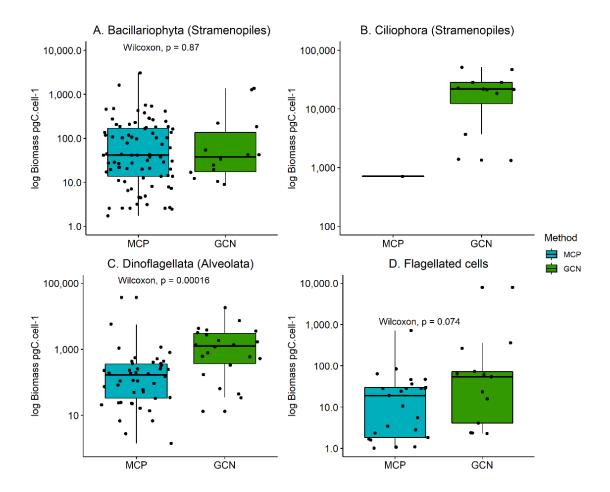
**Supplementary Table 1:** Summary of the different datasets utilized in this study regarding the identified number of species, cell C-content (proxy for biomass) and 18S rRNA GCNs, classified by microbial eukaryote groups determined in this study. MTB: Metabarcoding, MCP: Microscopy, GCN: Gene Copy Number, Sd: standard deviation.

	Species (Number)			Biomass [pg C.cell <sup>-1</sup> ]  (Median; mean ± Sd)			18S rRNA gene [GCN.cell <sup>-1</sup> ]  (Median; mean ± Sd)			
Dataset	МТВ	МСР	GCNdb	МТВ	МСР	GCNdb	MTB	МСР	GCNdb	
Bacillariophyta (Stramenopiles)	355	88	14	-	37.6 151 ± 376	37.9; 235 ± 462	-	-	166; 186688 ± 694605	
Ciliophora (Alveolata)	190	1	12	-	719; 719 ± 0	21991 ; 22365 ± 15947	-	-	71710; 97888 ± 86842	
Dinoflagellata (Alveolata)	506	47	22	-	181; 5581 ± 30871	1260 ; 2444 ± 3974	-	-	4919; 8170 ± 288688	
Flagellated cells	833	29	17	-	5.54; 42 ± 132	15.7 522 ± 1914	-	-	5.23 ; 70.2 ± 176	



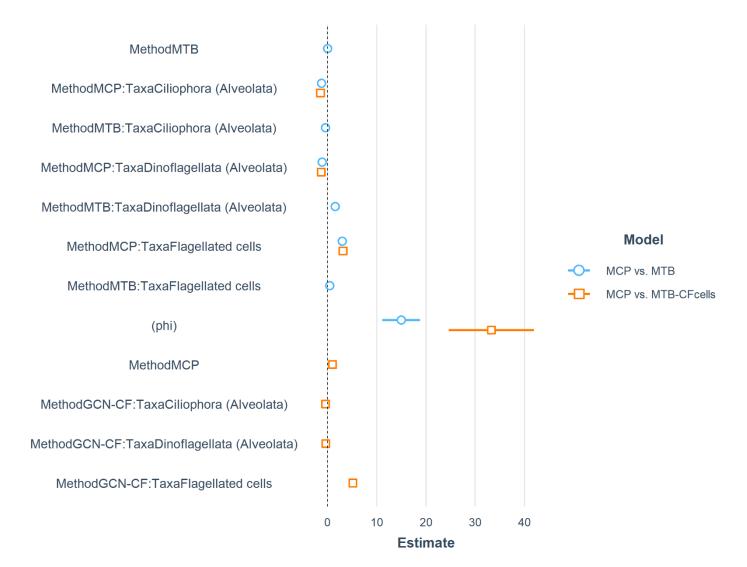
**Supplementary Figure 1:** Comparison of the cellular carbon content per taxa (pg C cell<sup>-1</sup>) between two-dataset used in this study: Microscopy (MCP) and Gene Copy Number dataset (GCN). Major single celled eucaryotic plankton groups described (A) Bacillariophyta (Stramenopiles), (C) Dinoflagellata, (B) Ciliophora and (D) Flagellated cells.

**Supplementary Table 2:** List of samples and sampling dates used in this study.

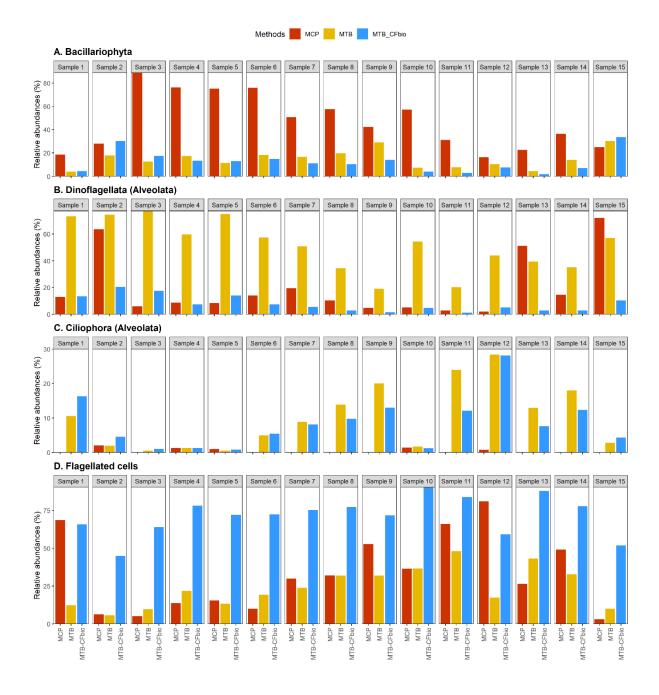
Sample Name	Sampling date (DD/MM/YYYY)					
Sample 1	02/05/2018					
Sample 2	25/07/2018					
Sample 3	29/08/2018					
Sample 4	25/09/2018					
Sample 5	24/10/2018					
Sample 6	12/11/2018					
Sample 7	18/12/2018					
Sample 8	29/01/2019					
Sample 9	20/02/2019					
Sample 10	11/03/2019					
Sample 11	08/04/2019					
Sample 12	24/04/2019					
Sample 13	08/05/2019					
Sample 14	22/05/2019					
Sample 15	04/06/2019					

**Supplementary Table 3:** Exponentially transformed results of the two beta regression models performed in this study to test the effect of CF for estimation of cell proportions. The taxonomic group percentages were modeled against the analysis method (A) Microscopy vs. metabarcoding and (B) Microscopy vs. corrected values using CF. The interaction between the analysis method and the specific phyla analyzed results are displayed as well. Estimations were calculated based on maximum likelihood. MCP: microscopy, MTB: metabarcoding, MTB\_CFcell: corrected metabarcoding values for cell proportions, CI: Confidence intervals.

	Cell relative abundances							
		[A] MCP vs. MT	[B] MCP vs. MTB_CFcell					
Predictors	Estimates	CI	p	Estimates	CI	p		
(Intercept)	0.22	0.16 - 0.30	<0.001	0.07	0.05 - 0.10	<0.001		
Method [MTB]	1.00	0.64 - 1.56	0.994					
Method [MCP]				2.74	1.86 - 4.04	<0.001		
[MTB] : Ciliophora	0.67	0.42 - 1.07	0.095					
[MCP] : Ciliophora	0.29	0.17 - 0.50	<0.001	0.24	0.16 - 0.37	<0.001		
[MTB_CFcell] : Ciliophora				0.66	0.41 - 1.07	0.090		
[MTB] : Dinoflagellata	4.78	3.19 - 7.17	<0.001					
[MCP] : Dinoflagellata	0.34	0.20 - 0.56	<0.001	0.29	0.19 - 0.43	<0.001		
[ MTB_CFcell ] * Dinoflagellata				0.71	0.44 - 1.13	0.150		
[MTB] : Flagellated cells	1.55	1.01 - 2.37	0.043					
[MCP] : Flagellated cells	19.72	12.62 – 30.83	<0.001	23.13	16.82 - 31.80	<0.001		
[ MTB_CFcell ] * Flagellated cel	lls			175.51	112.02 – 275.00	<0.001		
Observations		120			120			
$\mathbb{R}^2$		0.844		0.960				
Phi (φ)		14.960	1.57E <sup>-14</sup>		33.227	4.67E <sup>-14</sup>		



**Supplementary Figure 2:** Visualization of the results (Supplementary table 3) of the two generalized linear modeling based on beta distribution performed in this study to test the effect of CF for estimation of cell proportions. The taxonomic group percentages were modeled against the analysis method (A) Microscopy vs. metabarcoding and (B) Microscopy vs. corrected values using CF. The interaction between the analysis method and the specific phyla analyzed results are displayed as well. Estimations were calculated based on maximum likelihood. MCP: microscopy, MTB: metabarcoding, MTB\_CFcell: corrected metabarcoding values for cell proportions.



**Supplementary Figure 3:** Comparison between relative abundances (%) in each of the 15 marine water samples per plankton group estimated biomass from inverted microscopy (MCP Biomass [pgC.taxa<sup>-1</sup>]; red), reads from DNA metabarcoding (MTB; yellow), and the results of the corrected biomass to estimate the carbon content per taxa using MCP datasets mean C-biomass values (MTB\_CFbio). (A) Bacillariophyta, (B) Dinoflagellata and (C) Flagellated cells. MCP: Microscopy, MTB: Metabarcoding, MTB\_CFbio: metabarcoding corrected values for biomass.

**Supplementary Table 4:** Exponentially transformed results of the three beta regression models performed in this study to test the effect of CF for the estimations of C-biomass proportions. The taxonomic group percentages were modeled against the analysis method (A) Microscopy vs. metabarcoding and (B) Microscopy vs. corrected values using MTB median C-content and (C) Microscopy vs. corrected values using MCP median C-content. The interaction between the analysis method and the specific phyla analyzed results are displayed as well. Estimations were calculated based on maximum likelihood. MCP: microscopy, MTB: metabarcoding, MTB\_CFbio: corrected metabarcoding values for biomass proportions, CI: Confidence intervals.

## **Biomass Relative Abundances**

[A] MCP vs. MTB				[B] MCP vs. MT_CFbio			
Estimates	CI	p	Estimates	CI	p		
0.90	0.64 – 1.26	0.534	0.90	0.65 – 1.24	0.506		
0.28	0.16 – 0.47	<0.001					
0.10	0.06 - 0.18	<0.001					
0.71	0.40 – 1.26	0.235					
0.30	0.18 - 0.50	<0.001					
4.23	2.51 – 7.14	<0.001					
0.53	0.33 – 0.87	0.012					
1.48	0.86 – 2.55	0.153					
			0.23	0.14 - 0.38	<0.00		
			0.10	0.05 - 0.17	<0.00		
			0.29	0.18 – 0.48	<0.00		
			0.53	0.33 – 0.84	0.007		
			0.78	0.44 – 1.38	0.390		
			0.77	0.44 – 1.36	0.372		
			10.86	6.41 – 18.39	<0.00		
	120			120			
	0.590	ı		0.703	6.64E		
	Estimates 0.90 0.28 0.10 0.71 0.30 4.23 0.53 1.48	Estimates       CI         0.90       0.64 - 1.26         0.28       0.16 - 0.47         0.10       0.06 - 0.18         0.71       0.40 - 1.26         0.30       0.18 - 0.50         4.23       2.51 - 7.14         0.53       0.33 - 0.87         1.48       0.86 - 2.55	Estimates         CI         p           0.90         0.64 - 1.26         0.534           0.28         0.16 - 0.47         <0.001	Estimates         CI         p         Estimates           0.90         0.64 - 1.26         0.534         0.90           0.28         0.16 - 0.47         <0.001	Estimates         CI         p         Estimates         CI           0.90         0.64 - 1.26         0.534         0.90         0.65 - 1.24           0.28         0.16 - 0.47         <0.001		



**Supplementary Figure 4:** Visualization of the results (Supplementary table 4) of the two generalized linear modeling based on beta distribution performed in this study to test the effect CF for the estimations of C-biomass proportions. The taxonomic group percentages were modeled against the analysis method (A) Microscopy vs. metabarcoding and (B) Microscopy vs. corrected values using MCP median C-content. The interaction between the analysis method and the specific phyla analyzed results are displayed as well. Estimations were calculated based on maximum likelihood. MCP: microscopy, MTB: metabarcoding MTB\_CFbio: corrected metabarcoding values for biomass proportions.