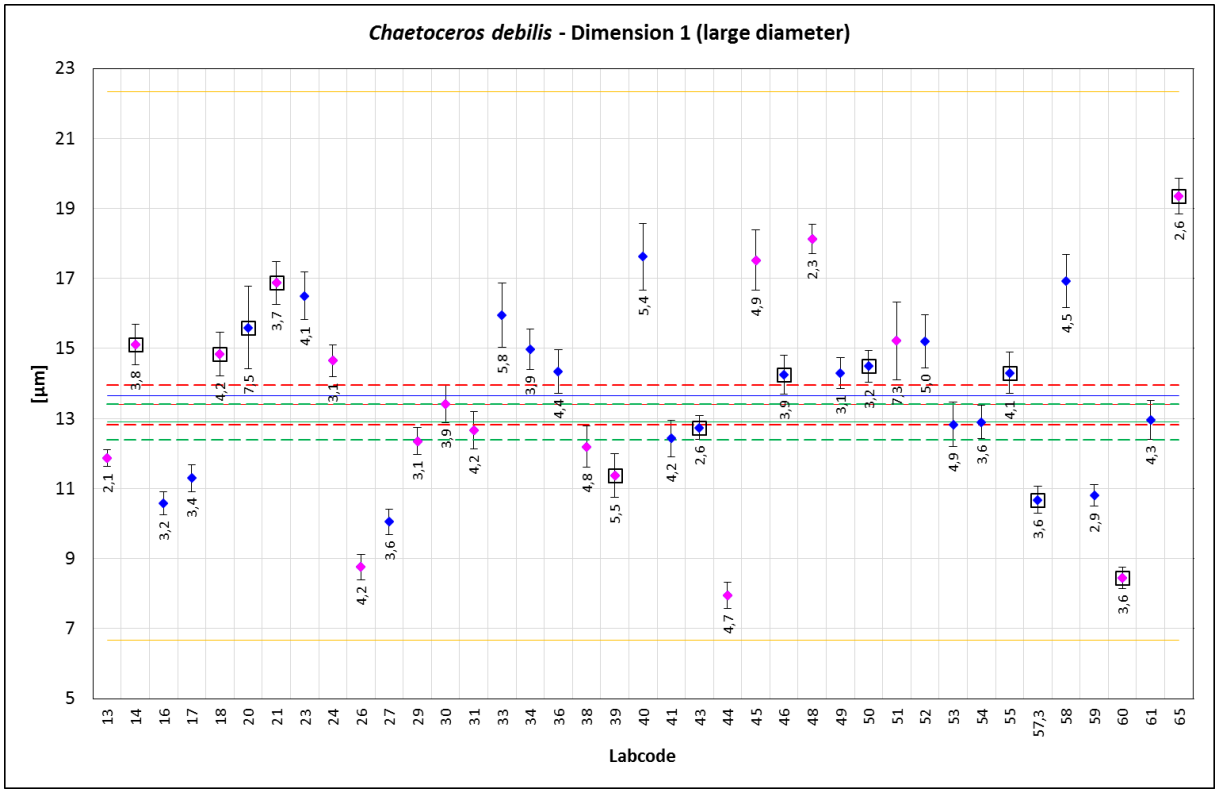

Figure 54: Results and some statistical characteristics for dimension 1 (large diameter) of *Chaetoceros debilis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

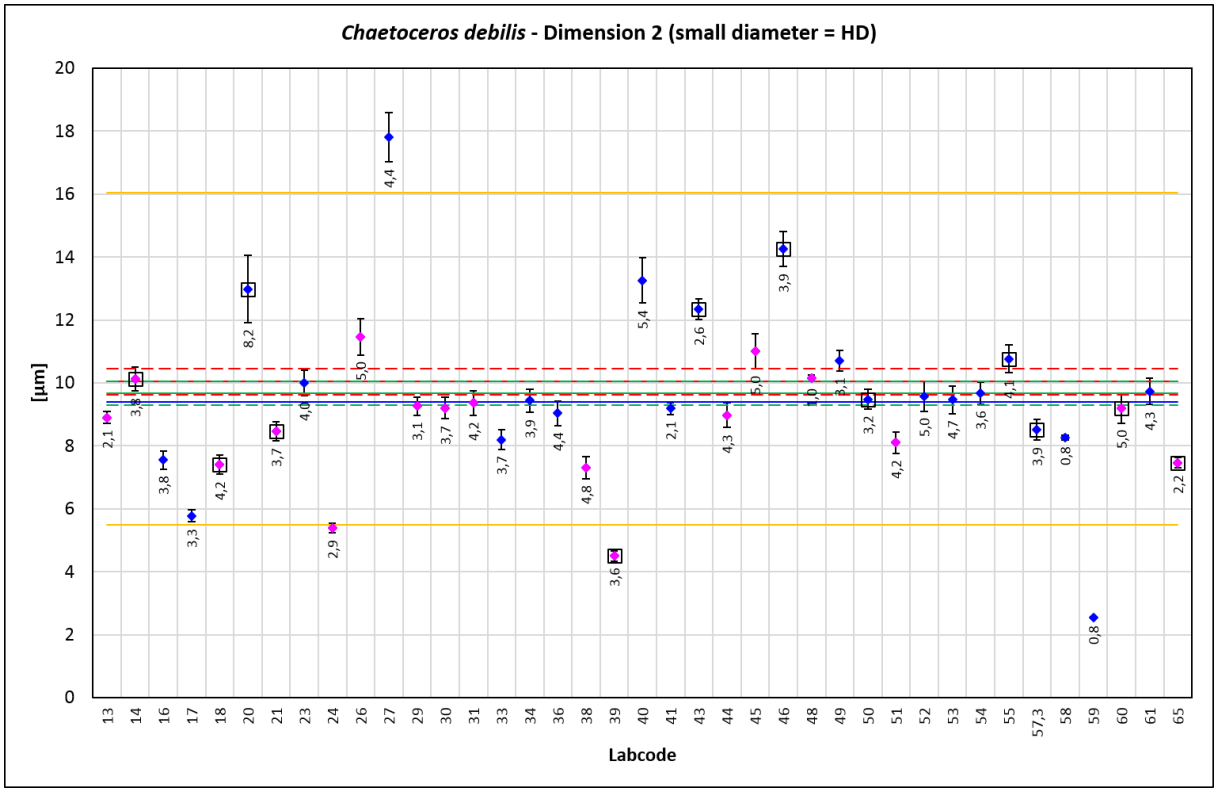

Figure 55: Results and some statistical characteristics for dimension 2 (small diameter = HD) of *Chaetoceros debilis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

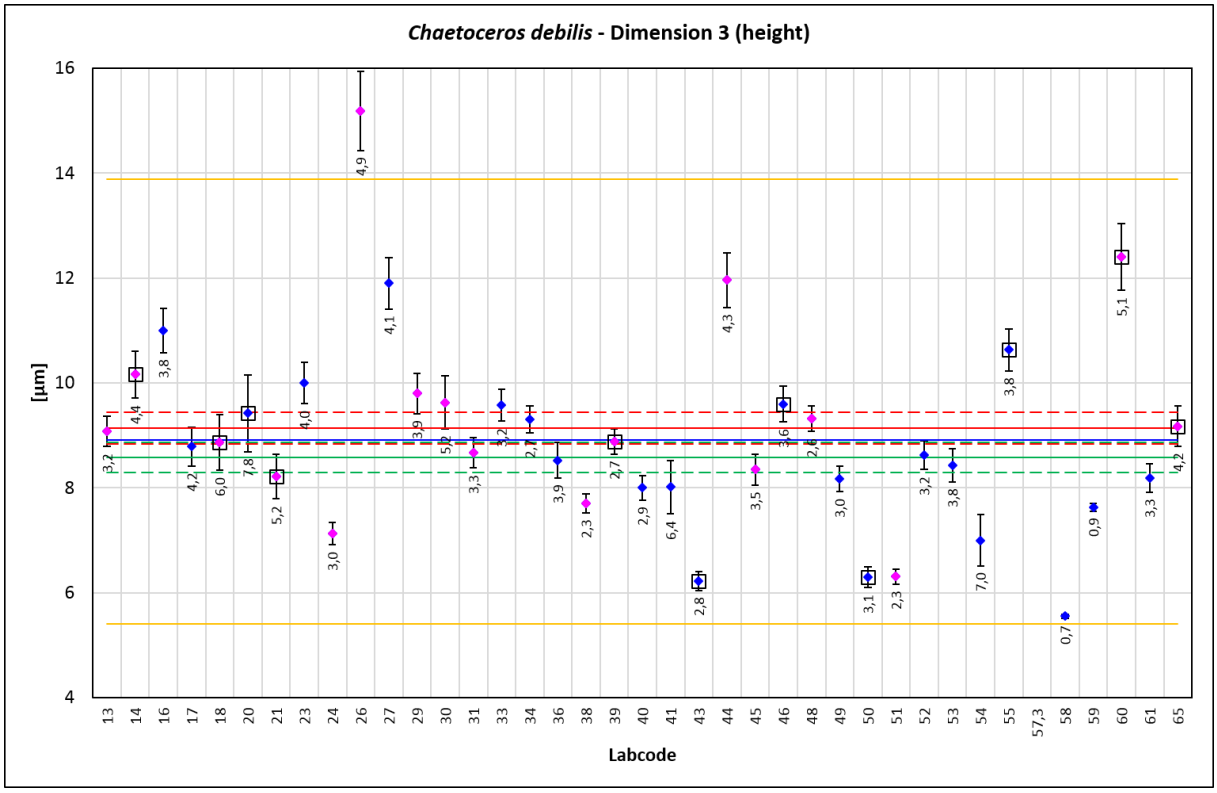

Figure 56: Results and some statistical characteristics for dimension 3 (height) of *Chaetoceros debilis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

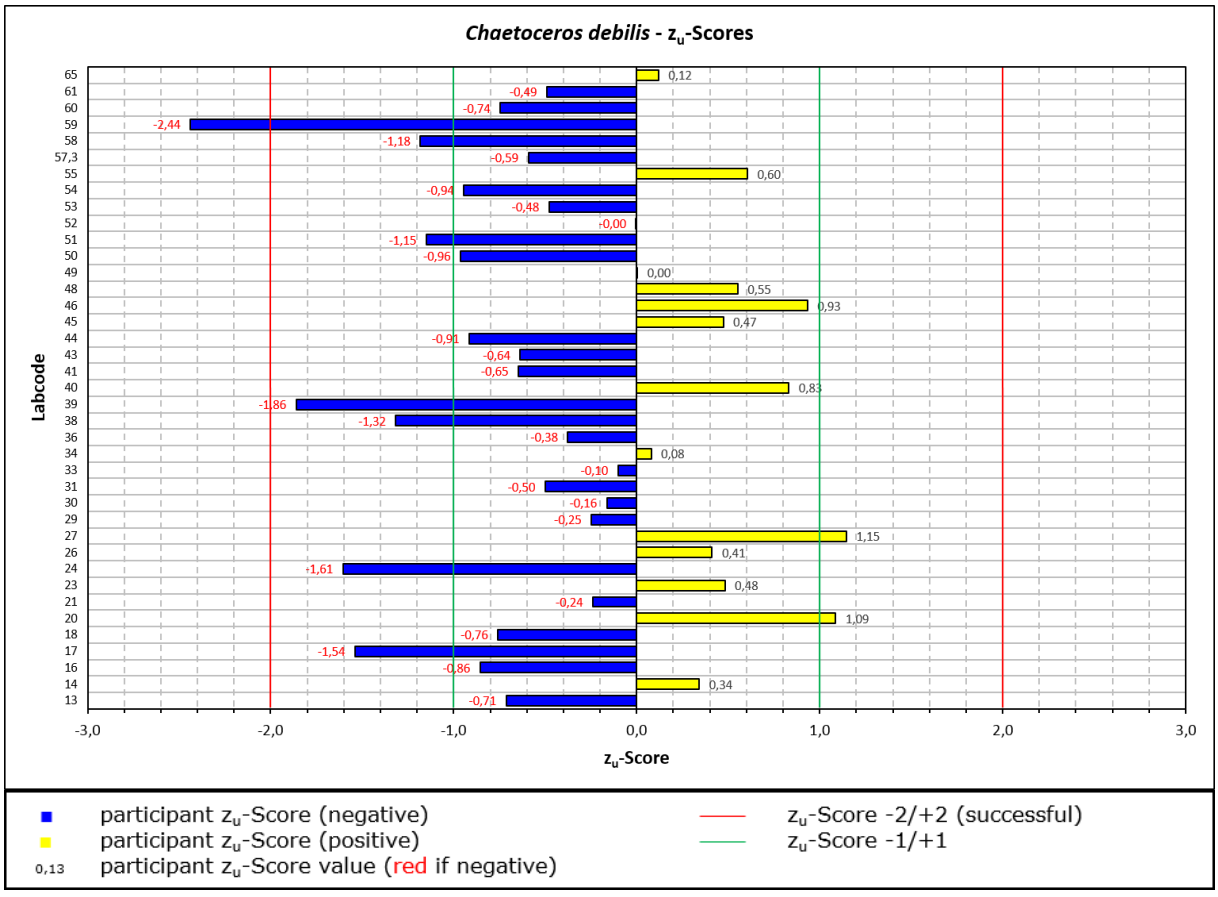

Figure 57: z_u scores of participants for *Chaetoceros debilis*. Please note: Comma used as decimal mark.

3.4.11 *Fragilaria crotonensis*

Fragilaria crotonensis is a freshwater diatom that forms ribbon-like colonies (Figure 58). All participants that have measured this species have correctly assigned the 'rhombic prism' as geometrical shape, according to the Standard Document (Table 13). Two participants have compared it with the special form of HELCOM 'half parallelepiped'. Almost half of the participants have not used the correction factor recommended by the Standard Document to adjust the biovolume of the real cell form to the biovolume of the geometrical shape. This correction factor has to be applied here, because the height of the cell is greater at the middle than at the ends. Using the extent measured in the middle of the cell, the correction factor downsizes the results of the equation to well-fitting values. The comparison of the results for the pervalvar axis (height, Figure 62) supported the assumption that some participants may have measured rather more to the ends of the cell than in the middle (Figure 58). The reference values lay in the upper part of the range (Figure 59). This underlines the necessity for clear rules defined from where to where the cells have to be measured exactly.

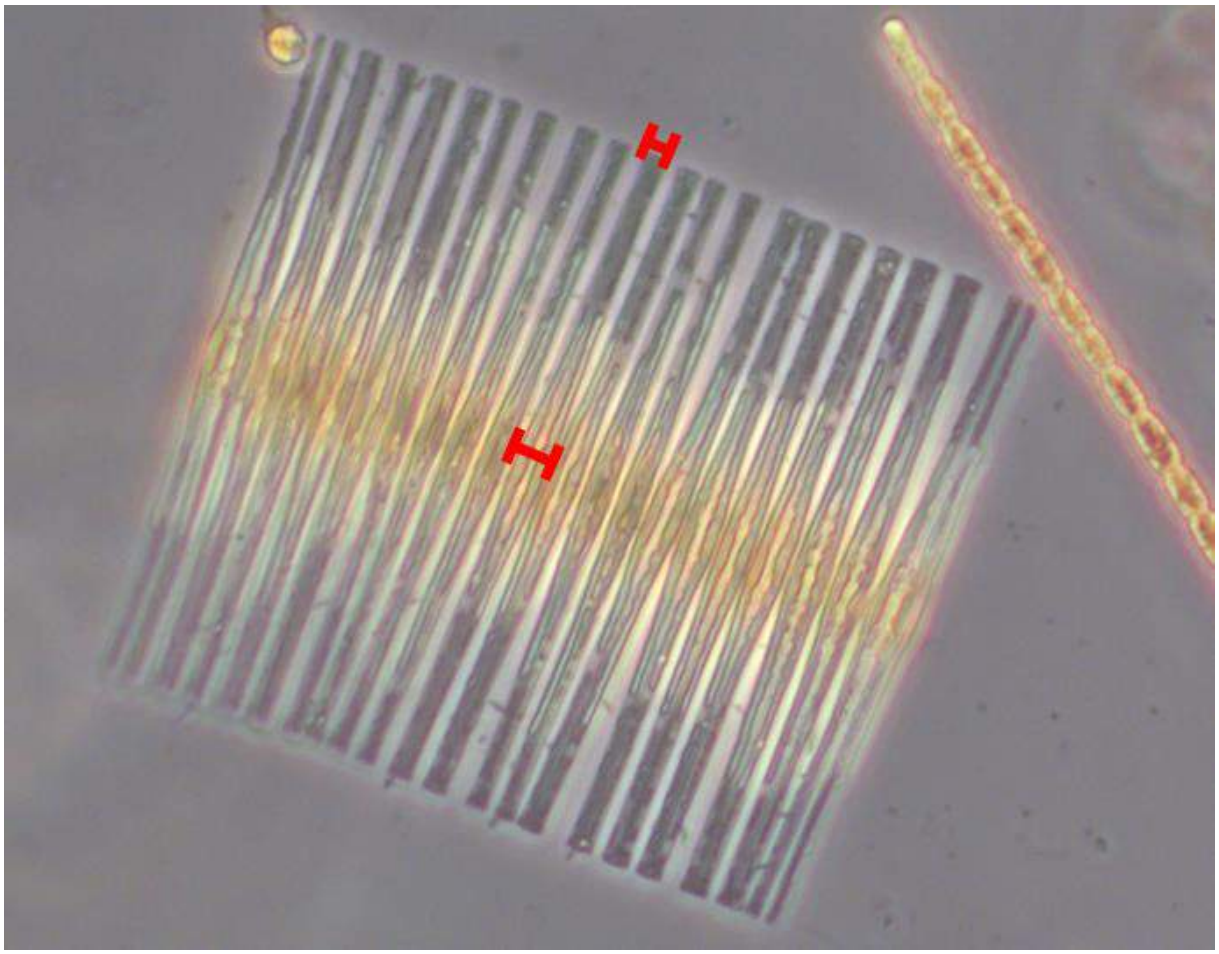

Figure 58: Microphotographic picture of chain forming *Fragilaria crotonensis* in the Scharmützelsee sample. Red lines display the discrepancy in the height in the middle and at the end of the cell.

Table 13: Inventory of the main results (except special statistics) for *Fragilaria crotonensis.*

General			
48	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 23 24 25 26 27 29 30 31 32 33 34 35 36 37 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
	1	participant has measured single cells AND colonies (chains) for comparison	
31	measurements with an eyepiece micrometre		
22	measurements with image analysis software		
Geometry and Dimensions			
50	measurements/calculations with a 'rhombic prism' assigned as geometrical shape according to the Standard Document		
	3	additional calculations with a 'half parallelepiped' assigned as geometrical shape notwithstanding to the Standard Document (according to HELCOM PEG-list) for comparison	
	1	additional measurement/calculation for 'rhombic prism' and 'half parallelepiped' each measuring the colony and calculating the average of the pervalvar axis by dividing by the number of cells.	
29	calculations with a correction factor of 0.9 used according to the Standard Document		
23	calculations with no correction factor used notwithstanding to the Standard Document		
3	calculations with 'large diameter' and 'height' being confused		
Statistics			
12-120	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	29	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		1	measurement with the biovolume standard error being ≥ 10 %
	3	total measurements with the biovolume standard error being ≥ 10 %	

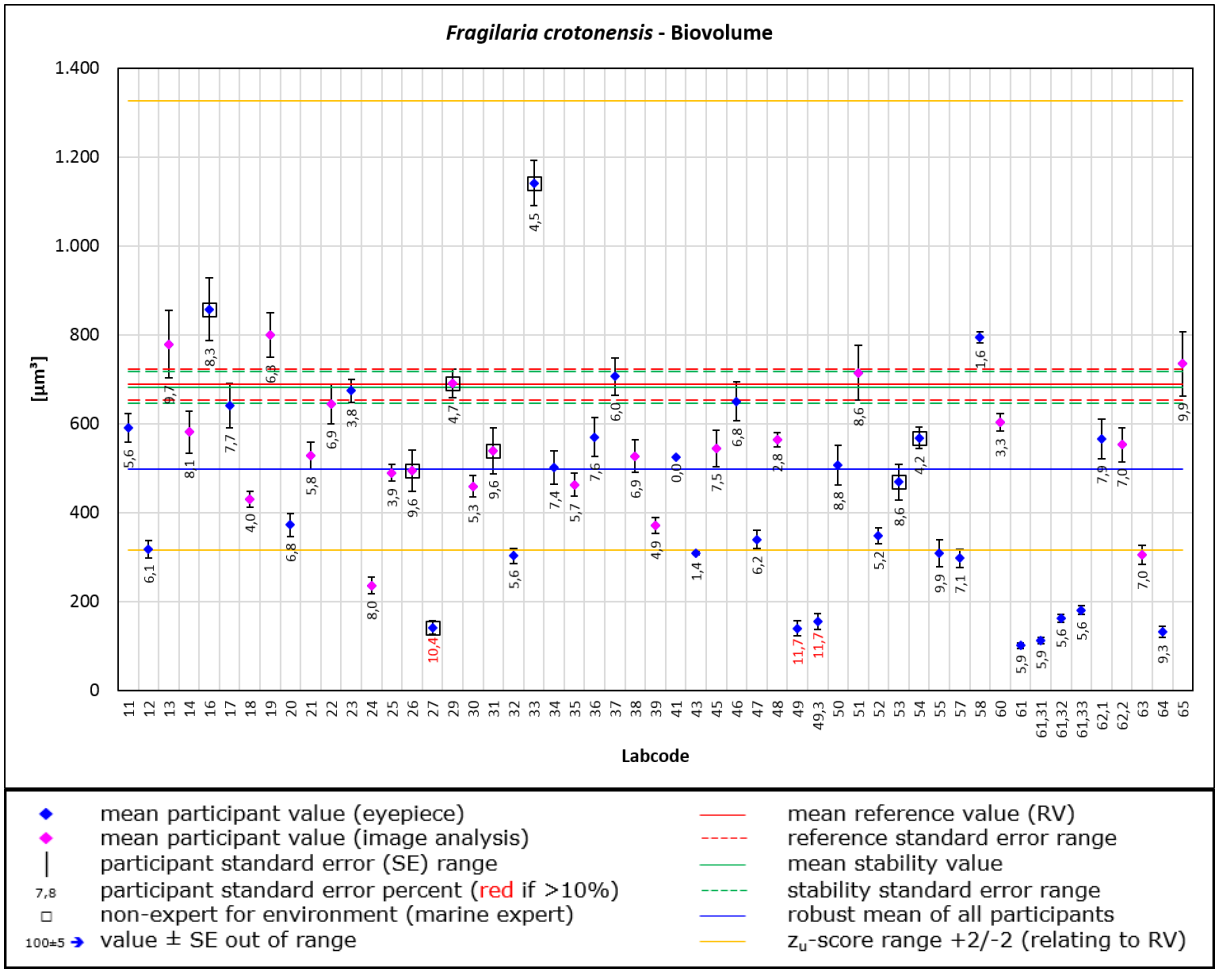

Figure 59: Results and some statistical characteristics for biovolume of *Fragilaria crotonensis*. Please note: Comma used as decimal mark, full stop used as thousands separator.

On average, the biovolume results of the participants were lower than the reference data. The biovolume data of the participants reached from 100 to 1,100 µm³ with a mean of about 500 µm³ while the reference data had a biovolume of 700 µm³. Compared to other species many participants missed the requested z_u range from -2 to +2, all of them being lower than -2. This might be due to the missing correction factor but a further look into the single dimensions revealed that the difference mainly came from the 'height', i.e. the pervalvar axis (Figure 60, Figure 61 and Figure 62). This is the hidden dimension which was set for reference data to have the same values as the transapical axis. The calculated data of the hidden dimension of the participants ranged from 0.01 to 2.3 with a mean of 1.16. Often the factor had been 1 as well (Table 25).

A clear tendency to highly negative z_u scores was obvious for this species (Figure 63). The explanation may be in the difference between the data to the reference data as a baseline for the calculation. Anyway, the reference data were regarded as reliable. Less than half of the participants which successfully had passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. Eleven participants even missed the z_u range from -2 to +2 and thus, did not pass the test for this species. Additionally, one had been excluded as outlier missing the z_u range from -3 to +3 (Table 26). This reflects a poor performance for the biovolume analysis of this species.

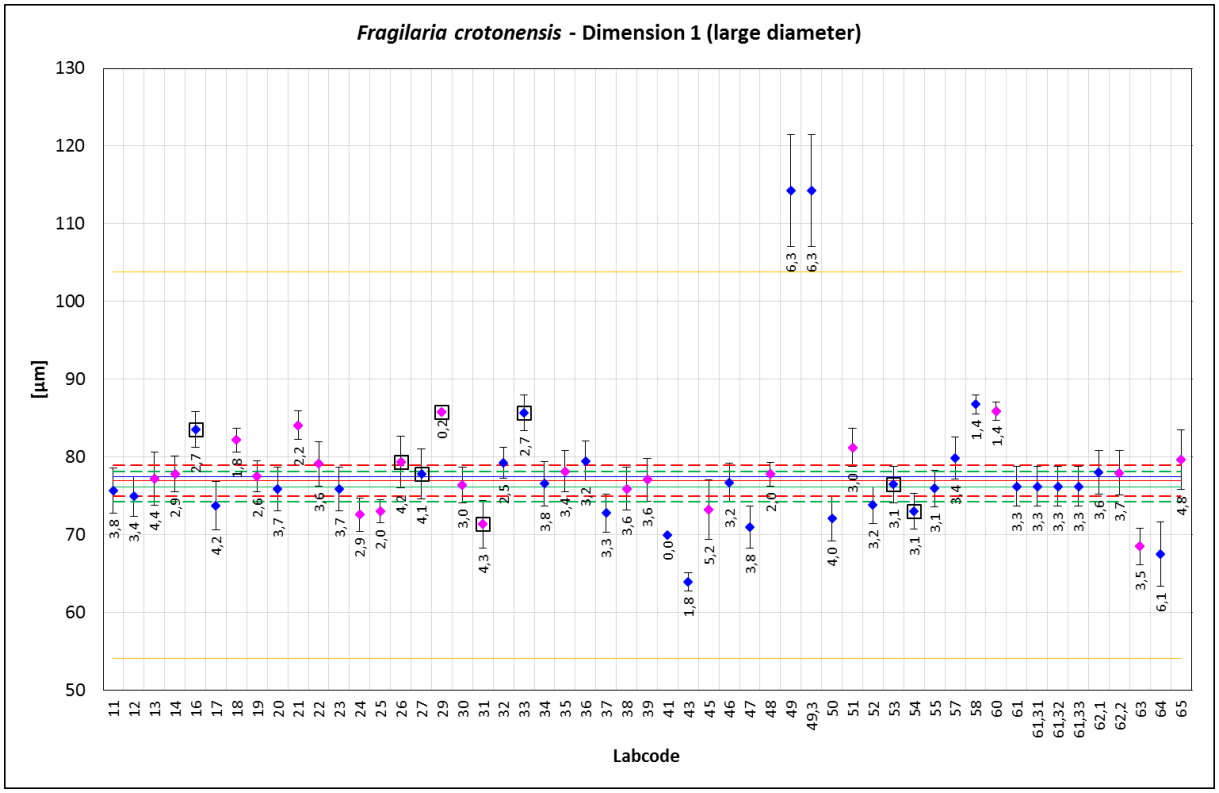

Figure 60: Results and some statistical characteristics for dimension 1 (large diameter) of *Fragilaria crotonensis*. Legend see biovolume chart. Please note: Comma used as decimal mark.

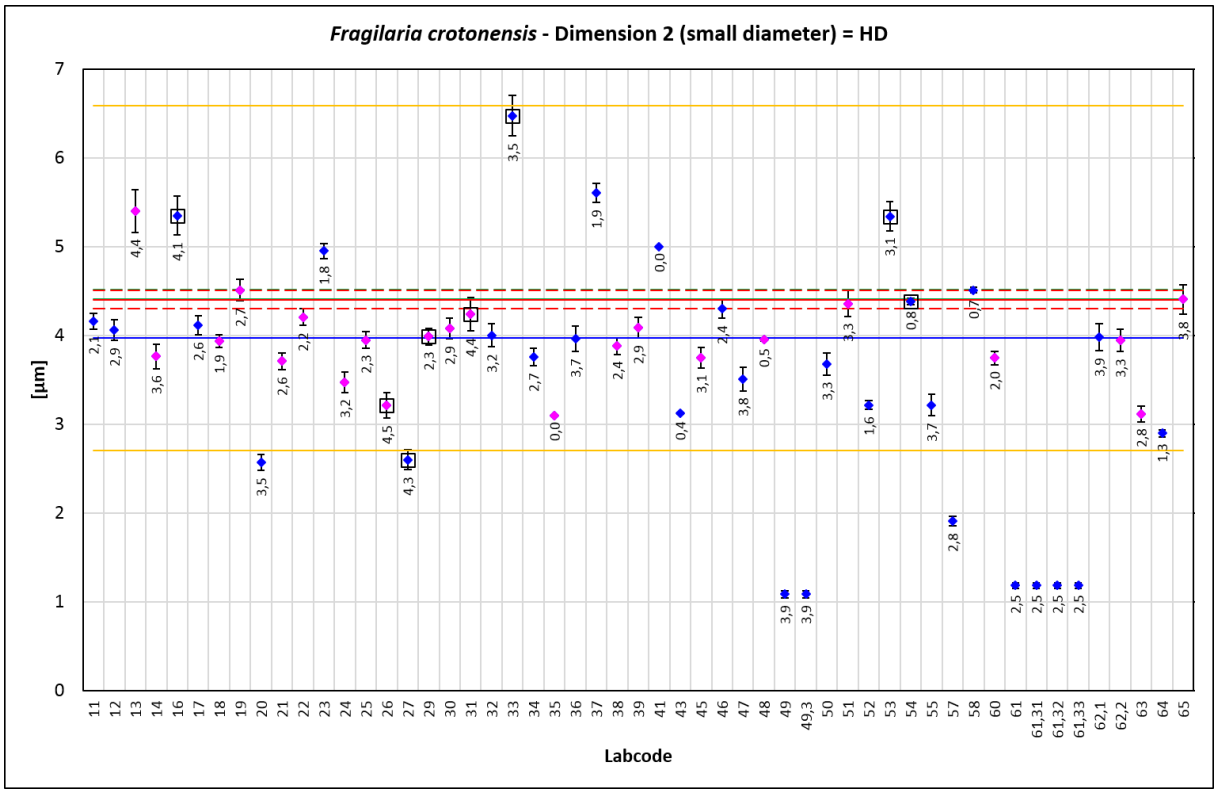

Figure 61: Results and some statistical characteristics for dimension 2 (small diameter) of *Fragilaria crotonensis*. Legend see biovolume chart. Please note: Comma used as decimal mark. Reference value ≈ Stability value.

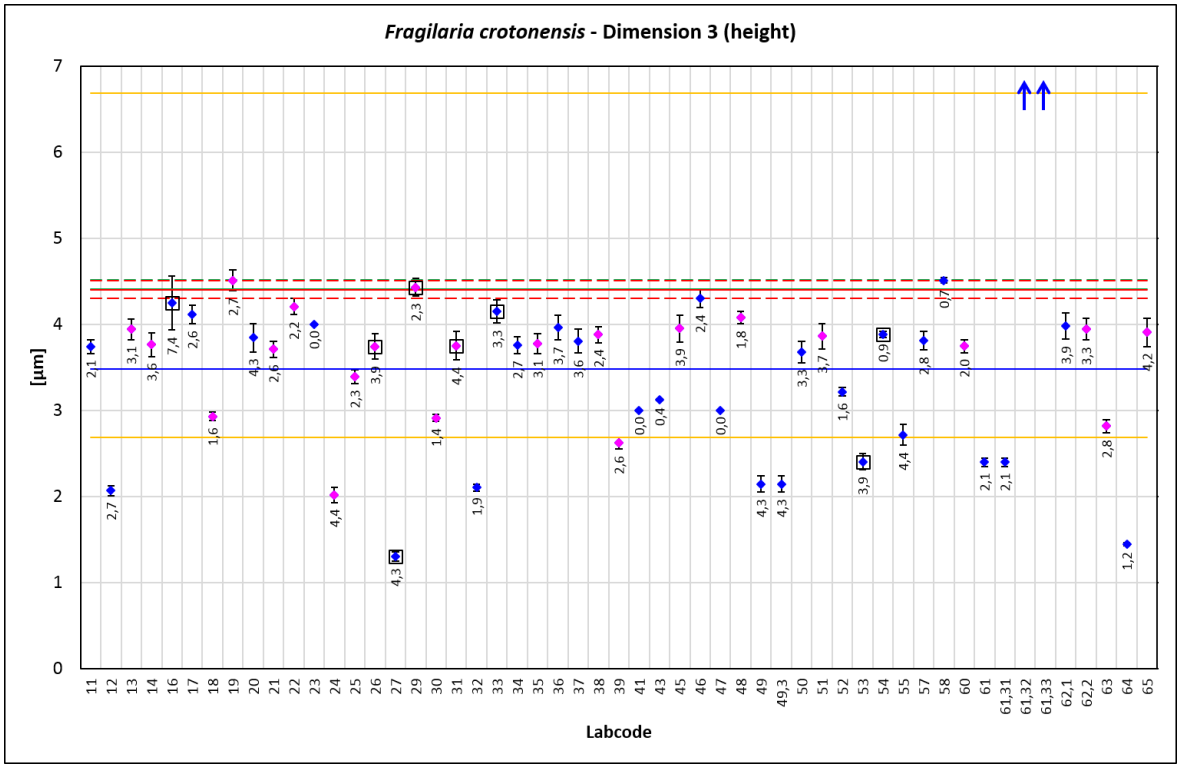

Figure 62: Results and some statistical characteristics for dimension 3 (height) of *Fragilaria crotonensis*. Legend see biovolume chart. Please note: Comma used as decimal mark. Reference value ≈ Stability value.

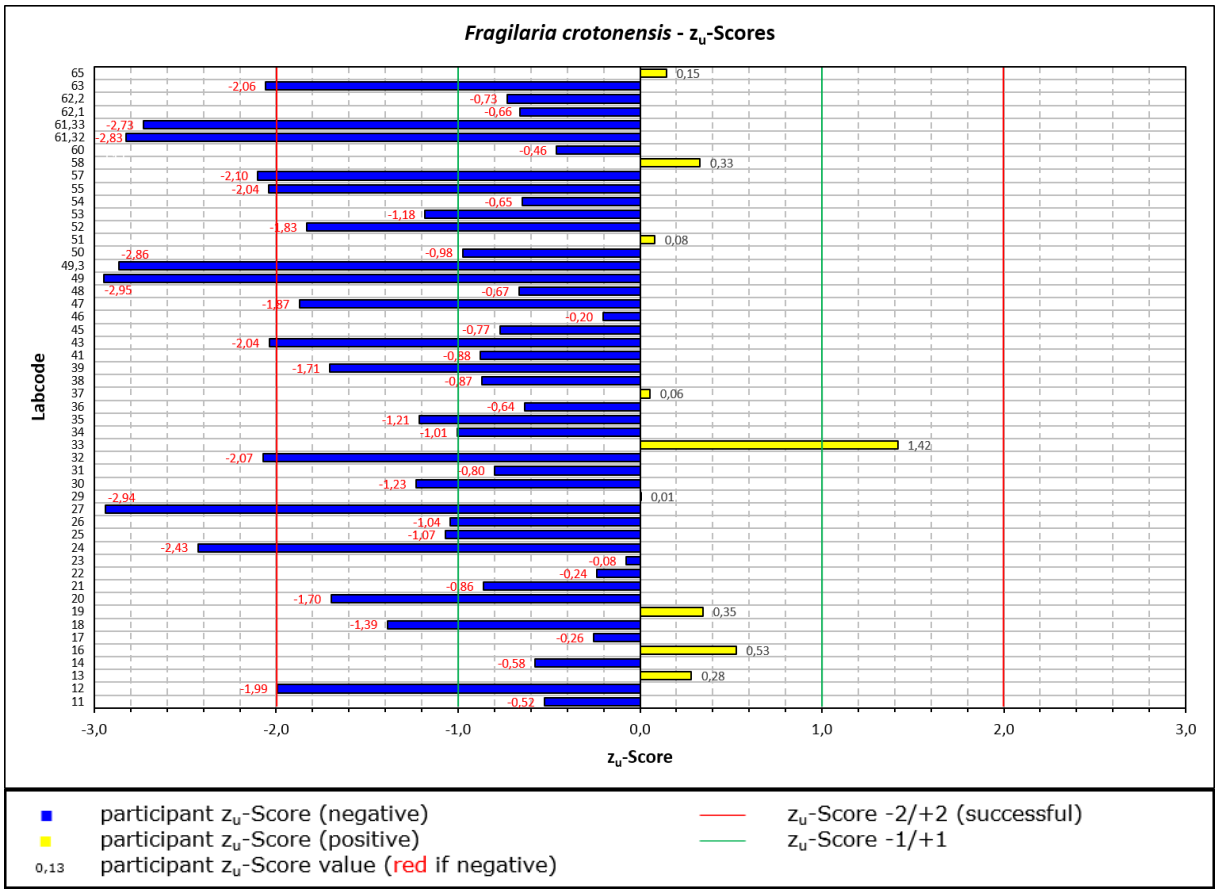

Figure 63: z_u scores of participants for *Fragilaria crotonensis*. Please note: Comma used as decimal mark.

3.4.12 *Tabellaria fenestrata*

Tabellaria fenestrata is a diatom that forms star-shaped colonies (Figure 64) or zigzag-bands. Most participants that measured this species have correctly assigned the 'cuboid' as geometrical shape (Table 14). Three participants have used the elliptic cylinder instead, but the results fit generally in with the range. One participant has used only two size classes for this species. The difference between the size classes was made by only one dimension out of three. This gives a hint that maybe all cells of a colony have been measured. In order to prevent unnecessary high variability, one criterion for selecting items should be to pick just one cell out of a colony.

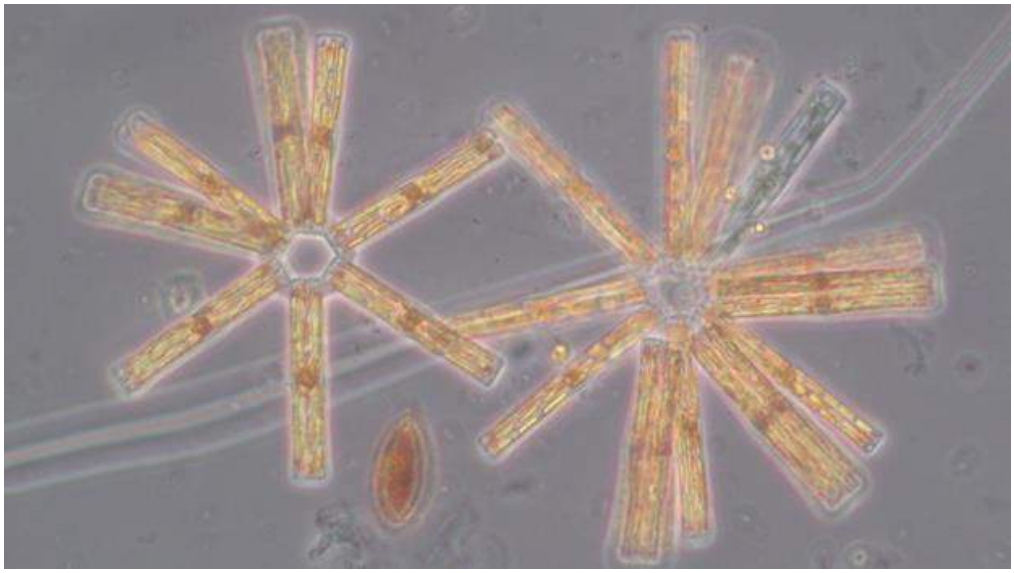

Figure 64: Microphotographic picture of colony forming *Tabellaria fenestrata* in the Scharmützelsee sample.

Average biovolume values ranged from about 1,000 to 4,000 µm^3 and most values lay between 1,000 and the reference values being a bit higher than 2,000 µm^3 (Figure 65). Most of the participants' results for the biovolume were within the requested z_u range from -2 to +2. There have been 4 outliers lying above and 2 outliers lying below that range. While the data of the participants and the reference data of first edge length have been comparable with each other, the other two dimensions showed a high deviation, especially in the hidden dimension (Figure 66, Figure 67, and Figure 68).

The hidden dimension is the transapical axis and has been calculated for the reference values as 0.1 multiplied by the length of the apical axis. When calculating the same relation for the participants it ranged between 0.06 and 0.39 with a mean of 0.15 (Table 25). It is not clear if the participants have used another relation as had been done for reference data. Thus, no clear statement about that factor can be made and the differences between reference data and data of participants might be caused also by measuring differences. This would especially be the case if most participants had calculated the hidden dimension as a relation of the height (Figure 68).

Table 14: Inventory of the main results (except special statistics) for *Tabellaria fenestrata.*

General			
47	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 23 24 25 26 27 29 30 31 32 33 34 35 36 37 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
27	measurements with an eyepiece micrometre		
21	measurements with image analysis software		
Geometry and Dimensions			
45	measurements/calculations with a 'cuboid' assigned as geometrical shape according to the Standard Document		
3	measurements/calculations with a 'elliptic cylinder' assigned as geometrical shape notwithstanding to the Standard Document		
48	calculations with no correction factor used according to the Standard Document		
1	calculation with 'apical' and 'pervalvar axis' being confused		
Statistics			
12-50	units have been measured		
	2	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	33	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		2	measurement with the biovolume standard error being ≥ 10 %
	2	total measurements with the biovolume standard error being ≥ 10 %	

Most z_u scores had negative values, but also high positive values have been calculated (Figure 69). Less than half of the participants which successfully had passed the repeated z_u assessment procedures did not lie within a z_u range from -1 to +1. Three participants even missed the z_u range from -2 to +2 and thus did not pass the test for this species. Additionally, three had been excluded as outliers missing the z_u range from -3 to +3 (Table 26). This reflects a moderate to poor performance for the biovolume analysis of this species.

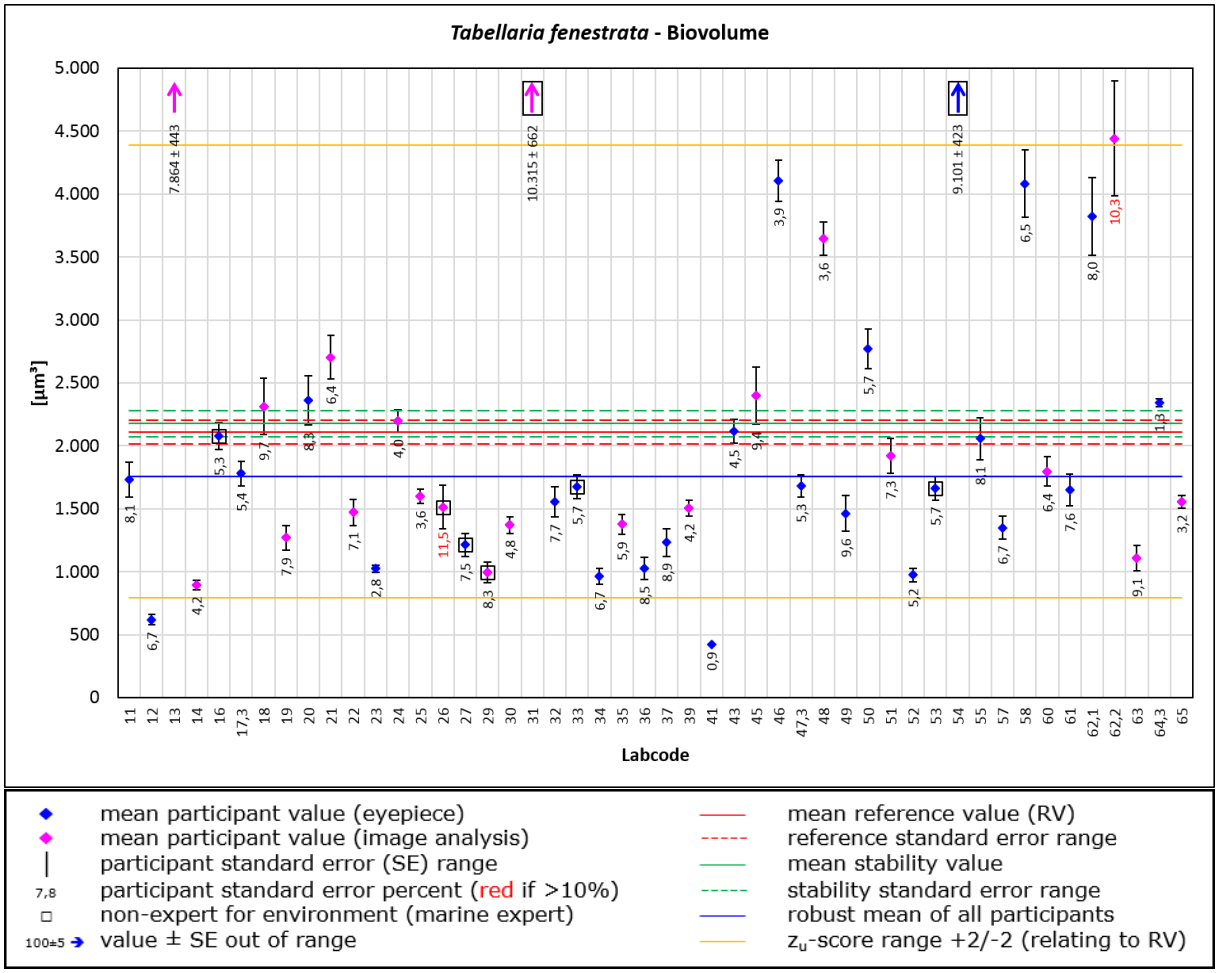

Figure 65: Results and some statistical characteristics for biovolume of *Tabellaria fenestrata*. Please note: Comma used as decimal mark, full stop used as thousands separator.

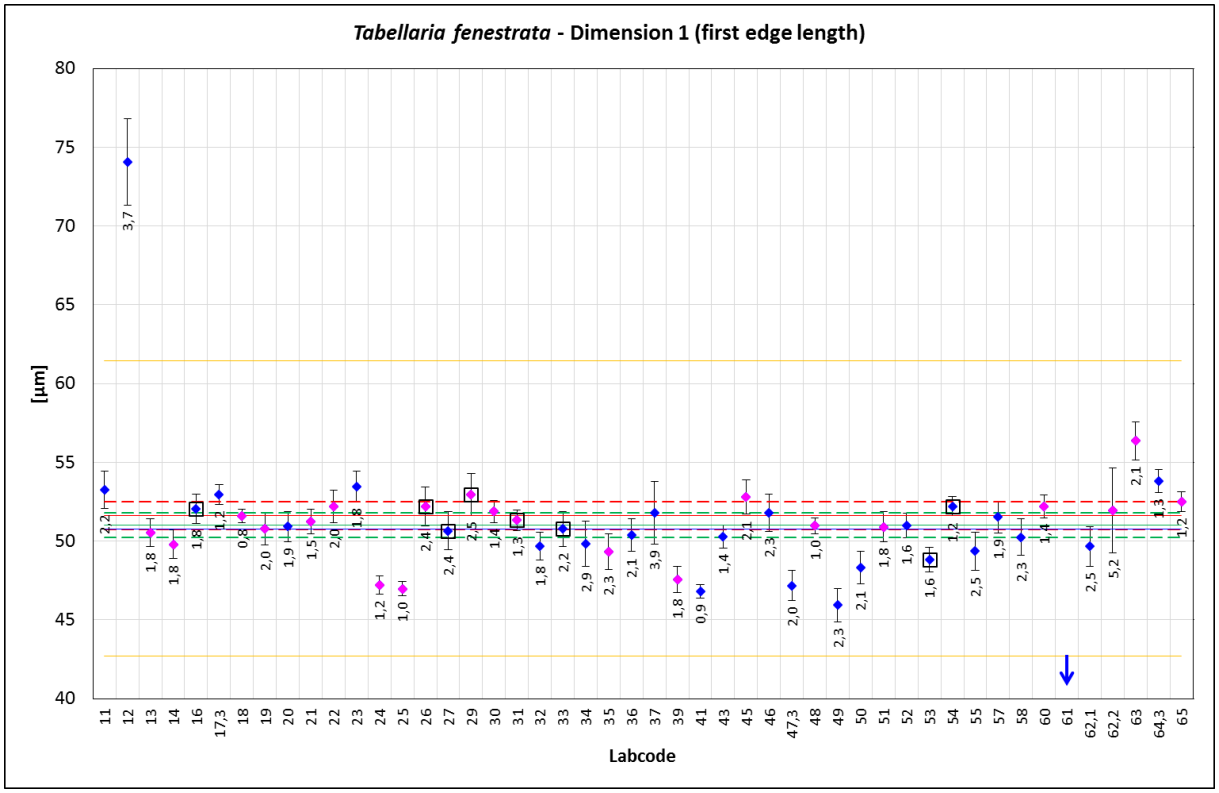

Figure 66: Results and some statistical characteristics for dimension 1 (first edge length) of *Tabellaria fenestrata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

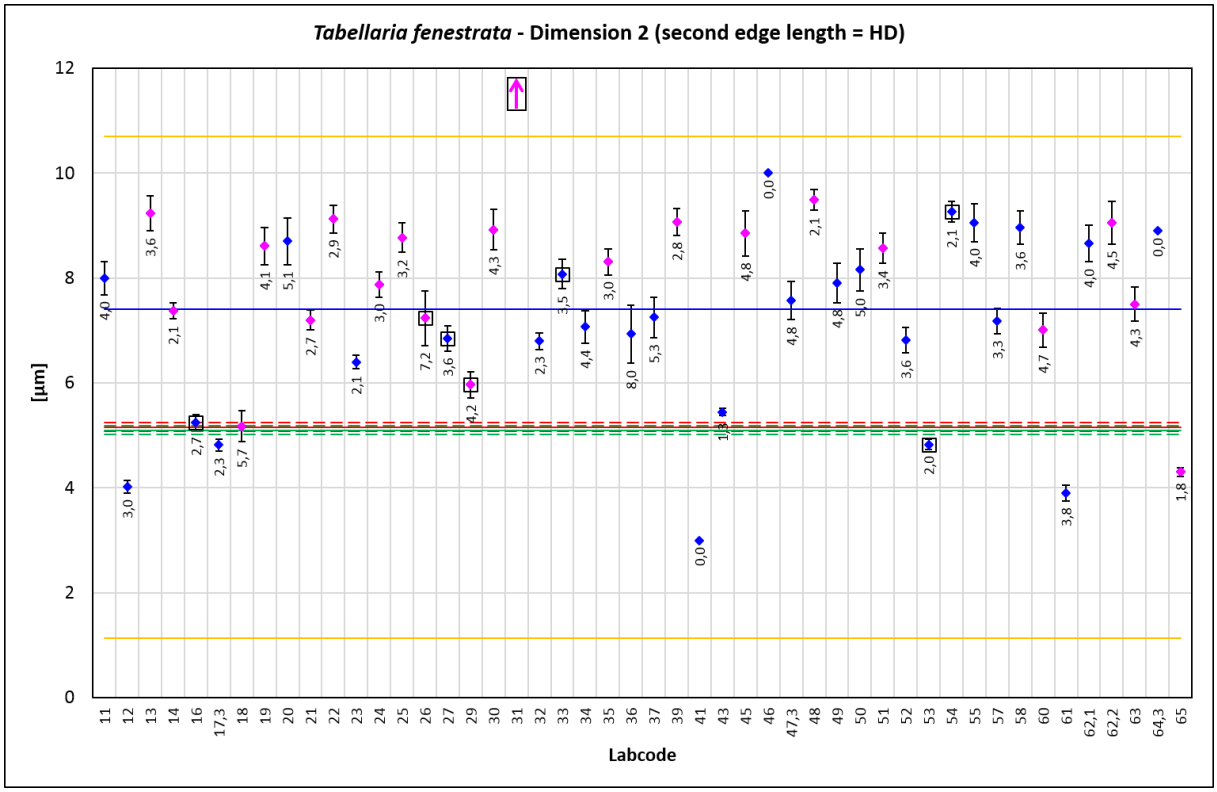

Figure 67: Results and some statistical characteristics for dimension 2 (second edge length = HD) of *Tabellaria fenestrata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

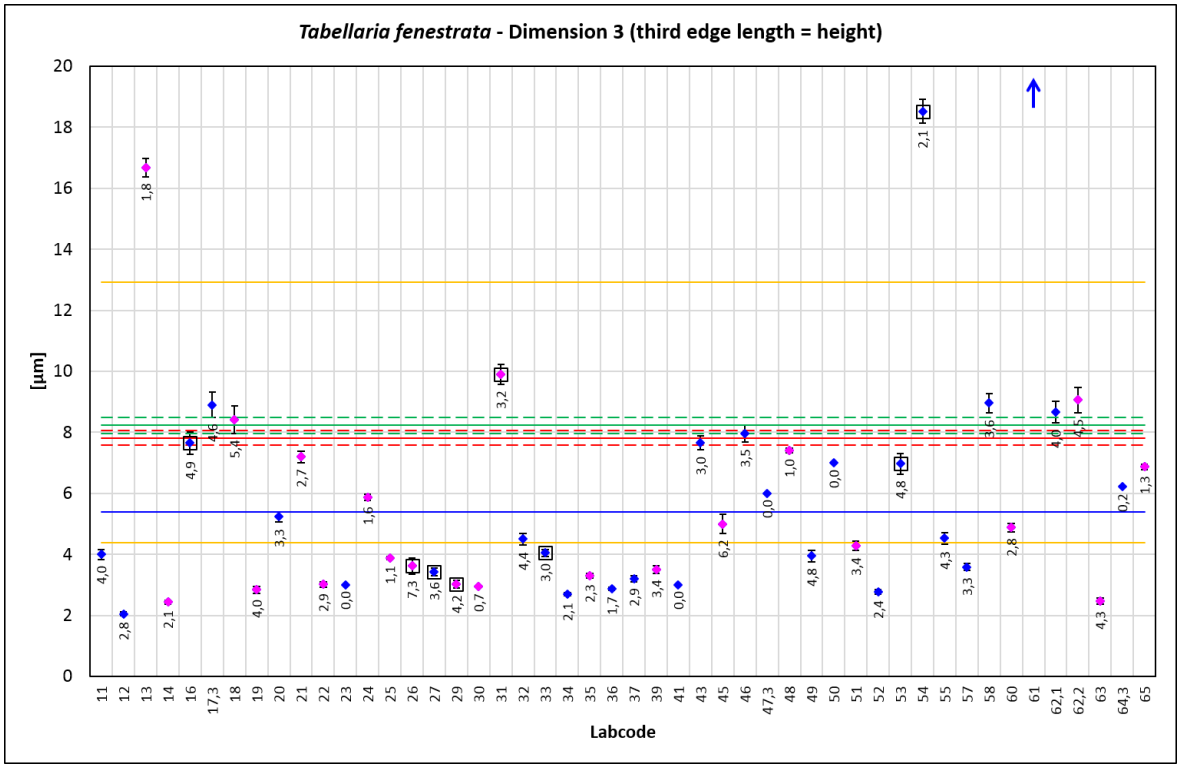

Figure 68: Results and some statistical characteristics for dimension 3 (third edge length = height) of *Tabellaria fenestrata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

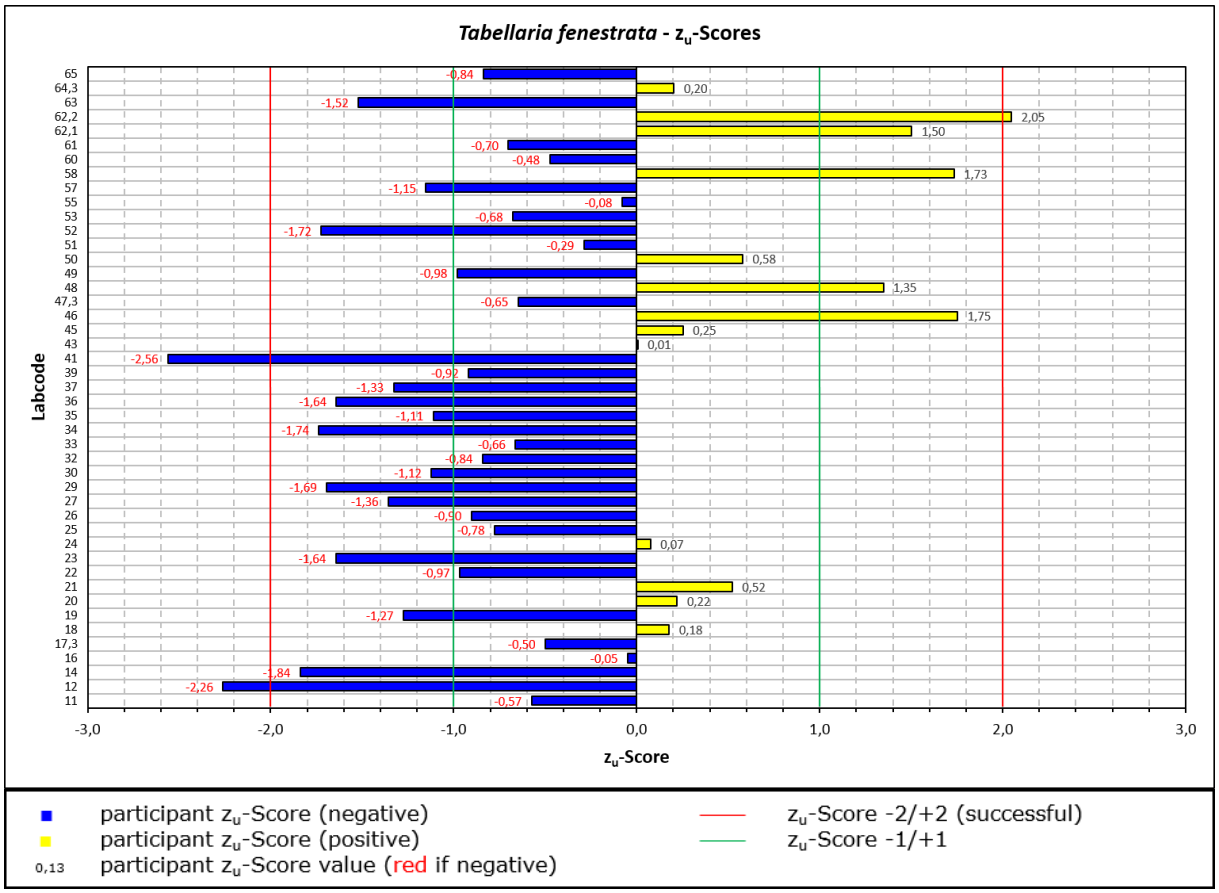

Figure 69: z_u scores of participants for *Tabellaria fenestrata*. Please note: Comma used as decimal mark.

3.4.13 *Aulacoseira granulata*

Aulacoseira granulata is a filamentous species consisting of cylindrical cells which can be identified easily (Figure 70). All participants that have measured this species have correctly assigned the 'cylinder' as geometrical shape, according to the Standard Document (Table 15). Most participants have measured cells, but some have measured filaments either in 100 µm sections or as the length of the filament (Figure 73). These values cannot be standardised and have been excluded from further analysis (Table 26). One participant has confused diameter and height (Figure 72), but the results have been correctly recalculated.

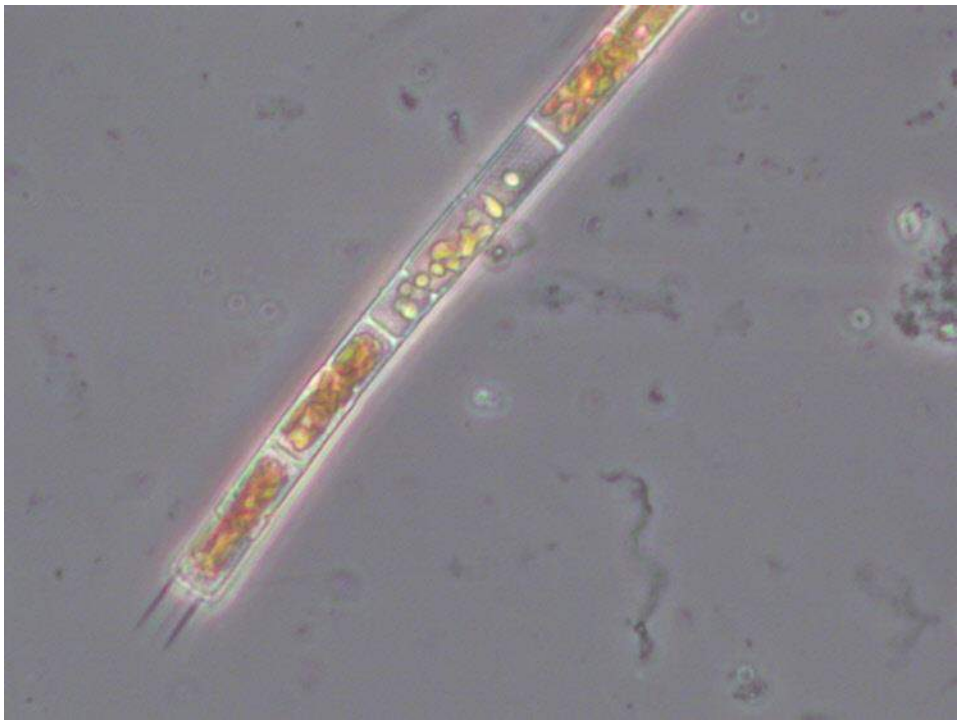

Figure 70: Microphotographic picture of chain forming *Aulacoseira granulata* in the Scharmützelsee sample.

The range of biovolume lay between 400 to 1,200 µm^3; mostly between 550 and 950 µm^3 and thus, higher in average than the reference values (Figure 71). This is mainly caused by the measurements of the diameter (Figure 72). There have been 6 outliers lying above the z_u range from -2 to +2, mainly due to the deviant way the height had been measured.

The z_u score values were mainly positive. Most of the participants, which successfully had passed the repeated z_u assessment procedures, lay within a z_u range from -1 to +1. Only four missed that range. All participants passed the test for this species (Figure 74). This reflects a very good performance for the biovolume analysis of this species.

Table 15: Inventory of the main results (except special statistics) *Aulacoseira granulata.*

General			
48	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 23 24 25 26 27 29 30 31 32 33 34 35 36 37 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
	1	participant has measured single cells AND colonies (chains) for comparison	
27	measurements with an eyepiece micrometre		
23	measurements with image analysis software		
Geometry and Dimensions			
50	measurements/calculations with a 'cylinder' assigned as geometrical shape according to the Standard Document		
50	calculations with no correction factor used according to the Standard Document		
1	calculation with 'diameter' and 'height' being confused		
3	measurements with 'height' measured in 100 µm sections		
3	measurements with 'height' measured as total chain length		
Statistics			
20-50	units have been measured		
	0	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	32	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		1	measurement with the biovolume standard error being ≥ 10 %
	2	total measurements with the biovolume standard error being ≥ 10 %	

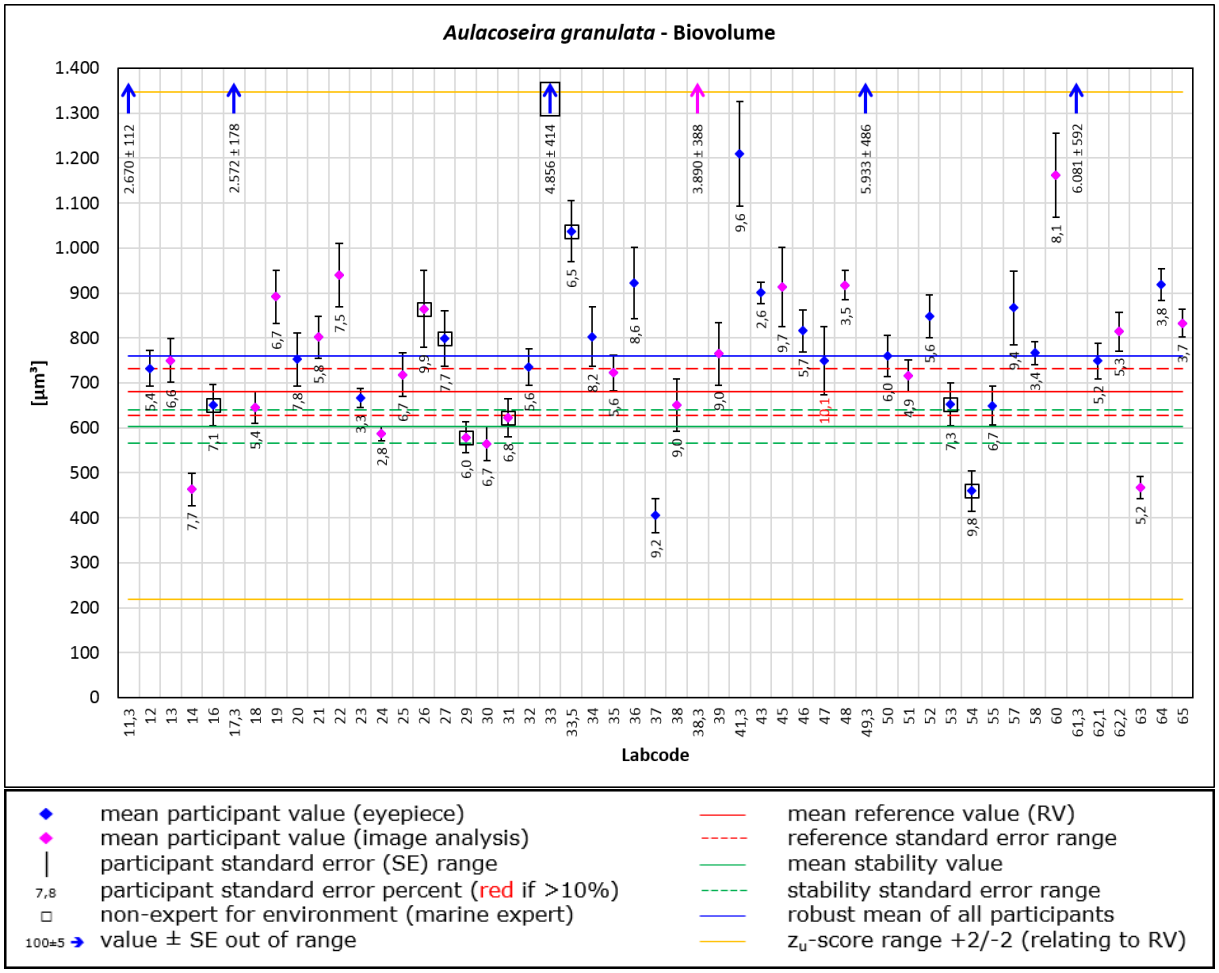

Figure 71: Results and some statistical characteristics for biovolume of *Aulacoseira granulata*. Please note: Comma used as decimal mark, full stop used as thousands separator.

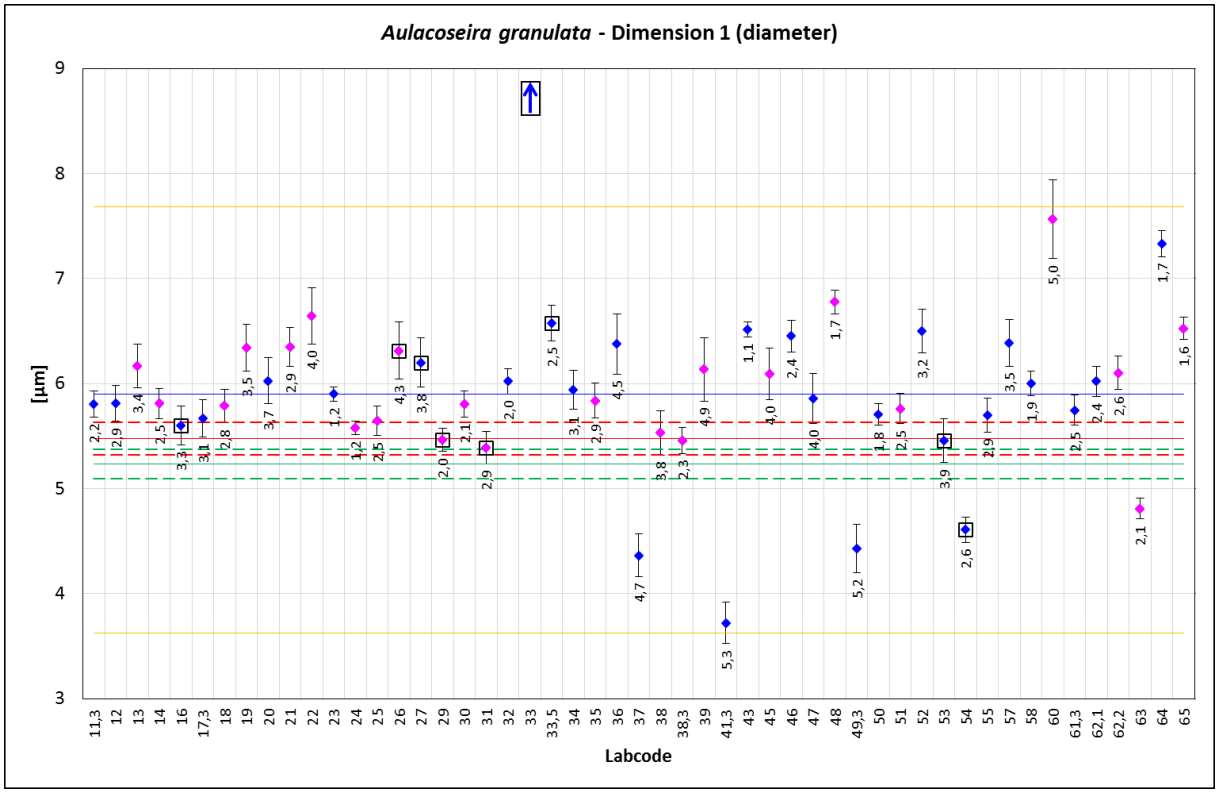

Figure 72: Results and some statistical characteristics for dimension 1 (diameter) of *Aulacoseira granulata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

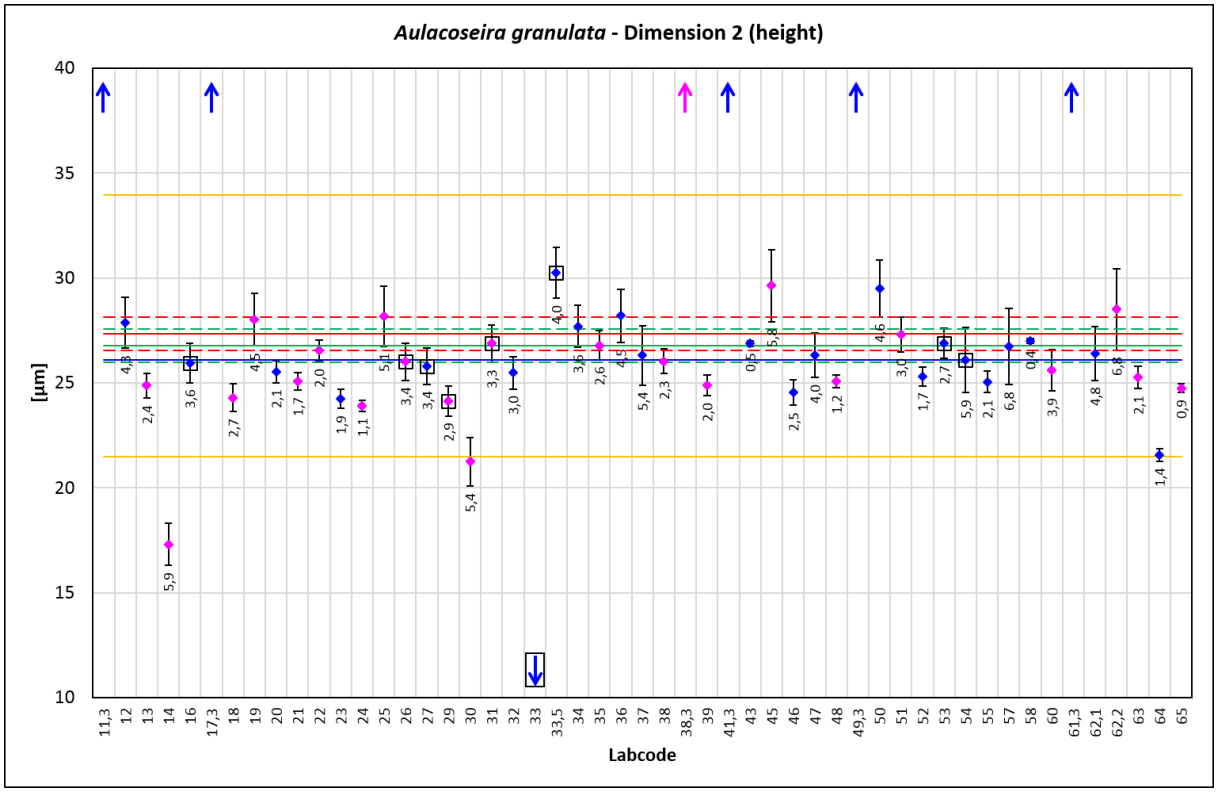

Figure 73: Results and some statistical characteristics for dimension 2 (height) of *Aulacoseira granulata*. Legend see biovolume chart. Please note: Comma used as decimal mark.

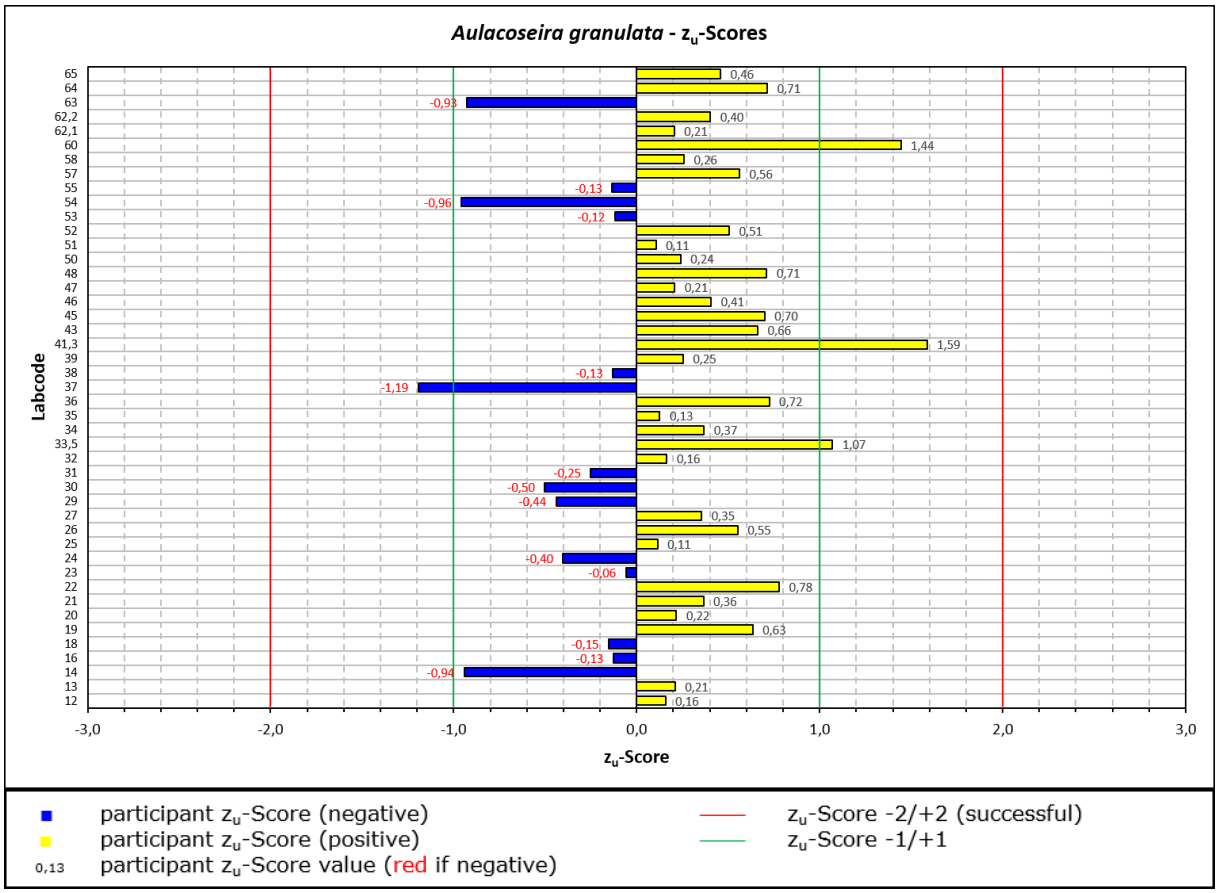

Figure 74: z_u scores of participants for *Aulacoseira granulata*. Please note: Comma used as decimal mark.

3.4.14 *Cryptomonas erosa*

Cryptomonas erosa is a large cryptophyte (Figure 75). All participants have correctly assigned the 'ellipsoid' as geometrical shape (Table 16). For reasons of comparison, one participant has assigned the 'prolate spheroid' as well. Additionally, one participant has used the same values for both diameters, which in reality converts the 'ellipsoid' to a 'prolate spheroid'. According to the Standard Document, no correction factor shall be applied. Nevertheless, two participants have used such a factor.

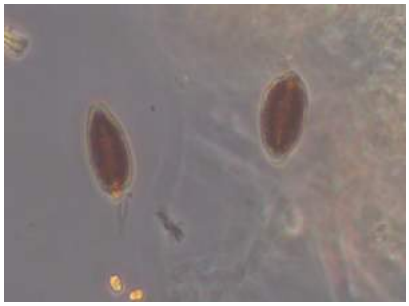

Figure 75: Microphotographic picture of *Cryptomonas erosa* in the Scharmützelsee sample.

Table 16: Inventory of the main results (except special statistics) for *Cryptomonas erosa.*

General			
48	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 23 24 25 26 27 29 30 31 32 33 34 35 36 37 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
27	measurements with an eyepiece micrometre		
23	measurements with image analysis software		
Geometry and Dimensions			
49	measurements/calculations with a 'ellipsoid' assigned as geometrical shape according to the Standard Document		
	1	measurement/calculation with a 'ellipsoid' assigned as geometrical shape according to the Standard Document but using the same values for 'large diameter' as for 'small diameter' which effectively is a 'prolate spheroid'	
	1	additional measurement/calculation with a 'prolate spheroid' assigned as geometrical shape notwithstanding to the Standard Document for comparison	
48	calculations with no correction factor used according to the Standard Document		
2	calculations with a correction factor of 0.8 used notwithstanding to the Standard Document		
3	calculations with' large diameter' and 'height' being confused		
Statistics			
20-50	units have been measured		
	0	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)	
	37	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		1	measurement with the biovolume standard error being ≥ 10 %
	2	total measurements with the biovolume standard error being ≥ 10 %	

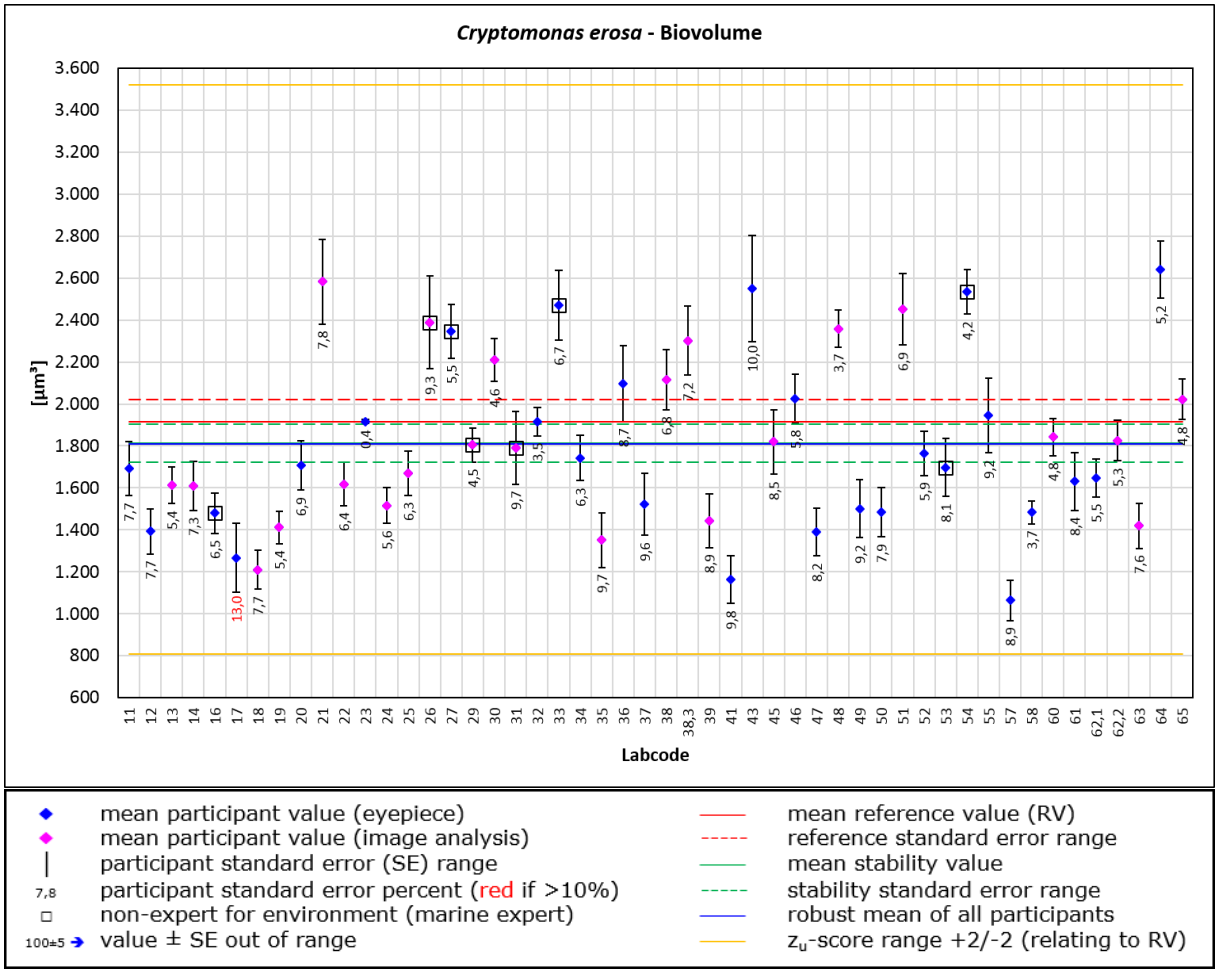

Figure 76: Results and some statistical characteristics for biovolume of *Cryptomonas erosa*. Please note: Comma used as decimal mark, full stop used as thousands separator.

The variability in the biovolume was high and values ranged from 1,100 to 2,600 µm³ (Figure 76). This could be explained by the high variability in the hidden dimension measurements (Figure 78). For reference values, the hidden dimension 'small diameter' has been calculated by multiplying 0.85 with the 'large diameter'. The values of the participants ranged from 0.44 to 1.99 with a mean of 0.79 (Table 25). The hidden dimensions seem to be the main cause for the deviation of the biovolumes calculated by the participants from reference biovolume data since the height is comparable (Figure 79) and the large diameter is even smaller in mean values (Figure 77). However, all of the participants' results for the biovolume were within the requested z_u range from -2 to +2.

Most z_u score values lay within the range between -1 and 1 with a tendency to negative values. Only five participants missed that range. All participants passed the test for this species (Figure 80). This reflects a very good performance for this species.

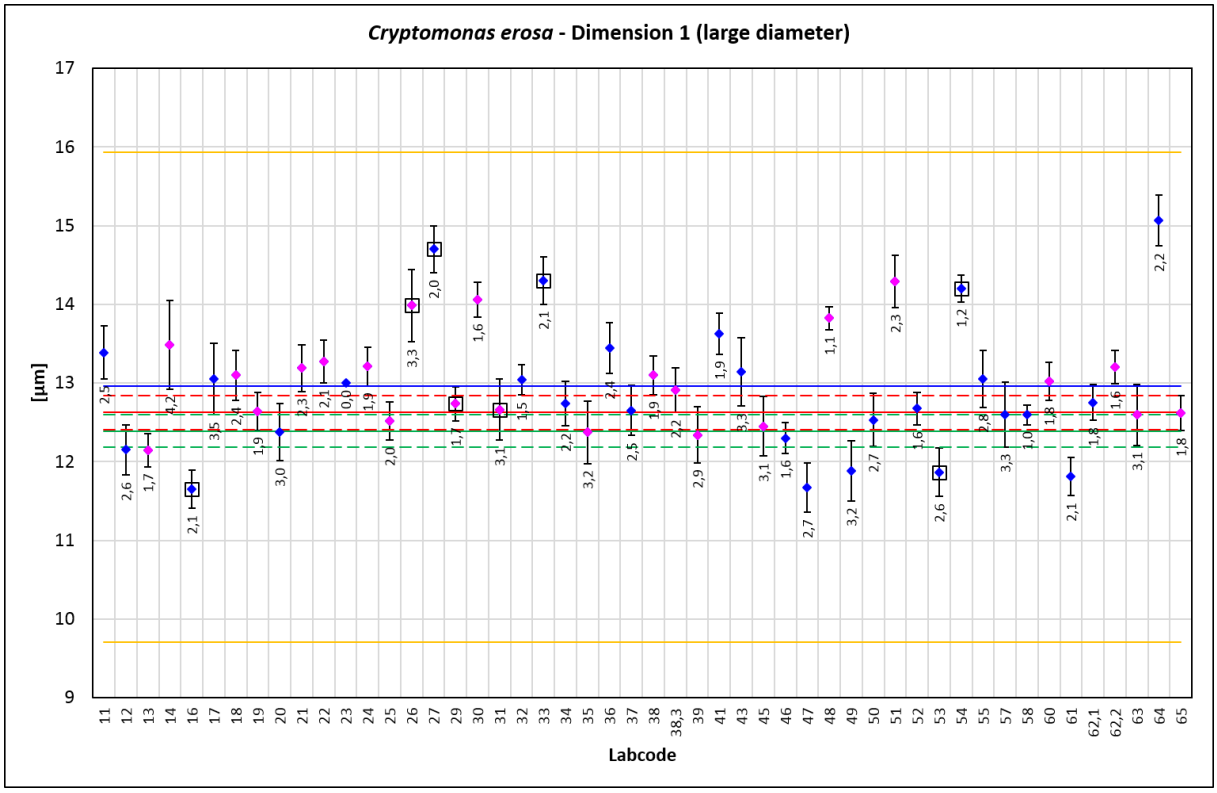

Figure 77: Results and some statistical characteristics for dimension 1 (large diameter) of *Cryptomonas erosa*. Legend see biovolume chart. Please note: Comma used as decimal mark.

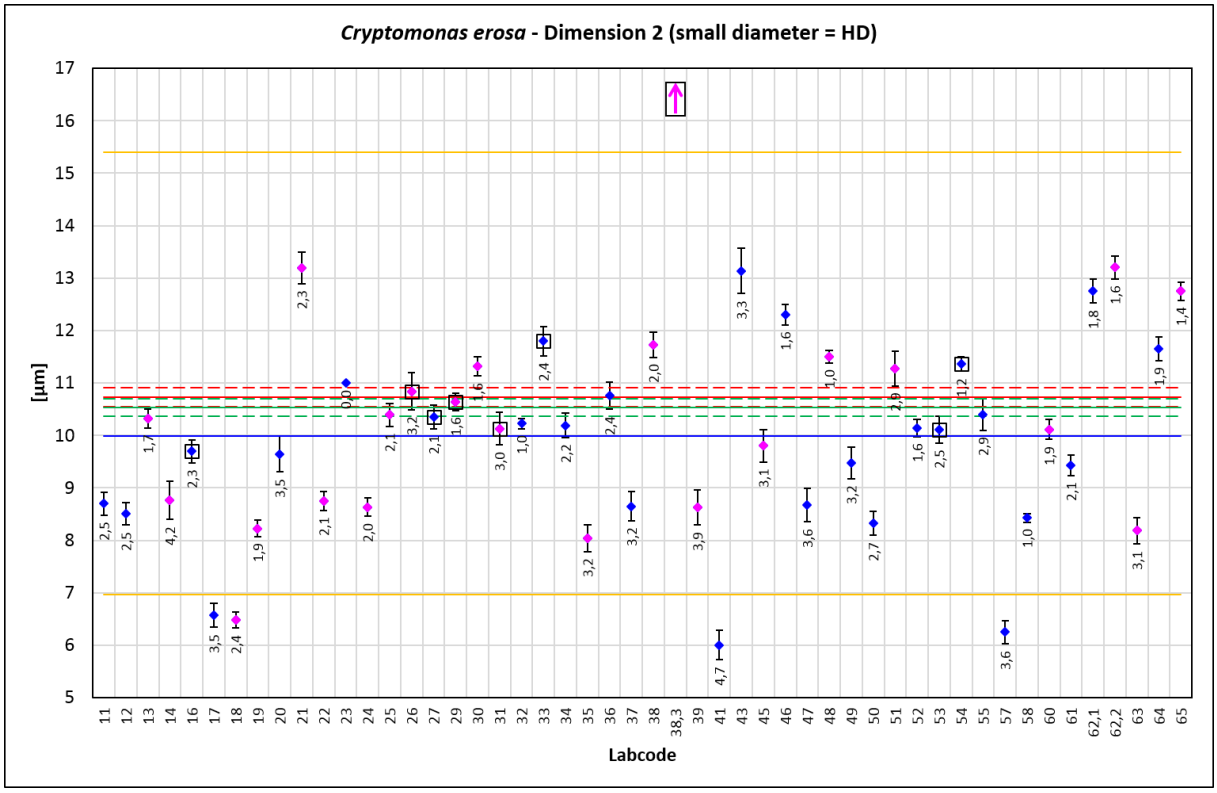

Figure 78: Results and some statistical characteristics for dimension 2 (small diameter = HD) of *Cryptomonas erosa*. Legend see biovolume chart. Please note: Comma used as decimal mark.

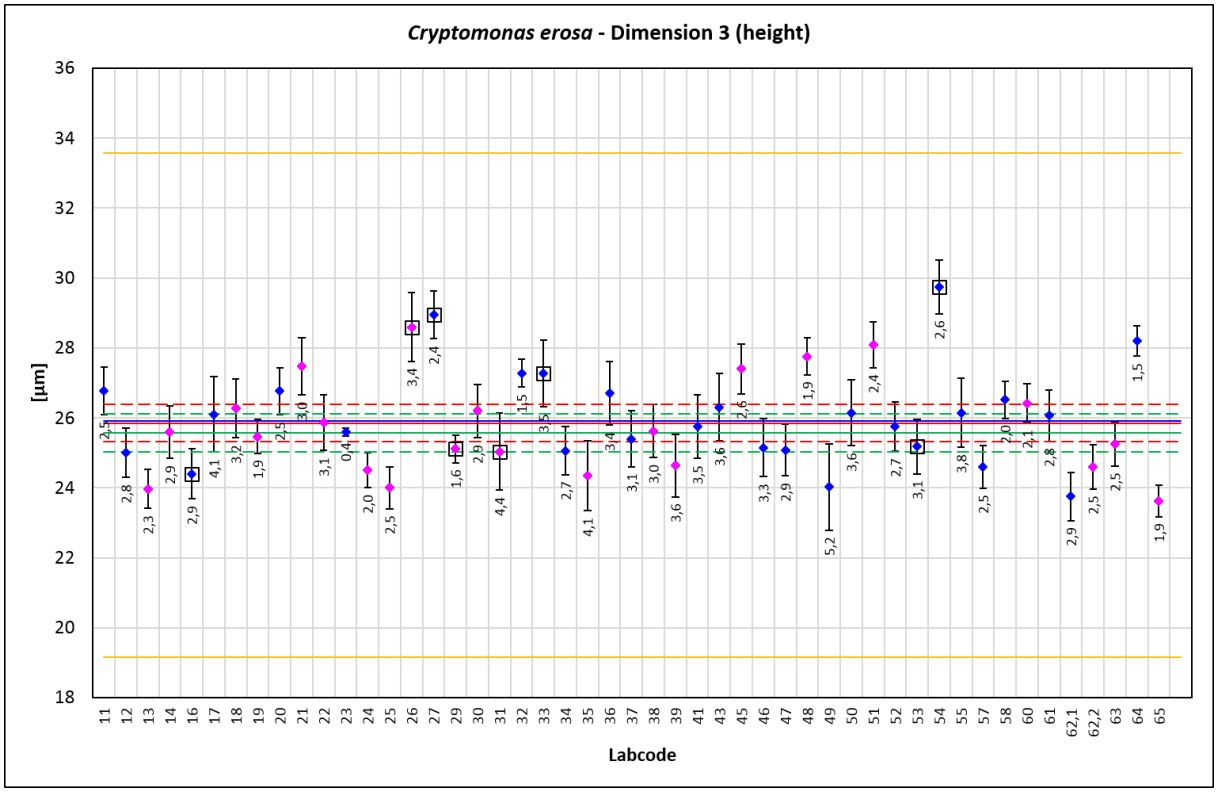

Figure 79: Results and some statistical characteristics for dimension 3 (height) of *Cryptomonas erosa*. Legend see biovolume chart. Please note: Comma used as decimal mark.

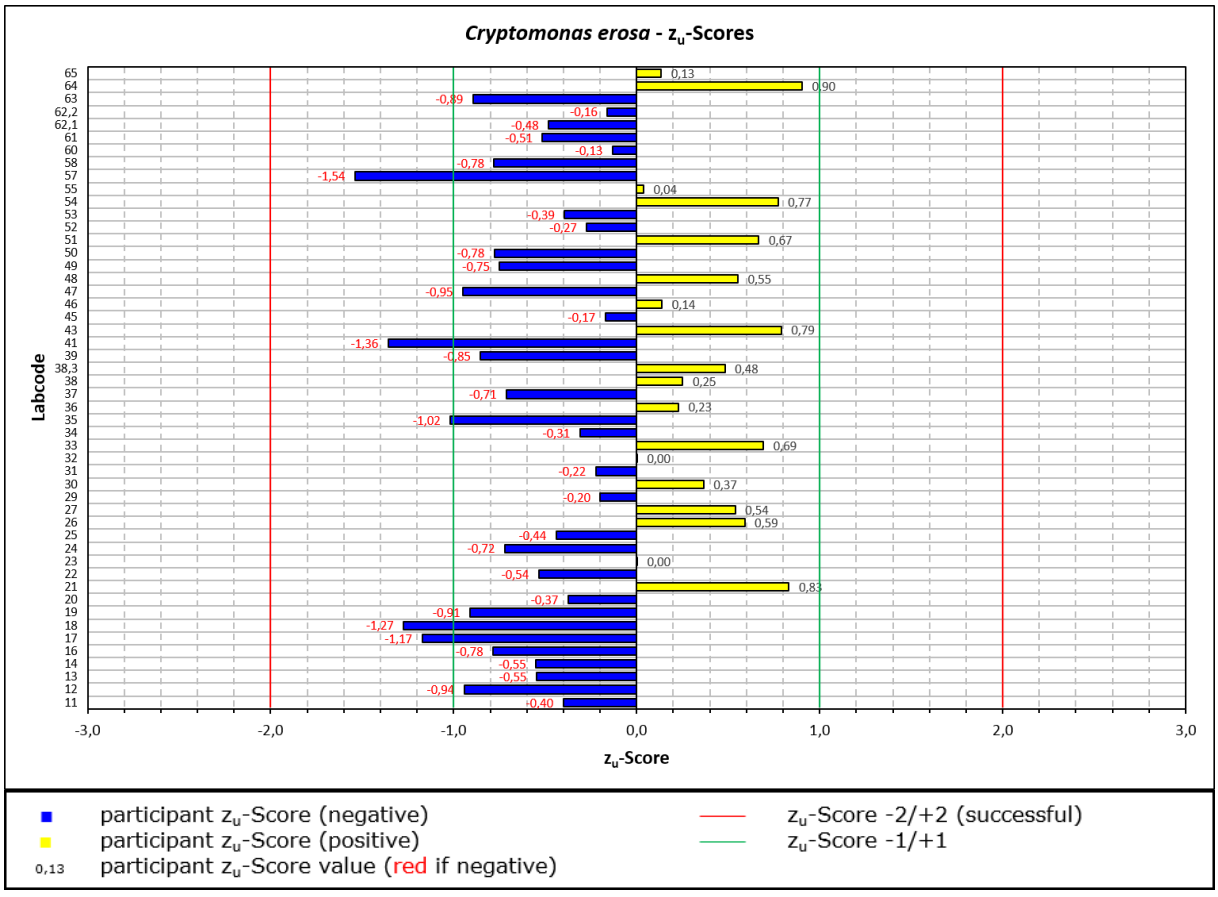

Figure 80: z_u scores of participants for *Cryptomonas erosa*. Please note: Comma used as decimal mark.

3.4.15 *Rhodomonas lacustris* var. *nannoplanctica*

Rhodomonas lacustris var. nannoplanctica is a small cryptophyte (Figure 81). Most participants have correctly assigned the 'cone with half sphere' as geometrical shape, according to the Standard Document (Table 17). One participant has used 'half cone' instead, while another has additionally calculated the biovolume for a 'cone' for comparison. Two participants have confused the dimensions, which has been corrected and shifted the respective biovolume values within the range of all participants (Figure 83, Figure 84).

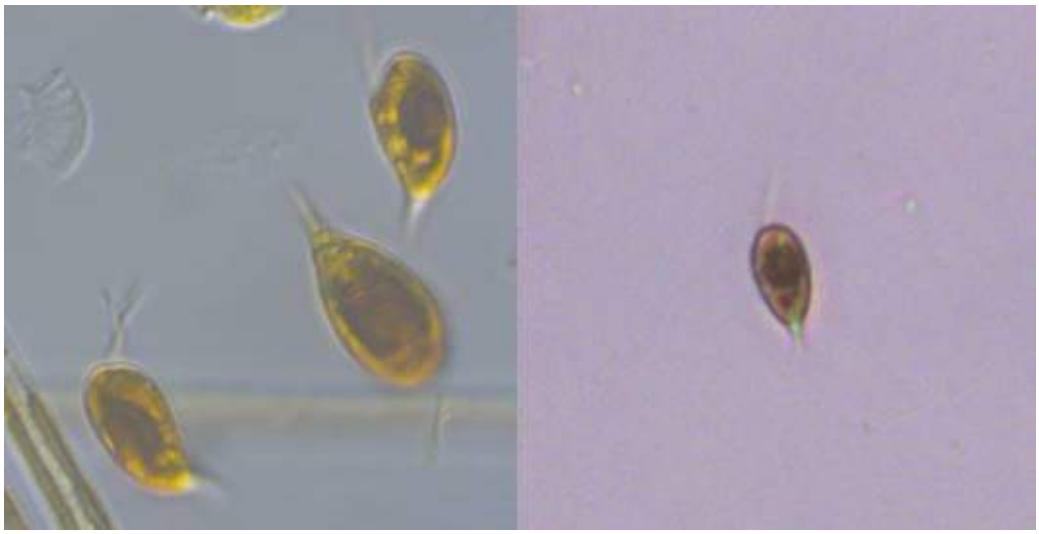

Figure 81: Microphotographic pictures of *Rhodomonas lacustris* var. *nannoplanctica* in the Scharmützelsee sample (left one provided by Pepita Nolla).

The range of biovolume, with some exceptions, lay between 60 and 150 µm^3 and was in good accordance with the reference values (Figure 82). Except one, all of the participants' results for the biovolume were within the requested z_u range from -2 to +2.

The z_u score values showed a high variability but most of the participants, which successfully had passed the repeated z_u assessment procedures, lay within a z_u range from -1 to +1. One participant did miss the z_u range from -2 to +2 and did not pass the test for this species (Figure 85). This reflects a good performance for the biovolume analysis of this species.

Table 17: Inventory of the main results (except special statistics) for *Rhodomonas lacustris* var. *nannoplanctica.*

General			
47	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 23 24 25 27 29 30 31 32 33 34 35 36 37 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
27	measurements with an eyepiece micrometre		
22	measurements with image analysis software		
Geometry and Dimensions			
48	measurements/calculations with a 'cone with half sphere' assigned as geometrical shape according to the Standard Document		
	1	additional measurement/calculation with a 'cone' assigned as geometrical shape notwithstanding to the Standard Document for comparison	
1	measurement/calculation with a 'half cone' assigned as geometrical shape notwithstanding to the Standard Document		
49	calculations with no correction factor used according to the Standard Document		
2	calculations with 'diameter' and 'total height' being confused		
Statistics			
15-120	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	33	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		1	measurement with the biovolume standard error being ≥ 10 %
	1	total measurements with the biovolume standard error being ≥ 10 %	

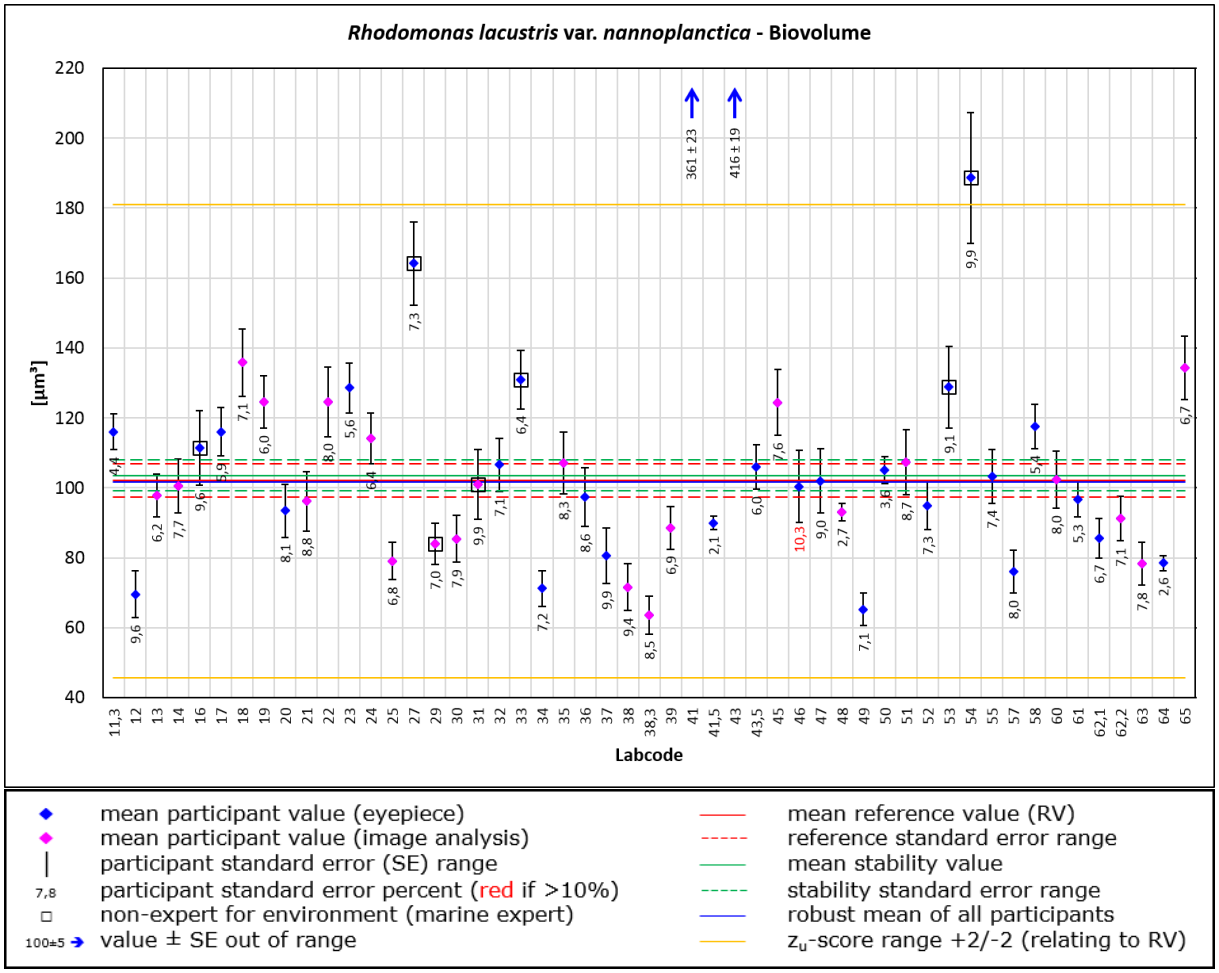

Figure 82: Results and some statistical characteristics for biovolume of *Rhodomonas lacustris* var. *nannoplanctica*. Please note: Comma used as decimal mark.

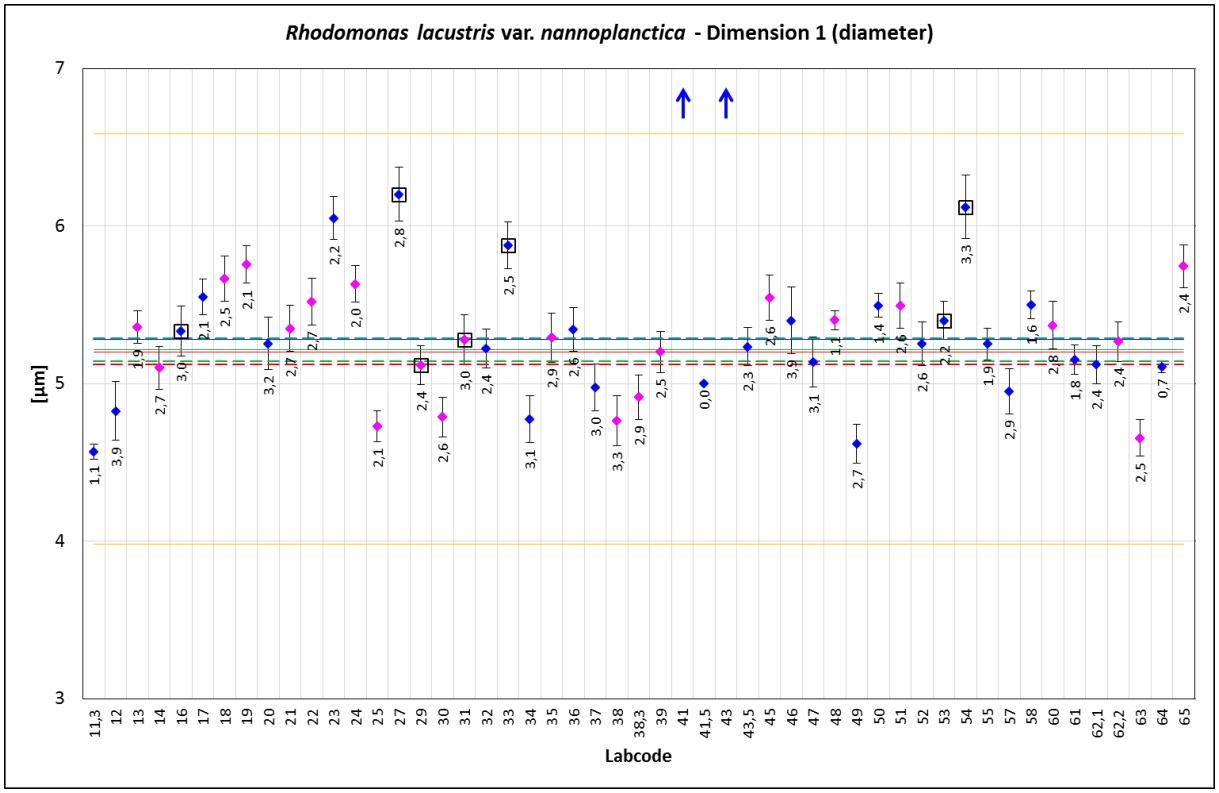

Figure 83: Results and some statistical characteristics for dimension 1 (diameter) of *Rhodomonas lacustris* var. *nannoplanctica*. Legend see biovolume chart. Please note: Comma used as decimal mark.

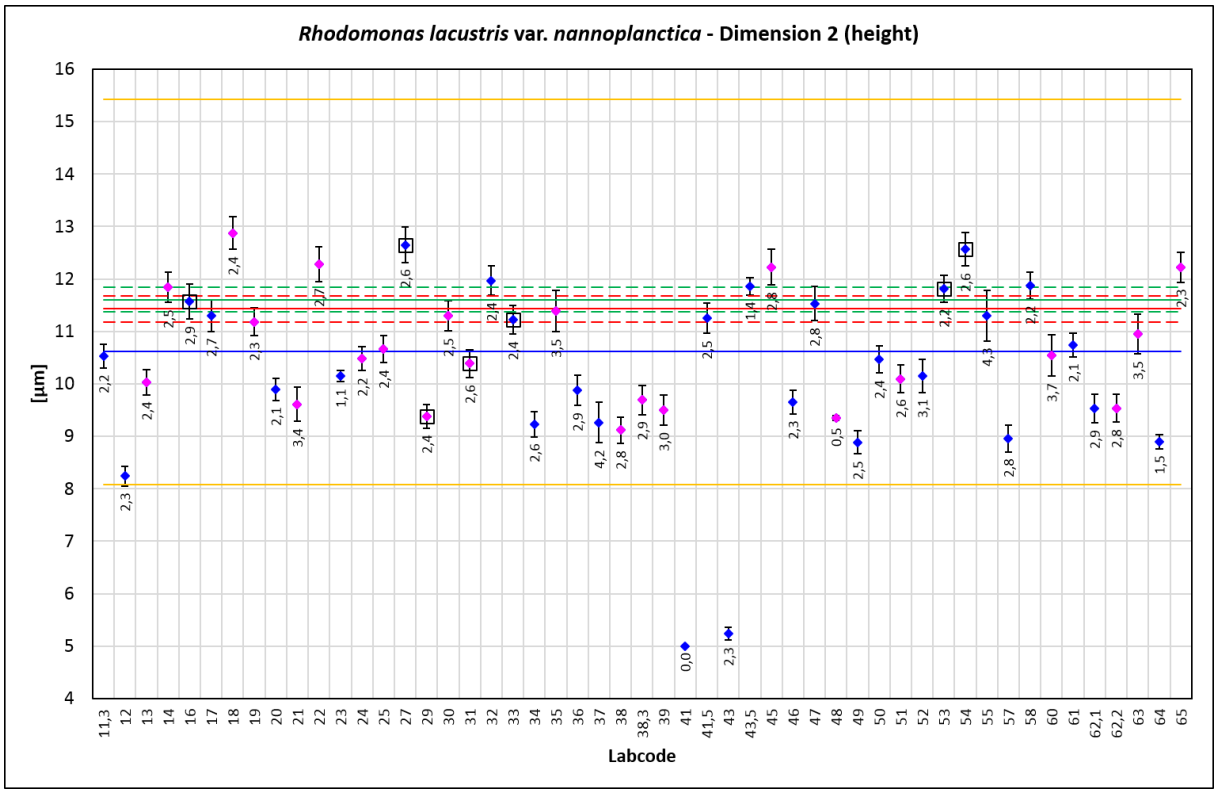

Figure 84: Results and some statistical characteristics for dimension 2 (height) of *Rhodomonas lacustris* var. *nannoplanctica*. Legend see biovolume chart. Please note: Comma used as decimal mark.

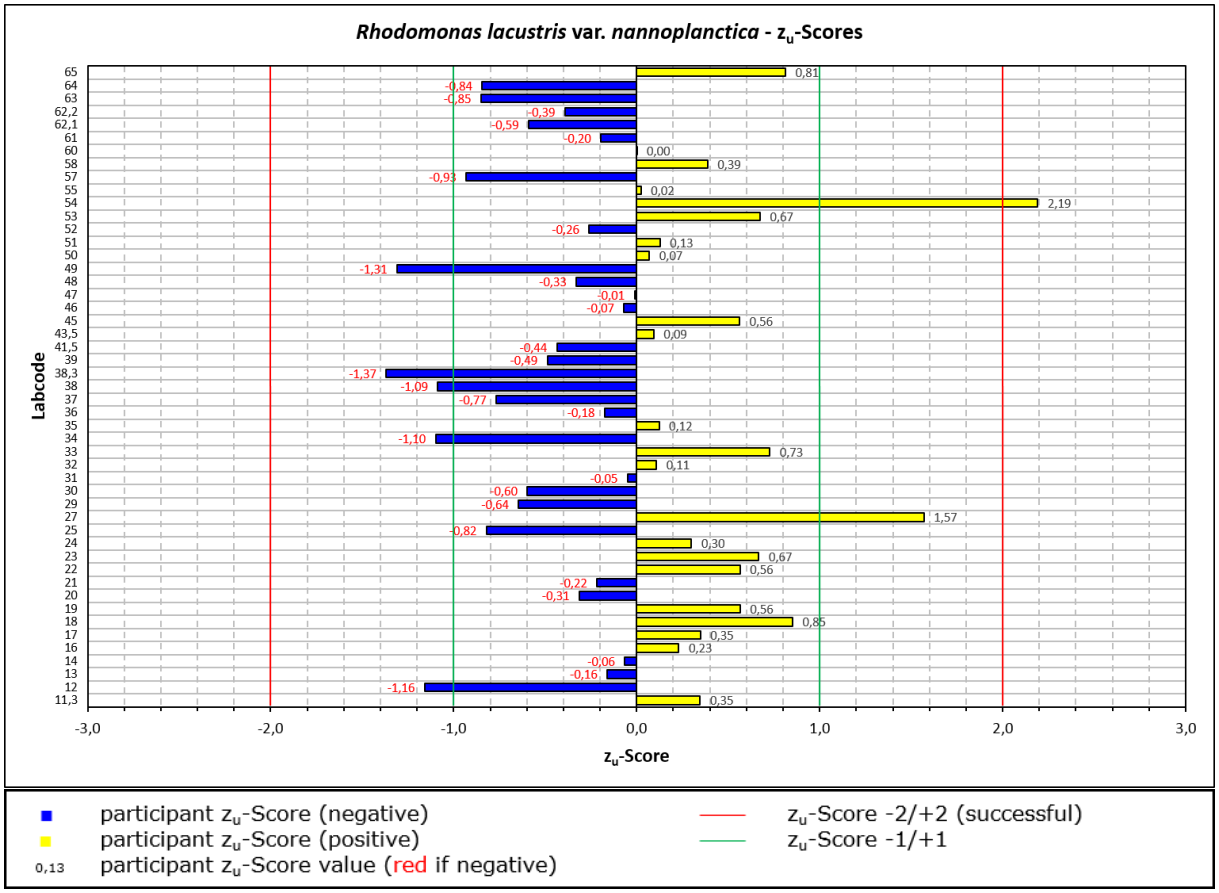

Figure 85: z_u scores of participants for *Rhodomonas lacustris* var. *nannoplanctica*. Please note: Comma used as decimal mark.

3.4.16 *Planktothrix agardhii*

Planktothrix agardhii is a filamentous species consisting of cylindrical cells which cannot be identified easily (Figure 86). All participants that have measured this species have correctly assigned the cylinder as geometrical shape, according to the Standard Document (Table 18). The participants have applied four different methods for the measurements. Most have measured filaments either in 10 or 100 µm sections, or the length of the filament in total or within a counting grid, others have measured cells (Figure 88). All methods are in accordance with the Standard Document, although the document did not explicitly mention pieces of 10 µm, which had been changed now. For the comparison, the calculations have been normalised by using the measured diameters and 100 µm as length for all participants.

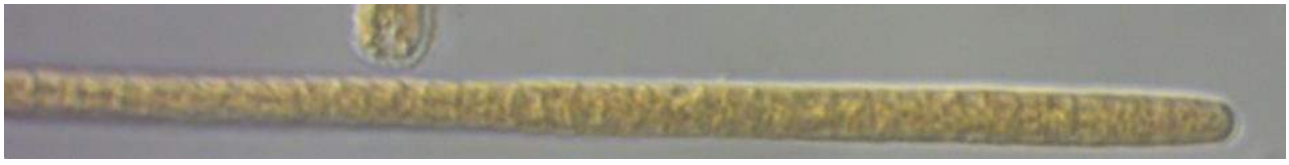

Figure 86: Microphotographic picture of *Planktothrix agardhii* in the Oldenburg sample (provided by Pepita Nolla).

Table 18: Inventory of the main results (except special statistics) for *Planktothrix agardhii.*

General			
45	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 24 25 27 29 30 31 32 33 34 35 36 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
25	measurements with an eyepiece micrometre		
22	measurements with image analysis software		
Geometry and Dimensions			
48	measurements/calculations with a 'cylinder' assigned as geometrical shape according to the Standard Document		
	1	participant has measured the total length and the length of a cell for comparison	
48	calculations with no correction factor used according to the Standard Document		
12	calculations using 100 µm pieces for length withstanding to the Standard Document		
26	calculations using total filament length withstanding to the Standard Document		
7	calculations using cell length withstanding to the Standard Document		
3	calculations using 10 µm pieces for length withstanding to the Standard Document		
Statistics			
20-120	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	27	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		1	measurement with the biovolume standard error being ≥ 10 %
	4	total measurements with the biovolume standard error being ≥ 10 %	

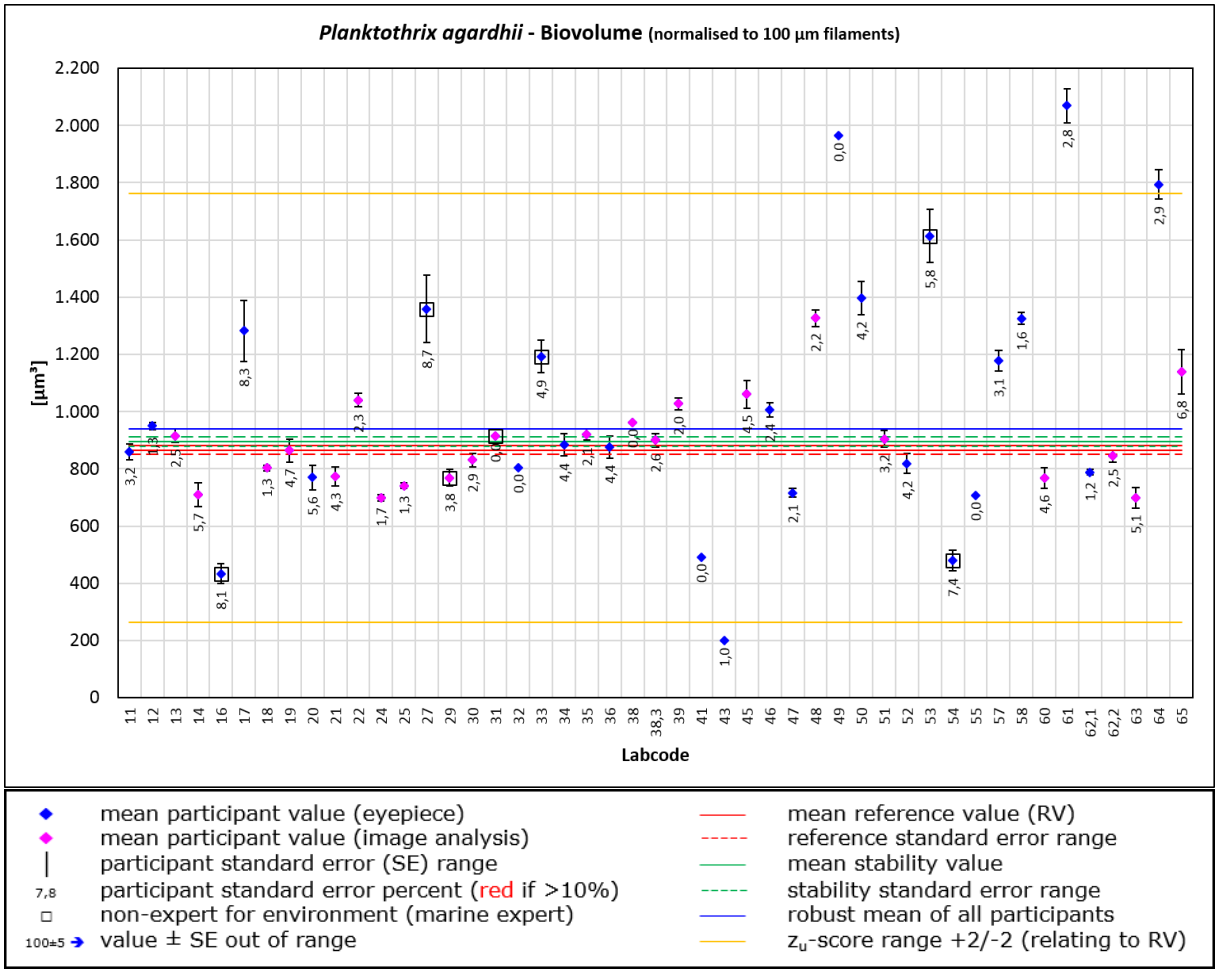

Figure 87: Results and some statistical characteristics for biovolume of *Planktothrix agardhii*. Please note: Comma used as decimal mark, full stop used as thousands separator.

Biovolume values lay in a range of 200 to 2,000 µm³, mostly grouped between 600 and 1,400 µm³ and being in good accordance with the reference data (Figure 87). Most of the participants' results for the biovolume were within the requested z_u range from -2 to +2. There have been 3 outliers lying above and 1 outlier lying below that range.

The z_u score values showed a high variability. Although most of the participants lay within a z_u range from -1 to +1, four did not pass the test for this species (Figure 89) missing the z_u range from -2 to +2. However, this reflects a good performance for this species.

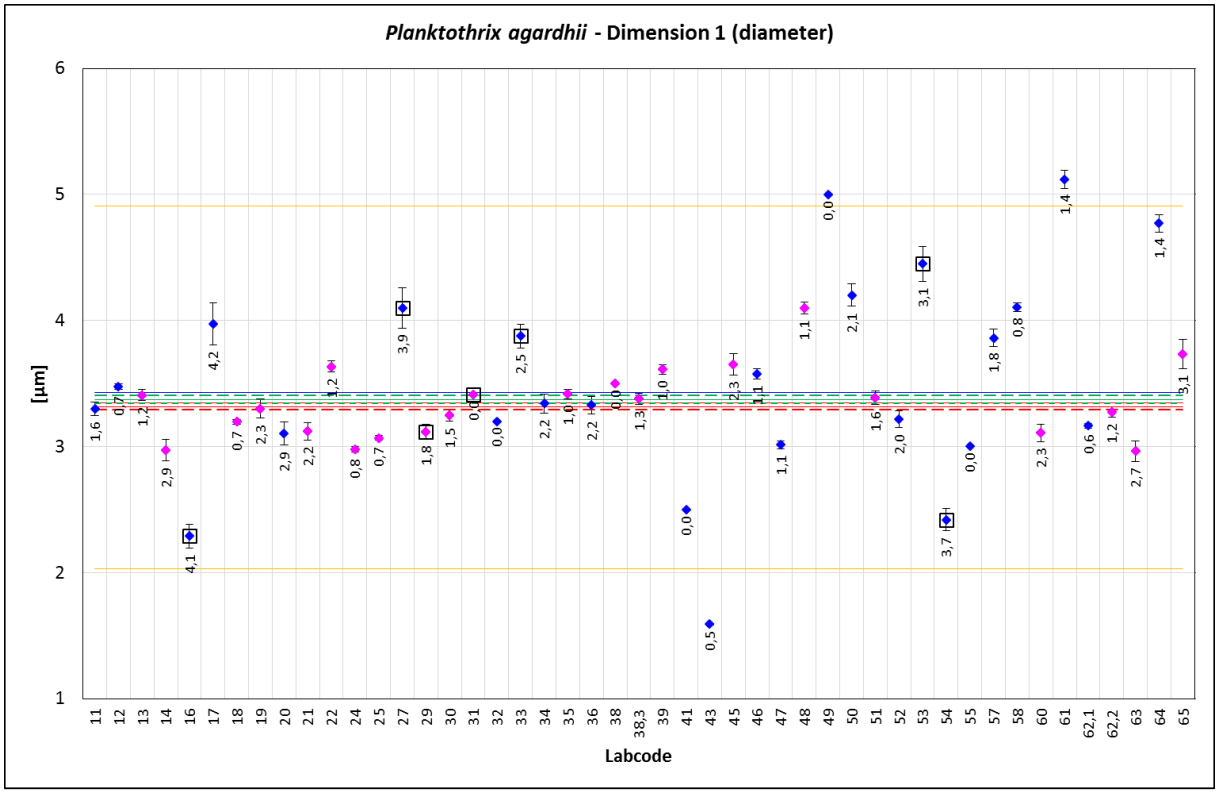

Figure 88: Results and some statistical characteristics for dimension 1 (diameter) of *Planktothrix agardhii*. Legend see biovolume chart. Please note: Comma used as decimal mark.

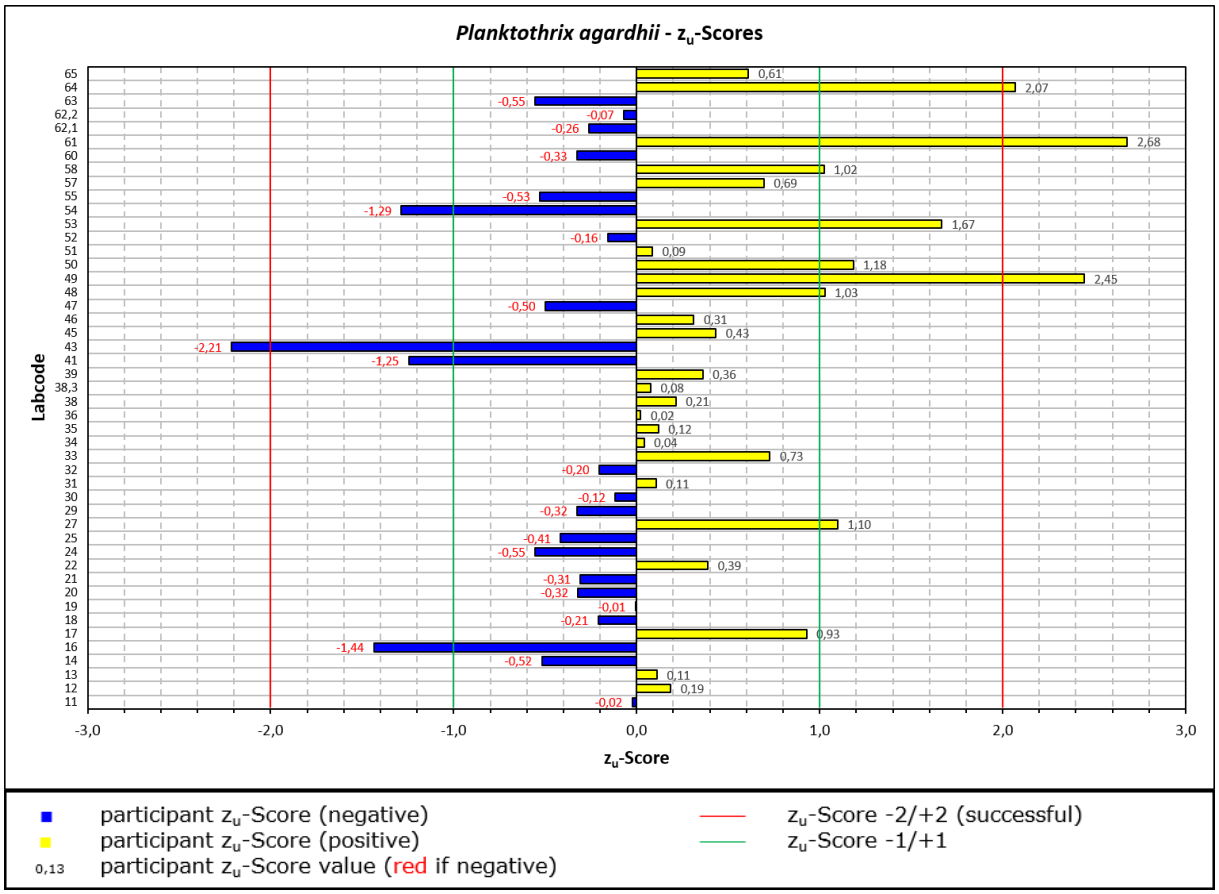

Figure 89: z_u scores of participants for *Planktothrix agardhii*. Please note: Comma used as decimal mark.

3.4.17 *Monoraphidium arcuatum*

Monoraphidium arcuatum is a curved species (Figure 90). Most participants that have measured this species have correctly assigned the 'spindle' as geometrical shape, according to the Standard Document (Table 19). However, some have used the 'monoraphidioid' shape either for comparison or in general, one participant has used 'double cone' for comparison.

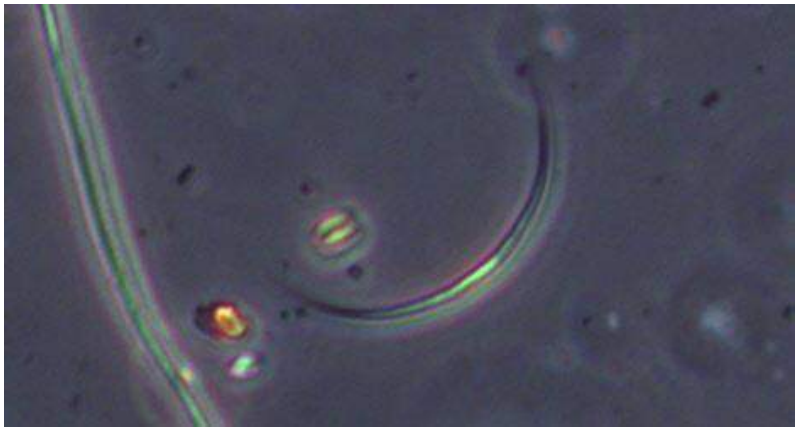

Figure 90: Microphotographic picture of *Monoraphidium arcuatum* in the Oldenburg sample.

For this species, it had been interesting how the length of a curved form was measured by the different participants. Some selected the 'monoraphidioid' instead of the 'spindle' shape. This may have been due to the name but also due to the easier way to measure the object. Although three dimensions had to be measured for the 'monoraphidioid' instead of two – as for the 'spindle' –, these measurements may be easier and quicker than to measure one curved line for the 'spindle'. In order to measure a curved line, the objective micrometre as well as the tool for image analysis have to be located several times on the object. In contrast to that, with the dimensions of the 'monoraphidioid' the length of the spindle can be calculated. This has been done for the reference data to assure that the data are as accurate as possible. Data of participants showed lower values in the 'height' (Figure 93) compared to the reference data. The diameter measurements were comparable between reference data and participants (Figure 92). This might be a hint that curved dimensions might be underestimated.

Most biovolume values including reference data were in the range between 25 to 100 µm^3, and this holds for all used geometrical shapes (Figure 91). Outliers can be found at the upper part of the z_u range from -2 to +2, one due the confusion of dimensions.

The z_u scores have a tendency to negative values. More than half of the participants, which successfully had passed the repeated z_u assessment procedures, lay within a z_u range from -1 to +1. Two participants missed the z_u range from -2 to +2 and thus, did not pass the test for this species (Figure 94). Monoraphidioids had been included in the test and passed as well. This reflects a good to moderate performance for the biovolume analysis of this species.

Table 19: Inventory of the main results (except special statistics) for *Monoraphidium arcuatum.*

General		
46	participants (labcode) have measured this species: 11 12 13 14 16 17 18 19 20 21 22 23 24 25 27 29 30 31 32 33 34 35 36 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65	
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison
28	measurements with an eyepiece micrometre	
22	measurements with image analysis software	
Geometry and Dimensions		
42	measurements/calculations with a 'spindle' assigned as geometrical shape according to the Standard Document	
	2	additional measurements/calculations with a 'monoraphidioid' assigned as geometrical shape notwithstanding to the Standard Document for comparison
	1	additional calculation with a 'double cone' assigned as geometrical shape notwithstanding to the Standard Document for comparison
5	measurements/calculations with a 'monoraphidioid' assigned as geometrical shape notwithstanding to the Standard Document	
50	calculations with no correction factor used according to the Standard Document	
1	calculation with' large diameter' and 'height' being confused	
Statistics		
19-59	units have been measured	
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)
	24	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)
	6	measurements with the biovolume standard error being ≥ 10 %
	9	total measurements with the biovolume standard error being ≥ 10 %

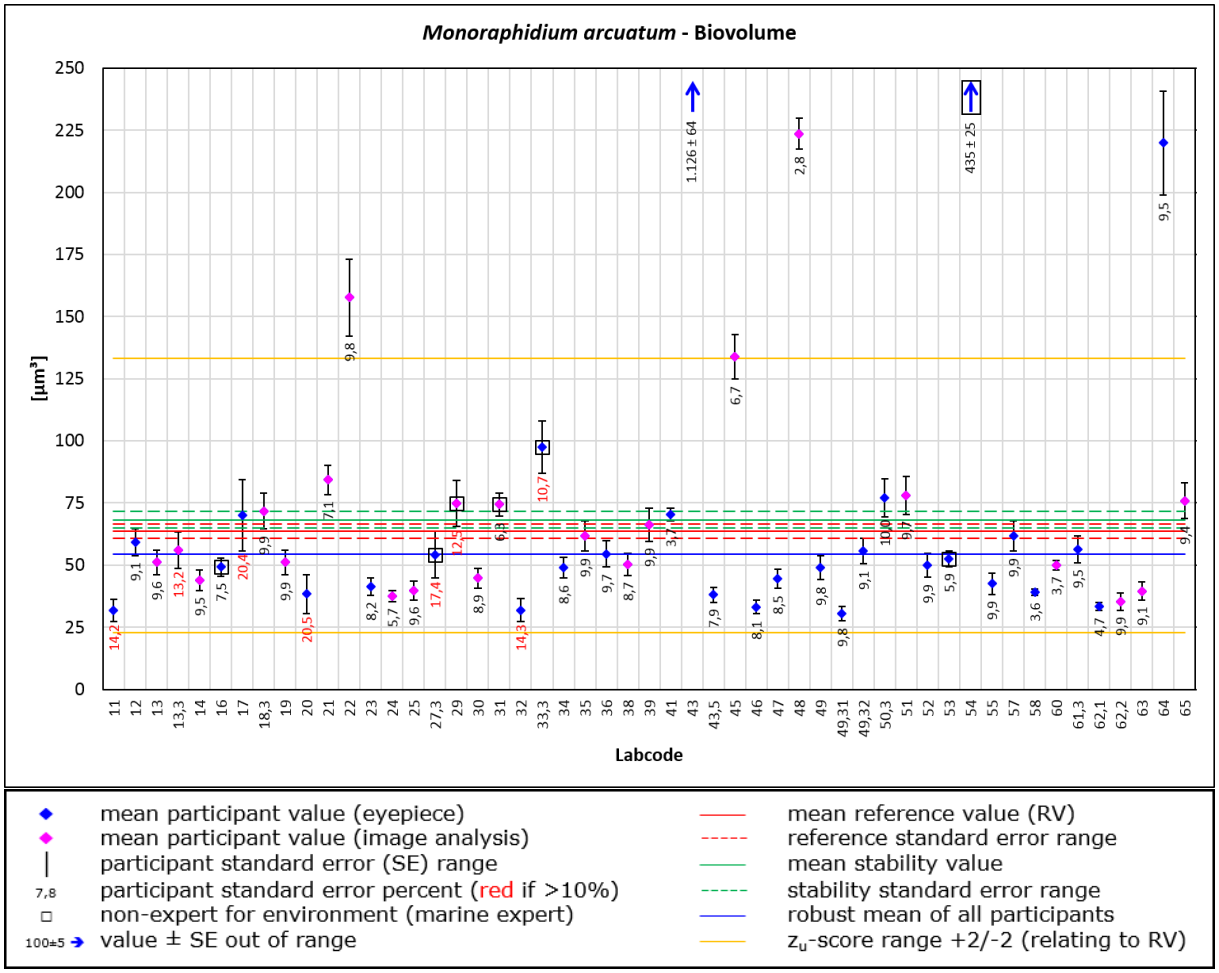

Figure 91: Results and some statistical characteristics for biovolume of *Monoraphidium arcuatum*. Please note: Comma used as decimal mark, full stop used as thousands separator.

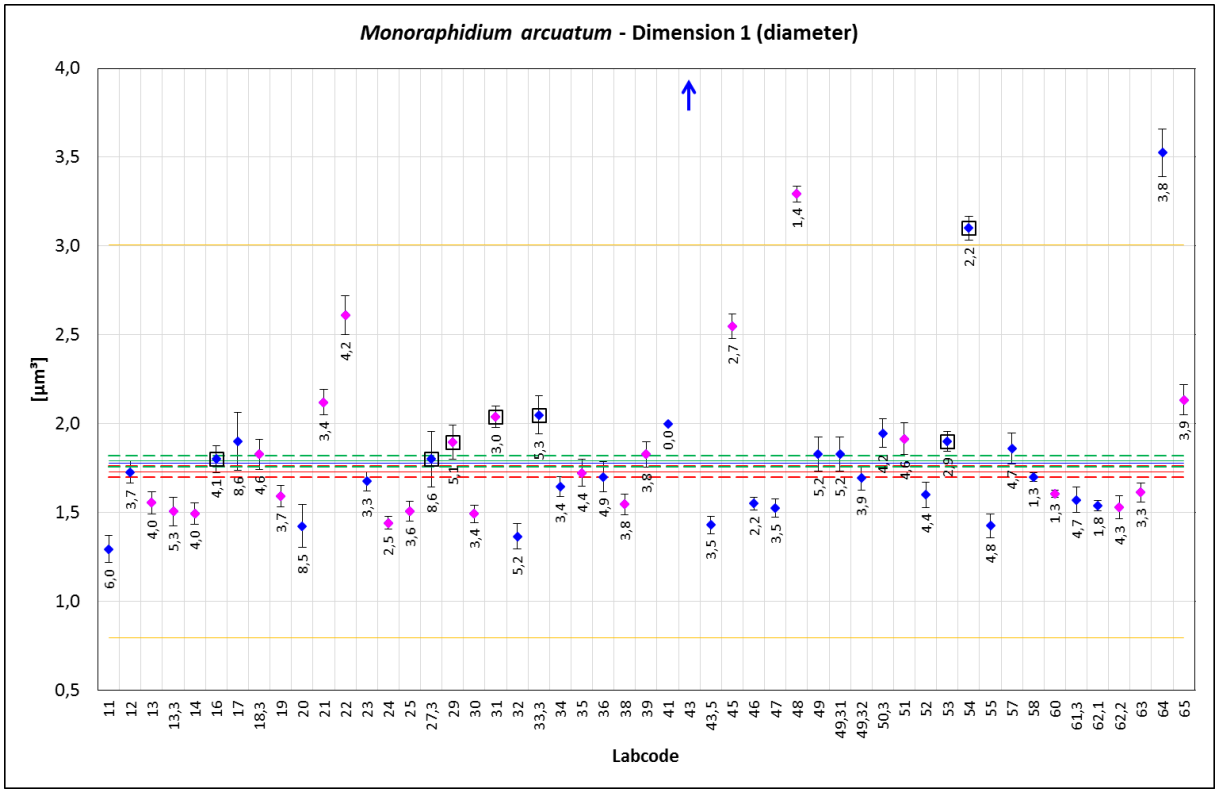

Figure 92: Results and some statistical characteristics for dimension 1 (diameter) of *Monoraphidium arcuatum*. Legend see biovolume chart. Please note: Comma used as decimal mark.

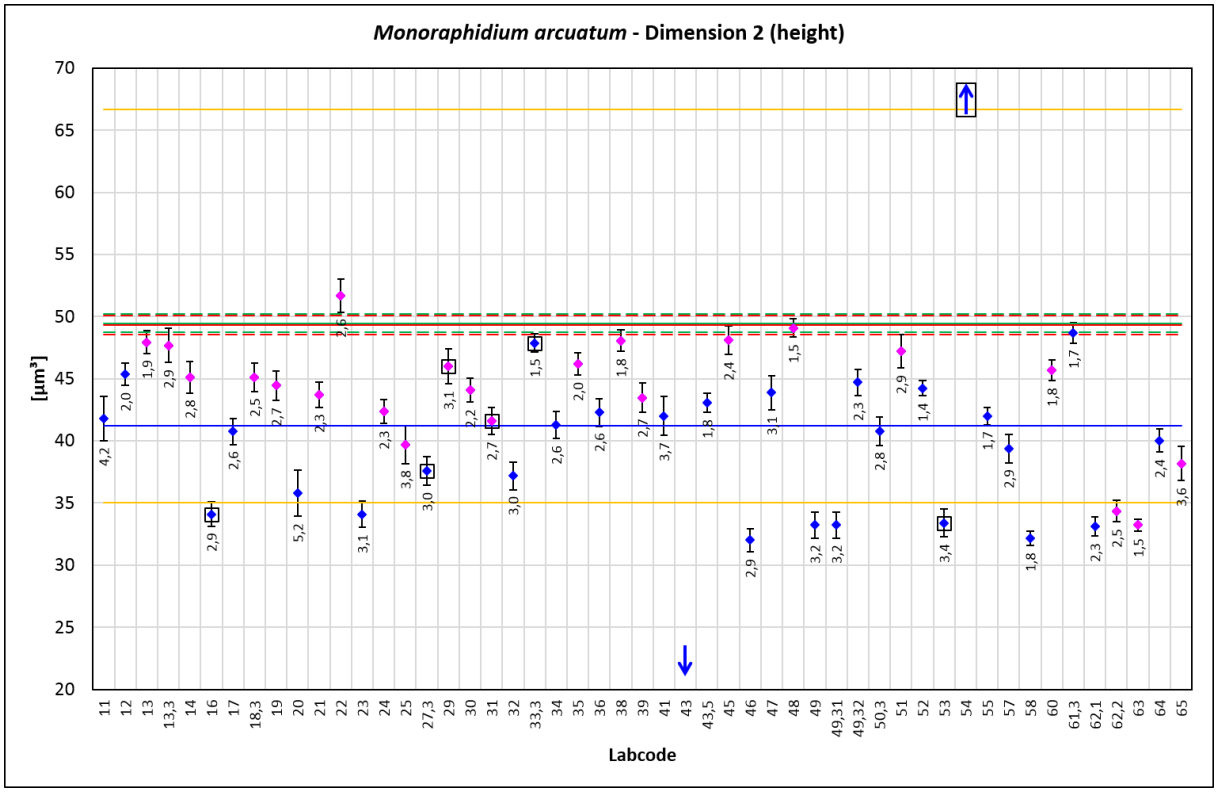

Figure 93: Results and some statistical characteristics for dimension 2 (height) of *Monoraphidium arcuatum*. Legend see biovolume chart. Please note: Comma used as decimal mark.

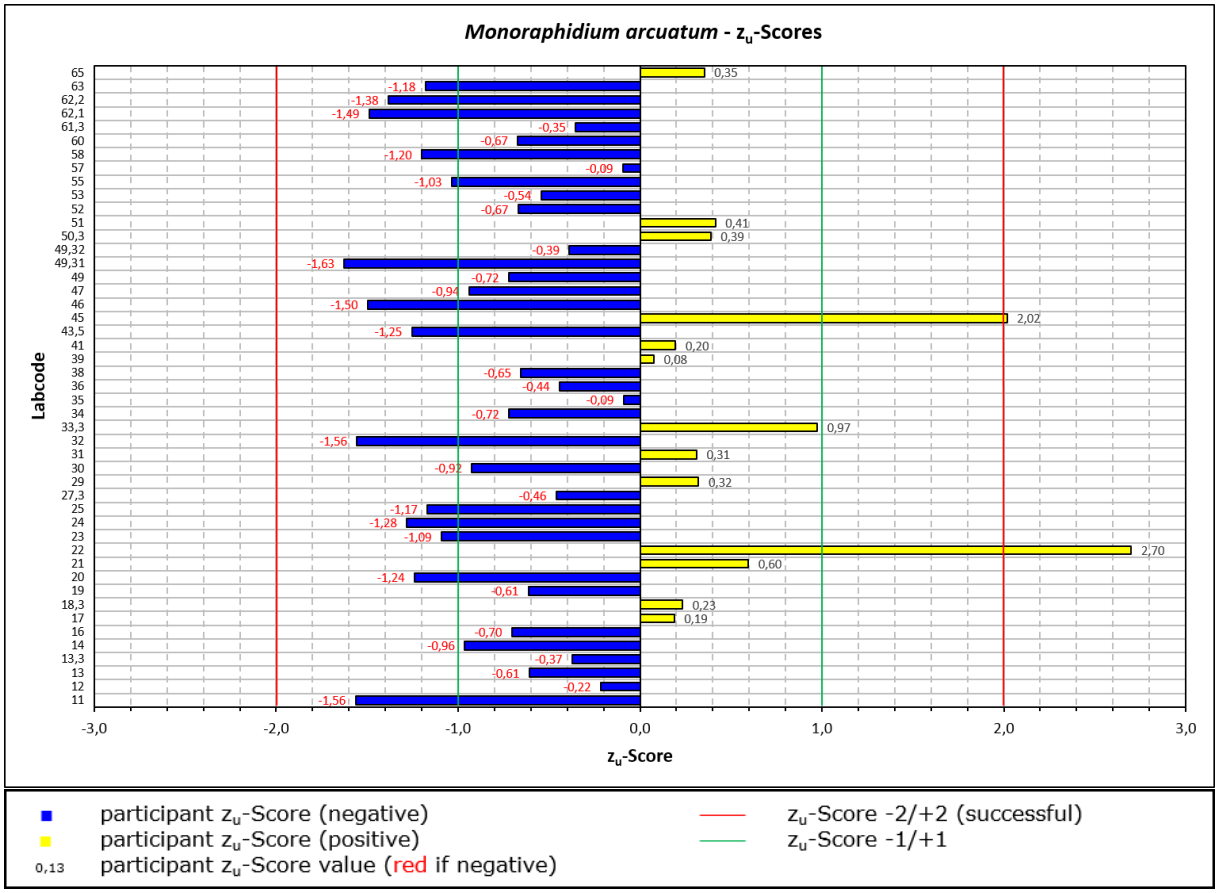

Figure 94: z_u scores of participants for *Monoraphidium arcuatum*. Please note: Comma used as decimal mark.

3.4.18 *Woronichinia naegeliana*

Woronichinia naegeliana is a colonial cyanophyte with small elliptic cells that form irregular spheroid colonies (Figure 95). All participants that have measured this species have correctly assigned the 'prolate spheroid' as geometrical shape (Table 20). One participant, however, has measured the colonies as a whole and not the single cells. The data of this participant have been excluded from further analysis (Table 26).

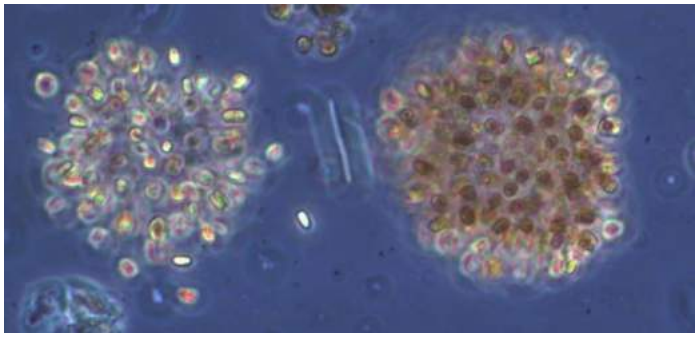

Figure 95: Microphotographic picture of the colony forming *Woronichinia naegeliana* in the Oldenburg sample.

Table 20: Inventory of the main results (except special statistics) for *Woronichinia naegeliana.*

General			
44	participants (labcode) have measured this species: 12 13 14 16 17 18 19 20 21 22 23 24 25 27 29 30 31 32 33 34 35 36 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 62 63 64 65		
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison	
24	measurements with an eyepiece micrometre		
21	measurements with image analysis software		
Geometry and Dimensions			
45	measurements/calculations with a 'prolate spheroid' assigned as geometrical shape according to the Standard Document		
45	calculations with no correction factor used according to the Standard Document		
1	measurement/calculation with the colony instead of the cell		
2	calculation with 'diameter' and 'height' being confused		
Statistics			
15-100	units have been measured		
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)	
	26	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)	
		0	measurement with the biovolume standard error being ≥ 10 %
	1	total measurement with the biovolume standard error being ≥ 10 %	

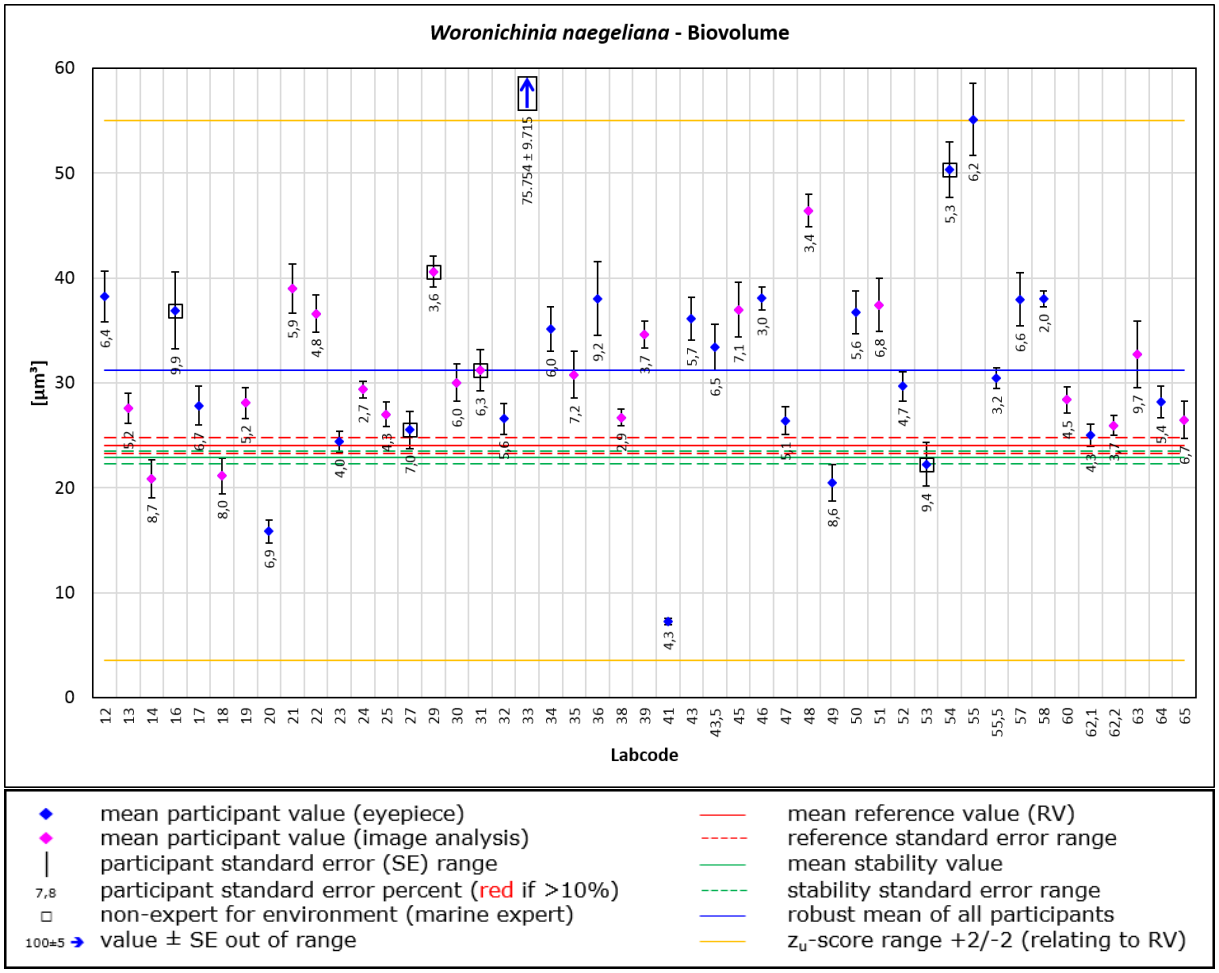

Figure 96: Results and some statistical characteristics for biovolume of *Woronichinia naegeliana*. Please note: Comma used as decimal mark, full stop used as thousands separator.

The biovolume values ranged from lower than 10 µm³ to more than 50 µm³, but most felt into the range from 20 to 40 µm³ (Figure 96). Most of the participants' results for the biovolume were within the requested z_u range from -2 to +2.

However, most values have been higher than the reference values. In contrast to the height (Figure 98), the diameter (Figure 97) measurements between participants and reference data diverged. An image analysis confirmed the measurements for the reference values. Cells in tight colonies are not easy to measure; performance of measurements is better with disintegrated cells. Thus, the small cell size, the form of the colonies and the selection process might have been the reasons for biovolumes being overestimated by the participants.

The z_u scores showed a clear tendency to positive values. Most of the participants, which successfully had passed the repeated z_u assessment procedures, lay within a z_u range from -1 to +1. One participant missed the z_u range from -2 to +2 and did not pass the test for this species (Figure 99).

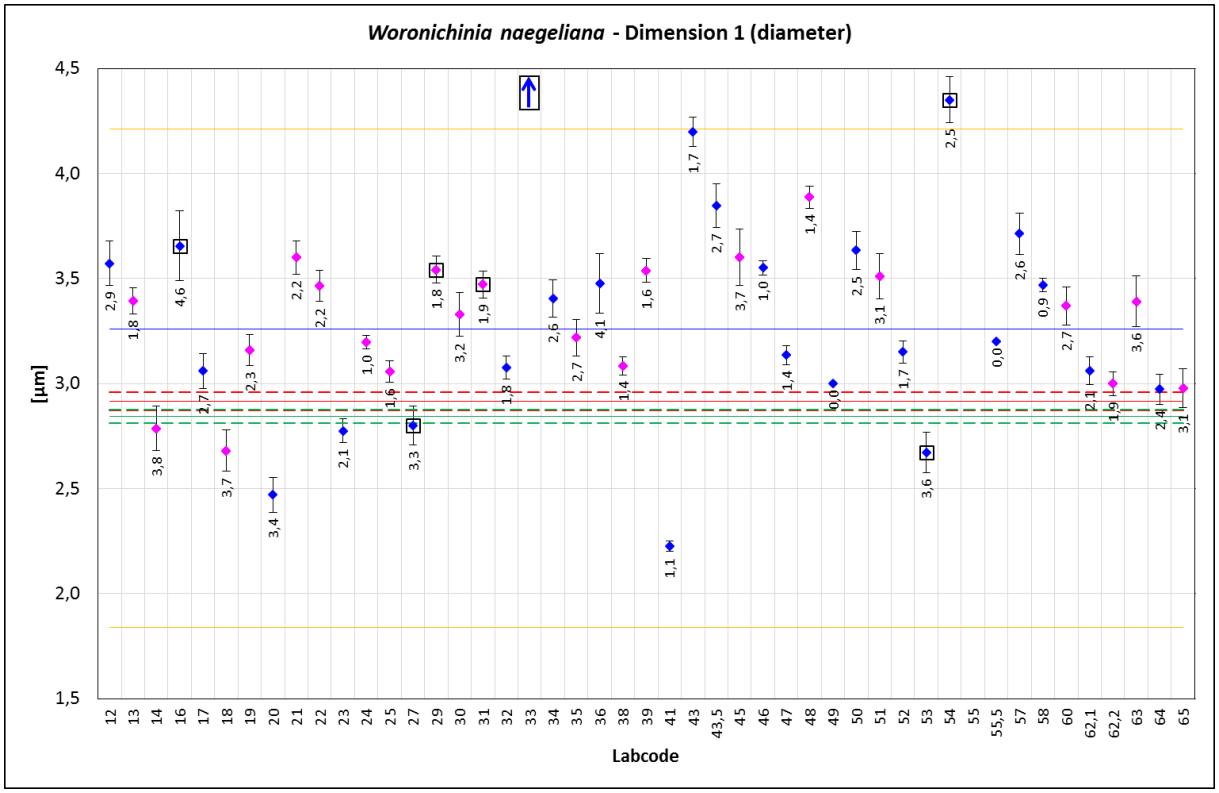

Figure 97: Results and some statistical characteristics for dimension 1 (diameter) of *Woronichinia naegeliana*. Legend see biovolume chart. Please note: Comma used as decimal mark.

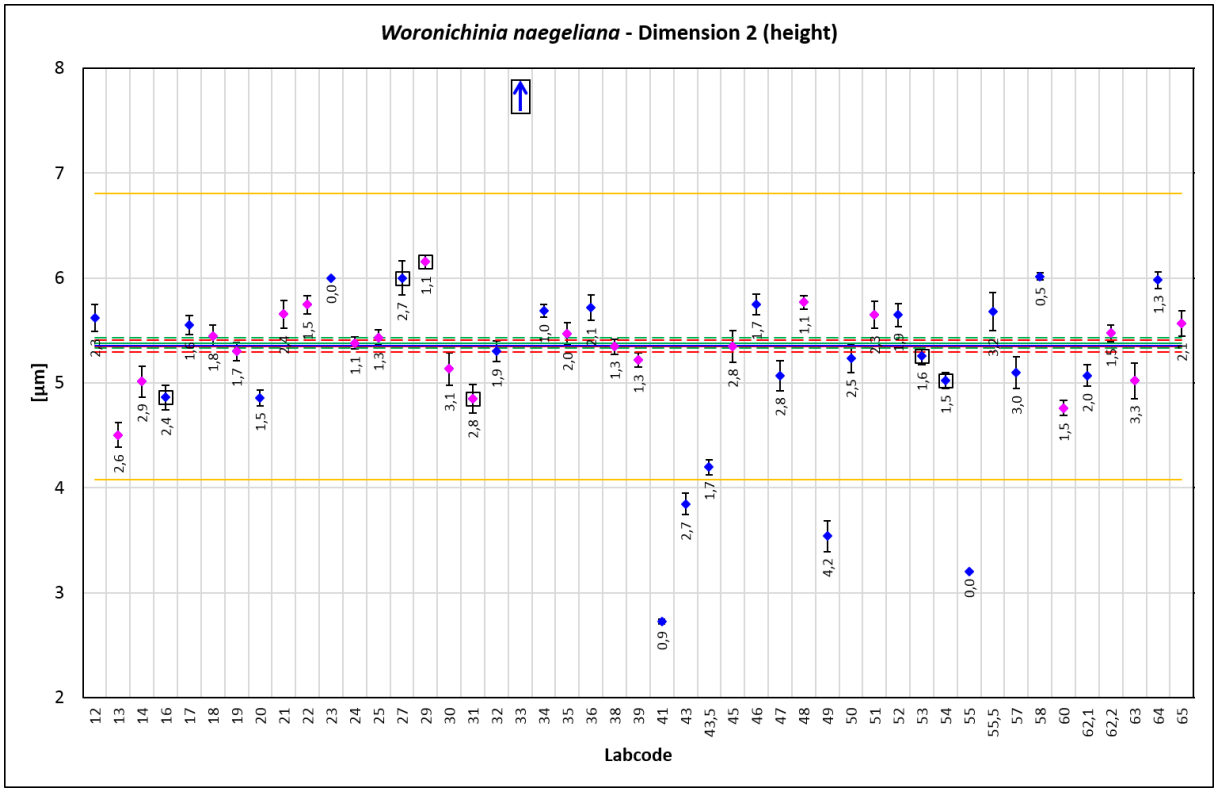

Figure 98: Results and some statistical characteristics for dimension 2 (height) of *Woronichinia naegeliana*. Legend see biovolume chart. Please note: Comma used as decimal mark.

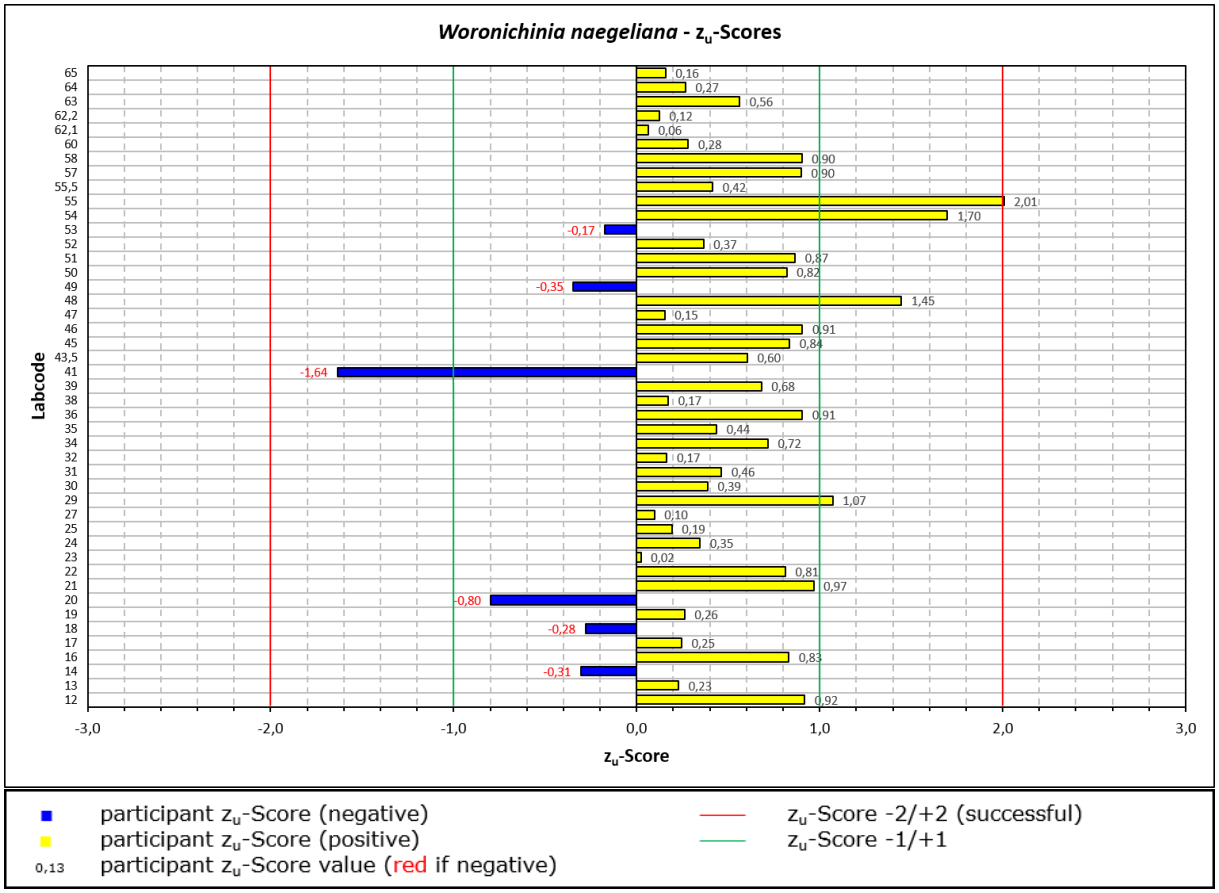

Figure 99: z_u scores of participants for *Woronichinia naegeliana*. Please note: Comma used as decimal mark.

3.4.19 *Cosmarium ocellatum*

Cosmarium ocellatum is a single celled desmid deeply constricted in the middle (Figure 100). Most of the participants that have measured this species have correctly assigned 'ellipsoid' as geometrical shape (Table 21). Additionally, 'double prolate spheroid' has been used for comparison, or the half cells were measured independently and the average of the half cells used for the calculation of the entire cell. The last has been done to take into account that the half cells have been different in size due to division. One participant used 'double prolate spheroid' instead of 'ellipsoid'. Further inspection of the data revealed that some participants used 'ellipsoid' but measured only one half cell (Figure 102). One participant did the same but used the correction factor of 2.0 for equalisation. It became obvious that the use of the 'ellipsoid' form for a species like this is not self-evident. The 'double prolate spheroid' or – because of the flattened form – the 'double ellipsoid', which was not included in the Standard Document, seemed to be more understandable and acceptable by the analysts. Several comments have been made on this. To reduce the number of geometrical shapes it had been decided to use 'prolate spheroid' or 'ellipsoid' for a half cell for these cases.

Table 21: Inventory of the main results (except special statistics) for *Cosmarium ocellatum.*

General										
45	participants (labcode) have measured this species: 12 13 14 16 17 18 19 20 21 22 23 24 25 27 29 30 31 32 33 34 35 36 38 39 41 43 45 46 47 48 49 50 51 52 53 54 55 57 58 60 61 62 63 64 65									
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison								
26	measurements with an eyepiece micrometre									
22	measurements with image analysis software									
Geometry and Dimensions										
45	measurements/calculations with a 'ellipsoid' assigned as geometrical shape according to the Standard Document									
1	measurement/calculation with a 'double prolate spheroid' assigned as geometrical shape notwithstanding to the Standard Document									
	1	additional calculation with a 'double prolate spheroid' assigned as geometrical shape notwithstanding to the Standard Document for comparison								
	1	additional calculation with a 'ellipsoid (separate)' assigned as geometrical shape notwithstanding to the Standard Document for comparison								
5	measurements assigned 'ellipsoid' as geometrical shape but measured one half of the cell									
48	calculations with no correction factor used according to the Standard Document									
1	calculation with a correction factor of 2 used notwithstanding to the Standard Document									
1	calculation with' large diameter' and 'height' being confused									
Statistics										
16-50	units have been measured									
	1	measurement has not fulfilled the minimum requirements according to the Standard Document (20 units)								
	29	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)								
		0	measurements with the biovolume standard error being ≥ 10 %							
	0	total measurements with the biovolume standard error being ≥ 10 %								

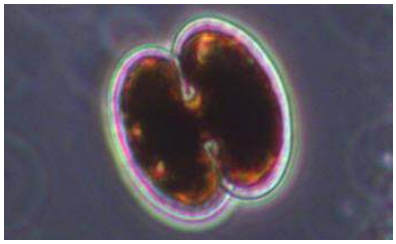

Figure 100: Microphotographic picture of *Cosmarium ocellatum* in the Oldenburg sample.

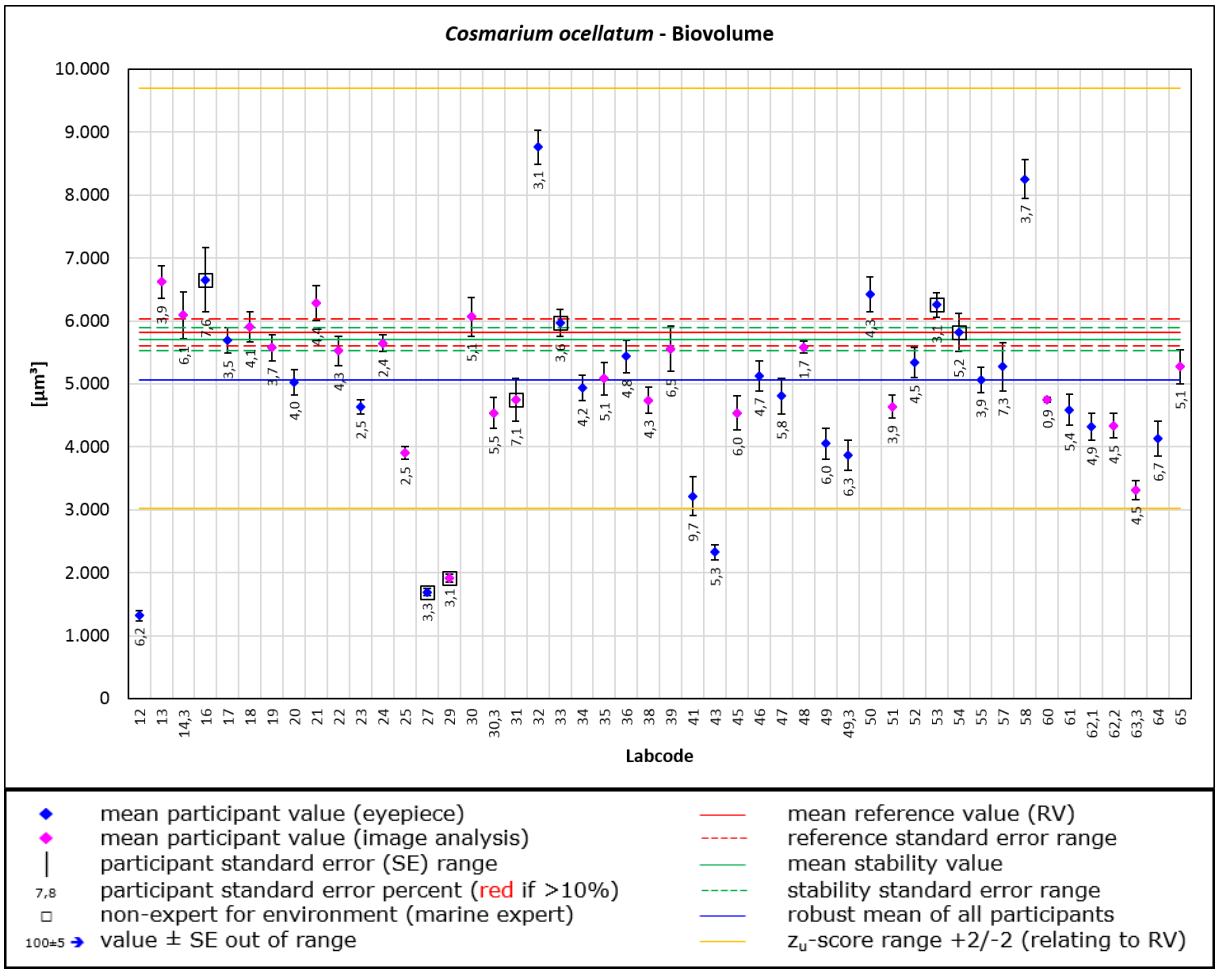

Figure 101: Results and some statistical characteristics for biovolume of *Cosmarium ocellatum*. Please note: Comma used as decimal mark, full stop used as thousands separator.

Most biovolume values ranged from 4,000 to 7,000 µm³ (Figure 101). Four outliers could be found at the lower part of the z_u range from -2 to +2. The reference values were high, compared to the average of the participants. An inspection of the dimensions has revealed that this has been mainly due to the large diameter (Figure 102, Figure 103 and Figure 104). An explanation could be that some participants did not measure the largest part of the cell but the isthmus.

The hidden dimension for the reference data was the 'small diameter'. It was calculated as 0.5 times the 'large diameter'. Using this relation and calculating back the data of the participants, the resulting factor ranged from 0.35 to 1.18 with a mean of 0.59 (Table 25).

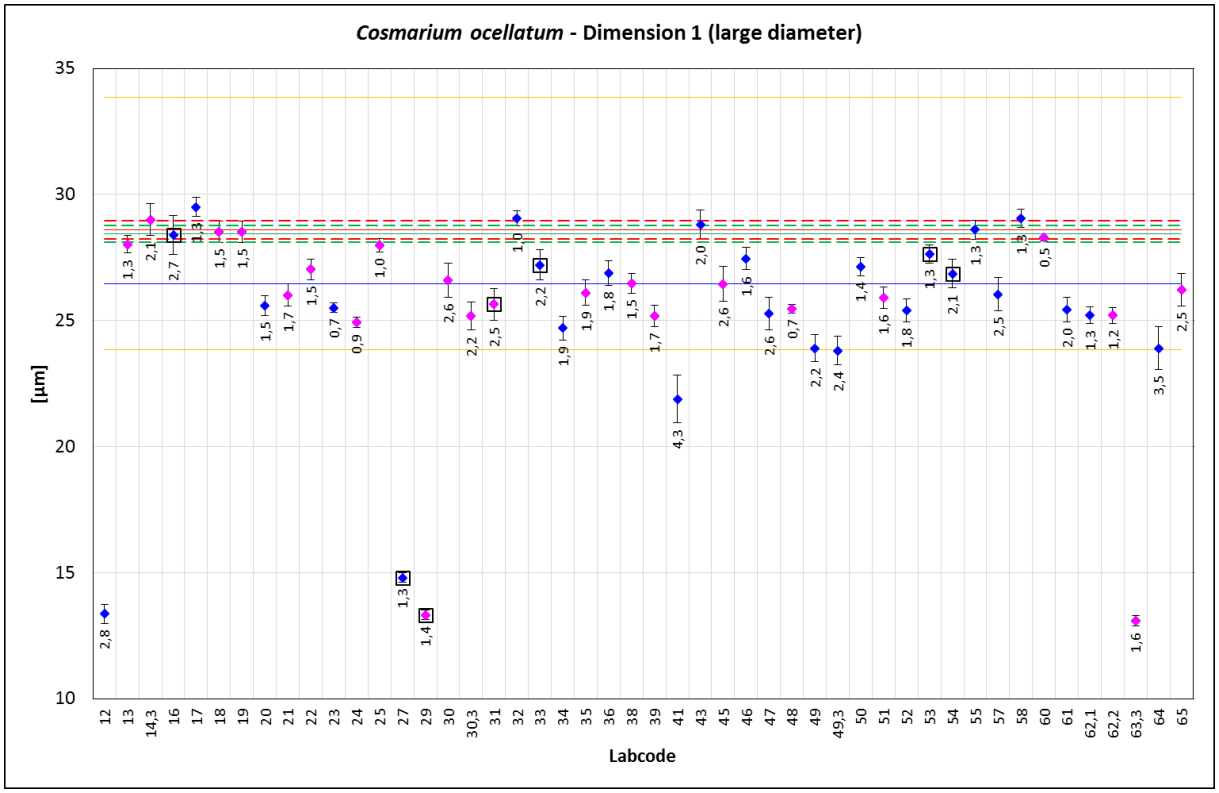

Figure 102: Results and some statistical characteristics for dimension 1 (large diameter) of *Cosmarium ocellatum*. Legend see biovolume chart. Please note: Comma used as decimal mark.

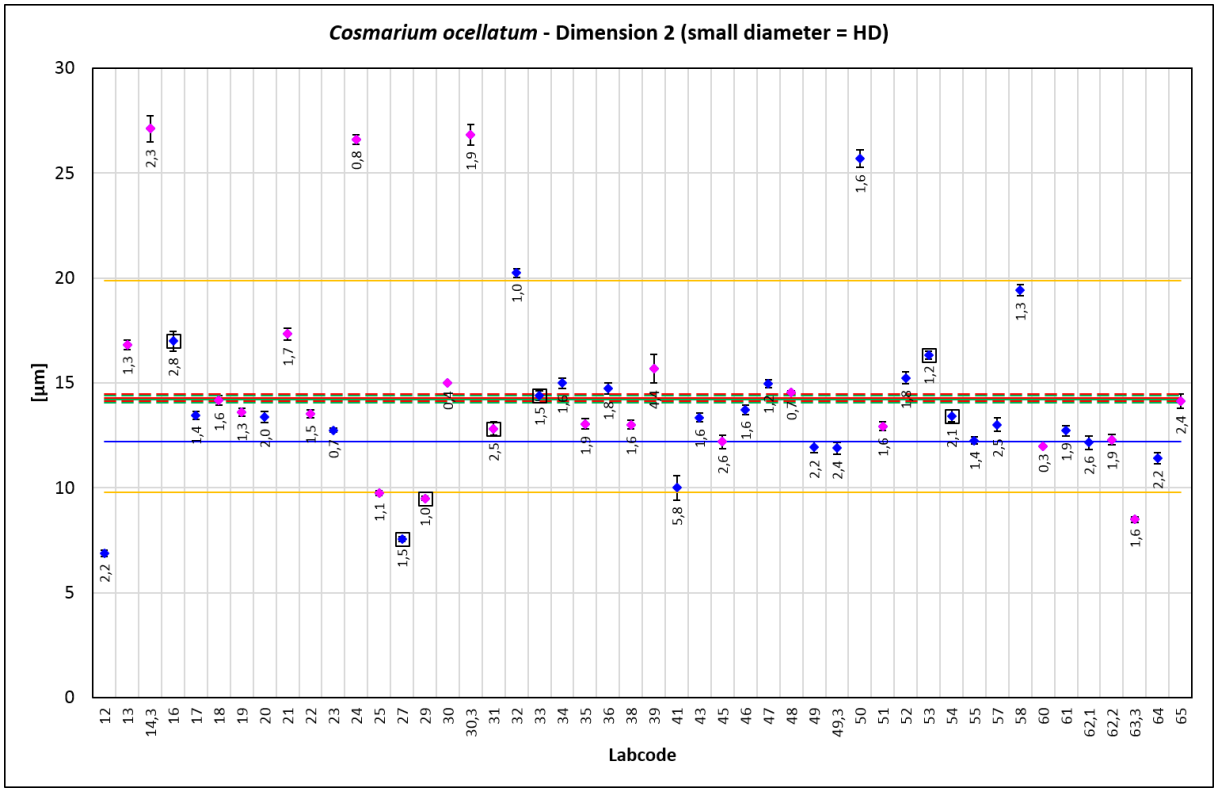

Figure 103: Results and some statistical characteristics for dimension 2 (small diameter = HD) of *Cosmarium ocellatum*. Legend see biovolume chart. Please note: Comma used as decimal mark.

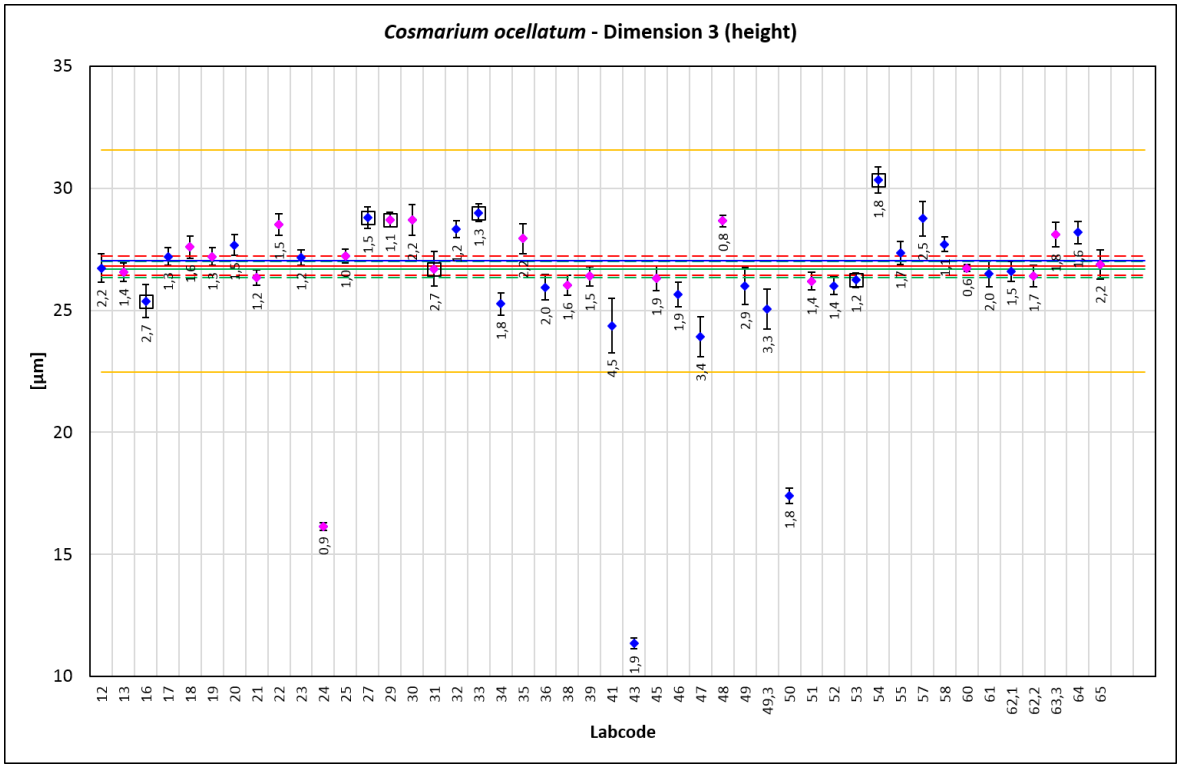

Figure 104: Results and some statistical characteristics for dimension 3 (height) of *Cosmarium ocellatum*. Legend see biovolume chart. Please note: Comma used as decimal mark.

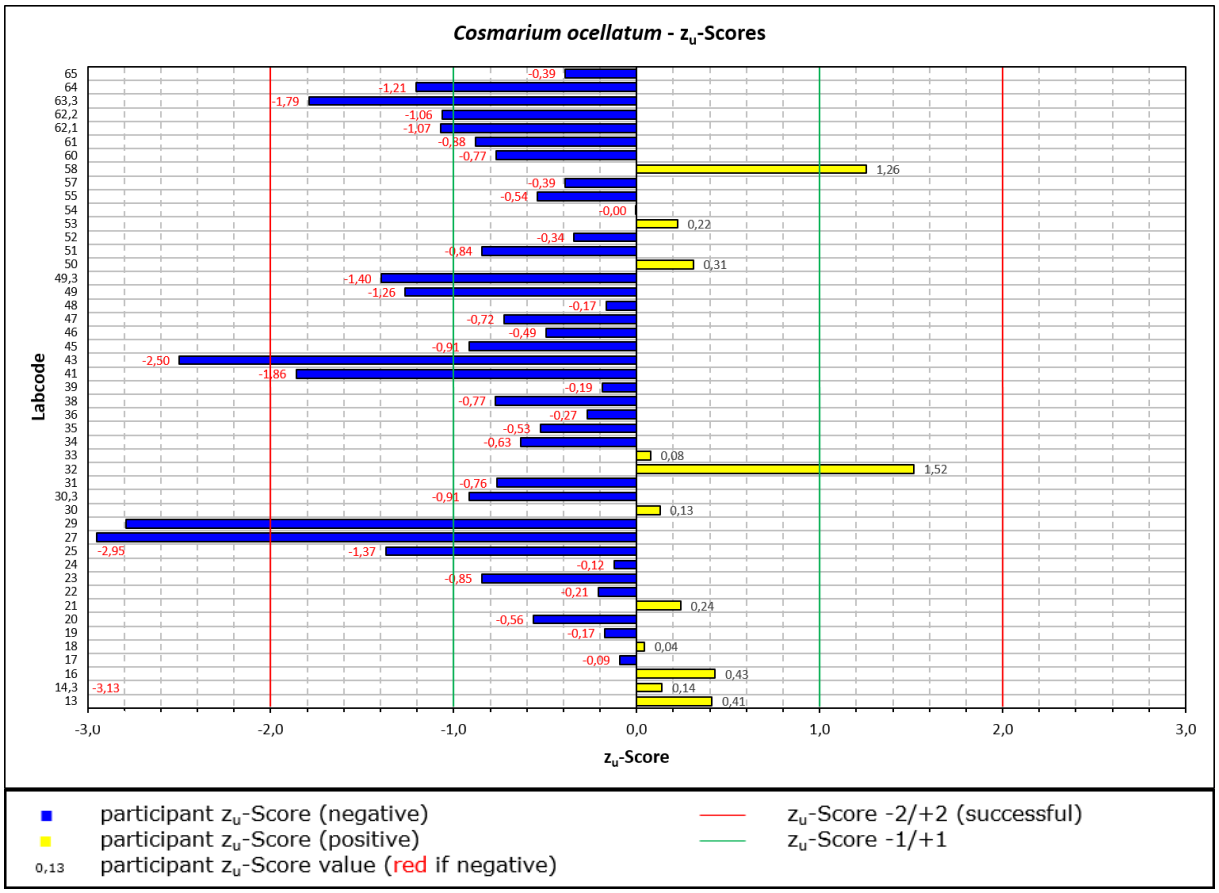

Figure 105: z_u scores of participants for *Cosmarium ocellatum*. Please note: Comma used as decimal mark.

Most z_u score values lay within the range between -1 and +1 with a tendency to negative values. 13 participants were not within that range. Three of these participants did not pass the test for this species by missing the z_u score range of -2 to +2 (Figure 105). One participant was excluded as an outlier due to missing even the range of -3 to +3 (Table 26). This reflects a moderate performance for the biovolume analysis of this species.

3.4.20 *Trachelomonas hispida*

Trachelomonas hispida is a green euglenoid flagellate (Figure 106). All participants that have measured this species have correctly assigned the 'prolate spheroid' as geometrical shape, according to the Standard Document (Table 22). However, two participants have confused the diameter and the height (Figure 108, Figure 109). It is very important to use only values correctly assigned to the variables of the equation, because the diameter is an exponential term in the equation.

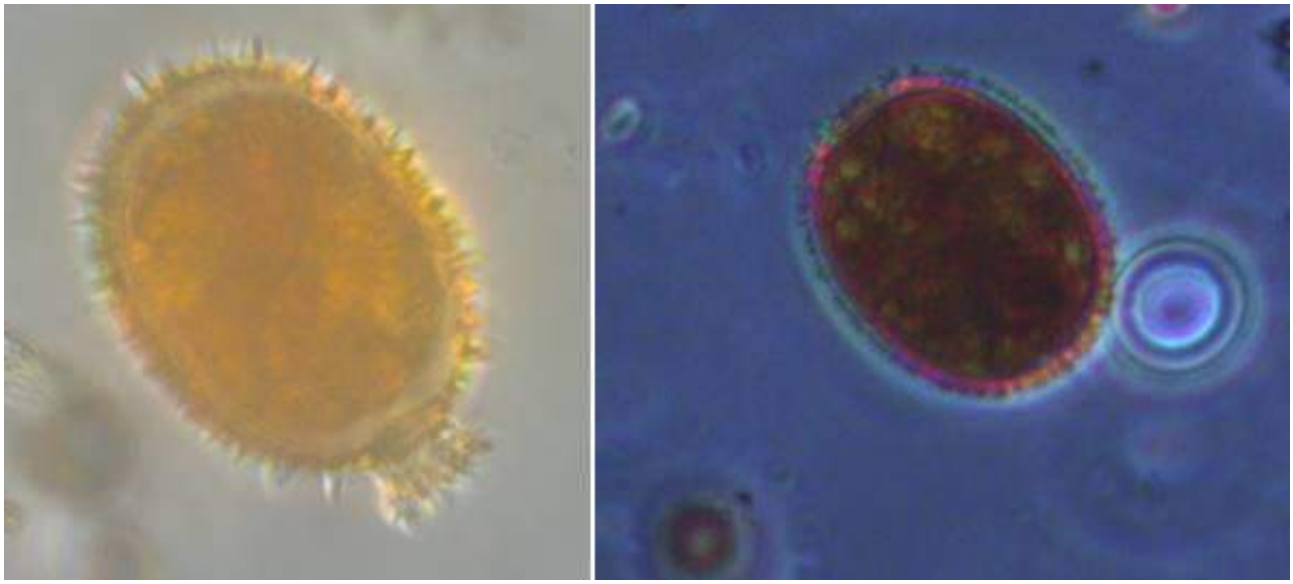

Figure 106: Microphotographic pictures of *Trachelomonas hispida* in the Oldenburg sample (left one provided by Pepita Nolla).

Biovolume values ranged from 4,000 to 10,000 µm^3 and were in accordance with the reference values (Figure 107). Except one outlier all of the participants' results for the biovolume were within the requested z_u range of -2 to +2.

The z_u scores were variable but often in the range between -1 and +1. One dataset missed the range between -3 and +3 and had to be excluded as an outlier (Table 26). All other participants passed the test for this species (Figure 110). This reflects a good performance for the biovolume analysis of this species.

Table 22: Inventory of the main results (except special statistics) for *Trachelomonas hispida*.

General										
45	participants (labcode) have measured this species:									
	12	13	14	16	17	18	19	20	21	22
	23	24	25	27	29	30	31	32	33	34
	35	36	38	39	41	43	45	46	47	48
	49	50	51	52	53	54	55	57	58	60
	61	62	63	64	65					
	1	participant has measured with an eyepiece micrometre AND image analysis software for comparison								
25	measurements with an eyepiece micrometre									
21	measurements with image analysis software									
Geometry and Dimensions										
46	measurements/calculations with a 'prolate spheroid' assigned as geometrical shape according to the Standard Document									
46	calculations with no correction factor used according to the Standard Document									
2	calculations with 'diameter' and 'height' being confused									
Statistics										
15-76	units have been measured									
	1	measurements have not fulfilled the minimum requirements according to the Standard Document (20 units)								
	30	measurements have exactly fulfilled the minimum requirements according to the Standard Document (20 units)								
		0	measurements with the biovolume standard error being ≥ 10 %							
	0	total measurements with the biovolume standard error being ≥ 10 %								

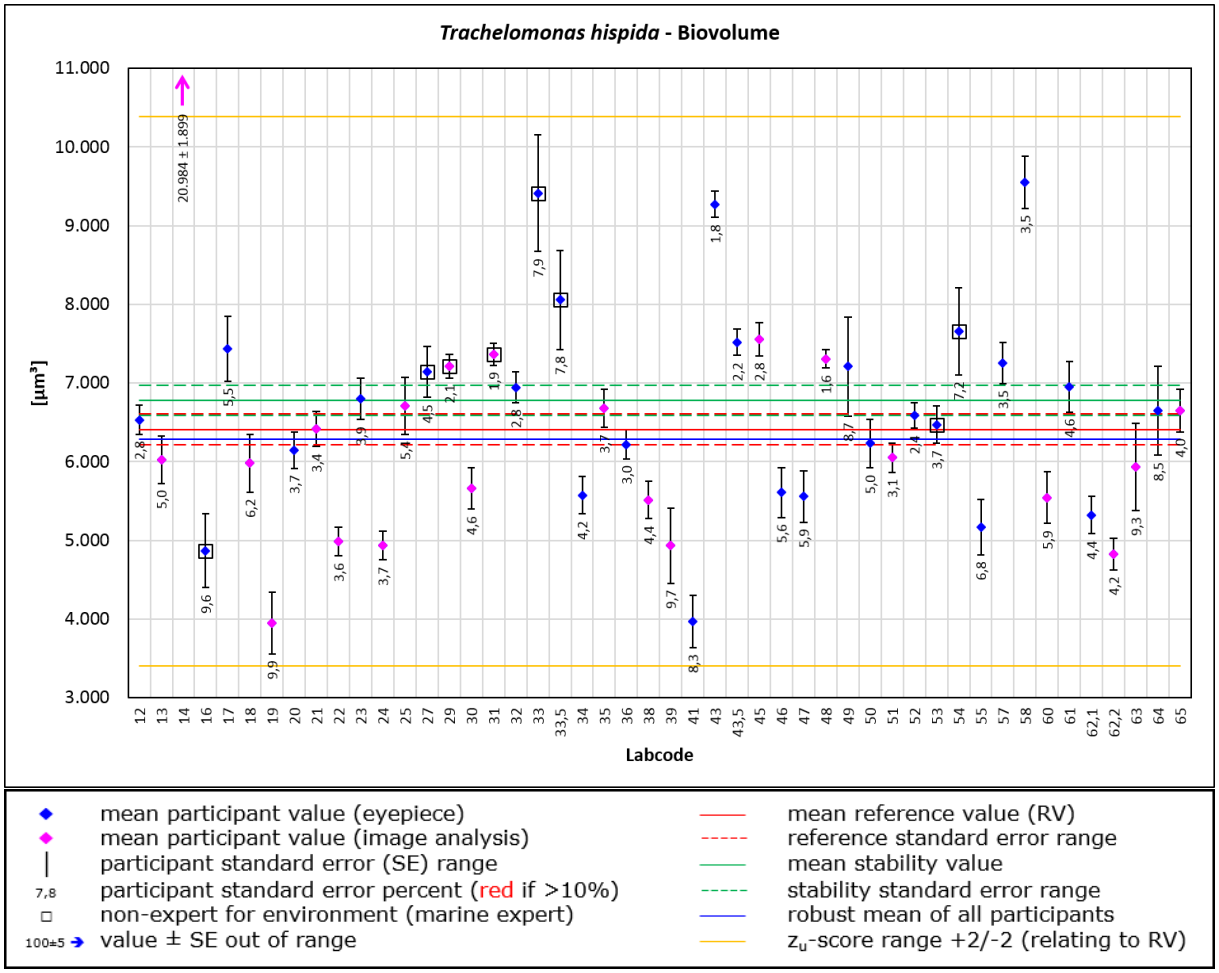

Figure 107: Results and some statistical characteristics for biovolume of *Trachelomonas hispida*. Please note: Comma used as decimal mark, full stop used as thousands separator.

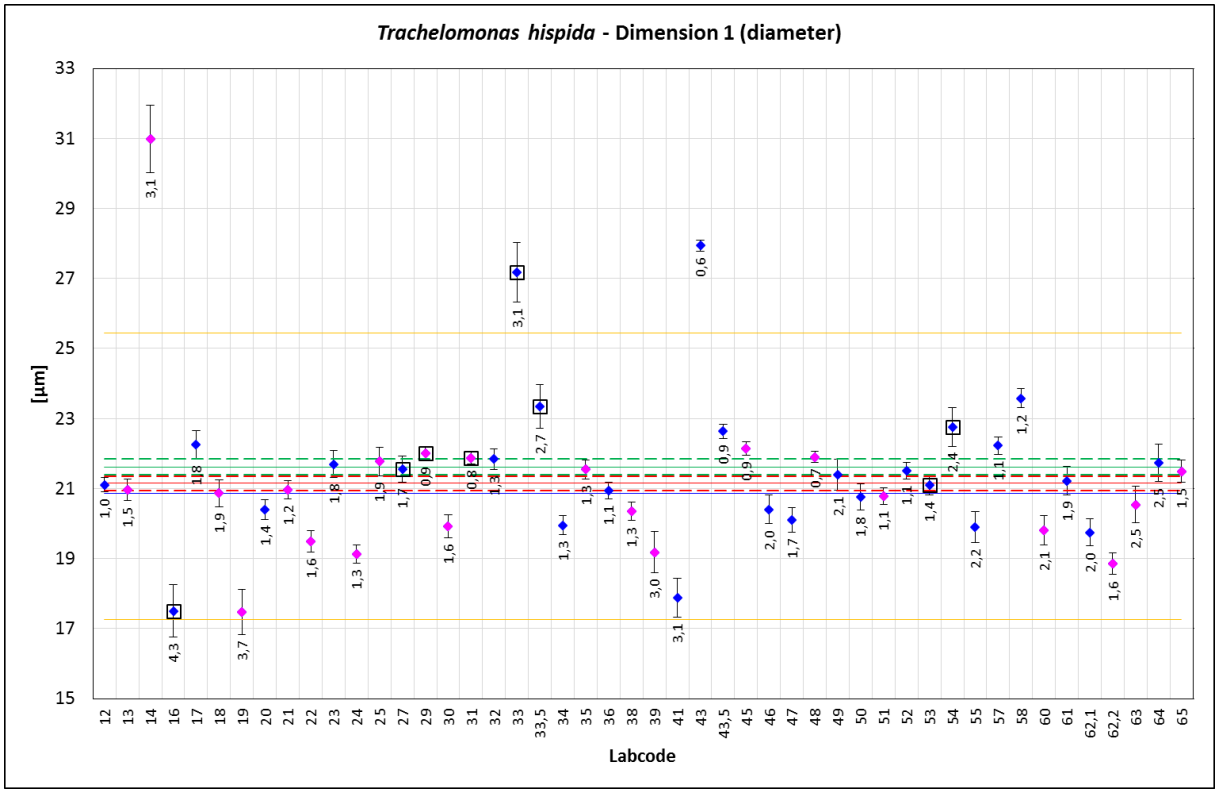

Figure 108: Results and some statistical characteristics for dimension 1 (diameter) of *Trachelomonas hispida*. Legend see biovolume chart. Please note: Comma used as decimal mark.

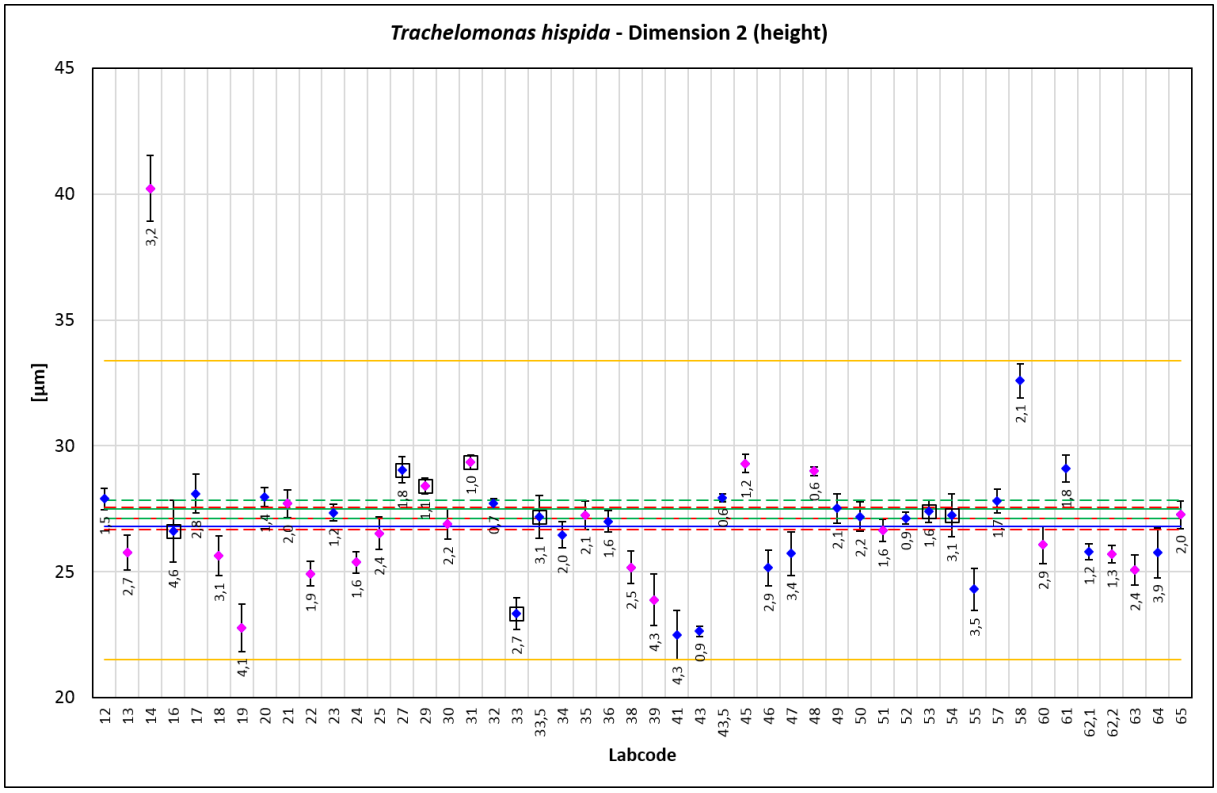

Figure 109: Results and some statistical characteristics for dimension 2 (height) of *Trachelomonas hispida*. Legend see biovolume chart. Please note: Comma used as decimal mark.

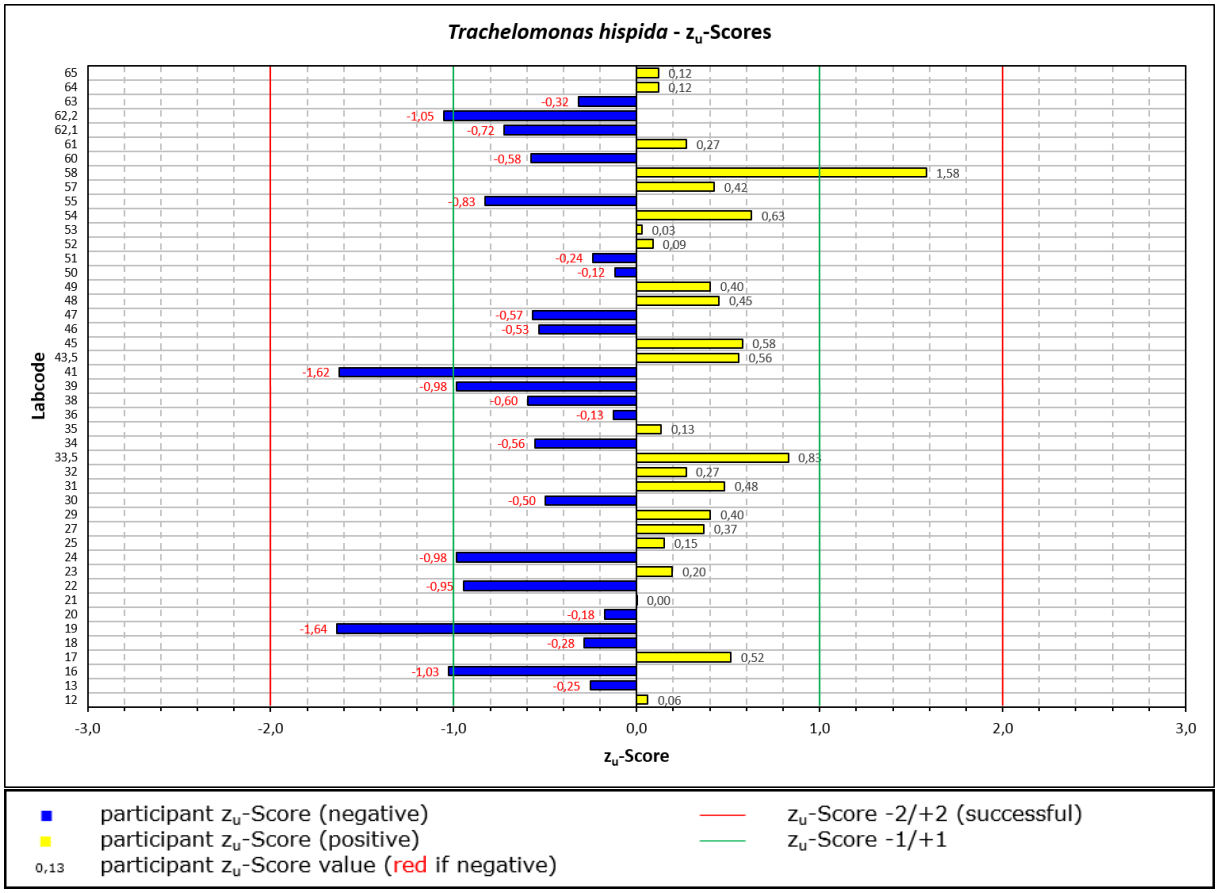

Figure 110: z_u scores of participants for *Trachelomonas hispida*. Please note: Comma used as decimal mark.

3.5 Summarising statistics

In the following part some summarising statistics are presented which do not refer to special species or give an overview for all species.

3.5.1 Magnifications

Information about the magnification used for each species had been requested and is presented in the following pie charts (Figure 111, Figure 112, Figure 113, Figure 114). Despite in general a high variability in magnifications can be found, it becomes obvious that the dominant magnification for all species had been 400 times. For most species the composition of magnifications used and their proportions was quite similar. As could be expected for larger species in all dimensions (as *Odontella* or *Ceratium*) or one dimension (as *Planktothrix*) the composition changed to smaller magnifications and for small species or cells (as *Cryptomonas* or *Woronichinia*) to higher magnifications.

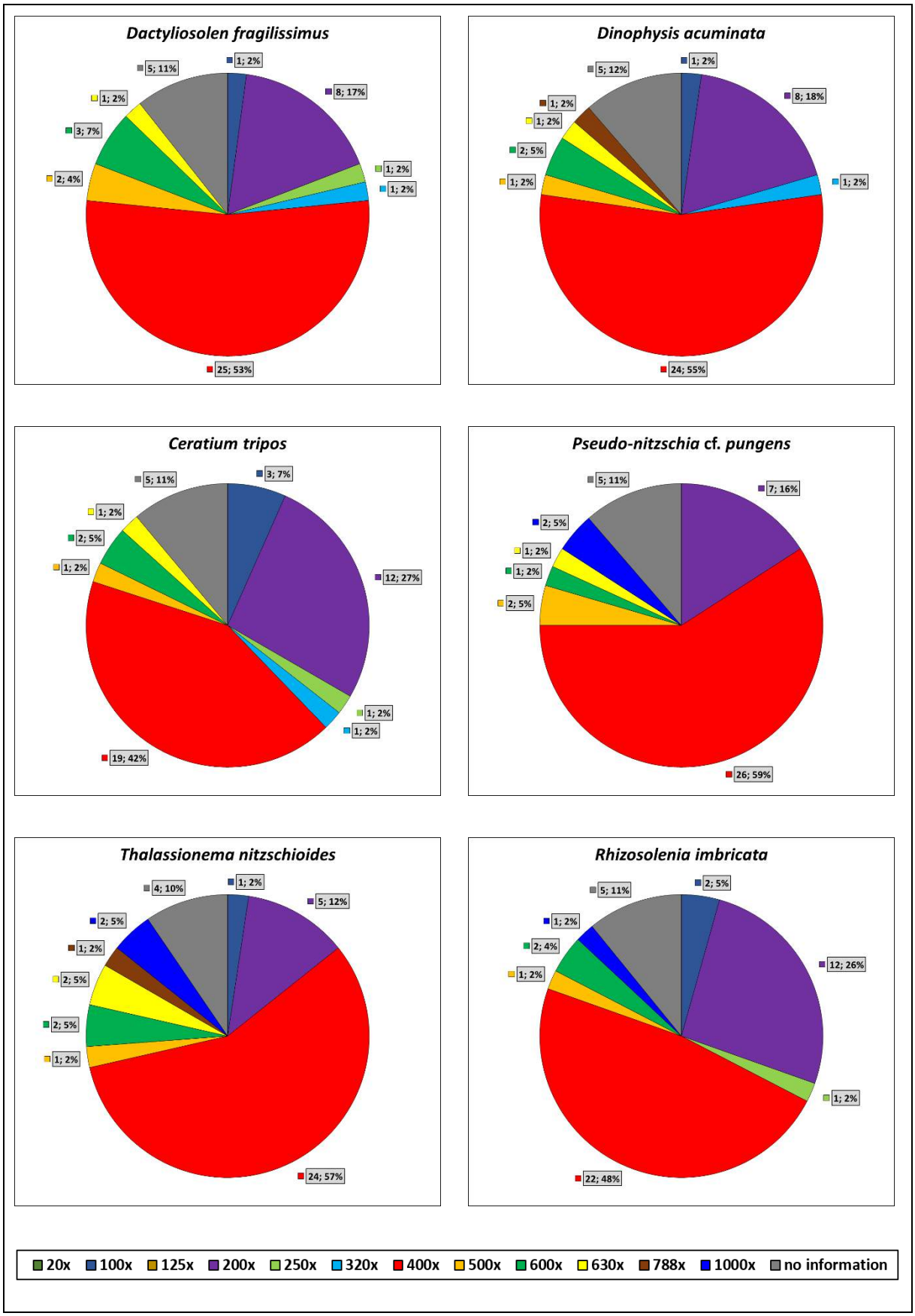

Figure 111: Overview of used total magnifications for measuring the species part 1.

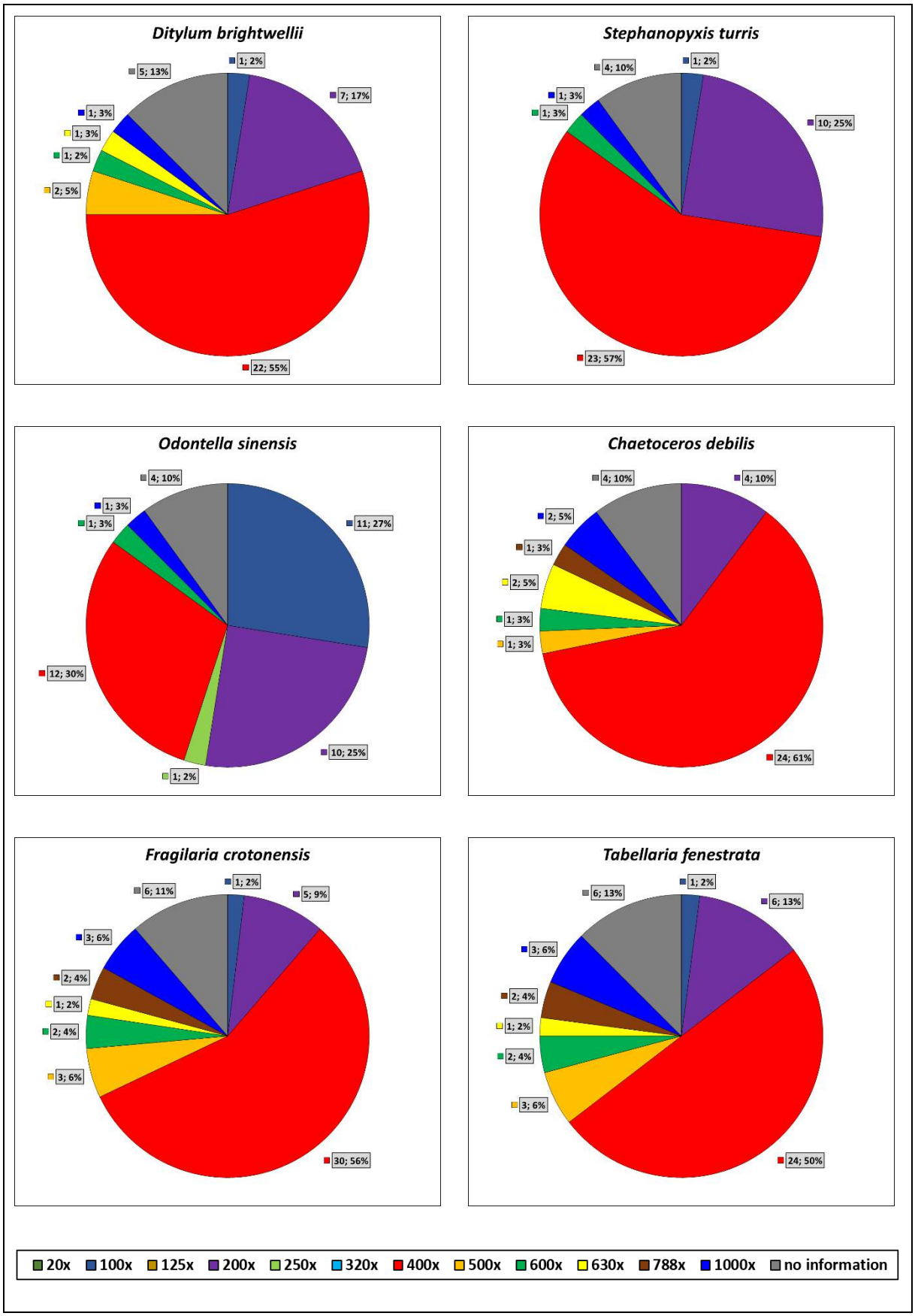

Figure 112: Overview of used total magnifications for measuring the species part 2.

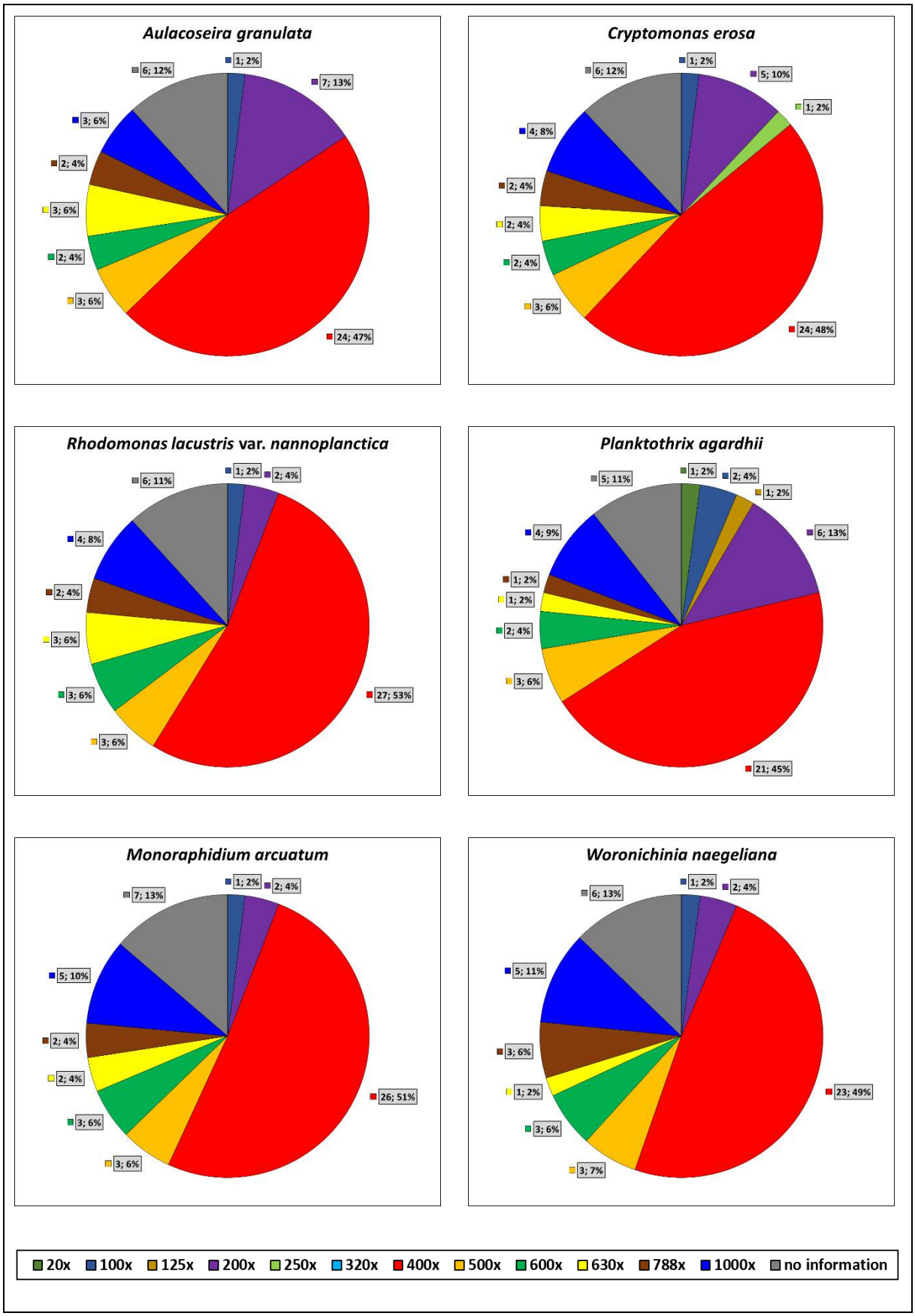

Figure 113: Overview of used total magnifications for measuring the species part 3.

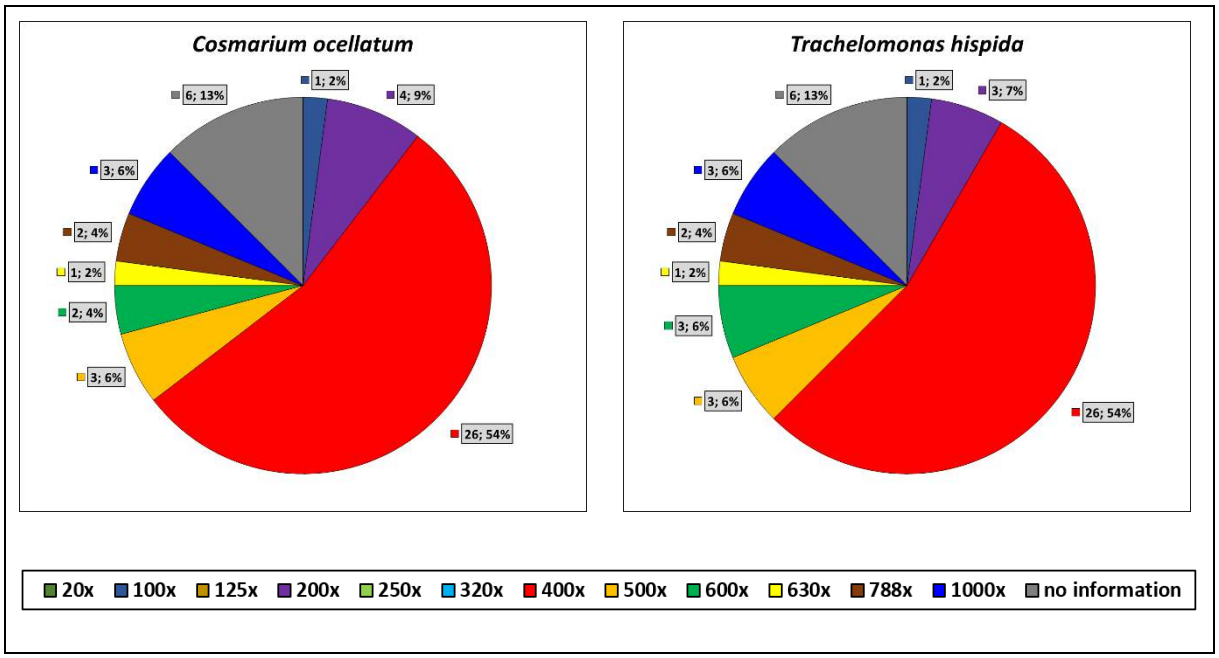

Figure 114: Overview of used total magnifications for measuring the species part 4.

3.5.2 Measurement Method

The analysis of the measurement methods (Table 23) revealed that no significant differences between the measurements with eyepiece micrometre and image analysis existed. However, image analysis might be helpful for measuring complicated shapes as curved or contorted dimensions.

Table 23: p-values of the analysis of the measurement method. Significant differences between the mean values derived either from eyepiece micrometre or from image analysis are indicated by bold numbers.

Species	**p**	**Species**	**p**
Dactyliosolen fragilissimus	0.832	*Fragilaria crotonensis*	0.065
Dinophysis acuminata	0.756	*Tabellaria fenestrata*	0.418
Ceratium tripos	0.458	*Aulacoseira granulata*	0.210
Pseudo-nitzschia cf. *pungens*	0.883	*Cryptomonas erosa*	0.601
Thalassionema nitzschioides	0.124	*Rhodomonas lacustris* var. *nannoplanctica*	0.282
Rhizosolenia imbricata	0.277	*Planktothrix agardhii*	0.176
Ditylum brightwellii	0.137	*Monoraphidium arcuatum*	0.465
Stephanopyxis turris	0.089	*Woronichinia naegeliana*	0.365
Odontella sinensis	0.702	*Cosmarium ocellatum*	0.872
Chaetoceros debilis	0.370	*Trachelomonas hispida*	0.114

3.5.3 Expertise

Experience in the identification and measurement might be an important matter in determining phytoplankton biovolume. However, for most of the analysed species no significant differences due to the expertise of the participants could be found (Table 24). The experience in working with marine or freshwater phytoplankton seemed to be of less importance.

There have been two exceptions. The first was *Ditylum brightwellii*. This species was included in the sample that had been less well preserved to test for the importance of the preservation. Indeed, *Ditylum* was hardly to be found in a good condition; it was shrunken in the middle part of the cell and had lost its shape. For experienced analysts it might be more obvious what dimensions had to be measured in such cases in order to obtain reasonable results.

The second example was *Stephanopyxis turris*. Here it was obvious that the mean biovolume results of the participants deviated from the reference values. A further look into the structure showed that the difference was higher for non-experienced analysts than for experienced participants in the marine field (Figure 42). For this species, the expertise seemed to have been quite important. Although *Stephanopyxis* was in the same sample as *Ditylum*, its shape was much better preserved. Thus, rather a methodological problem might be the reason for the difference.

The trend in some p-values of other species might indicate that the experience could be of relevance for other species as well, but it is obviously species-specific.

Table 24: p-values for the analysis of the expertise of the participants. Significant differences between mean values from specialist and non-specialist groups are indicated by bold numbers.

Species	**p**	**Species**	**p**
Dactyliosolen fragilissimus	0.213	*Fragilaria crotonensis*	0.068
Dinophysis acuminata	0.386	*Tabellaria fenestrata*	0.051
Ceratium tripos	0.758	*Aulacoseira granulata*	0.296
Pseudo-nitzschia cf. *pungens*	0.688	*Cryptomonas erosa*	0.055
Thalassionema nitzschioides	0.643	*Rhodomonas lacustris* var. *nannoplanctica*	0.153
Rhizosolenia imbricata	0.246	*Planktothrix agardhii*	0.977
Ditylum brightwellii	**0.032**	*Monoraphidium arcuatum*	0.630
Stephanopyxis turris	**0.006**	*Woronichinia naegeliana*	0.330
Odontella sinensis	0.991	*Cosmarium ocellatum*	0.592
Chaetoceros debilis	0.429	*Trachelomonas hispida*	0.083

3.5.4 Hidden dimensions

The analysis of hidden dimensions was not a requested part of this intercalibration test, because it has not been a topic of the mandate. For the analysis, the participants of the intercomparison have been asked to use their own or 'home' methods to calculate the dimensions they could not measure. In this way, the complete scope of fluctuation concerning that topic should be included in the test. The data analysis revealed that the dimensions defined as hidden often seemed to be the main reason for the high variability in biovolumes. Therefore, a questionnaire about the methods used by the participants had been sent around. Unfortunately, the return rate has been low and thus, information is still poor.

However, it became obvious that all kinds of methods were used, i.e. calculation of factors, use of constants or even attempts to measure the hidden dimensions in a way. In this report, hidden dimension factors have also been calculated from the data of the participants. Assuming that all participants had always selected the same dimension as hidden with regard to a particular species, the same factor that had been used for the reference data has been calculated as a relation of two dimensions. Certainly, this assumption will not reflect the real proceeding in all cases, but nevertheless a comparison seemed possible. The varieties of values for the hidden dimension factors for each species are shown in Table 25. The variability was generally high, but the sorted values gave an overview on distributions and comparability within a certain range. For example, the values ranged from 0.5 to 1.49 for *Thalassionema*, but almost half of the participants had used the factor 1. Furthermore, it turned out that many participants had calculated the hidden dimension for *Ditylum* via the trigonometric function (value 0.87). Another noticeable outcome of this analysis was the fact that the means calculated from all mean values of the participants were very close to the values used for the reference data, although the ranges of values of the participants were very broad and the respective variability high (see the last two lines of Table 25).

Table 25: Hidden dimension factors of participants calculated from their data. The factors have been calculated for participant and each item and are shown as mean by participant in ascending order for each species. The mean for each species (calculated by the mean of each participant) is shown at the end and compared with the hidden dimension factor used for the reference data. Note the different number of rows for the species due to the different number of participants that measured the species.

	Dinophysis	*Ceratium*	*Pseudo-nitzschia*	*Thalassionema*	*Ditylum*	*Odontella*	*Chaetoceros*	*Fragilaria*	*Tabellaria*	*Cryptomonas*	*Cosmarium*
Hidden dimension factors of participants in ascending order for each species	0.30	0.20	0.59	0.50	1.00	0.12	0.24	0.01	0.06	0.44	0.35
	0.31	0.33	0.61	0.57	0.55	0.21	0.38	0.01	0.06	0.49	0.42
	0.43	0.33	0.63	0.59	0.87	0.22	0.39	0.49	0.08	0.50	0.43
	0.44	0.33	0.65	0.60	0.87	0.23	0.41	0.49	0.08	0.50	0.45
	0.47	0.35	0.67	0.65	0.87	0.24	0.50	0.50	0.09	0.65	0.46
	0.49	0.36	0.75	0.70	0.87	0.24	0.50	0.51	0.10	0.65	0.46
	0.49	0.39	0.77	0.72	0.87	0.24	0.51	0.51	0.10	0.65	0.48
	0.50	0.41	0.92	0.74	0.87	0.25	0.51	0.68	0.10	0.65	0.48
	0.50	0.42	0.97	0.77	0.87	0.25	0.54	0.84	0.11	0.65	0.48
	0.50	0.42	0.97	0.78	0.87	0.28	0.57	0.86	0.11	0.65	0.49
	0.50	0.44	1.00	0.79	0.87	0.29	0.59	0.90	0.12	0.66	0.49
	0.50	0.45	1.00	0.80	0.87	0.29	0.60	0.96	0.13	0.66	0.50
	0.50	0.47	1.00	0.81	0.89	0.30	0.63	0.98	0.13	0.67	0.50
	0.50	0.50	1.00	0.86	0.97	0.33	0.63	1.00	0.14	0.68	0.50
	0.50	0.50	1.00	0.90	0.99	0.33	0.63	1.00	0.14	0.70	0.50
	0.50	0.50	1.00	0.94	1.00	0.35	0.63	1.00	0.14	0.70	0.50
	0.51	0.50	1.00	0.98	1.00	0.39	0.63	1.00	0.14	0.70	0.50
	0.51	0.50	1.00	1.00	1.00	0.40	0.67	1.00	0.14	0.74	0.50
	0.60	0.50	1.00	1.00	1.00	0.40	0.67	1.00	0.14	0.77	0.50
	0.62	0.52	1.09	1.00	1.00	0.40	0.69	1.00	0.14	0.78	0.50
	0.65	0.53	1.17	1.00	1.00	0.40	0.71	1.00	0.14	0.78	0.50
	0.65	0.56	1.22	1.00	1.00	0.40	0.74	1.00	0.14	0.78	0.50
	0.66	0.58	1.24	1.00	1.00	0.42	0.74	1.00	0.15	0.79	0.50
	0.67	0.60	1.25	1.00	1.00	0.44	0.75	1.00	0.15	0.79	0.51
	0.67	0.61	1.25	1.00	1.00	0.44	0.75	1.00	0.16	0.79	0.52
	0.67	0.61	1.27	1.00	1.00	0.44	0.75	1.00	0.16	0.80	0.52
	0.67	0.62	1.33	1.00	1.00	0.45	0.75	1.00	0.17	0.80	0.53
	0.67	0.62	1.33	1.00	1.00	0.45	0.75	1.00	0.17	0.80	0.54
	0.69	0.65	1.33	1.00	1.00	0.45	0.75	1.00	0.17	0.80	0.55
	0.69	0.65	1.36	1.00	1.00	0.45	0.75	1.11	0.17	0.80	0.57
	0.70	0.65	1.38	1.00	1.00	0.47	0.78	1.11	0.17	0.80	0.57
	0.70	0.65	1.39	1.00	1.00	0.50	0.81	1.13	0.17	0.80	0.59
	0.70	0.67	1.39	1.00	1.00	0.50	0.83	1.13	0.17	0.80	0.60
	0.70	0.67	1.48	1.00	1.00	0.50	0.97	1.13	0.17	0.80	0.60
	0.70	0.67	1.63	1.00	1.00	0.50	1.00	1.13	0.17	0.83	0.60
	0.71	0.72	1.66	1.00	1.00	0.50	1.09	1.16	0.17	0.83	0.60
	0.71	0.75	1.82	1.00	1.00	0.50	1.17	1.17	0.18	0.83	0.61
	0.74	0.75	1.98	1.01	1.08	0.56	1.35	1.19	0.18	0.83	0.62
	0.74	0.76	1.98	1.02	1.32	0.60	1.82	1.24	0.18	0.84	0.65

	Dinophysis	*Ceratium*	*Pseudo-nitzschia*	*Thalassionema*	*Ditylum*	*Odontella*	*Chaetoceros*	*Fragilaria*	*Tabellaria*	*Cryptomonas*	*Cosmarium*
	0.78	0.78	1.98	1.06	1.74	0.91		1.35	0.18	0.85	0.67
	0.78	0.82	1.98	1.14				1.39	0.18	0.85	0.67
	0.82	1.00	2.00	1.49				1.40	0.18	0.85	0.70
	0.85	1.00	2.00					1.43	0.19	0.90	0.72
	0.98	1.00	2.00					1.50	0.19	1.00	0.93
		1.83						1.57	0.19	1.00	0.95
								1.61	0.19	1.00	1.07
								1.67	0.20	1.00	1.07
								1.78	0.39	1.00	1.18
								1.90		1.01	
								1.96		1.99	
								2.00			
								2.00			
								2.30			
Mean of participants	0.61	0.60	1.25	0.91	0.98	0.39	0.72	1.12	0.15	0.79	0.59
Reference data	0.60	0.65	1.00	1.00	0.87	0.40	0.75	1.00	0.10	0.85	0.50

3.5.5 Compilation of participants results

As a general rule, participants who had not selected the correct geometric shape or whose measurements were apparently completely out of range or whose absolute z_u score values were > 2 did not pass the test for the respective species.

However, summarising the results it can be stated that most participants have passed the intercalibration test for most of the species they had measured (75-100 %). Table 26 gives a detailed overview over the results.

Since the intercalibration exercise was carried out only as a validation test for the biovolume method as well as for a suitable handling of the Standard Document, the overall number of species analysed by the individual participant had no effect on his result. Within this context, it has been very much appreciated that most of the participants have analysed all species and furthermore, some of them have provided additional data for a comparison of measuring methods or geometric shapes.

Table 26: Overview of all species and all participants. Species not measured by a participant are marked with an x. for the others the z_u score values are shown. The z_u scores are in red when the value is out of the range to pass the test (<-2 to >2). Some data had been excluded before the analysis. These are marked with an 'out' (in blue if excluded because of measuring not comparable parts/geometry. in red if excluded from further analysis after testing for outliers). Fields highlighted with light orange show values of z_u scores that would pass the test, but the participants had selected the incorrect geometric shape according to the Standard Document and therefore did not pass for this species. Highlighted in light yellow are two fields where intentional and in agreement the 'girdle diameter' had been selected instead of the required shape. Additional measurements/calculations are not shown, except for the comparison of the two measurement methods by labcode 62. For each participant statistics about number of species measured and the fraction of passed species are shown.

Labcode	*Dactyliosolen*	*Dinophysis*	*Ceratium*	*Pseudo-nitzschia*	*Thalassionema*	*Rhizosolenia*	*Ditylum*	*Stephanopyxis*	*Odontella*	*Chaetoceros*	*Fragilaria*	*Tabellaria*	*Aulacoseira*	*Cryptomonas*	*Rhodomonas*	*Planktothrix*	*Monoraphidium*	*Woronichinia*	*Cosmarium*	*Trachelomonas*	species done	Species passed	passed in % of all species	passed in % of species done
11	x	x	x	x	x	-1.80	x	x	x	x	-0.52	-0.57	out	-0.40	0.35	-0.02	-1.56	x	x	x	8	6	30	75
12	0.64	0.25	-0.51	-1.76	0.24	0.14	0.53	x	x	x	-1.99	-2.26	0.16	-0.94	-1.16	0.19	-0.22	0.92	out	0.06	17	15	75	88
13	0.00	-0.19	-0.62	-1.10	0.32	0.76	-0.48	-0.34	0.00	-0.71	0.28	out	0.21	-0.55	-0.16	0.11	-0.61	0.23	0.41	-0.25	20	19	95	95
14	-0.20	1.05	0.40	-0.67	x	0.36	-1.15	-1.04	-0.83	0.34	-0.58	-1.84	-0.94	-0.55	-0.06	-0.52	-0.96	-0.31	0.14	out	19	16	80	84
15	-0.48	-0.85	-2.68	-0.49	0.24	0.13	x	x	-0.71	x	x	x	x	x	x	x	x	x	x	x	7	6	30	86
16	-0.06	-0.95	1.54	0.82	0.19	-0.38	0.06	-0.41	-0.54	-0.86	0.53	-0.05	-0.13	-0.78	0.23	-1.44	-0.70	0.83	0.43	-1.03	20	20	100	100
17	0.07	0.00	2.64	-1.49	0.34	x	-0.04	-0.39	0.06	-1.54	-0.26	-0.50	out	-1.17	0.35	0.93	0.19	0.25	-0.09	0.52	19	16	80	84
18	0.15	1.21	2.08	-1.42	-0.29	0.14	0.40	0.10	1.04	-0.76	-1.39	0.18	-0.15	-1.27	0.85	-0.21	0.23	-0.28	0.04	-0.28	20	18	90	90
19	x	x	x	x	x	x	x	x	x	x	0.35	-1.27	0.63	-0.91	0.56	-0.01	-0.61	0.26	-0.17	-1.64	10	10	50	100

Labcode	*Dactyliosolen*	*Dinophysis*	*Ceratium*	*Pseudo-nitzschia*	*Thalassionema*	*Rhizosolenia*	*Ditylum*	*Stephanopyxis*	*Odontella*	*Chaetoceros*	*Fragilaria*	*Tabellaria*	*Aulacoseira*	*Cryptomonas*	*Rhodomonas*	*Planktothrix*	*Monoraphidium*	*Woronichinia*	*Cosmarium*	*Trachelomonas*	species done	Species passed	passed in % of all species	passed in % of species done
20	0.26	1.46	1.05	-0.89	-0.65	-0.26	-1.59	-1.91	-2.10	1.09	-1.70	0.22	0.22	-0.37	-0.31	-0.32	-1.24	-0.80	-0.56	-0.18	20	19	95	95
21	-0.07	-0.83	0.58	1.36	0.53	0.71	-0.84	-1.59	-0.17	-0.24	-0.86	0.52	0.36	0.83	-0.22	-0.31	0.60	0.97	0.24	0.00	20	20	100	100
22	x	x	x	x	x	x	x	x	x	x	-0.24	-0.97	0.78	-0.54	0.56	0.39	2.70	0.81	-0.21	-0.95	10	9	45	90
23	0.30	-0.34	-2.11	-0.18	0.16	0.17	0.13	-0.63	-1.00	0.48	-0.08	-1.64	-0.06	0.00	0.67	x	-1.09	0.02	-0.85	0.20	19	18	90	95
24	-0.42	-0.69	-0.76	-1.87	-0.06	0.18	-0.87	-0.65	0.16	-1.61	-2.43	0.07	-0.40	-0.72	0.30	-0.55	-1.28	0.35	-0.12	-0.98	20	19	95	95
25	-0.08	0.12	0.66	0.33	x	0.67	x	-1.29	x	x	-1.07	-0.78	0.11	-0.44	-0.82	-0.41	-1.17	0.19	-1.37	0.15	16	16	80	100
26	0.31	0.68	-0.83	0.90	1.55	1.85	0.25	0.00	-0.56	0.41	-1.04	-0.90	0.55	0.59	x	x	x	x	x	x	14	14	70	100
27	-0.18	0.12	-1.06	-1.91	-0.65	0.80	0.35	-0.11	-0.36	1.15	-2.94	-1.36	0.35	0.54	1.57	1.10	-0.46	0.10	-2.95	0.37	20	17	85	85
29	0.11	0.43	-1.04	-0.24	-0.67	0.29	-0.40	-0.46	-1.56	-0.25	0.01	-1.69	-0.44	-0.20	-0.64	-0.32	0.32	1.07	-2.79	0.40	20	19	95	95
30	-0.43	-0.65	-1.84	-1.01	-0.52	-0.51	-1.97	-0.88	-0.64	-0.16	-1.23	-1.12	-0.50	0.37	-0.60	-0.12	-0.92	0.39	0.13	-0.50	20	20	100	100
31	-0.22	0.59	-0.72	-0.55	0.44	0.46	-0.84	-0.71	-0.80	-0.50	-0.80	out	-0.25	-0.22	-0.05	0.11	0.31	0.46	-0.76	0.48	20	19	95	95
32	x	x	x	x	x	x	x	x	x	x	-2.07	-0.84	0.16	0.00	0.11	-0.20	-1.56	0.17	1.52	0.27	10	9	45	90
33	0.16	-0.69	-1.39	0.08	0.65	0.72	0.05	-1.11	-1.02	-0.10	1.42	-0.66	1.07	0.69	0.73	0.73	0.97	out	0.08	0.83	20	18	90	90
34	-0.32	-0.27	-0.41	-0.08	-0.30	0.20	-1.09	-0.99	0.13	0.08	-1.01	-1.74	0.37	-0.31	-1.10	0.04	-0.72	0.72	-0.63	-0.56	20	20	100	100
35	x	x	x	x	x	x	x	x	x	x	-1.21	-1.11	0.13	-1.02	0.12	0.12	-0.09	0.44	-0.53	0.13	10	10	50	100

Labcode	*Dactyliosolen*	*Dinophysis*	*Ceratium*	*Pseudo-nitzschia*	*Thalassionema*	*Rhizosolenia*	*Ditylum*	*Stephanopyxis*	*Odontella*	*Chaetoceros*	*Fragilaria*	*Tabellaria*	*Aulacoseira*	*Cryptomonas*	*Rhodomonas*	*Planktothrix*	*Monoraphidium*	*Woronichinia*	*Cosmarium*	*Trachelomonas*	species done	Species passed	passed in % of all species	passed in % of species done
36	-0.32	-0.21	-0.24	0.36	0.10	0.24	-0.45	-0.80	0.14	-0.38	-0.64	-1.64	0.72	0.23	-0.18	0.02	-0.44	0.91	-0.27	-0.13	20	20	100	100
37	0.09	x	-0.69	x	x	x	x	x	x	x	0.06	-1.33	-1.19	-0.71	-0.77	x	x	x	x	x	7	7	35	100
38	0.14	0.02	-0.77	-1.25	-0.58	-0.27	-1.04	-0.37	0.44	-1.32	-0.87	x	-0.13	0.25	-1.09	0.21	-0.65	0.17	-0.77	-0.60	19	19	95	100
39	-0.29	-2.37	-0.48	-1.47	-1.21	-0.30	-2.18	-1.06	-0.07	-1.86	-1.71	-0.92	0.25	-0.85	-0.49	0.36	0.08	0.68	-0.19	-0.98	20	18	90	90
40	0.36	-0.10	-1.64	0.38	0.47	0.66	0.62	0.20	-0.56	0.83	x	x	x	x	x	x	x	x	x	x	10	10	50	100
41	-0.89	-1.16	-2.54	-1.81	-0.58	-0.73	-1.13	-0.27	-0.99	-0.65	-0.88	-2.56	out	-1.36	-0.44	-1.25	0.20	-1.64	-1.86	-1.62	20	17	85	85
43	-0.42	-2.94	-1.88	-0.76	0.30	0.16	-0.39	out	-0.56	-0.64	-2.04	0.01	0.66	0.79	0.09	-2.21	-1.25	0.60	-2.50	0.56	20	15	75	75
44	0.39	-0.31	-1.17	-1.37	0.87	0.77	-0.47	-0.14	-0.89	-0.91	x	x	x	x	x	x	x	x	x	x	10	10	50	100
45	0.17	-0.65	-0.36	0.09	1.22	0.73	-1.08	-0.56	-0.26	0.47	-0.77	0.25	0.70	-0.17	0.56	0.43	2.02	0.84	-0.91	0.58	20	19	95	95
46	-0.31	-0.34	-0.22	-1.32	0.05	-0.38	-1.10	out	-1.42	0.93	-0.20	1.75	0.41	0.14	-0.07	0.31	-1.50	0.91	-0.49	-0.53	20	19	95	95
47	x	x	x	x	x	x	x	x	x	x	-1.87	-0.65	0.21	-0.95	-0.01	-0.50	-0.94	0.15	-0.72	-0.57	10	9	45	90
48	1.35	0.91	0.03	-0.59	0.75	0.85	-0.18	-0.80	-0.33	0.55	-0.67	1.35	0.71	0.55	-0.33	1.03	out	1.45	-0.17	0.45	20	19	95	95
49	-0.68	0.04	-0.87	-1.86	-0.30	0.35	-0.77	-2.17	0.04	0.00	-2.95	-0.98	out	-0.75	-1.31	2.45	-0.72	-0.35	-1.26	0.40	20	16	80	80
50	-0.15	1.58	-0.80	-1.16	-1.10	-0.48	-1.26	-1.10	0.62	-0.96	-0.98	0.58	0.24	-0.78	0.07	1.18	0.39	0.82	0.31	-0.12	20	19	95	95
51	0.06	0.06	-1.94	0.28	0.30	1.36	-0.81	-1.48	-0.81	-1.15	0.08	-0.29	0.11	0.67	0.13	0.09	0.41	0.87	-0.84	-0.24	20	20	100	100

Labcode	*Dactyliosolen*	*Dinophysis*	*Ceratium*	*Pseudo-nitzschia*	*Thalassionema*	*Rhizosolenia*	*Ditylum*	*Stephanopyxis*	*Odontella*	*Chaetoceros*	*Fragilaria*	*Tabellaria*	*Aulacoseira*	*Cryptomonas*	*Rhodomonas*	*Planktothrix*	*Monoraphidium*	*Woronichinia*	*Cosmarium*	*Trachelomonas*	species done	Species passed	passed in % of all species	passed in % of species done
52	-0.37	-0.30	-0.71	1.14	0.07	0.19	-1.23	-1.00	-0.11	0.00	-1.83	-1.72	0.51	-0.27	-0.26	-0.16	-0.67	0.37	-0.34	0.09	20	19	95	95
53	0.46	-0.58	1.00	2.01	0.90	0.21	-0.46	-0.55	-0.40	-0.48	-1.18	-0.68	-0.12	-0.39	0.67	1.67	-0.54	-0.17	0.22	0.03	20	19	95	95
54	0.03	0.39	1.48	-1.25	0.79	0.11	-0.77	-2.35	-1.76	-0.94	-0.65	out	-0.96	0.77	2.19	-1.29	out	1.70	0.00	0.63	20	16	80	80
55	-0.26	0.31	-1.11	0.39	-0.40	0.04	-0.23	x	0.40	0.60	-2.04	-0.08	-0.13	0.04	0.02	-0.53	-1.03	0.42	-0.54	-0.83	20	18	90	90
57	0.01	0.57	-1.29	-2.26	-0.47	0.85	-1.36	-1.83	-0.34	-0.59	-2.10	-1.15	0.56	-1.54	-0.93	0.69	-0.09	0.90	-0.39	0.42	20	17	85	85
58	0.36	2.17	out	-0.25	-0.93	0.64	0.16	-1.36	0.58	-1.18	0.33	1.73	0.26	-0.78	0.39	1.02	-1.20	0.90	1.26	1.58	20	18	90	90
59	0.04	0.71	-2.06	0.06	0.32	0.44	-0.97	-0.12	-0.80	-2.44	x	x	x	x	x	x	x	x	x	x	10	8	40	80
60	0.27	-0.83	-1.63	-1.23	out	-0.37	-0.89	-0.93	0.01	-0.74	-0.46	-0.48	1.44	-0.13	0.00	-0.33	-0.67	0.28	-0.77	-0.58	20	19	95	95
61	0.24	-0.07	-0.21	-1.79	0.37	0.68	0.01	-0.70	2.25	-0.49	-2.83	-0.70	out	-0.51	-0.20	2.68	-0.35	x	-0.88	0.27	19	14	70	74
62.1	x	x	x	x	x	x	x	x	x	x	-0.66	1.50	0.21	-0.48	-0.59	-0.26	-1.49	0.06	-1.07	-0.72	10	10	50	100
62.2	x	x	x	x	x	x	x	x	x	x	-0.73	2.05	0.40	-0.16	-0.39	-0.07	-1.38	0.12	-1.06	-1.05	10	9	45	90
63	x	x	x	x	x	x	x	x	x	x	-2.06	-1.52	-0.93	-0.89	-0.85	-0.55	-1.18	0.56	-1.79	-0.32	10	9	45	90
64	1.39	0.47	-0.87	x	x	x	x	x	x	x	out	0.20	0.71	0.90	-0.84	2.07	out	0.27	-1.21	0.12	13	9	45	69
65	0.16	out	-1.61	0.18	0.31	-0.13	-1.01	-0.36	-1.33	0.12	0.15	-0.84	0.46	0.13	0.81	0.61	0.35	0.16	-0.39	0.12	20	20	100	100

3.5.6 Biovolume results

Table 27 shows the summarised biovolume results of the overall analysis of the participants for each species. Only the results of laboratories with $|z_u| \leq 3$ for the respective analysis of a species have been included in the assessment calculations.

Table 27: Aggregated biovolume results and statistic values for the analysed species; columns show the number of labs and overall measurements for each species being included in the assessment, furthermore, the mean values including the robust mean from the Hampel statistics, s_R / CV_R – reproducibility standard deviation / relative in %), s_r / CV_r - repeatability standard deviation / relative in %) as results from the Q method (DIN 38402-45)

	Biovolume participants [µm³]				Hampel Schaetzer				
Species	**no labs**	**no meas**	**median**	**mean arith**	**robust mean**	**s_R**	**CV_R**	**s_r**	**CV_r**
Dactyliosolen fragilissimus	45	1,225	6,911	7,045	7,033	2,566	36.5%	2,497	35.5%
Dinophysis acuminata	43	992	16,334	17,241	17,190	6,906	40.2%	3,940	22.9%
Ceratium tripos	42	955	85,380	89,007	86,162	34,406	39.9%	12,723	14.8%
Pseudo-nitzschia cf.pungens	43	1,024	1,039	1,130	1,111	519	46.7%	339	30.5%
Thalassionema nitzschioides	41	1,006	555	536	535	218	40.7%	161	30.0%
Rhizosolenia imbricata	42	1,154	16,089	17,031	16,988	8,001	47.1%	6,982	41.1%
Ditylum brightwellii	40	1,114	18,753	20,080	20,080	8,571	42.7%	7,689	38.3%
Stephanopyxis turris	39	1,015	77,951	73,860	74,663	36,347	48.7%	25,710	34.4%
Odontella sinensis	39	1,082	1,550,550	1,561,527	1,558,185	765,669	49.1%	711,148	45.6%
Chaetoceros debilis	39	982	854	938	937	468	49.9%	328	35.0%
Fragilaria crotonensis	53	1,391	507	481	476	257	54.0%	81	17.1%
Tabellaria fenestrata	45	1,009	1,648	1,863	1,758	880	50.1%	463	26.3%
Aulacoseira granulata	45	1,032	753	761	760	272	35.9%	216	28.4%
Cryptomonas erosa	50	1,133	1,725	1,809	1,809	655	36.2%	545	30.2%
Rhodomonas lacustris var.	49	1,249	100	103	102	33	32.4%	31	30.8%
Planktothrix agardhii	45	1,215	876	923	915	332	36.3%	66	7.3%
Monoraphidium arcuatum	45	1,204	50	53	53	25	47.8%	19	35.0%
Woronichinia naegeliana	45	1,152	30	31	31	12	39.4%	8	25.5%
Cosmarium ocellatum	48	1,079	5,073	4,992	5,008	1,680	33.6%	962	19.2%
Trachelomonas hispida	46	1,088	6,441	6,370	6,348	1,756	27.7%	1,329	20.9%

The number of laboratories being included in the single assessment varies from 39 to 53, the number of measurements from 955 to 1,391. The various mean values in the table calculated on different bases showed a fairly good agreement. The calculated standard deviations were in the same range as being found in former ring tests (Schilling 2010).

Table 28 lists the mean values and the calculated limits for $|z|$ and $|z_u| = 2$ for the different species. The data clearly shows that lower and upper limits for z_U values are significantly higher than these of the respective z scores. This is due to applying a weighting described in DIN 38402-45: If the standard deviation of the results is relatively high, the normal symmetrical z-scoring can lead to lower limits being < 0 for z = -2. This would unduly prefer very low or zero values of the results. For that reason, the z_U scoring method can be applied, shifting the scoring range to more positive values. On the other hand, this will mean that relatively high values might also get an approval even if they normally were excluded by applying the usual z score limits.

Table 28: Mean values of biovolume results as well as upper and lower tolerance limits for $|z|$ and $|z_U| = 2$ for all species

	Biovolume participants [µm³]					Tolerance limits / z-scores			
Species	no labs	no meas	median	mean arith	robust mean	z-score (+2)	z-score (-2)	z_U (+2)	z_U (-2)
Dactyliosolen fragilissimus	45	1,225	6,911	7,045	7,033	11,956	1,691	13,127	2,497
Dinophysis acuminata	43	992	16,334	17,241	17,190	30,530	2,906	34,002	5,353
Ceratium tripos	42	955	85,380	89,007	86,162	177,973	40,347	195,176	52,439
Pseudo-nitzschia cf.pungens	43	1,024	1,039	1,130	1,111	2,394	317	2,687	549
Thalassionema nitzschioides	41	1,006	555	536	535	926	54	1,037	133
Rhizosolenia imbricata	42	1,154	16,089	17,031	16,988	29,981	-2,021	34,516	1,600
Ditylum brightwellii	40	1,114	18,753	20,080	20,080	41,197	6,913	45,736	10,230
Stephanopyxis turris	39	1,015	77,951	73,860	74,663	172,335	26,948	193,328	44,322
Odontella sinensis	39	1,082	1,550,550	1,561,527	1,558,185	3,316,799	254,125	3,761,218	625,813
Chaetoceros debilis	39	982	854	938	937	1,958	86	2,232	319
Fragilaria crotonensis	53	1,391	507	481	476	1,203	174	1,358	320
Tabellaria fenestrata	45	1,009	1,648	1,863	1,758	3,871	350	4,387	791
Aulacoseira granulata	45	1,032	753	761	760	1,225	135	1,347	219
Cryptomonas erosa	50	1,133	1,725	1,809	1,809	3,225	604	3,521	808
Rhodomonas lacustris var.	49	1,249	100	103	102	168	37	181	46
Planktothrix agardhii	45	1,215	876	923	915	1,530	202	1,681	306
Monoraphidium arcuatum	45	1,204	50	53	53	114	13	129	25
Woronichinia naegeliana	45	1,152	30	31	31	48	0	54	4
Cosmarium ocellatum	48	1,079	5,073	4,992	5,008	9,180	2,459	9,875	2,944
Trachelomonas hispida	46	1,088	6,441	6,370	6,348	9,923	2,897	10,485	3,335

3.5.7 Performance data

Table 30 through Table 33 show the overall performance data of all laboratories with $|z_u$ score$| < 3$ listed for each analysed species separately. The relative reproducibility standard deviations CV_R for the biovolumes lie within reasonable ranges. Another study based on a ring test and carried out by Schilling (2010) has also assessed various biovolume results of different phytoplankton species. Some of these species have been analysed in the current ring trial as well. These species included *Dactyliosolen fragilissimus*, *Pseudo-nitzschia* cf. *pungens* and *Thalassionema nitzschioides*.

Since the standard deviations for reproducibility and repeatability of the biovolume measurements have been calculated in the same way – according to DIN 38402 A45 – these values could be well compared, as will be shown in Table 29.

Table 29: Comparison of standard deviations for reproducibility and repeatability for three species between the interlaboratory test from 2007 and 2014.

	Ring trial 2007		Ring trial 2014	
Species	CV_R	CV_r	CV_R	CV_r
Dactyliosolen fragilissimus	40.8%	33.7%	36.5%	35.5%
Pseudo-nitzschia cf.pungens	59.6%	26.6%	46.7%	30.5%
Thalassionema nitzschioides	39.7%	32.9%	40.7%	30.0%

It can clearly be stated that the performance values for these 3 species showed a very good agreement; only the reproducibility values for *Pseudo-nitzschia* sp. were about 13 % lower in 2014 compared to 2007.

Table 30: Performance data for measurements of first dimension in biovolume analysis of phytoplankton species in natural samples. (d) diameter; (l) length of triangle side; (a) first edge length.

Taxon name	*l*	*n*	$X_{assigned}$ µm	X_{median} µm	X_{mean} µm	X_{HS} µm	s_R µm	CV_R %	s_r µm	CV_r %
Dactyliosolen fragilissimus (d)	45	1,225	11.74	11.93	12.06	12.04	2.01	16.7	1.80	14.9
Dinophysis acuminata (d_1)	42	961	33.48	33.90	34.07	34.07	4.24	12.5	3.23	9.5
Ceratium tripos (d_1)	44	998	66.79	65.93	66.74	66.66	6.12	9.2	4.56	6.8
Pseudo-nitzschia cf. *pungens* (d_1)	44	1,054	111.87	111.69	111.12	111.19	12.43	11.2	11.18	10.1
Thalassionema nitzschioides (d_1)	42	1,013	32.95	32.82	33.15	33.15	4.29	13.0	4.25	12.8
Rhizosolenia imbricata (d)	42	1,154	9.68	10.19	10.16	10.19	2.49	24.4	2.14	21.0
Ditylum brightwellii (l)	40	1,114	26.76	23.83	23.64	23.69	4.89	20.7	4.18	17.6
Stephanopyxis turris (d)	37	964	43.15	40.35	39.96	39.96	7.60	19.0	7.07	17.7
Odontella sinensis (d_1)	40	1,106	167.28	163.87	175.38	174.21	40.62	23.3	33.02	19.0
Chaetoceros debilis (d_1)	39	982	13.39	14.25	13.65	13.65	3.84	28.2	2.77	20.3
Fragilaria crotonensis (d_1)	53	1,391	76.94	76.58	78.15	77.47	12.51	16.2	14.27	18.4
Tabellaria fenestrata (a)	47	1,052	51.62	50.91	50.77	50.77	4.77	9.4	4.20	8.3
Aulacoseira granulata (d)	50	1,183	5.48	5.88	5.89	5.90	1.02	17.3	0.84	14.2
Cryptomonas erosa (d_1)	50	1,133	12.63	12.83	12.96	12.96	1.58	12.2	1.32	10.2
Rhodomonas lacustris (d)	49	1,249	5.20	5.27	5.28	5.28	0.66	12.5	0.71	13.5
Planktothrix agardhii (d)	47	1,260	3.32	3.34	3.44	3.42	0.72	21.0	0.15	4.3
Monoraphidium arcuatum (d)	42	1,085	1.73	1.69	1.83	1.78	0.54	30.3	0.32	17.8
Woronichinia naegeliana (d)	44	1,184	2.92	3.27	3.26	3.26	0.60	18.3	0.39	12.0
Cosmarium ocellatum (d_1)	43	977	28.60	26.21	26.44	26.46	2.54	9.6	1.85	7.0
Trachelomonas hispida (d)	45	1,068	21.15	20.96	20.86	20.87	2.08	10.0	1.75	8.4

l	number of laboratories with $\|z_u$ score$\| < 3$
n	number of total individual test results of all laboratories with $\|z_u$ score$\| < 3$
$X_{assigned}$	assigned reference value based on 7 replicates with each having a minimum of 30 single measurements
X_{median}	interlaboratory* median value
X_{mean}	interlaboratory* arithmetic mean value
X_{HS}	interlaboratory* robust mean value (Hampel Schaetzer)
s_R	reproducibility standard deviation
CV_R	relative reproducibility standard deviation
s_r	repeatability standard deviation
CV_r	relative repeatability standard deviation
*	based on arithmetic means of individual laboratory measurements; only laboratories with $\|z_u$ score$\| < 3$) included

Table 31: Performance data for measurements of first dimension in biovolume analysis of phytoplankton species in natural samples. (h) height; (d) diameter; (m) height of triangle; (b) second edge length.

Taxon name	*l*	*n*	$X_{assigned}$ µm	X_{median} µm	X_{mean} µm	X_{HS} µm	s_R µm	CV_R %	s_r µm	CV_r %
Dactyliosolen fragilissimus (h)	45	1,225	60.61	59.69	59.32	59.32	12.01	20.3	11.86	20.0
Dinophysis acuminata (d_2)	43	994	20.09	19.45	20.58	20.32	5.58	27.5	1.90	9.4
Ceratium tripos (d_2)	45	1,018	43.41	38.00	38.53	38.09	11.82	31.0	2.12	5.6
Pseudo-nitzschia cf. *pungens* (d_2)	43	1,024	4.81	4.51	4.59	4.58	1.01	22.0	0.84	18.4
Thalassionema nitzschioides (d_2)	42	1,013	3.99	4.35	4.26	4.23	0.99	23.4	0.77	18.2
Rhizosolenia imbricata (h)	42	1,154	188.20	201.34	200.16	200.46	45.13	22.5	41.59	20.7
Ditylum brightwellii (m)	40	1,114	23.17	22.99	22.87	22.96	4.47	19.5	4.51	19.7
Stephanopyxis turris (h)	37	964	66.83	59.20	58.30	58.30	10.36	17.8	9.62	16.5
Odontella sinensis (d_2)	40	1,106	66.91	64.27	64.17	63.63	20.62	32.4	11.50	18.1
Chaetoceros debilis (d_2)	38	959	10.04	9.31	9.53	9.40	2.59	27.6	1.59	16.9
Fragilaria crotonensis (d_2)	47	1,171	4.40	3.96	3.99	3.98	0.96	24.2	0.23	5.9
Tabellaria fenestrata (b)	47	1,052	5.16	7.57	7.39	7.41	2.33	31.4	1.07	14.4
Aulacoseira granulata (h)	43	966	27.33	26.04	26.11	26.11	3.16	12.1	2.68	10.3
Cryptomonas erosa (d_2)	49	1,113	10.73	10.13	9.95	9.99	2.10	21.1	0.95	9.5
Rhodomonas lacustris (h)	49	1,249	11.43	10.53	10.61	10.61	1.85	17.4	1.67	15.7
Planktothrix agardhii	-	-	-	-	-	-	-	-	-	-
Monoraphidium arcuatum (h)	41	1,065	49.31	42.00	41.25	41.25	7.92	19.2	4.98	12.1
Woronichinia naegeliana (h)	43	1,077	5.35	5.38	5.33	5.36	0.69	12.9	0.49	9.2
Cosmarium ocellatum (d_2)	43	956	14.30	13.39	13.52	12.19	2.52	20.7	0.87	7.2
Trachelomonas hispida (h)	45	1,068	27.12	27.13	26.83	26.80	3.01	11.2	2.35	8.8

l	number of laboratories with $\|z_u$ score$\| < 3$
n	number of total individual test results of all laboratories with $\|z_u$ score$\| < 3$
$X_{assigned}$	assigned reference value based on 7 replicates with each having a minimum of 30 single measurements
X_{median}	interlaboratory* median value
X_{mean}	interlaboratory* arithmetic mean value
X_{HS}	interlaboratory* robust mean value (Hampel Schaetzer)
s_R	reproducibility standard deviation
CV_R	relative reproducibility standard deviation
s_r	repeatability standard deviation
CV_r	relative repeatability standard deviation
*	based on arithmetic means of individual laboratory measurements; only laboratories with $\|z_u$ score$\| < 3$) included

Table 32: Performance data for measurements of third dimension in biovolume analysis of phytoplankton species in natural samples. (h) height; (c) third edge length.

Taxon name	*l*	*n*	$X_{assigned}$ µm	X_{median} µm	X_{mean} µm	X_{HS} µm	s_R µm	CV_R %	s_r µm	CV_r %
Dactyliosolen fragilissimus	-	-	-	-	-	-	-	-	-	-
Dinophysis acuminata (h)	42	961	46.69	48.71	48.88	48.77	5.38	11.0	4.58	9.4
Ceratium tripos (h_1)	42	938	40.33	42.67	41.39	41.44	6.27	15.1	3.34	8.1
Pseudo-nitzschia cf. *pungens* (h)	44	1,054	4.81	4.06	4.14	4.12	1.51	36.7	0.66	16.1
Thalassionema nitzschioides (h)	42	1,013	4.59	4.69	4.77	4.76	1.00	21.0	0.78	16.4
Rhizosolenia imbricata	-	-	-	-	-	-	-	-	-	-
Ditylum brightwellii (h)	40	1,114	74.17	70.10	71.00	70.88	17.30	24.4	17.01	24.0
Stephanopyxis turris	-	-	-	-	-	-	-	-	-	-
Odontella sinensis (h)	40	1,106	195.30	180.70	175.04	175.34	47.20	26.9	42.85	24.4
Chaetoceros debilis (h)	38	954	9.14	8.83	8.99	8.91	2.10	23.6	1.57	17.6
Fragilaria crotonensis (h)	49	1,291	4.40	3.76	3.48	3.48	0.98	28.2	0.30	8.6
Tabellaria fenestrata (c)	41	929	7.82	4.88	5.47	5.40	2.06	38.2	0.70	13.0
Aulacoseira granulata	-	-	-	-	-	-	-	-	-	-
Cryptomonas erosa (h)	49	1,113	25.85	25.75	25.91	25.91	3.64	14.1	3.53	13.6
Rhodomonas lacustris	-	-	-	-	-	-	-	-	-	-
Planktothrix agardhii	-	-	-	-	-	-	-	-	-	-
Monoraphidium arcuatum	-	-	-	-	-	-	-	-	-	-
Woronichinia naegeliana	-	-	-	-	-	-	-	-	-	-
Cosmarium ocellatum (h)	46	1,014	26.82	26.86	27.04	27.04	2.32	8.6	2.12	7.8
Trachelomonas hispida	-	-	-	-	-	-	-	-	-	-

l	number of laboratories with $\|z_u$ score$\| < 3$
n	number of total individual test results of all laboratories with $\|z_u$ score$\| < 3$
$X_{assigned}$	assigned reference value based on 7 replicates with each having a minimum of 30 single measurements
X_{median}	interlaboratory* median value
X_{mean}	interlaboratory* arithmetic mean value
X_{HS}	interlaboratory* robust mean value (Hampel Schaetzer)
s_R	reproducibility standard deviation
CV_R	relative reproducibility standard deviation
s_r	repeatability standard deviation
CV_r	relative repeatability standard deviation
*	based on arithmetic means of individual laboratory measurements; only laboratories with $\|z_u$ score$\| < 3$) included

Table 33: Performance data for biovolume analysis of phytoplankton species in natural samples.

Taxon name	*l*	*n*	$X_{assigned}$ µm³	X_{median} µm³	X_{mean} µm³	X_{HS} µm³	s_R µm³	CV_R %	s_r µm³	CV_r %
Dactyliosolen fragilissimus	45	1,225	6,824	6,911	7,045	7,033	2,566	36.5	2,497	35.5
Dinophysis acuminata	43	992	16,718	16,334	17,241	17,190	6,906	40.2	3,940	22.9
Ceratium tripos	44	998	109,160	85,983	95,007	88,516	37,516	42.4	13,216	14.9
Pseudo-nitzschia cf. *pungens*	44	1,054	1,355	1,058	1,167	1,135	541	47.7	348	30.7
Thalassionema nitzschioides	41	1,006	490	555	536	535	218	40.7	161	30.0
Rhizosolenia imbricata	42	1,154	13,980	16,089	17,031	16,988	8,001	47.1	6,982	41.1
Ditylum brightwellii	40	1,114	24,055	18,753	20,080	20,080	8,571	42.7	7,689	38.3
Stephanopyxis turris	37	964	99,642	80,331	77,611	77,611	34,081	43.9	27,694	35.7
Odontella sinensis	40	1,106	1,785,462	1,560,584	1,624,194	1,586,715	785,689	49.5	723,632	45.6
Chaetoceros debilis	39	982	1,022	854	938	937	468	49.9	328	35.0
Fragilaria crotonensis	50	1,311	688	526	503	498	247	49.7	85	17.1
Tabellaria fenestrata	45	1,009	2,111	1,648	1,863	1,758	880	50.1	463	26.3
Aulacoseira granulata	45	1,032	680	753	761	760	272	35.9	216	28.4
Cryptomonas erosa	50	1,133	1,914	1,725	1,809	1,809	655	36.2	545	30.2
Rhodomonas lacustris	49	1,249	102	100	103	102	33	32.4	31	30.8
Planktothrix agardhii	47	1,260	866	884	969	941	362	38.4	68	7.2
Monoraphidium arcuatum	47	1,246	64	51	57	55	27	49.5	19	35.1
Woronichinia naegeliana	45	1,151	24	30	31	31	12	39.7	8	25.3
Cosmarium ocellatum	47	1,057	5,820	5,083	5,071	5,070	1,624	32.0	987	19.5
Trachelomonas hispida	45	1,068	6,410	6,414	6,302	6,287	1,716	27.3	1,309	20.8

l	number of laboratories with $\|z_u$ score$\| < 3$
n	number of total individual test results of all laboratories with $\|z_u$ score$\| < 3$
$X_{assigned}$	assigned reference value based on 7 replicates with each having a minimum of 30 single measurements
X_{median}	interlaboratory* median value
X_{mean}	interlaboratory* arithmetic mean value
X_{HS}	interlaboratory* robust mean value (Hampel Schaetzer)
s_R	reproducibility standard deviation
CV_R	relative reproducibility standard deviation
s_r	repeatability standard deviation
CV_r	relative repeatability standard deviation
*	based on arithmetic means of individual laboratory measurements; only laboratories with $\|z_u$ score$\| < 3$) included

Table 30 through Table 33 show the overall performance data of all laboratories with $|z_u$ score$| < 3$ listed for each analysed species separately. The relative reproducibility standard deviations CV_R for the biovolumes lie within reasonable ranges (compare Schilling 2007).

4 Conclusions

The interlaboratory comparison test has revealed some general objectives that have been taken into account for the revision of the Standard Document and been implemented in the final version.

In the analysis, sometimes dimensions have been confused. This seems to be a general problem of really understanding and correctly working with geometrical shapes. Furthermore, this problem is not related to the question whether the analyst is working with a predefined computer programme (a counting programme such as OrgaCount or similar) or simply filling in equations in a spread sheet (as in Excel or similar). Especially the transfer of dimensions from an image of a geometrical shape to the orientation of a cell in space (for example, under the microscope) is often not trivial and an important source of errors. This had been supported by the results of some participants. Different species might have different proportions of dimensions but the same geometrical form. A good example is the elliptic cylinder, where the height might be the smallest or one of the smallest dimensions (*Tabellaria fenestrata*) or the largest dimension (e.g. *Odontella sinensis*), or similar to other dimensions (*Chaetoceros debilis*). Dependent on the proportions and on other characteristics such as forming colonies, the orientation of the cell and the hidden dimension differs. However, experienced analysts should be well able to assign the correct forms and dimensions.

The importance of correct assignment of dimensions to the equation is dependent on the geometry and the respective equation. Especially when exponents are used, incorrect assignments can lead to large errors. Thus, carefulness is needed and routines will have to be changed in some cases. Additionally, some dimensions need to be measured as accurate as possible because slight differences in just one dimension can lead to high variances in the biovolume, while other dimensions may not have such a big effect.

The hidden dimension is an important topic that has to be discussed. It had not been part of the project in the beginning, but during the course of the exercise it became obvious that hidden dimensions will have to be regarded in the Standard Document. In general, different methods are used for calculating hidden dimensions. Factors multiplicated with a measurable dimension are applied or diverse constants exist for different sizes. Sometimes, these factors have been deduced from intense measurement data sets, sometimes not. Whenever possible, the hidden dimensions should be measured when not hidden, and the measurements should be transferred and stored. There is a strong need to standardise the biovolume measurements and for that reason suggestions for hidden dimensions are now included in the Standard Document for most of the taxa.

Applying correction factors for geometrical shapes does not seem to be a common procedure, because in both examples of this study up to half of the participants have not applied these factors when required. Furthermore, it has turned out that the participants partly have mixed up the correction factors of the Standard Document with the correction factors for the hidden dimensions, as for example in the HELCOM monitoring. This is now clarified in the Standard Document and both factors are implemented in the taxa list which should avoid confusion in the future.

For some species, it will be necessary to discuss and decide how the measurements should be performed in detail. Especially species with horns, wings and other special characteristics fall into that category (*Ceratium tripos*, *Dinophysis acuminata*). For others (*Rhizosolenia imbricata*), this should be self-evident, but the interlaboratory comparison test has given evidence that this is not the case. For both categories, examples are now shown in the Standard Document with images or drawings exactly illustrating from where to where the dimensions have to be measured, in order to support the analyst as far as possible.

Although the Standard Document gives advice on the unit to be measured (cell or filament), the test has shown that the respective column in Annex B has not always been consulted (*Aulacoseira granulata*). For some species, several different methods exist which all have been applied (*Planktothrix agardhii*) by the participants within this study. Generally, the Standard Document describes all methods, although in the Annex D only one method per species is listed. It would be interesting to compare all the methods concerning the total biovolume in a sample, but this has been beyond the scope of this project since it will require counting data.

Selection criteria for the items to be measured can have an effect on the biovolume results. Cells shall randomly be chosen for the measurements, but the cell of interest must facilitate the identification and correct measurement of the dimensions (for example, cells should lie straight for the analysis). In colonies, only one cell has to be picked to avoid pseudo-replication. This is also described in the Standard Document.

The number of size classes used for a species has been quite variable among the participants. Some, especially the participants using image analysis, have had almost as many size classes as units measured, others had just two or three for species that may be variable in size. The Standard Document generally approves working with size classes; however, size classes have to be reasonable. Experienced analysts may identify the correct size class easily, although rechecking the standard size class values and its application from time to time is recommended.

The grade of accuracy required for calculating the biovolume definitely depends on the needs of the project or monitoring programme. The Standard Document provides the most precise methods and descriptions, which require at the same time as less effort as necessary. Depending on the objective of a project or monitoring programme, the grade of accuracy and effort can easily be adapted to the requirements.

The performance data from this ring trial (Table 30 through Table 33) to be published in the Standard Document clearly show that the errors in measuring the different dimensions strongly vary with the species to be analysed and with the respective assigned geometric body. This of course has partly a strong impact on the calculation of the biovolume, depending on the formula which merges the different dimensions into the final biovolume.

Summarising the results of the intercalibration exercise, it became obvious that working with the Standard Document has not been as easy as expected. At least from the results and the communication with the participants, we got the impression that some participants had not consulted the document at all. The question for the reasons of doing so remains open. Wrongly applied geometrical forms (if not additionally applied), missing correction factors, rules that were not followed (e.g. the minimum number of items to

be counted) as well as questions via e-mail and phone have raised the question why the information in the Standard Document and the instructions had not been used. The description of the measuring process in the document is short and all needed information is provided in the text or in the Annexes. Maybe different routines of experienced analysts hamper the introduction and application of new methods. But if standardisation for the near future is desired, then an accurate application of the content of the Standard Document will be necessary. However, in order to simplify the process of determining the biovolume as far as possible, the number of geometrical shapes to be applied to the different species has been reduced as much as possible. Consequently, the final taxa list includes mostly genera and species only when they differ from the genus in the geometric shape, the geometric shape correction factor, or the hidden dimension factor.

5 Bibliography

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Annex A: List of Participants

Name	Institution	Country
Jytte Hørning	Orbicon A/S	Denmark
Heidi Timm	Orbicon A/S	Denmark
Charlotte Moeller	Grontmij Denmark	Denmark
Kirsten Olrik	Laboratory of Environmental Biology Aps	Denmark
Sanna Autio	Water and Environment Research of South-West Finland	Finland
Reija Jokipii	Finnish Environment Institute (SYKE)	Finland
Satu Zwerver	Company Zwerver	Finland
Jeremy Auboin	AQUABIO	France
Wolfgang Arp	LimPlan	Germany
Susanne Busch	Leibniz Institut für Ostseeforschung	Germany
Regina Hansen	Leibniz Institut für Ostseeforschung	Germany
Anja Hansen	Leibniz Institut für Ostseeforschung	Germany
Matthias Greyer	Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Germany
Gabriele Hanke	Landesamt für Umwelt. Naturschutz und Geologie	Germany
Eberhard Hoehn	LBH	Germany
Anja Hoffmann	Landeslabor Berlin Brandenburg	Germany
Sybill Jaschinski	GEOMAR	Germany
Caroline Kaiser	Umweltbundesamt	Germany
Eva Schmidt	Umweltbundesamt	Germany
Juliane Kasten	Lüttig & Friends GbR	Germany
Ute Kieb	Biologische Anstalt Helgoland	Germany
Silvia Peters	Alfred-Wegener-Institut/ Biologische Anstalt Helgoland	Germany
Jan Köhler	Institute of Freshwater Biology and Inland Fisheries	Germany
Nicole Liebeke	Landesamt für Umwelt. Naturschutz und Geologie	Germany
Jens Meisel	Institute for applied freshwater ecology GmbH	Germany
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Margit Schönberger	Landesamt für Umwelt. Naturschutz und Geologie	Germany
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Murizio Maierna	ARPA Lombardia	Italy
Elena Stanca	University of Salento	Italy
Grasilda Gudziunaite	Environmental Protection Agency	Lithuania
Aleksandra Korz	Environmental Protection Agency	Lithuania
Irina Olenina	Environmental Protection Agency	Lithuania
Birger Skjelbred	Norwegian Institute for Water Research (NIVA)	Norway
Justyna Kobos	Institute of Ocanography. University of Gdańsk	Poland
Agnieszka Napiórkowska-Krzebietke	Inland Fisheries Institute	Poland
Elsa Alverca	Agência Portuguesa do Ambiente (APA)	Portugal
Carina Menezes	Quimiteste. Engenharia e Tecnologia. Lda	Portugal
Sérgio Paulino	Instituto Nacional de Saúde Dr. Ricardo Jorge	Portugal
Pepita Nolla	Phytolab Control	Spain
Francesc Peters	Institute of Marine Sciences	Spain
Lars Edler	Weaq Lab	Sweden

Name	**Institution**	**Country**
Chatarina Karlsson	Umeå Marine research center. Umeå University	Sweden
Isabel Quintana	Swedish University of Agricultural Sciences	Sweden
Gina Henderson	ENSIS Ltd. University College London	United Kingdom
Pauline Lang	Scottish Environment Protection Agency (SEPA)	United Kingdom
Sarah Pritchard	Beacon Biological	United Kingdom
Cordula Scherer	Agri-Food and Bioscience Institute	United Kingdom
Claire Widdicombe	Plymouth Marine Laboratory	United Kingdom
Helen Woods	Centre for Ecology & Hydrology	United Kingdom

Annex B: Instructions for participants

The following instructions have been send around to all participants at the beginning of the interlaboratory comparison.

CEN/TC 230 - Mandate M/424
Development or Improvement of Standards in Support of the Water Framework Directive - WP 7: Guidance on the estimation of algal biovolume

European interlaboratory comparison for determination of phytoplankton biovolume

Instructions

On behalf of the European Committee of Standardization (CEN) within the above mentioned programme AquaEcology is compiling a Standard Document with the title 'Guidance on the Estimation of algal Biovolume'. This document will also include a table of geometrical shapes with information being necessary for volume calculation, as well as an extended taxonomical list with assigned geometries.

Part of the work package is the realisation of a European interlaboratory comparison as validation of the method based on the current version of the standard protocol. The aim is to calculate the biovolume of different species according to the Standard Document. No counting or taxonomical determination will be necessary.

All participants will receive four different samples preserved with Lugol solution from which the predetermined species shall be measured. Each sample will contain a sufficient number of individuals for the species. In rare cases, a volume of more than 3 mL or 10 mL will have to be prepared in a bigger counting chamber or in two to three smaller chambers, in order to achieve a sufficient number of cells.

All participants shall carry out the determination of the average biovolumes of the predetermined taxa according to the procedures of the Standard Document, because the comparison will serve as a validation for the methods. The document is attached as a PDF file. This will not be the final version of the intended European Standard Document, since it has to pass different CEN boards before it will be circulated among experts for first comments. Annex B contains a comprehensive taxonomic list, in which a specific geometric body is assigned to each genus, species, variety or form. Information on the geometric bodies is to be found in Annex A of the document. For a better handling Annex B here is provided as a separate PDF file.

Within the different working groups, various methods for the determination of the biovolume of the species are applied. For example, the HELCOM group is using standard size classes. In order to compare the prescribed standard method with these 'home-made' methods, a parallel determination of the biovolume by additionally applying these methods would be preferable.

Recording and transmission of the measured data will be achieved by using a special module of the counting software OrgaCount, which will be directly accessible via a browser – preferably Firefox. We will also attach a detailed guidance document as a PDF file. During the registration process for the intercalibration exercise, each participant has already received an individual login with username and password.

Within the 'Biovolume' window of the software, some statistical information on the measurements will be displayed that will be updated whenever some geometric data have been entered. As soon as the mandatory precision prescribed in the Standard Document is reached, the field showing the standard error will be highlighted in a green colour.

Participants additionally using their 'home-made' method for determining the biovolume can enter this measurement into the software as an additional sample. Please, refer also to the respective information in the User Guide. The same holds for participants who will carry out measurements with an image-analysing software as well as with an ocular scale at the same time.

For some of the species to be determined, the assignment of a geometric body maybe still in discussion – according to Annex B of the current Standard Document. If a participant would like to assign a geometric body being different from the assigned body prescribed in the Standard Document, she or he might of course do that as an additional measurement. In this case, an additional sample with an unambiguous name will have to be created (see User Guide).

If additional remarks, notes or comments are required for the 'treatment' of specific taxa, this information can be entered into additional comment fields (see User Guide). All data entered into the software system can be exported to respective Excel files. The detailed procedure will be described in the User Guide.

The results of all measurements will have to be entered into the OrgaCount software before 11th April. After that date, the software module will not be accessible any longer.

Description of the samples

As far as possible, the validation of the methods for determining the biovolume shall be carried out under real conditions. For that reason, only natural samples have been selected, in order to identify possible problems occurring in 'real' samples. Half of the taxa to be measured have a marine origin (two samples), the other half is coming from freshwater systems (two samples). The four samples will be briefly described in the

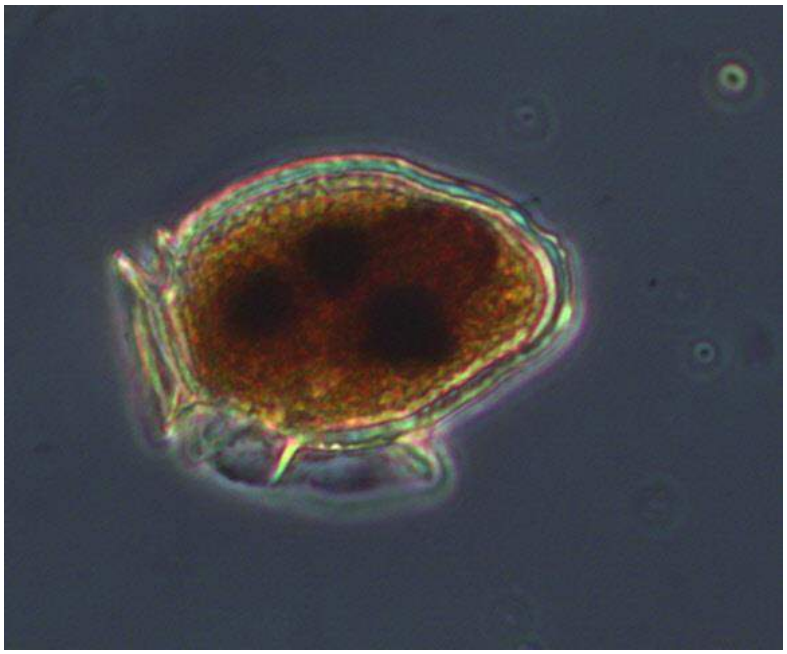
following and the species to be measured will be named. For each species, one or two photographic image will be attached.

1. Baltic Sea Sample Stralsund

This sample has been taken near the island of Ruegen by colleagues from the State Agency for Environment. Nature Conservation and Geology Mecklenburg-Vorpommern (LUNG) in Stralsund in September 2013. Within this sample, the average biovolume of the following species shall be determined:

Dactyliosolen fragilissimus

Dinophysis acuminata

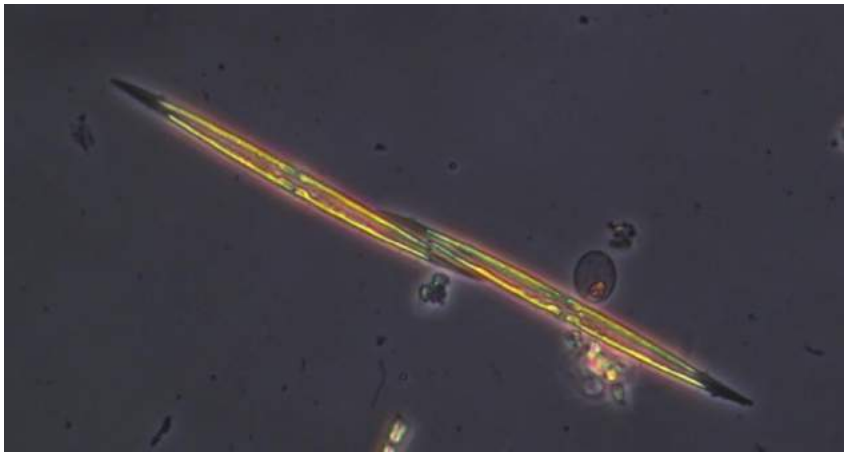


Ceratium tripos

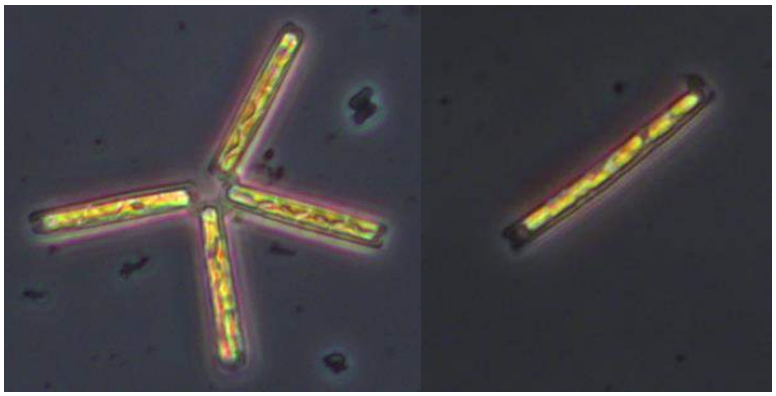
2. North Sea Sample Helgoland

This is a mixed sample from two samplings at the island of Helgoland from August and October 2013. The sample has been taken by colleagues from the Alfred-Wegener-Institute for Polar Research on the island of Helgoland. The sample has first been preserved with not-acidified Lugol solution and has later been treated with acidified Lugol. For that reason, the status of many cells in that sample is no longer optimal. Nevertheless, there are number of samples during routine analysis showing such conditions. Within this sample, the average biovolume of the following species shall be determined:

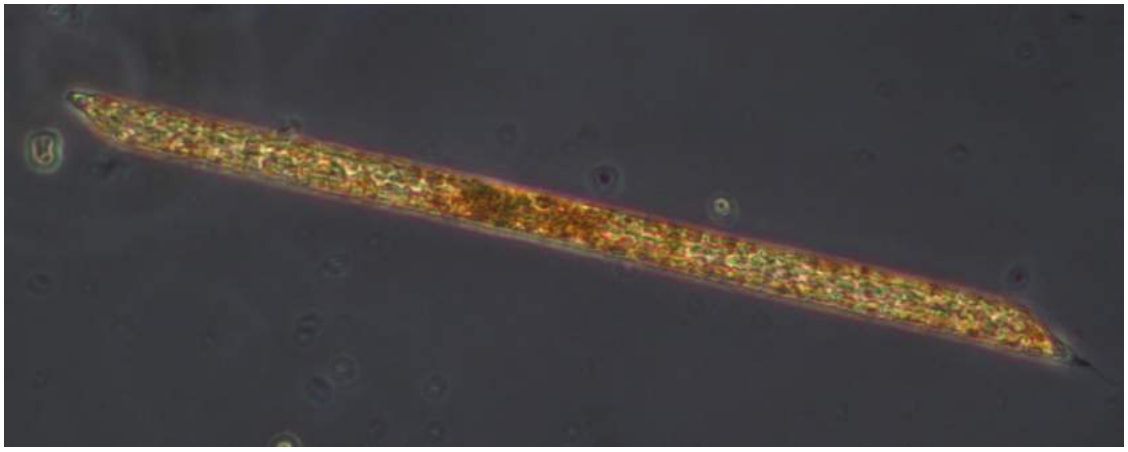
Pseudo-nitzschia cf. pungens



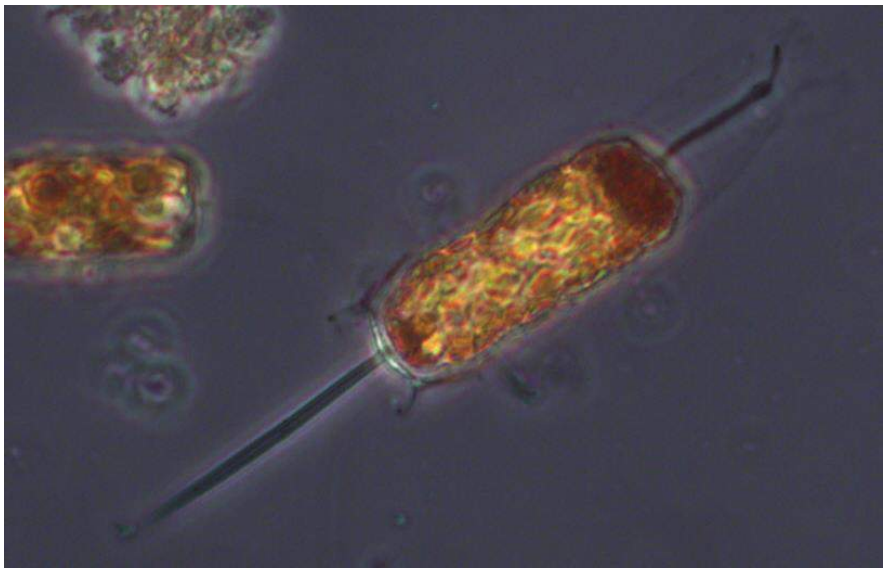
Thalassionema nitzschioides



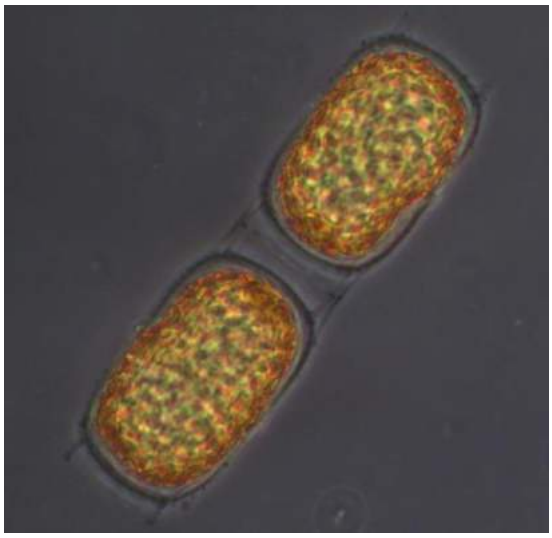
Rhizosolenia imbricata



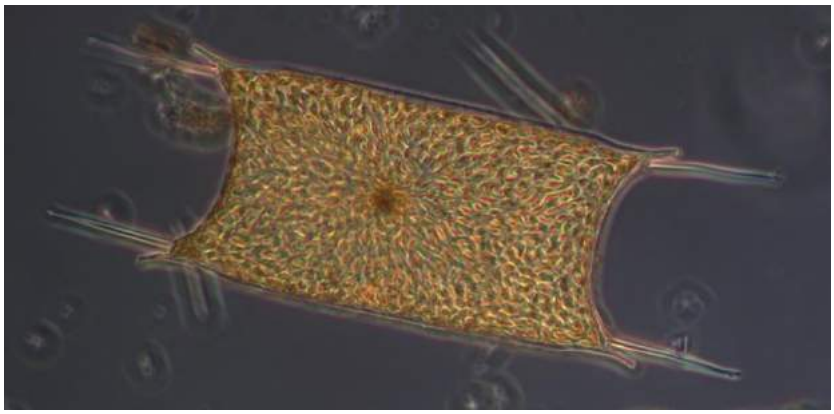
Ditylum brightwellii



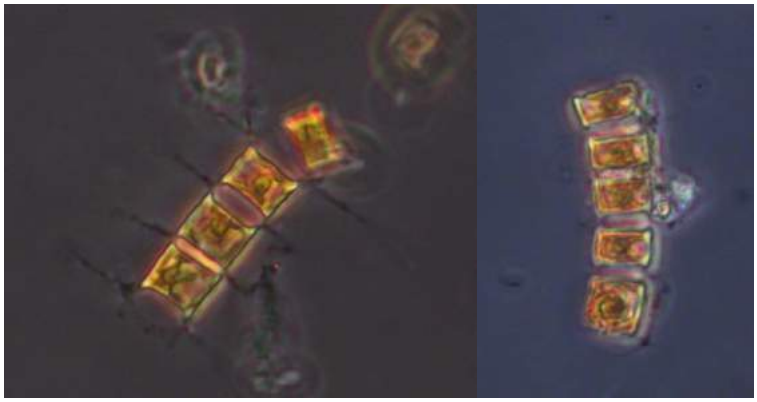
Stephanopyxis turris



Odontella sinensis



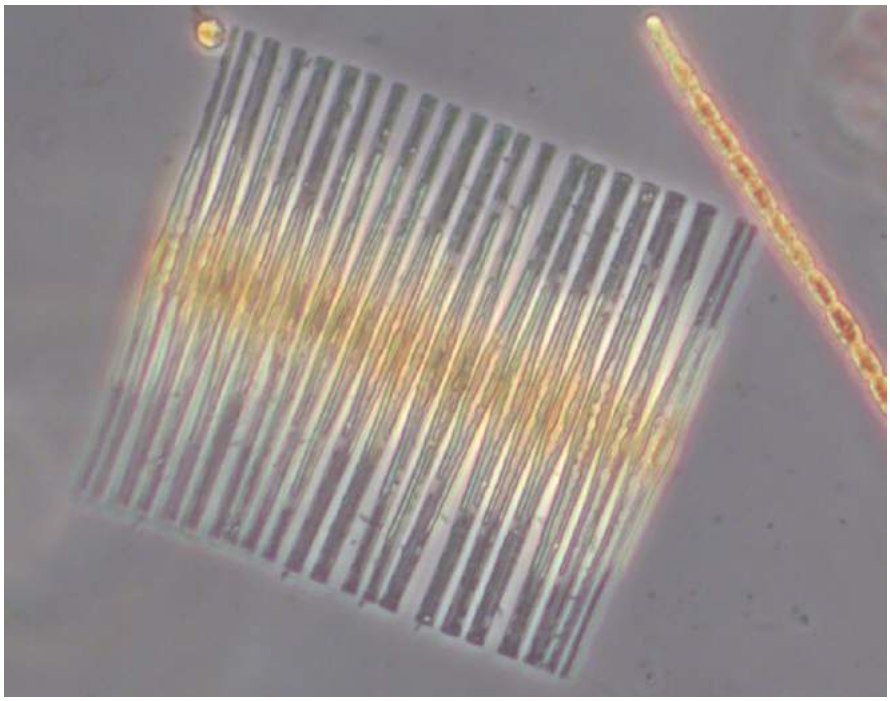
Chaetoceros debilis



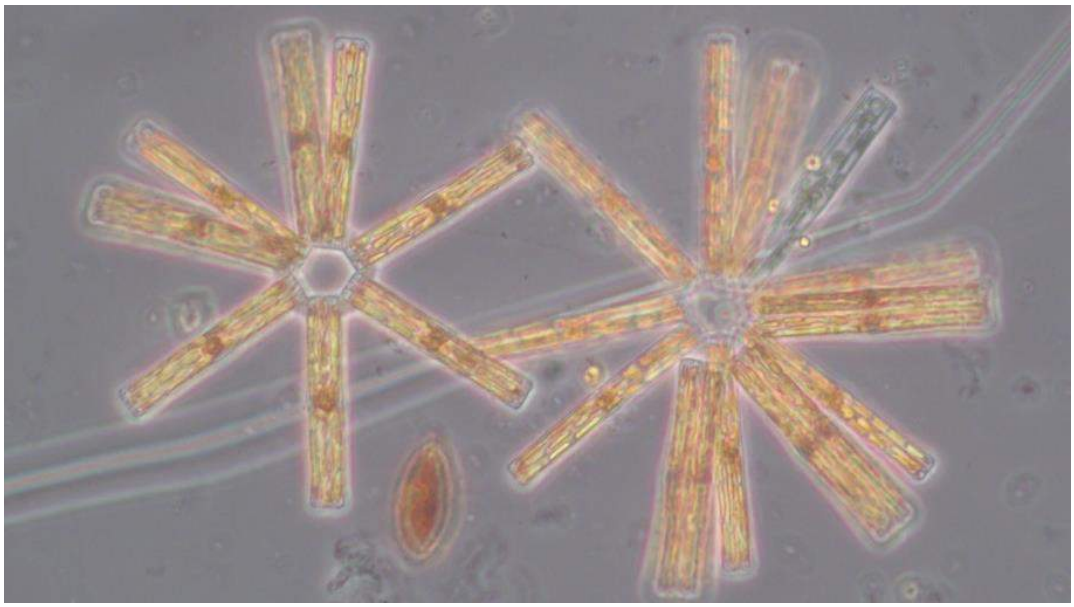
3. Freshwater Sample Scharmützelsee

This sample has been taken in October 2013 by colleagues from the Research Station Bad Saarow (Brandenburg Technical University of Cottbus-Senftenberg) in the lake Scharmützelsee in the east of Germany. Within this sample, the average biovolume of the following species shall be determined:

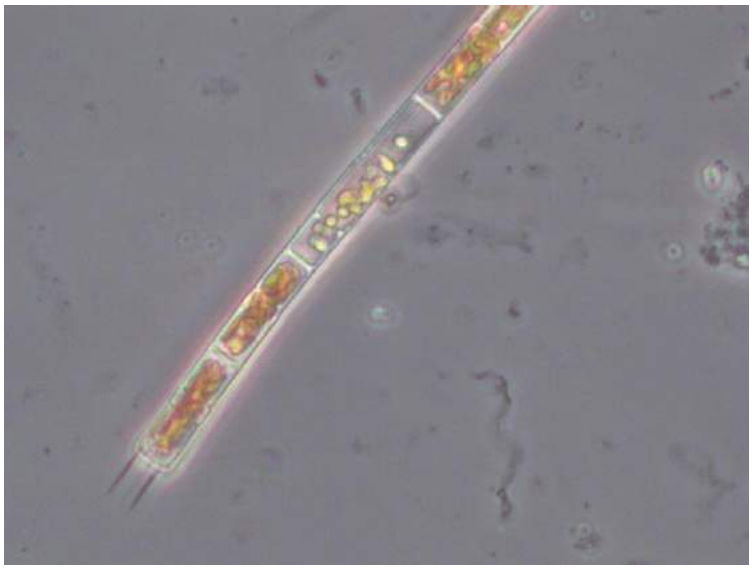
Fragilaria crotonensis



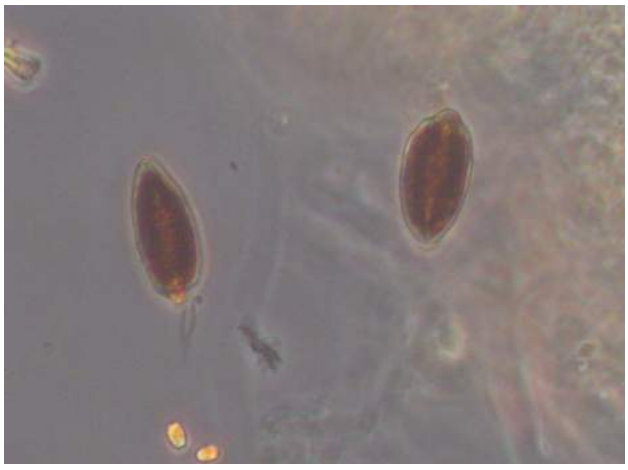
Tabellaria fenestrata



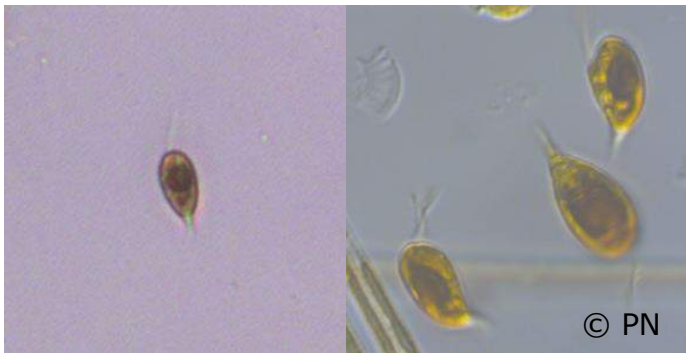
Aulacoseira granulata



Cryptomonas erosa



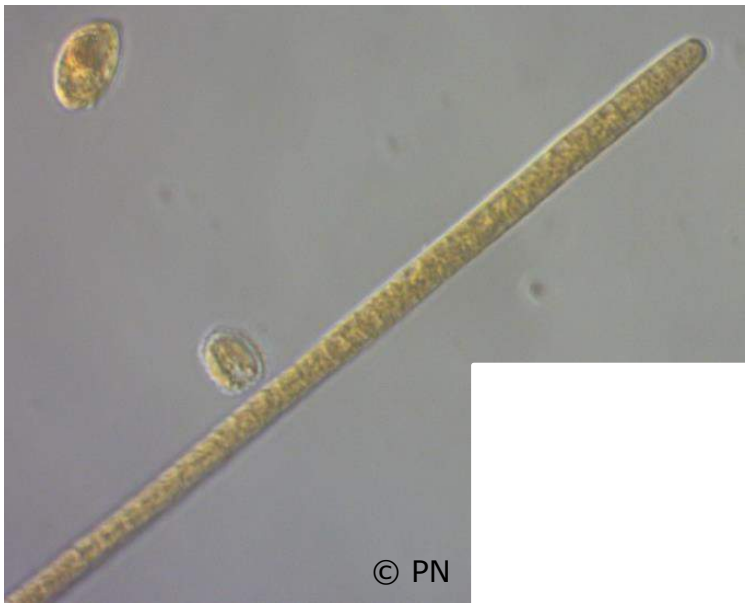
Rhodomonas lacustris var. nannoplanctica



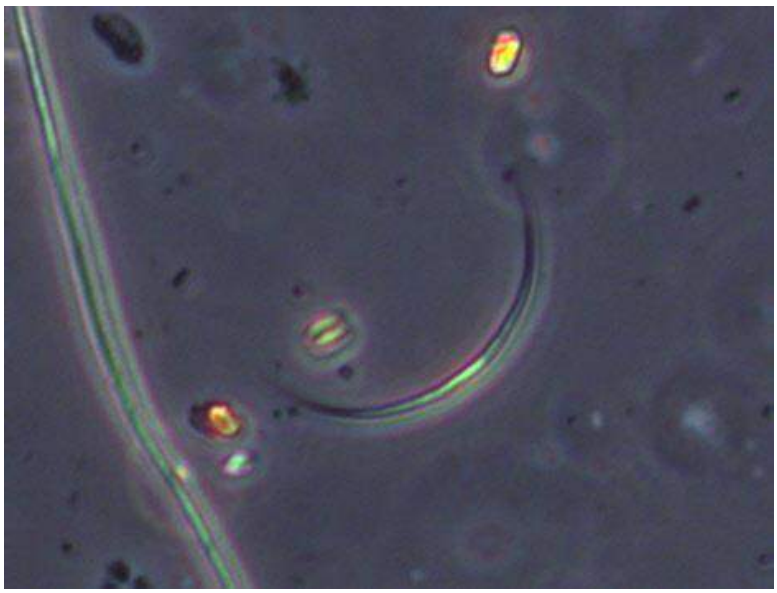
4. Freshwater Sample Oldenburg

This sample is a mixed sample from two smaller lakes near Oldenburg in the northwest of Germany. Within this sample, the average biovolume of the following species shall be determined:

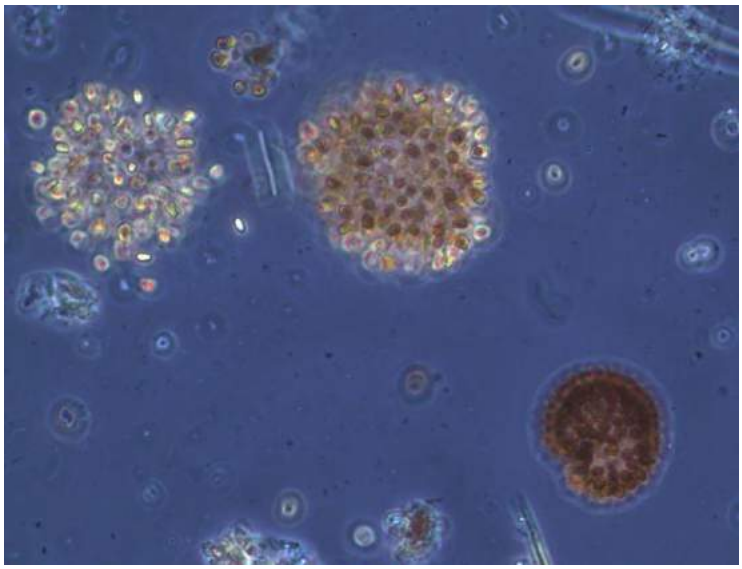
Planktothrix agardhii



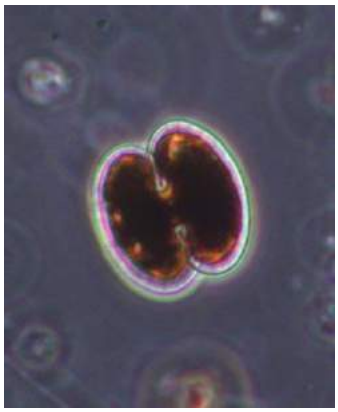
Monoraphidium arcuatum



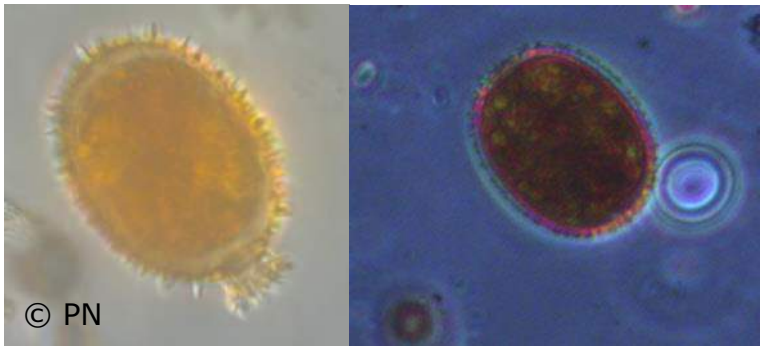
Woronichinia naegeliana



Cosmarium ocellatum

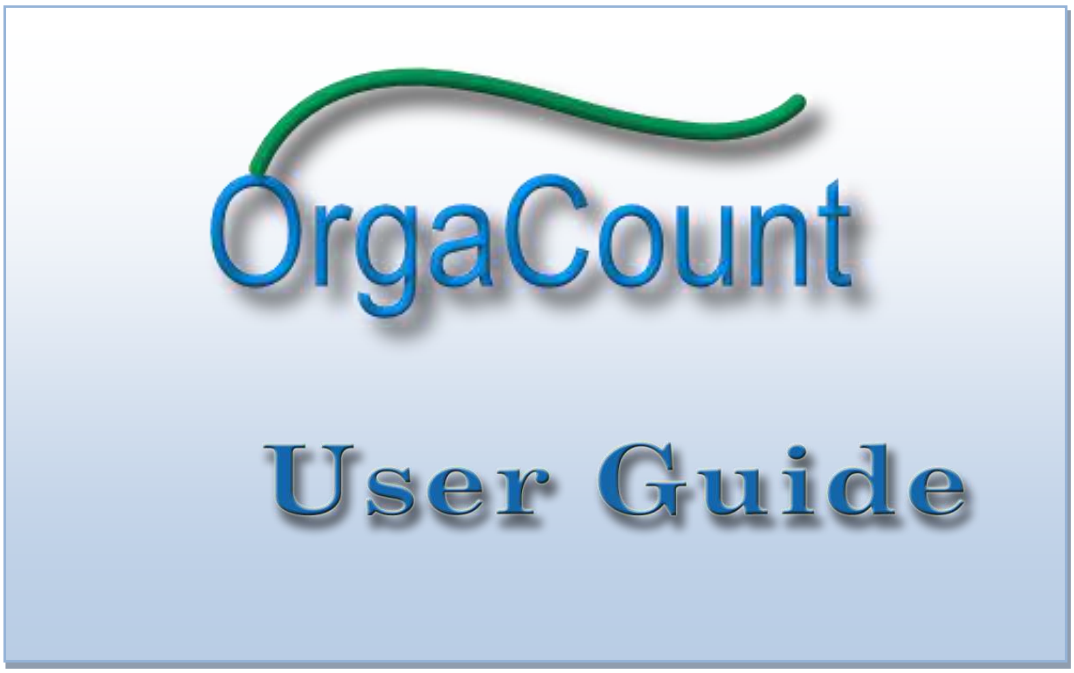


Trachelomonas hispida



Annex C: OrgaCount User Guide for CEN Intercomparison Test Phytoplankton Biovolume

The following user guide for the online software module of OrgaCount have been send around to all participants at the beginning of the interlaboratory comparison.



CEN Intercomparison Test Phytoplankton Biovolume

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Setting up the samples..... 5
Setting up the sessions..... 6
Adding taxa to the counting list and counter..... 9
Measuring the Items for Biovolume 11
Reports 14

OrgaCount is a web-based software for counting organisms. The application is optimised and tested with the Firefox browser. Other browsers may be used but no guarantee can be given for a correct performance.

For the scheduled intercomparison exercise, a modified mode of OrgaCount for measuring and counting phytoplankton will have to be used. Please, note that the software offers more features than being necessary for the exercise. For that reason, only the functions needed for the test will be explained in the following, others are omitted. Further information you will find in the detailed user guide for OrgaCount, accessible by the 'Help' button in the pick list at the left side or in the lowest panel of the programme called 'Search documents'.

For questions please address cen2014@aquaecology.de.

The OrgaCount environment is organised in various modules related to each other in a tree structure. Information is stored by three main levels of organisation, from general to specific: projects, samples, and sessions.

The branches are labelled and can be expanded by clicking on the plus sign located to the left side of the branch label.

In the following, the process will be explained step-by-step.

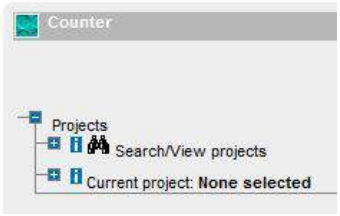
Open webpage https://orgacount.com/apps/orgacount_cenbiovol/index.php

Log in with your username and your password you specified within the registration process.

1 Setting up the project

Click on the sign of the *Projects* branch:

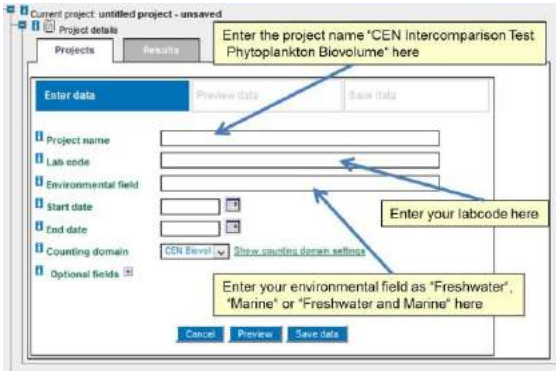
Click on the sign of the *Search/View projects* branch



to open the project window:

Set up a new project by clicking on the item *Add new project.*

Fill out the project form:



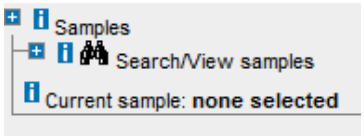
Please note that other information in that form is optional.

Click on Save data to save the project and confirm in the next window with the OK button.

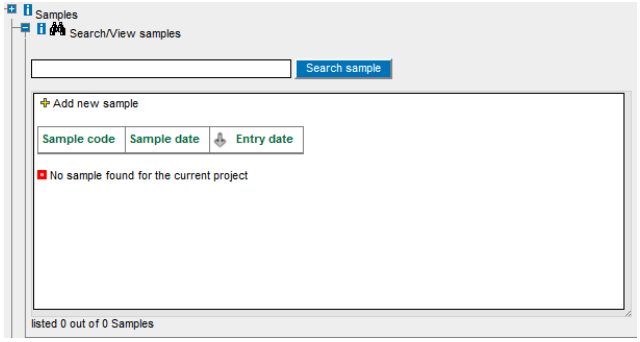
2 Setting up the samples

Samples in OrgaCount hold information about all data that are associated to a real sample, such as codes, information on the collecting site and the local conditions, counting procedures etc. For that reason, please, set up a new sample at least for each of the delivered samples. If you are using more than one measuring method or additionally your 'home method', set up an additional sample and add a suffix or prefix to the name that enables distinguishing between the measurements.

Click on the ⊞ sign of the *Search/View samples* branch

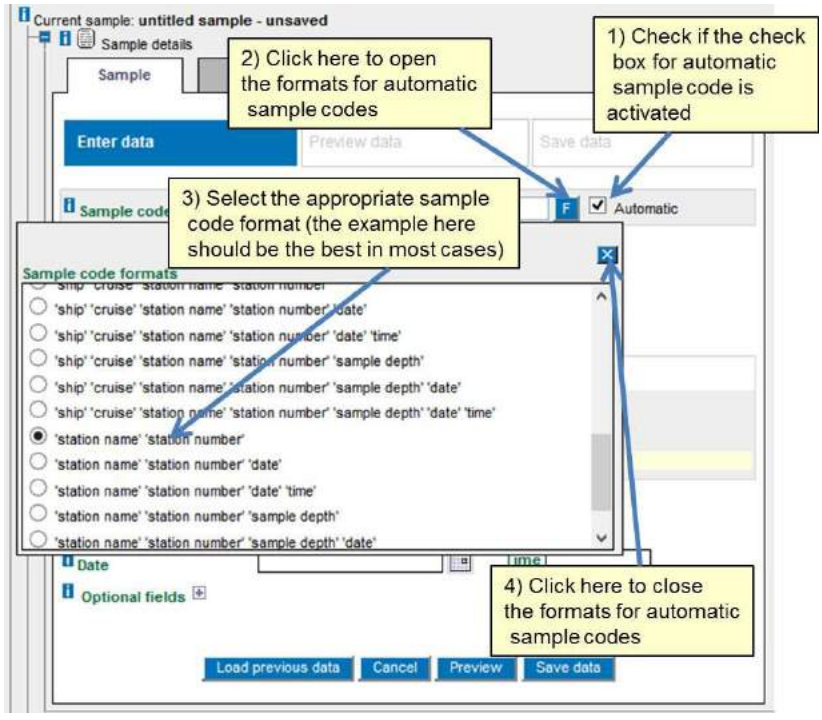


to open the sample window:



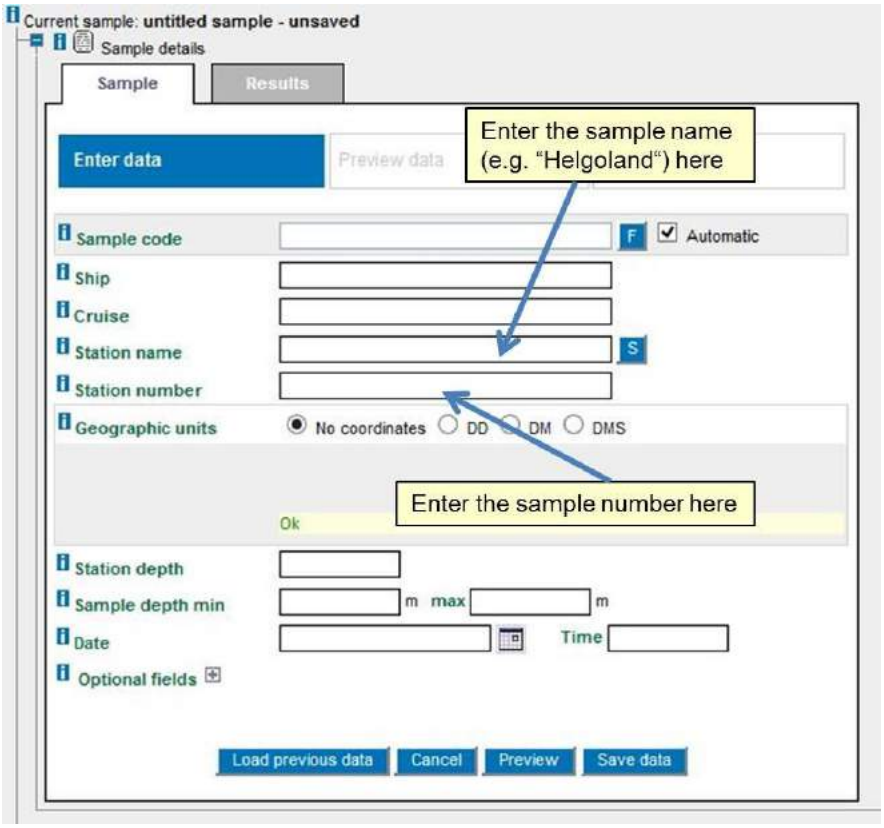
Set up a new sample by clicking on the item ✚ *Add new sample*.

Select the 'automatic' sample code format:



Please note: This has to be done only once for all samples, but please, verify that the checkbox is activated whenever a new sample is set up.

Now fill out the sample form:



Please note that other information in that form is optional.

Click on Save data to save the sample and confirm in the next window with the OK button.

3 Setting up the sessions

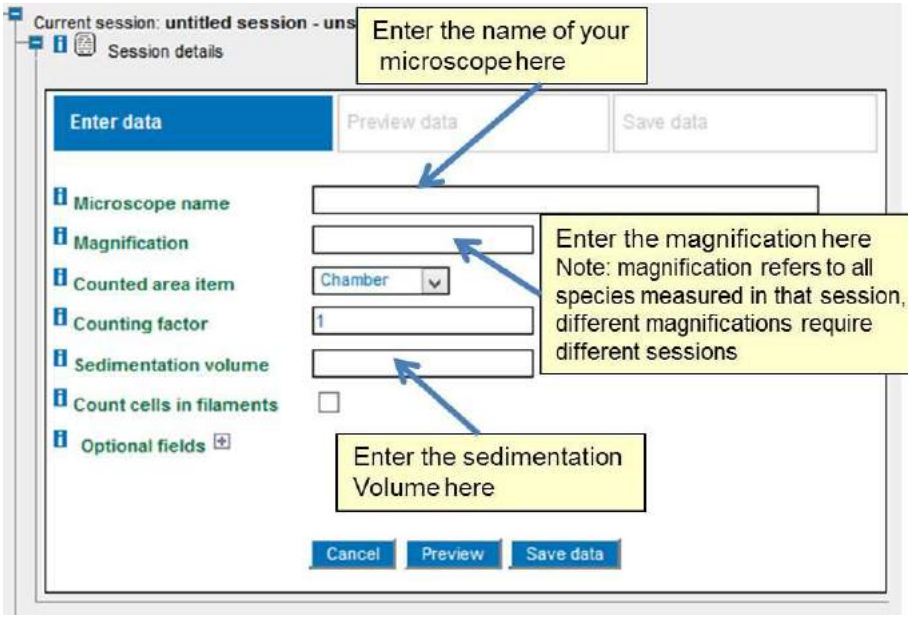
Sessions in OrgaCount contain those kinds of information related to organism counting processes, such as the microscope settings, the area counted, etc. One sample can contain more than one session.

The session window should be open already, otherwise click on the ⊞ sign of the *Search/View sessions* branch.

Set up a new session by clicking on the item *Add new session*

In the session form, some information is set by default, such as the counted area item and the counting factor. They are used only for calculations concerning counting and therefore are irrelevant for this test.

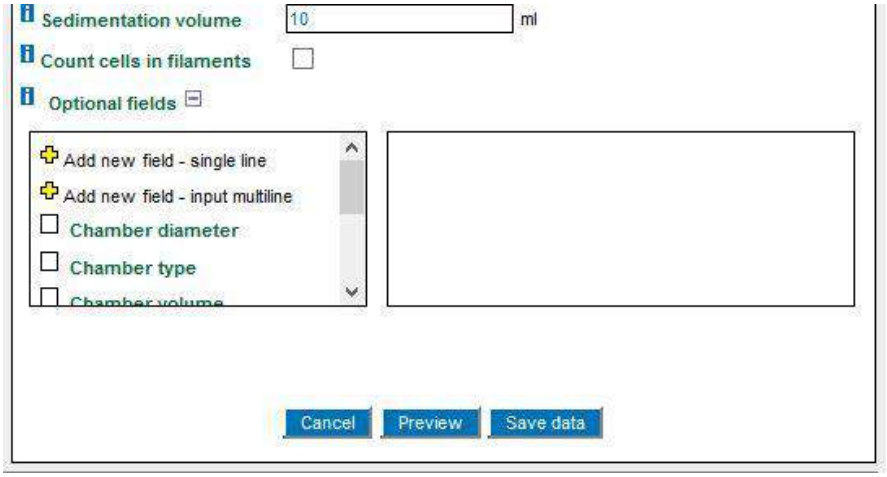
Fill out the session form:



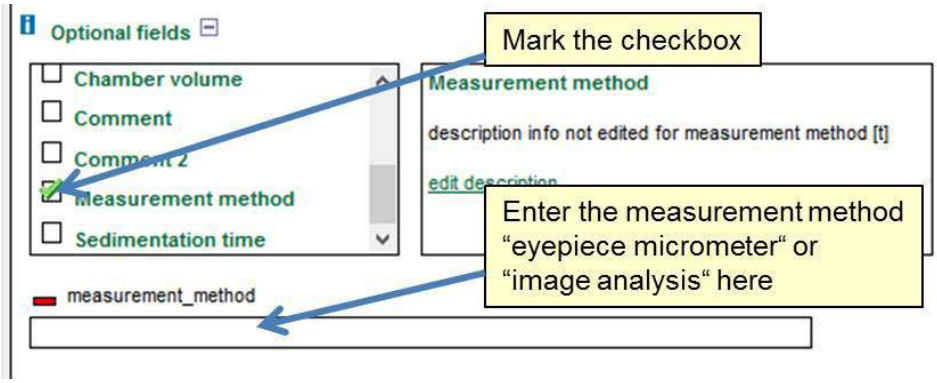
Please note: Set up different sessions for different magnifications or sedimentation volumes.

Additional information (see instruction) has to be stored in the *'Optional fields'*, the last item of the session form.

Click on the ⊞ sign at the right side of the label named '*Optional fields'* to open the optional fields panel.



Scroll down in the scroll bar to '*Measurement method'*.

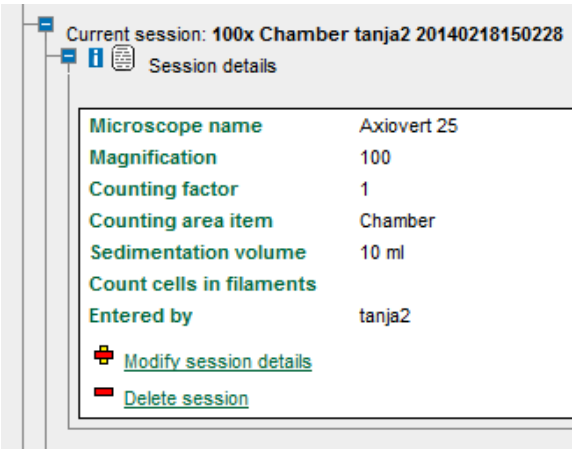


If you are using more than one measuring method, set up an additional sample and add a suffix or prefix to the name that enables distinguishing between the measurements.

Do as described for '*Comments'* or '*Comments 2'* as well, if you have any comments.

Click on Save data to save the session.

Please note: You can add information to the optional fields at any time. Select the session and click on the + sign of the branch labelled *session details*, then the window with the session details will open:

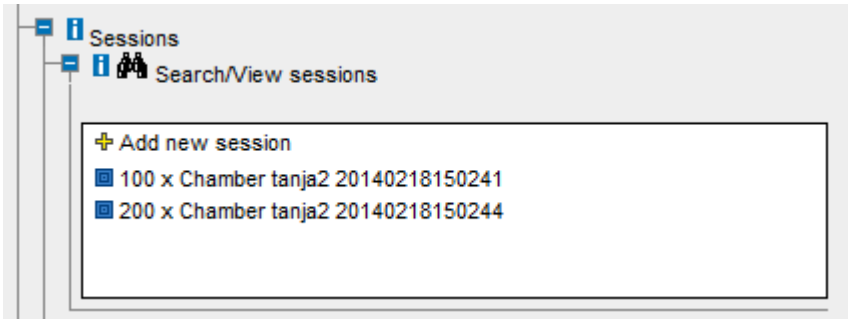


Click on Modify session details to open the session form and go on as described above.

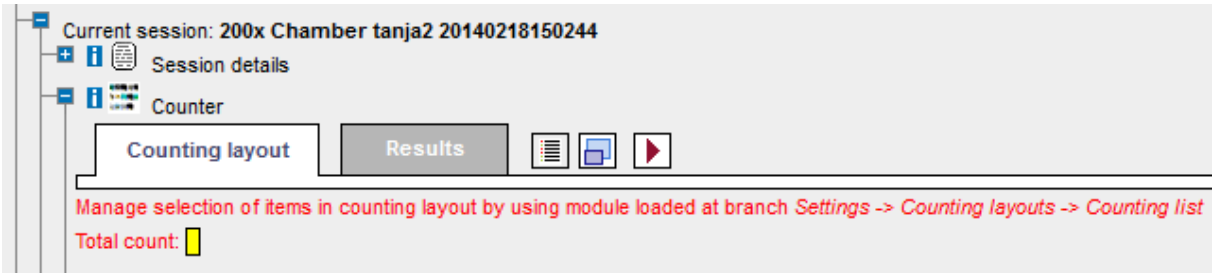
Editing will be possible at each level of OrgaCount. With regard to this intercomparison test, this should not be necessary when following the instructions. However, for further information see the detailed user guide of OrgaCount available by clicking the 'Help' button in the menu on the left side or at the 'Search documents' panel in the lower area of the programme window. Here, you will have the possibility to search within the document by using keywords.

4 Adding taxa to the counting list and counter

Click on a session in the session textbox

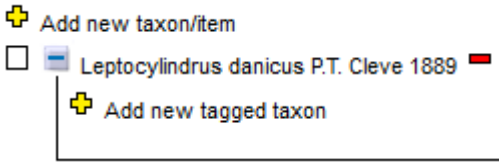


to open the counter which is used for the biovolume measurements as well:



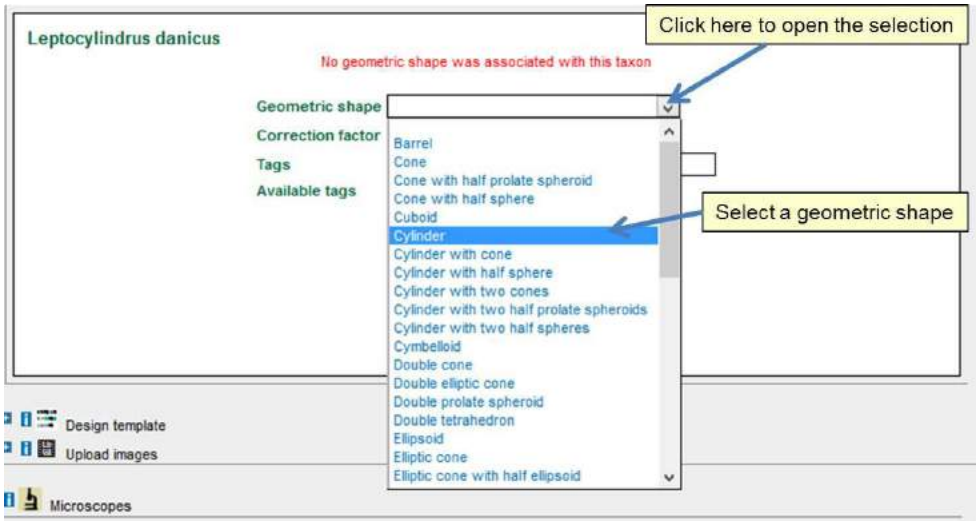
The species listed in alphabetical order in the 'List of all items/taxa' of the counting list panel. To find a certain species, just click on the initial letter of the name; for example, for *Rhizosolenia imbricata* click on the R in the list:

Select the species you would like to measure and click on the + sign at the left side of the species name. The branch will open for adding a tagged taxon to the counting list. Please, pay attention not to mark the check box on its left side.

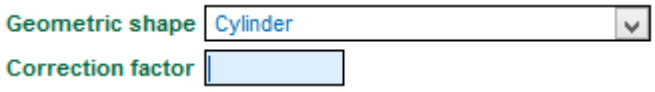


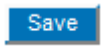
Click on ✚ *Add new tagged taxon.*

A window will open to add the geometric shape to the taxon:

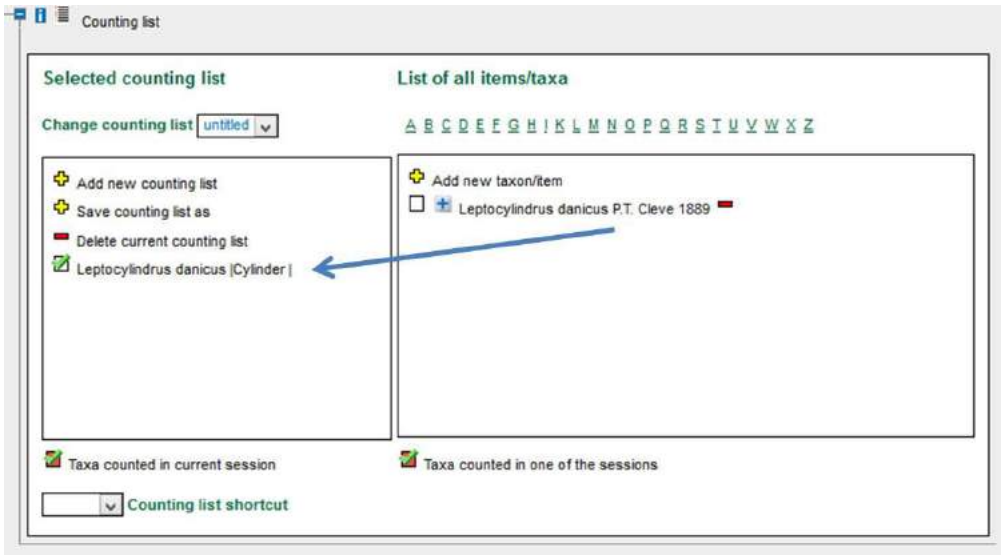


Additionally. you can enter a correction factor:



Click on 

; the geometry (and the correction factor) will then be assigned to the taxon and the species name with assigned geometric shape will appear in the counting list at the left side of the counting list panel:



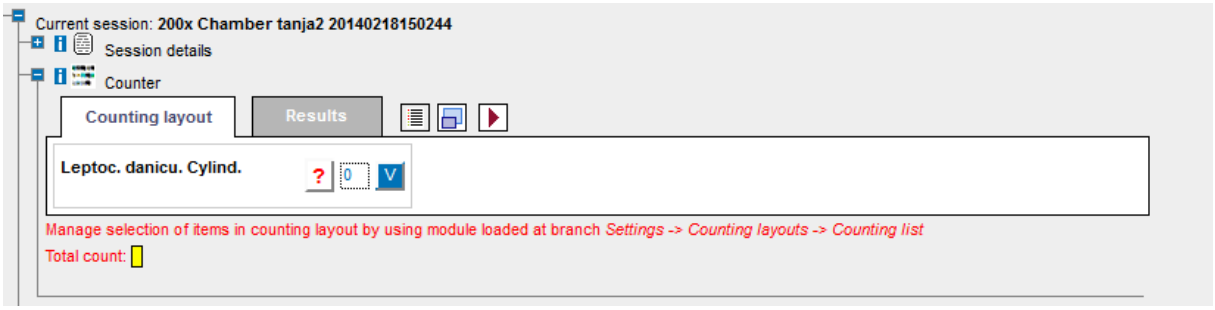
You can either add all geometric shapes to all species now or start with measuring for the first taxon and add the remaining taxa later.

Please note: By using this method, all species will appear in the counting list and in the counter (for further information about species lists see the detailed user guide for OrgaCount).

If you prefer a geometry and/or correction factor different from the one in the Standard Document, set up an additional sample with a suffix or prefix to the name that enables distinguishing between the measurements and assign the preferred geometric form. Afterwards, carry out the measurements for both samples for comparison. If the form is not available or you have another comment, put this information into one of the comment fields of the *'Optional fields'*.

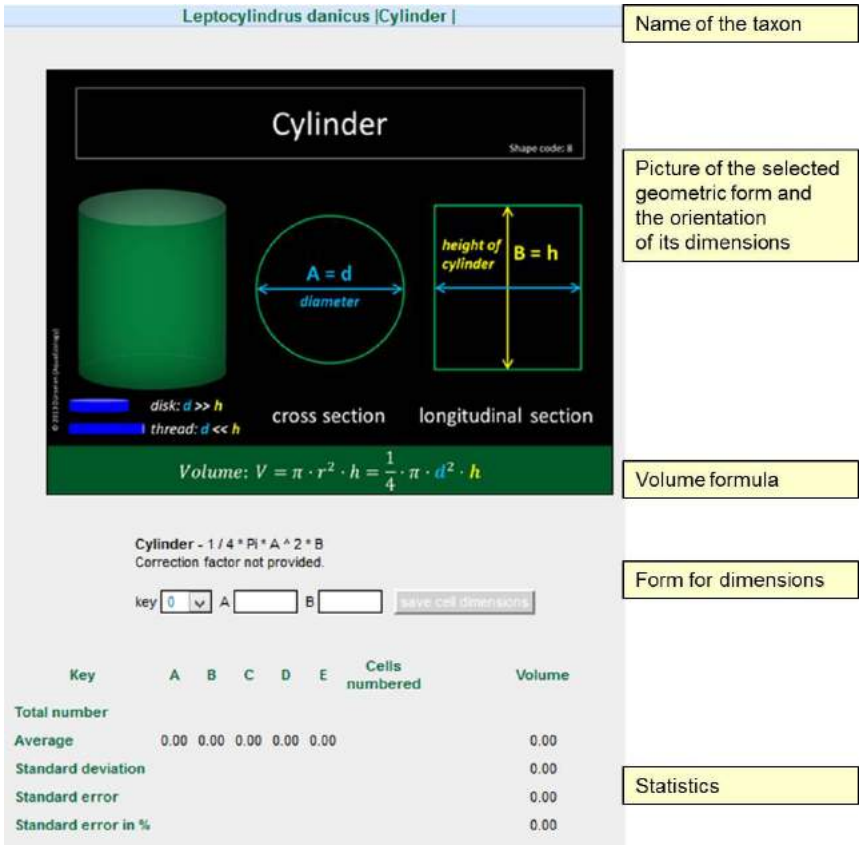
5 Measuring the Items for Biovolume

While assigning a geometric shape to a taxon and thereby adding it to the counting list it simultaneously appears in the counter. In the counter, abbreviations for the taxon name and the assigned geometric forms are used.



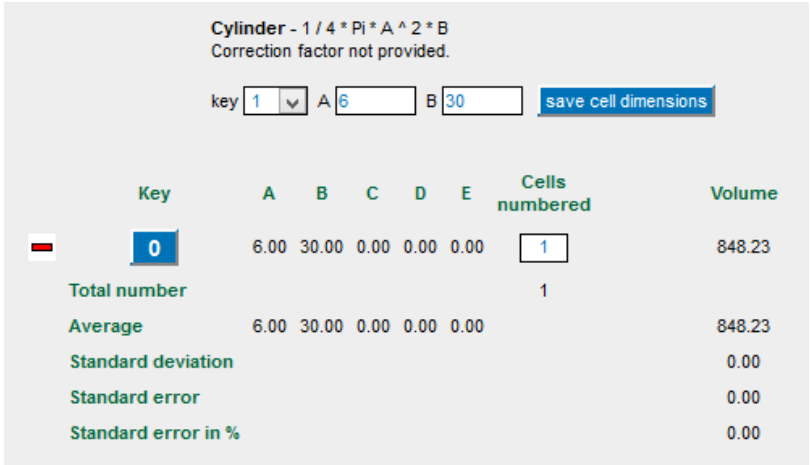
Click on the *run counting* button near the *'Results'* tab. Please, make always sure that the counting is active before working in the biovolume popup (next step).

Click on the V button next to the species you want to measure. The biovolume popup will open:

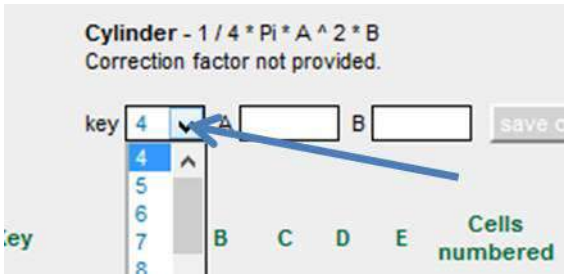


Enter your dimensions of the cell in the form and click on the button save cell dimensions, which turns blue when each of the fields is filled in. You can easily identify which dimension belongs to which field by considering the capital letters being assigned, respectively.

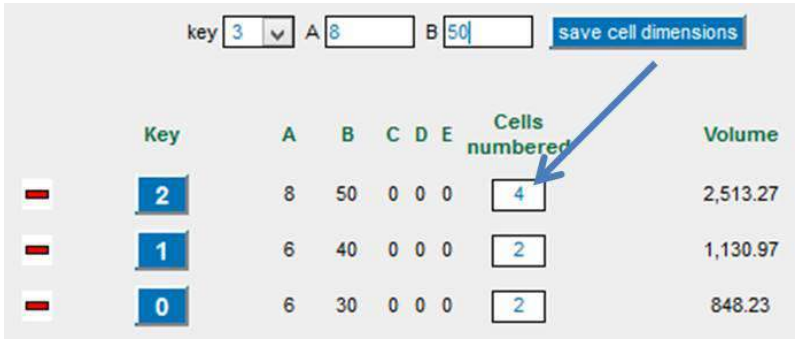
The set of dimensions will then be transferred to the list and the volume for a cell with these dimensions will be simultaneously calculated:



Each set of dimensions will get a key assigned automatically. Alternatively, you can select a key from the pick list:

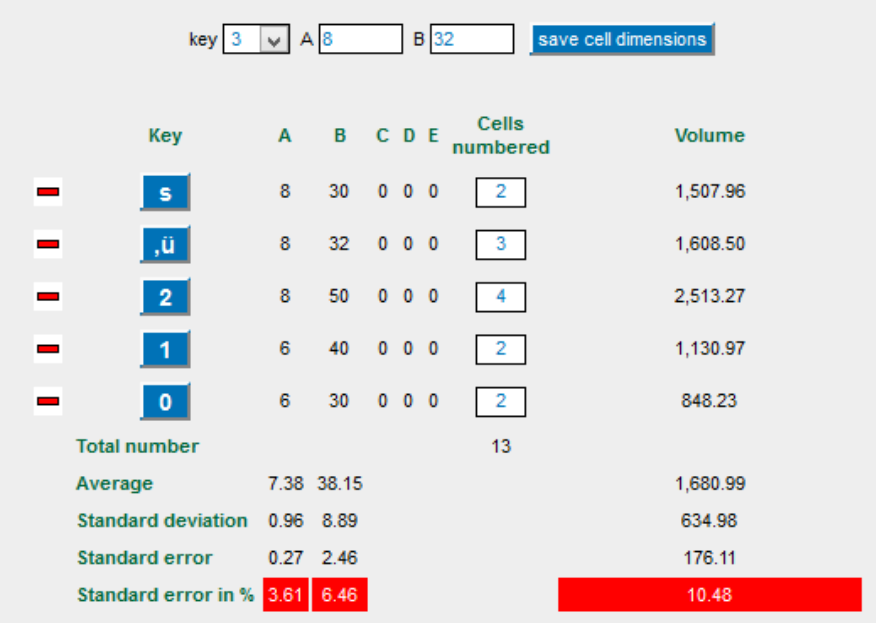


The key refers to the key on your keyboard. If you measure a set of dimensions twice, you can either click on the button for this key or hit the key on your keyboard (for combinations such as ,ü hit both keys simultaneously). If you fill out the form instead and click on save cell dimensions, this will increase the value of the respective set of dimensions automatically by 1:

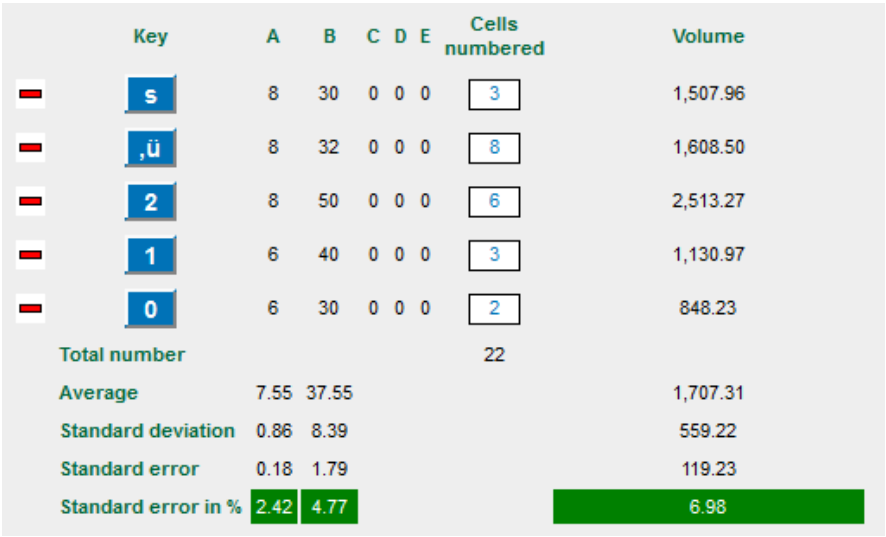


Please, make sure that the cursor is in an entry field when filling in measurements. Otherwise, if number keys are assigned to species, you will then continue counting for the assigned sets of dimensions instead of entering the value of a measurement!

Below this list, you will find the statistics being recalculated immediately if a new entry is added:



When the statistic requirements for the measurements (see Standard Document) are fulfilled. the field of the '*Standard error in %'* will automatically turn from red to green:



<u>You can stop measuring</u> if this is the case for the *'Standard error in %'* for the '*Volume'* column.

Please note: Below this statistical information, you will find some further statistical items, which are included in the OrgaCount software, but are not essential for this test.

If you want to delete a set of dimensions, just hit the delete button at the left side and confirm with OK.

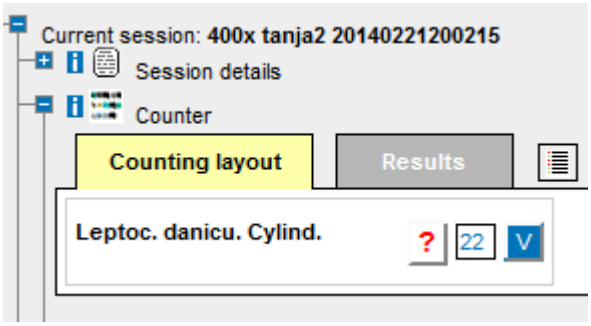
To change the number of cells for a set of dimensions, you can overwrite the value in the field '*Cells numbered*'. The biovolume popup will reload with the next click and update the displayed information.

Special instruction for HELCOM:

If you are additionally working with the size class method of HELCOM, fill out the form with the dimension of your size class and count the items in that size class as described above.

6 Reports

You may want to store your results. That is possible at session and sample level by selecting the '*Results'* tab. At the sample level, the results of all sessions within that sample will be integrated.



In the '*Results'* tab. you will find the button

. Click on it and then you will be able to save an excel sheet with your measurements for the respective taxa, the statistics and the additional information onto your local computer.

Thank you for your co-operation and your participation in the exercise.