Journal of Limnology
CNR - Water Research Institute (IRSA) - Verbania Pallanza - Italy

SUPPLEMENTARY MATERIALS

Transparent exopolymer particles (TEP), phytoplankton and picocyanobacteria along a littoral to pelagic depth gradient in a large subalpine lake

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Key words: TEP horizontal distribution, foams, picocyanobacteria and phytoplankton distribution, Lake Maggiore, littoral-pelagic transects

Tab. 1S. Results of the chemical analyses performed on water samples collected at different stations and depths.

Station	Depth m	pH	Cond. µS cm^{-1}	T. Alk. meq L^{-1}	DIC mg L^{-1}	Cl mg L^{-1}	SO_4 mg L^{-1}	$N\text{-}NO_3$ µg L^{-1}	$N\text{-}NH_4$ µg L^{-1}	RP µg L^{-1}	TP µg L^{-1}	TN mg L^{-1}	Si mg L^{-1}
21 May 2019													
St. 0	0.5	7.90	144	0.797	12.99	3.4	27.3	698	21	2	8	1.03	1.07
St. 2	0.5	7.90	142	0.803	12.74	3.2	27.2	696	8	<LOD	7	0.83	1.08
St. 2	5	7.84	143	0.797	12.49	3.2	27.4	697	8	<LOD	7	0.82	1.09
St. 2	10	7.68	143	0.804	13.06	3.2	27.6	706	10	1	6	0.81	1.11
St. 2	15	7.58	143	0.787	12.78	3.1	27.8	706	14	1	7	0.84	1.26
St. 2	29	7.56	145	0.811	13.16	3.3	27.8	727	14	2	6	0.92	1.13
St. 4	0.5	7.87	142	0.801	12.68	3.2	27.3	697	7	1	7	0.87	1.07
St. 4	5	7.84	142	0.798	12.37	3.2	27.4	696	7	1	7	0.88	1.08
St. 4	10	7.83	142	0.793	12.61	3.2	27.5	700	10	<LOD	6	0.88	1.11
St. 4	15	7.59	142	0.779	12.35	3.0	27.8	685	17	1	8	0.92	1.46
St. 4	80	7.37	143	0.820	13.6	3.2	28.9	770	4	4	9	0.88	1.54
St.6	0.5	7.85	143	0.798	12.61	3.3	27.3	708	17	1	8	1.07	1.07
St.6	5	7.81	142	0.797	12.74	3.2	27.1	691	7	<LOD	7	0.85	1.14
St.6	10	7.69	144	0.807	12.71	3.2	27.7	710	7	<LOD	6	0.86	1.10
St.6	15	7.62	144	0.804	12.82	3.2	28.0	719	13	1	6	0.88	1.14
St.6	120	7.21	152	0.837	14.75	3.2	29.8	775	5	9	16	0.87	1.98
16 July 2019													
St. 0	0.5	8.6	133	0.765	12.14	2.8	25.1	473	13	1	6	0.62	0.16
St. 2	0.5	8.8	132	0.761	11.18	2.9	25.1	460	10	<LOD	5	0.61	0.15
St. 2	5	8.7	131	0.739	10.98	2.6	25.2	485	14	1	6	0.65	0.26
St. 2	10	8.2	133	0.739	11.57	2.2	26.7	444	15	<LOD	8	0.61	0.74
St. 2	15	7.7	134	0.748	11.95	2.5	26,0	559	22	1	6	0.74	0.87
St. 2	20	7.4	145	0.807	13.39	3.2	27.6	759	5	1	6	0.87	1.20
St. 4	0.5	8.7	131	0.756	11.17	2.7	25.2	458	10	1	5	0.63	0.14
St. 4	5	8.6	132	0.756	11.24	2.5	25.7	452	13	1	8	0.64	0.26
St. 4	10	8.4	132	0.744	12.05	2.3	26.3	440	14	1	9	0.60	0.58
St. 4	15	7.6	138	0.774	12.62	2.8	26.2	659	13	1	7	0.83	0.99
St. 4	87	7.3	149	0.837	14.07	3.2	28.8	766	5	4	9	0.90	1.57
St.6	0.5	8.7	132	0.762	11.29	2.7	25.2	466	11	1	6	0.63	0.13
St.6	5	8.7	132	0.756	11.34	2.6	25.5	459	16	1	10	0.65	0.22
St.6	10	8.1	134	0.748	11.70	2.3	27.2	454	24	1	10	0.64	0.85
St.6	15	7.7	137	0.770	12.58	2.9	25.9	651	20	1	7	0.85	0.96
St.6	132	7.3	152	0.853	14.10	3.2	29.3	763	5	12	17	0.90	1.90
18 September 2019													
St. 0	0.5	8.7	138	0.793	12.43	2.8	26.8	432	5	<LOD	8	0.65	0.56
St. 2	0.5	8.8	137	0.784	12.35	2.7	26.8	413	5	1	6	0.63	0.55
St. 2	5	8.8	138	0.787	11.88	2.8	26.9	418	7	1	7	0.62	0.58
St. 2	10	8.0	151	0.806	12.76	2.3	32.6	447	21	1	10	0.60	1.23
St. 2	15	7.7	143	0.770	12.52	2.8	29.4	554	24	1	7	0.68	1.03
St. 2	20	7.4	143	0.796	11.7	3.1	26.9	759	5	1	5	0.87	1.14
St. 4	0.5	8.6	137	0.781	11.71	2.7	26.8	412	6	<LOD	6	0.60	0.54
St. 4	5	8.7	137	0.781	12.62	2.7	26.8	409	6	1	8	0.62	0.56
St. 4	10	8.0	148	0.792	12.7	2.3	31.7	452	21	1	9	0.63	1.14
St. 4	15	7.7	140	0.771	13.26	2.6	27.8	554	27	<LOD	7	0.73	0.94
St. 4	87	7.6	148	0.825	11.48	3.2	28.1	759	7	2	8	0.89	1.52
St.6	0.5	8.6	137	0.780	11.37	2.7	26.9	410	7	1	6	0.61	0.55
St.6	5	8.8	137	0.788	12.5	2.7	26.9	409	7	<LOD	6	0.61	0.55
St.6	10	8.0	146	0.793	12.37	2.4	30.7	456	22	1	8	0.64	1.08

St.6	15	7.8	140	0.772	13.68	2.6	27.7	540	30	1	6	0.68	0.91
St.6	132	7.4	152	0.846	11.89	3.1	28.7	750	8	11	16	0.89	1.96
LOD			0.5	0.005		0.02	0.06	15	4	1	3	0.03	0.015
RSD		0.4	1	2		4	3	5	8	15	18	8	3

Cond.: electrical conductivity at 20°C. T. Alk.: total alkalinity. RP: soluble reactive phosphorus. TP: total phosphorus. TN: total nitrogen. Si: reactive silica. LOD: Limit of Detection. RSD: relative standard deviation.

Tab. 2S. Output of the linear models of the effect of sampling depth and sampling station (vertical and horizontal distribution) on the measured parameters for each of the sampling dates.

		21 May 2019					16 July 2019					18 September 2019				
Variable		Sum Sq	DF	f-value	p-value		Sum Sq	DF	f-value	p-value		Sum Sq	DF	f-value	p-value	
TEP	*station*	102.4	1	0.4338	0.5157		466.7	1	2.1133	0.1576		45673	1	1.1426	0.2946	
	depth	4018.3	1	17.02	0.0003	***	14255.3	1	64.5545	1E-08	***	684331	1	17.12	0.0003	***
TOC	*station*	62869	1	20.009	0.0001	***	97412	1	4.7323	0.0385	*	82202	1	1.9779	0.171	
	depth	96390	1	30.678	7E-06	***	232286	1	11.2845	0.0023	**	303831	1	7.3107	0.0117	*
Chl tot	*station*	0.1103	1	1.052	0.3142		3.5617	1	4.4322	0.0447	*	0.0151	1	0.0631	0.8036	
	depth	5.5671	1	53.097	8E-08	***	30.0804	1	37.4321	2E-06	***	8.0099	1	33.4941	4E-06	***
Cytometer		Sum Sq	DF	f-value	p-value		Sum Sq	DF	f-value	p-value		Sum Sq	DF	f-value	p-value	
PE	*station*	1.9E+08	1	0.9339	0.3424		1.7E+08	1	0.0925	0.7634		2.9E+10	1	1.1322	0.2967	
	depth	1.2E+10	1	56.559	4E-08	***	3.3E+10	1	18.1666	0.0002	***	5.2E+11	1	20.3389	0.0001	***
PC	*station*	293457	1	0.4666	0.5004		177089	1	0.0171	0.897		878261	1	0.0496	0.8255	
	depth	1.2E+07	1	18.784	0.0002	***	1.4E+08	1	13.4219	0.0011	**	1.8E+08	1	10.3107	0.0034	**
Cyano Col/Fil	*station*	11160	1	0.0895	0.7671		125736	1	0.9412	0.3406		5388	1	0.6918	0.4129	
	depth	3811359	1	30.576	7E-06	***	427648	1	3.2011	0.0848		82076	1	10.5372	0.0031	**
EUK	*station*	1.4E+11	1	1.4095	0.2455		5059822	1	0.2194	0.6433		6.2E+10	1	0.24	0.6282	
	depth	3.5E+12	1	34.904	3E-06	***	5.4E+08	1	23.3288	5E-05	***	2.3E+13	1	90.54	4E-10	***
Bacteria	*station*	0.012	1	0.0089	0.9254		7.4E+11	1	8.9628	0.0058	**	0.012	1	0.0089	0.9254	
	depth	40.549	1	29.528	1E-05	***	5.6E+12	1	67.6289	8E-09	***	40.549	1	29.5276	1E-05	***
Fluoroprobe data		Sum Sq	DF	f-value	p-value		Sum Sq	DF	f-value	p-value		Sum Sq	DF	f-value	p-value	
Green	*station*	0.0242	1	0.0509	0.8236		0.01	1	0.1434	0.708		0.4435	1	0.8582	0.3628	
	depth	3.3772	1	7.1038	0.0138	*	0.03	1	0.5884	0.4499		0.0041	1	0.0079	0.9296	
CyanoPE	*station*	0.00405	1	0.5294	0.4742		0.00	1	2.2083	0.1493		3.4E-05	1	1.066	0.3114	
	depth	0.00387	1	0.5064	0.4838		0.00	1	0.0881	0.7689		2.5E-05	1	0.776	0.3864	
Brown	*station*	0.5298	1	1.2418	0.2766		0.34	1	0.0781	0.7821		0.8769	1	1.0465	0.3157	
	depth	2.8684	1	6.7237	0.0163	*	6.73	1	1.5464	0.2248		11.7392	1	14.0107	0.0009	***
Cryptophytes	*station*	0.00086	1	0.0332	0.857		0.00	1	0.5561	0.4625		1.8E-05	1	0.0192	0.891	
	depth	0.04327	1	1.6679	0.2094		0.01	1	1.0129	0.3235		0.00156	1	1.7062	0.2029	

*<0.05, **<0.01, ***<0.001. For the abbreviations see the text.

Tab. 3S. Output of the statistics of the linear models testing the effect of PE, PC cyanobacteria and eukaryotes (EUK) on the concentration of TEP in the whole dataset and for each of the sampling dates.

TEP vs number (flow cytometry) whole study

	Estimate	Std. Error	t- value	p-value	
(Intercept)	-294.798	200.29	-1.472	0.18469	
PC	-27.546	32.426	-0.849	0.39816	
PE	85.855	25.194	3.408	0.00101	**
EUK	-23.119	20.028	-1.154	0.26601	

TEP vs number (flow cytometry) May

	Estimate	Std. Error	t value	p-value	
(Intercept)	-132.53	182.382	-0.727	0.474	
PC	-12.219	8.77	-1.393	0.175	
PE	15.856	9.37	1.692	0.103	
EUK	6.934	16.288	0.426	0.674	

TEP vs number (flow cytometry) July

	Estimate	Std. Error	t value	p-value	
(Intercept)	-71.886	10.629	-6.763	3.54E-07	***
PC	15.041	5.165	2.912	0.00728	**
PE	-6.181	3.243	-1.906	0.06779	
EUK	9.271	2.967	3.124	0.00434	**

TEP vs number (flow cytometry) September

	Estimate	Std. Error	t value	p-value	
(Intercept)	734.35	2068.497	0.355	0.7254	
PC	1.523	66.559	0.023	0.9819	
PE	174.885	66.322	2.637	0.0139	*
EUK	-167.813	188.582	-0.89	0.3817	

*<0.05, **<0.01, ***<0.001

Tab. 4S. Output of the statistics of the linear models testing the effect of total Chl on TEP concentration in the whole dataset and for each of the sampling dates. With asterisks the significant values.

TEP in relation to total chlorophyll in the whole study

	Estimate	Std. Error	t value	p-value	
(Intercept)	39.002	114.224	0.341	0.763	
total Chla	62.255	15.092	4.125	8.43E-05	***

TEP in relation to total chlorophyll in May

	Estimate	Std. Error	t value	p-value	
(Intercept)	20.12	7.023	2.865	0.00783	**
total Chla	18.459	5.632	3.277	0.0028	**

TEP in relation to total chlorophyll in July

	Estimate	Std. Error	t value	p-value	
(Intercept)	13.661	5.369	2.545	0.0167	*
total Chla	17.463	1.76	9.924	1.14E-10	***

TEP in relation to total chlorophyll in Sept

	Estimate	Std. Error	t value	p-value	
(Intercept)	7.331	67.275	0.109	0.914	
total Chla	249.395	44.733	5.575	5.78E-06	***

*<0.05, **<0.01, ***<0.001

Tab. 5S. Output of the statistics of the linear models testing the effect of chlorophyll of the different algal groups (measured by FluoroProbe) on the concentration of TEP in the whole dataset and for each of the sampling dates.

TEP in relation to different algae groups (fluoroprobe data) in the whole study

	Estimate	Std. Error	t- value	p-value	
(Intercept)	3.59602	0.65813	5.464	0.020102	*
Green	0.18375	0.17081	1.076	0.285372	
CyanoPE	0.58877	1.94607	0.303	0.763053	
Brown	0.27347	0.07041	3.884	0.000212	***
Cryptophytes	0.40458	0.95762	0.422	0.673822	

TEP in relation to different algae groups (fluoroprobe data) in May

	Estimate	Std. Error	t value	p-value	
(Intercept)	3.22008	0.19675	16.366	1.98E-13	***
Green	0.28964	0.12805	2.262	0.0344	*
CyanoPE	0.61278	1.32836	0.461	0.6493	
Brown	0.23449	0.14591	1.607	0.123	
Cryptophytes	-0.08522	0.5836	-0.146	0.8853	

TEP in relation to different algae groups (fluoroprobe data) in July

	Estimate	Std. Error	t value	p-value	
(Intercept)	3.7661	0.3017	12.483	5.50E-12	***
Green	-0.64262	0.49853	-1.289	0.21	
CyanoPE	0.66763	2.93718	0.227	0.822	
Brown	0.07647	0.05862	1.304	0.204	
Cryptophytes	-0.44892	1.35886	-0.33	0.744	

TEP in relation to different algae groups (fluoroprobe data) in September

	Estimate	Std. Error	t value	p-value	
(Intercept)	2.7774	0.515	5.393	1.54E-05	***
Green	1.1182	0.3346	3.342	0.00272	**
CyanoPE	-22.168	37.7322	-0.588	0.56235	
Brown	1.3066	0.2207	5.92	4.15E-06	***
Cryptophytes	3.862	7.1754	0.538	0.59537	

Green: Chlorophyceae, Euglenophyceae, Prasinophyceae; *Cyano PE*: Cyanophyceae phycoerythryn-rich. *Brown:* Dinophyceae, Bacillariophyceae, Fragillariophyceae, Mediophyceae, Chrysophyceae; *Cryptophytes:* Cryptophyceae; *<0.05, **<0.01, ***<0.001,

Tab. 6S. Eigenvalues and multiple coefficient correlations of Canonical Correspondence Analysis of environmental variables versus microbial groups for all the data 2019.

Axes	**1**	**2**	**3**
Eigenvalues	0.0186	0.0015	0.0003
Species-environment correlations	92.1	78	70
Cumulative percentage variance of species data	73.9	80.2	81.5
Cumulative percentage variance of species-environment relation	90.7	98.4	1

Total inertia 0.0252

Tab. 7S. Significant variables selected for May, July, September and all three months considered in the study. The Table contains the Akaike information criterion (AIC), the F statistic and the Probability.

May

Variable	Df	AIC	F	Pr(>F)
TEP	1	110.41	8.1904	0.010989
Temperature	1	110.99	8.7936	0.010989
TP	1	115.26	13.9262	0.003996
PAR	1	120.80	22.9903	0.000999
pH	1	123.85	29.5114	0.000999
Oxygen	1	126.22	35.4938	0.000999
Chl.tot	1	127.97	40.5405	0.000999

July

Variable	Df	AIC	F	Pr(>F)
DIC	1	140.70	5.5970	0.018981
pH	1	144.28	9.0122	0.007992
Depth	1	144.99	9.7827	0.005994
TOC	1	147.01	12.1763	0.005994
TP	1	147.34	12.5934	0.002997
PAR	1	153.11	21.5374	0.001998
TN	1	149.46	15.5124	0.000999

September

Variable	Df	AIC	F	Pr(>F)
TOC	1	156.40	4.5211	0.04695
PAR	1	158.29	5.9708	0.03996
Chl.tot	1	158.01	5.7394	0.03497
N.NO3	1	158.67	6.2774	0.03297
Oxygen	1	160.17	7.5847	0.02198
pH	1	161.24	8.5917	0.01598
TN	1	161.92	9.2685	0.01399

All data

Variable	Df	AIC	F	Pr(>F)
TP	1	460.63	5.4234	0.016983
Temperature	1	461.14	5.9239	0.009990
PAR	1	462.38	7.1481	0.005994
N.NO3	1	463.96	8.7604	0.002997
Chl.tot	1	465.39	10.2693	0.002997
TEP	1	479.58	27.8960	0.000999

Tab. 8S. Eigenvalues and multiple coefficient correlations of Canonical Correspondence Analysis of environmental variables *versus* microbial groups in May 2019.

Axes	**1**	**2**	**3**
Eigenvalues	4.072e-03	8.955e-05	7.004e-05
Species-environment correlations	99	94.5	76.5
Cumulative percentage variance of species data	93.2	95.2	96.8
Cumulative percentage variance of species-environment relation	96.2	98.3	1

Total inertia 0.0043

Tab. 9S. Eigenvalues and multiple coefficient correlations of Canonical Correspondence Analysis of environmental variables *versus* microbial groups in July 2019.

Axes	**1**	**2**	**3**
Eigenvalues	0.008407	0.001651	0.0001145
Species-environment correlations	96.1	95.5	87.2
Cumulative percentage variance of species data	76	90.9	92
Cumulative percentage variance of species-environment relation	82.6	98.8	1

Total inertia 0.0110

Tab. 10S. Eigenvalues and multiple coefficient correlations of Canonical Correspondence Analysis of environmental variables versus microbial groups in September 2019.

Axes	**1**	**2**	**3**
Eigenvalues	1.669e-02	1.879e-04	6.706e-06
Species-environment correlations	96.1	48.7	54.1
Cumulative percentage variance of species data	88.5	89.53	89.57
Cumulative percentage variance of species-environment relation	98.8	1	1

Total inertia 0.0188

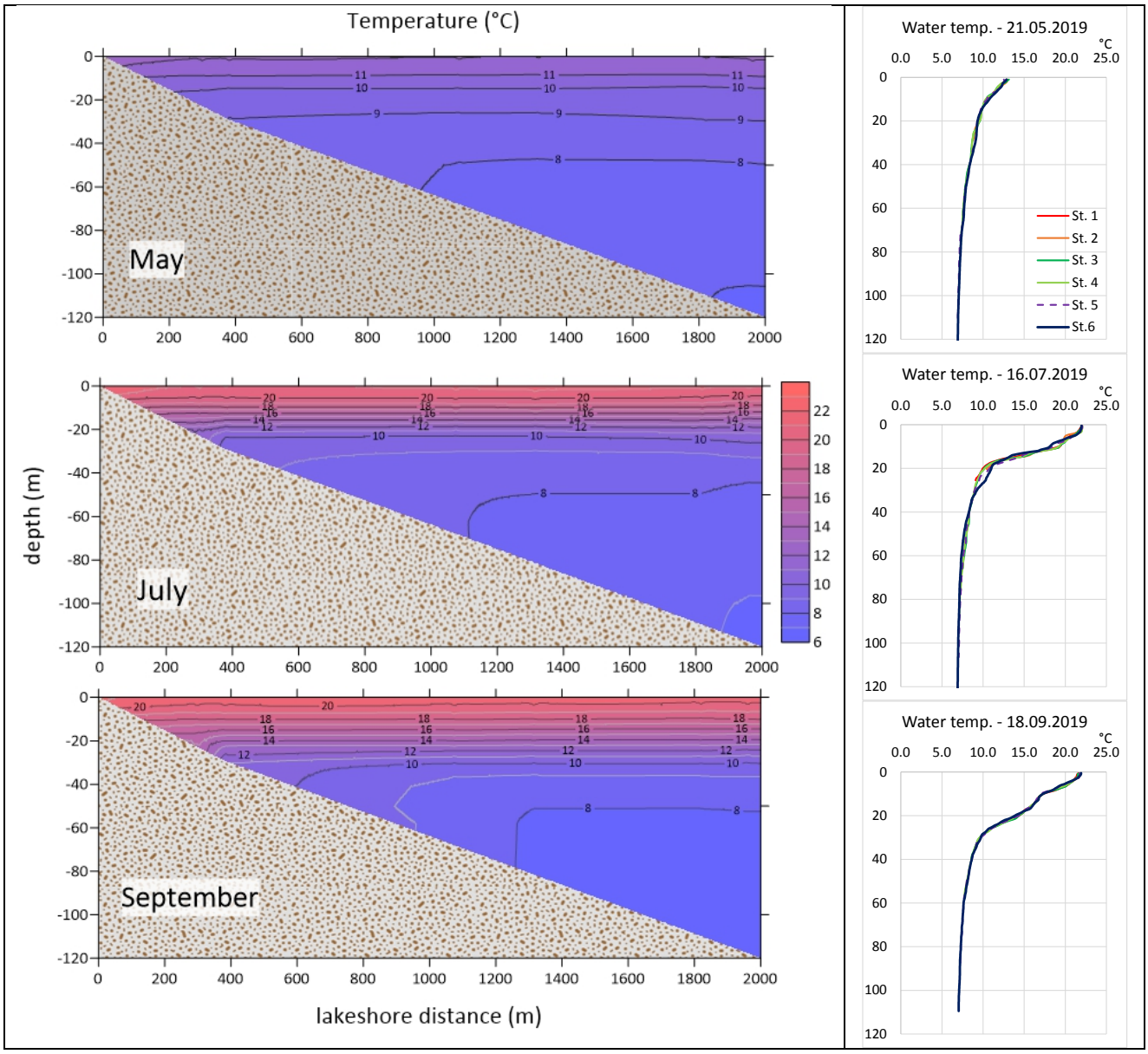

Fig. 1S. Temperature maps and profiles in May, July and September 2019 in the Borromeo basin of Lake Maggiore.

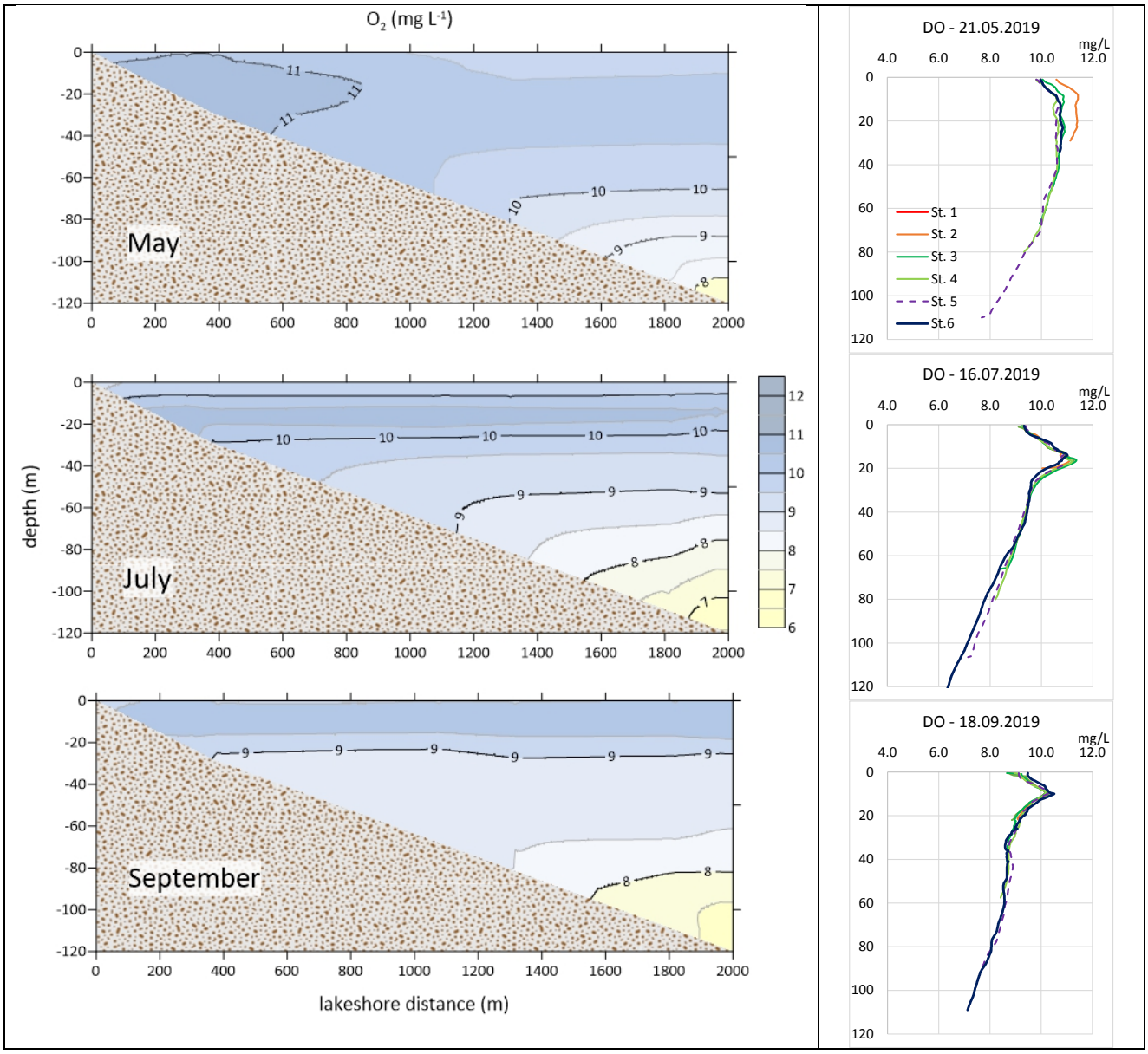

Fig. 2S. Dissolved oxygen (DO) maps and profiles in May, July and September 2019 in the Borromeo basin of Lake Maggiore.

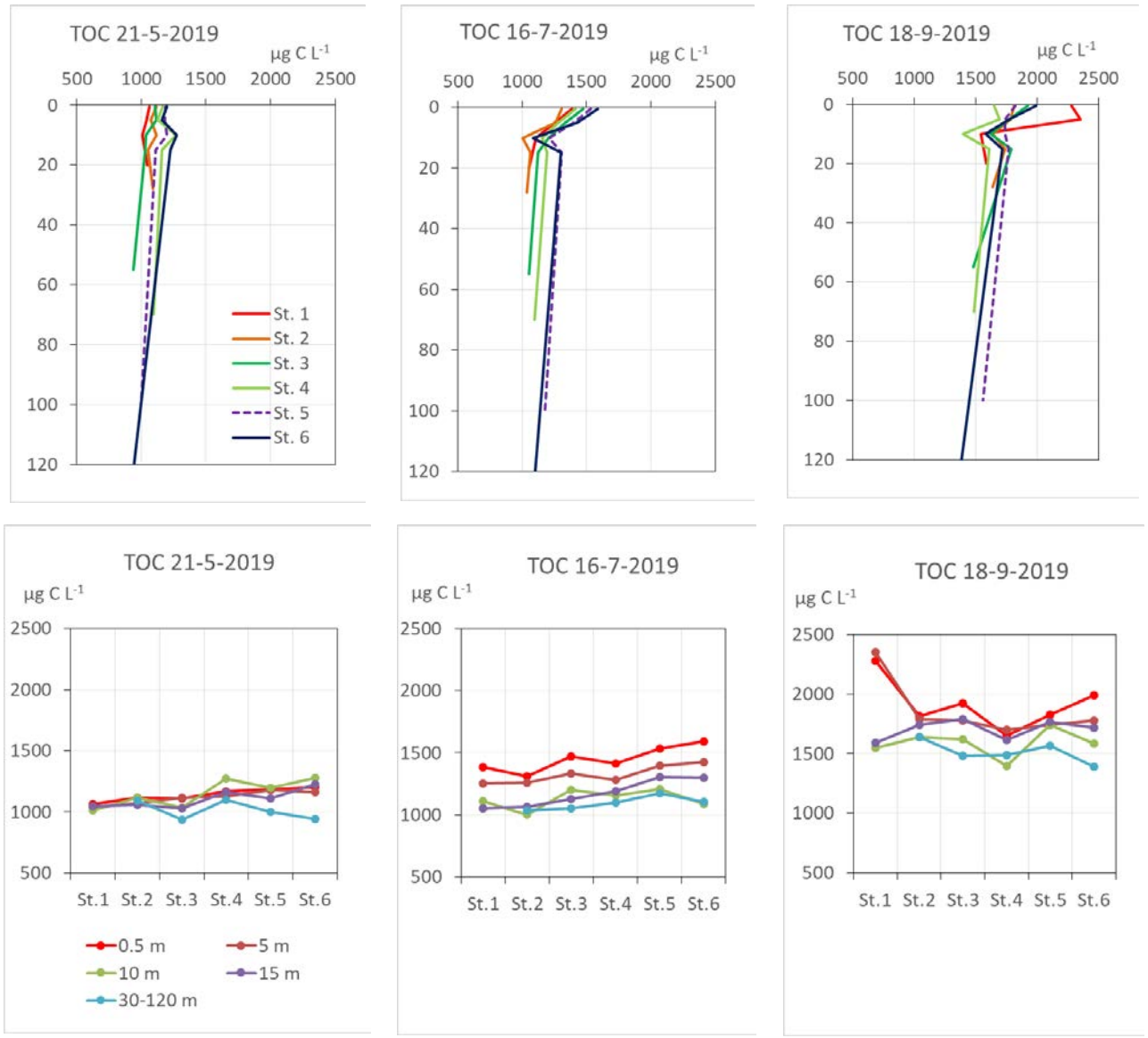

Fig. 3S. Vertical and horizontal profiles Total Organic Carbon (TOC) concentrations along the transect in Lake Maggiore, 2019.

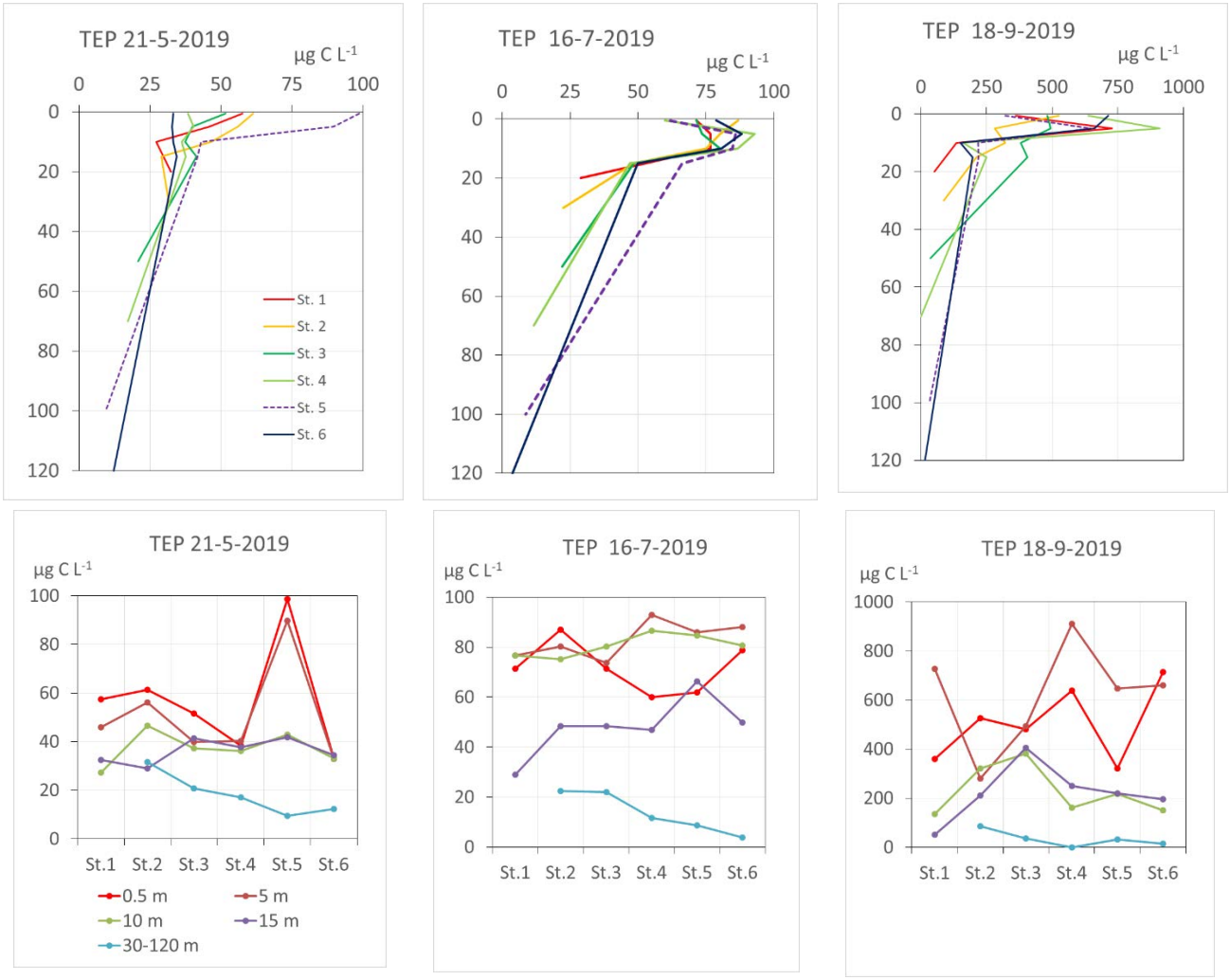

Fig. 4S. Vertical and horizontal profiles of Transparent Exopolymer Particles (TEP) concentrations along the transect in Lake Maggiore, 2019.

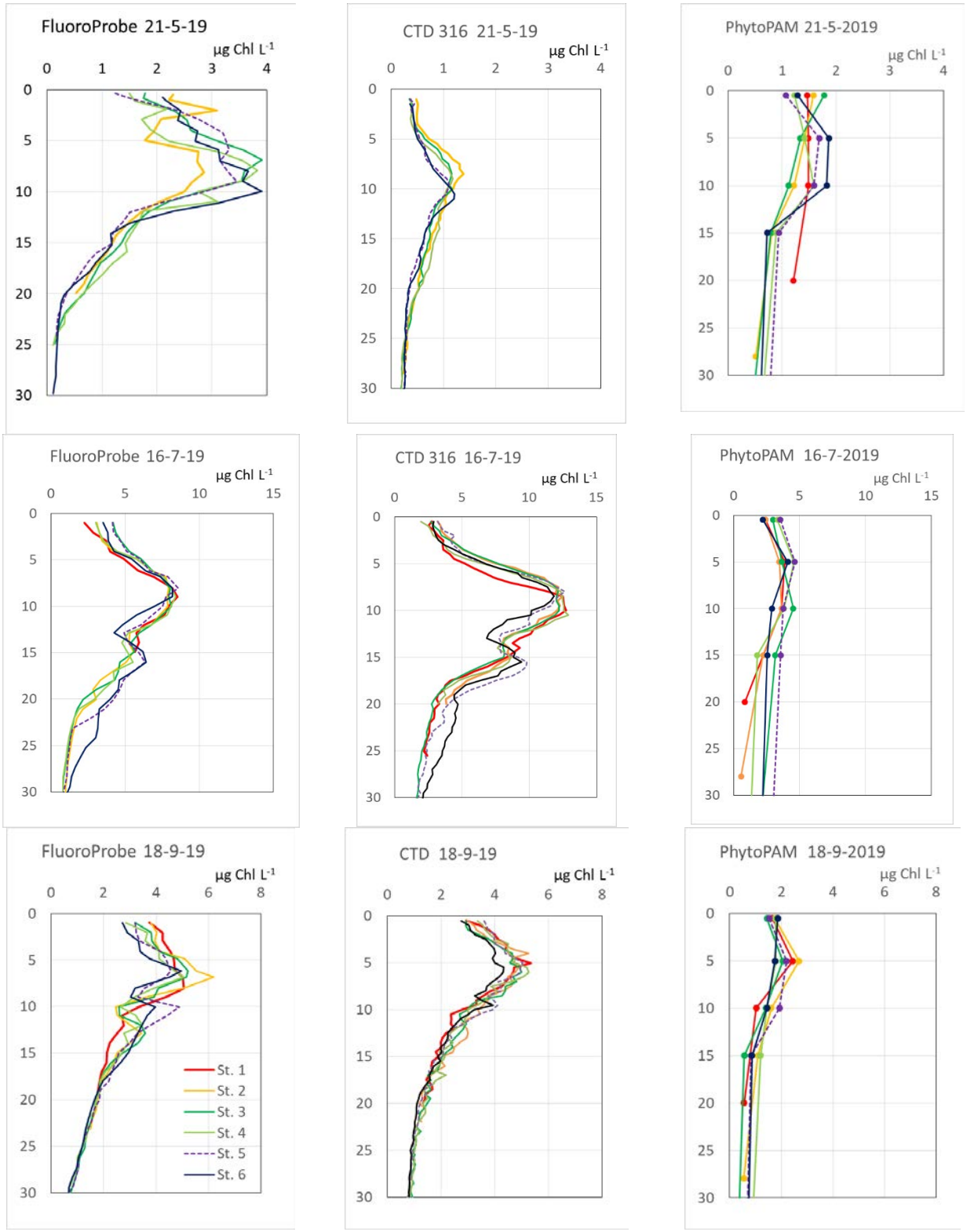

Fig. 5S. Results of the chlorophyll-*a* (Chl) measurements performed by different methods. The two *in situ* profiles by Fluoroprobe BBE and probe CTD 316 reveals a substantial similar vertical pattern but with different concentration values. In May, there was an overestimation of Chl values by the Fluoroprobe respect to the CTD 316, in July the opposite pattern was found and in September the two methods were comparable. These results indicate how the actual value of the Chl concentration is difficult to obtain with fluorescence *in situ* devices. The measures performed in

the laboratory by PhytoPAM are for discrete depths and consequently has the disadvantage to skip the eventual concentration maxima. In any case, the PhytoPAM gives generally an underestimation of the Chl concentration with respect to the *in situ* probes, particularly evident in July.

Green: Chlorophyceae, Euglenophyceae, Prasinophyceae

Brown: Dinophyceae, Bacillariophyceae, Fragillariophyceae, Mediophyceae, Chrysophyceae

Cryptophyta: Cryptophyceae

Cyano PE: Cyanophyceae phycoerythryn-rich

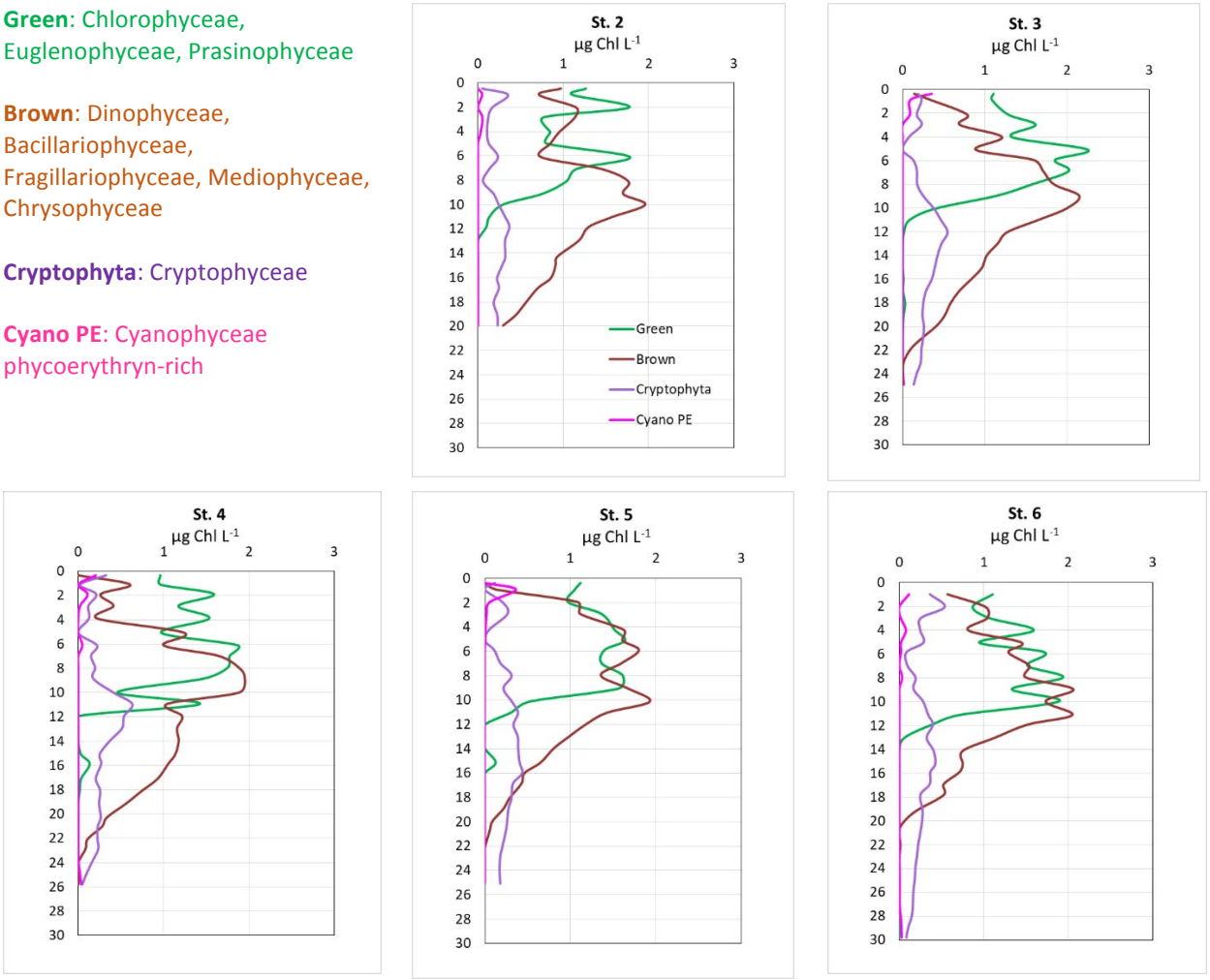

Fig. 6S. Profiles of Chlorophyll-*a* concentrations derived from the different algal groups (see legend) in the six stations along the transect, in Lake Maggiore on 21-5-2019.

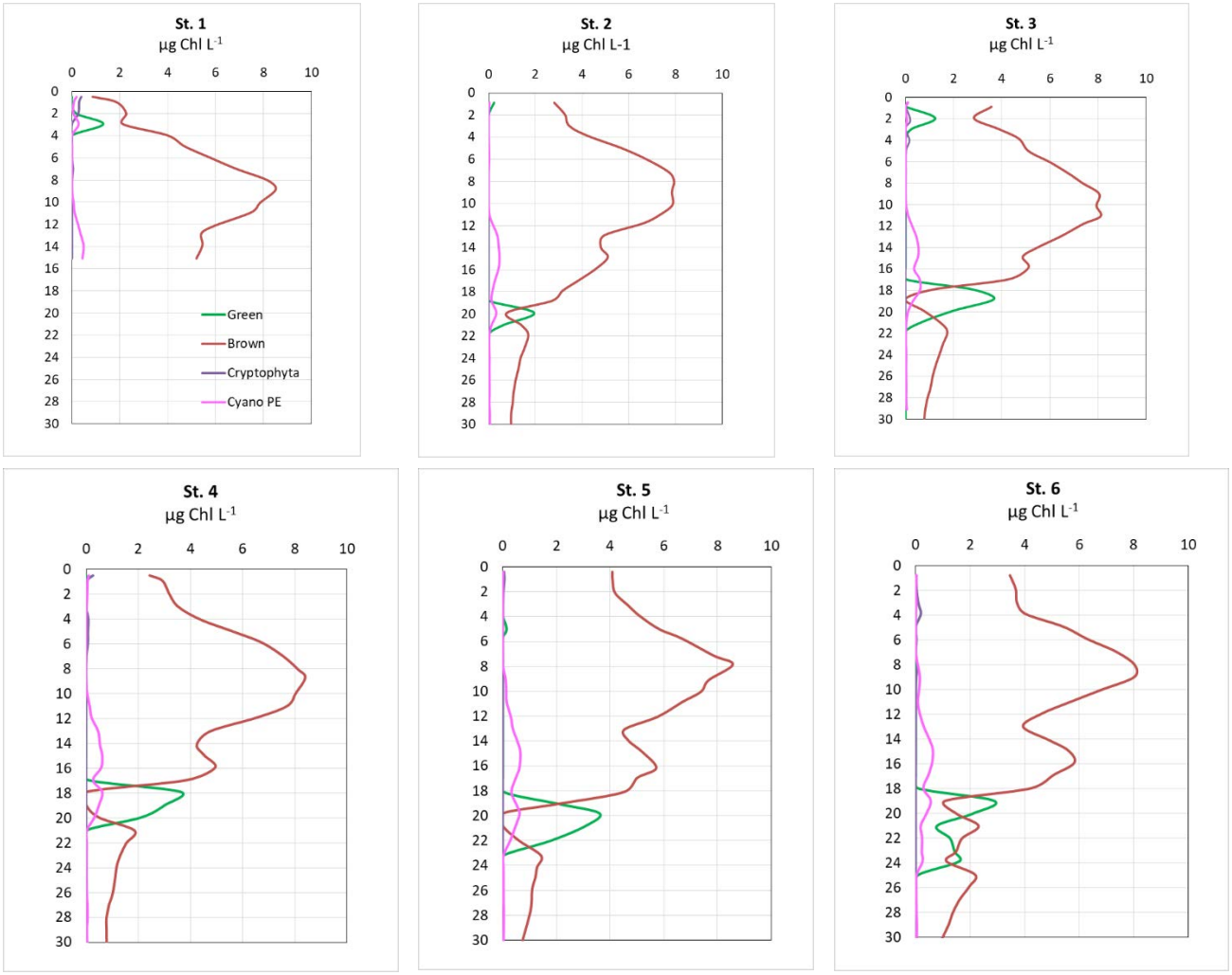

Fig. 7S. Profiles of Chlorophyll-*a* concentrations derived from the different algal groups (see legend of Fig. 5) in the six stations along the transect, in Lake Maggiore on 16-7-2019.

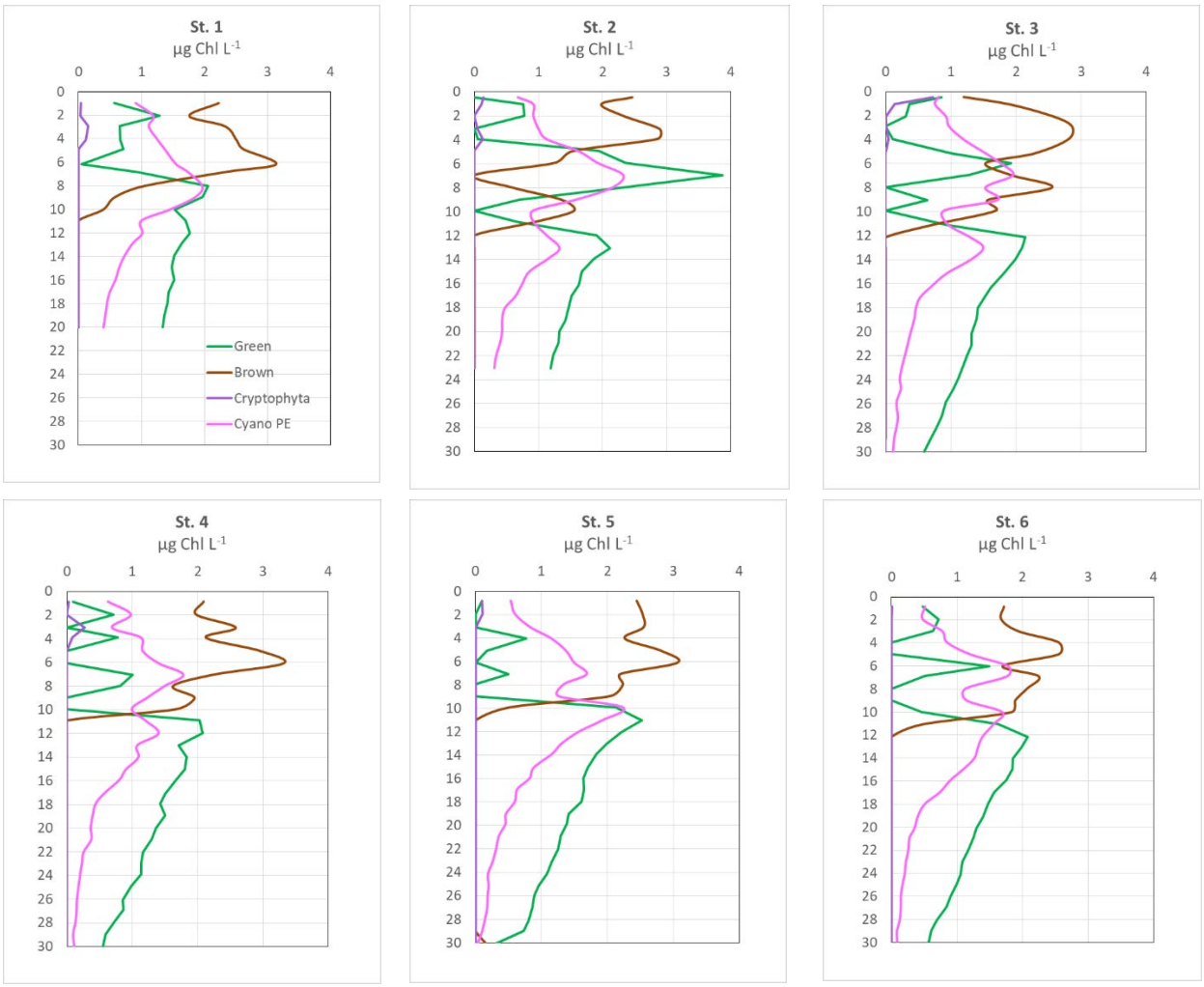

Fig. 8S. Profiles of Chlorophyll-*a* concentrations derived from the different algal groups (see legend of Fig. 5) in the six stations along the transect, in Lake Maggiore on 18-9-2019.

Fig. 9S. Microscope counts of phytoplankton taxa done at 5 m and 15 m in June 2019 (not much later than the first transect of May), in July and September at the transect (St. 1: 10m and St. 5: 15 m). The counts were done to evaluate the results of the FluoroProbe in the community composition. The presence of Chrysophyceae and Diatoms in spring, of Diatoms, Chlorophyceae and Cryptophyceae in July and the dominance of Cyanophyceae and Cryptophyceae in September validates the results obtained with Fluoroprobe.

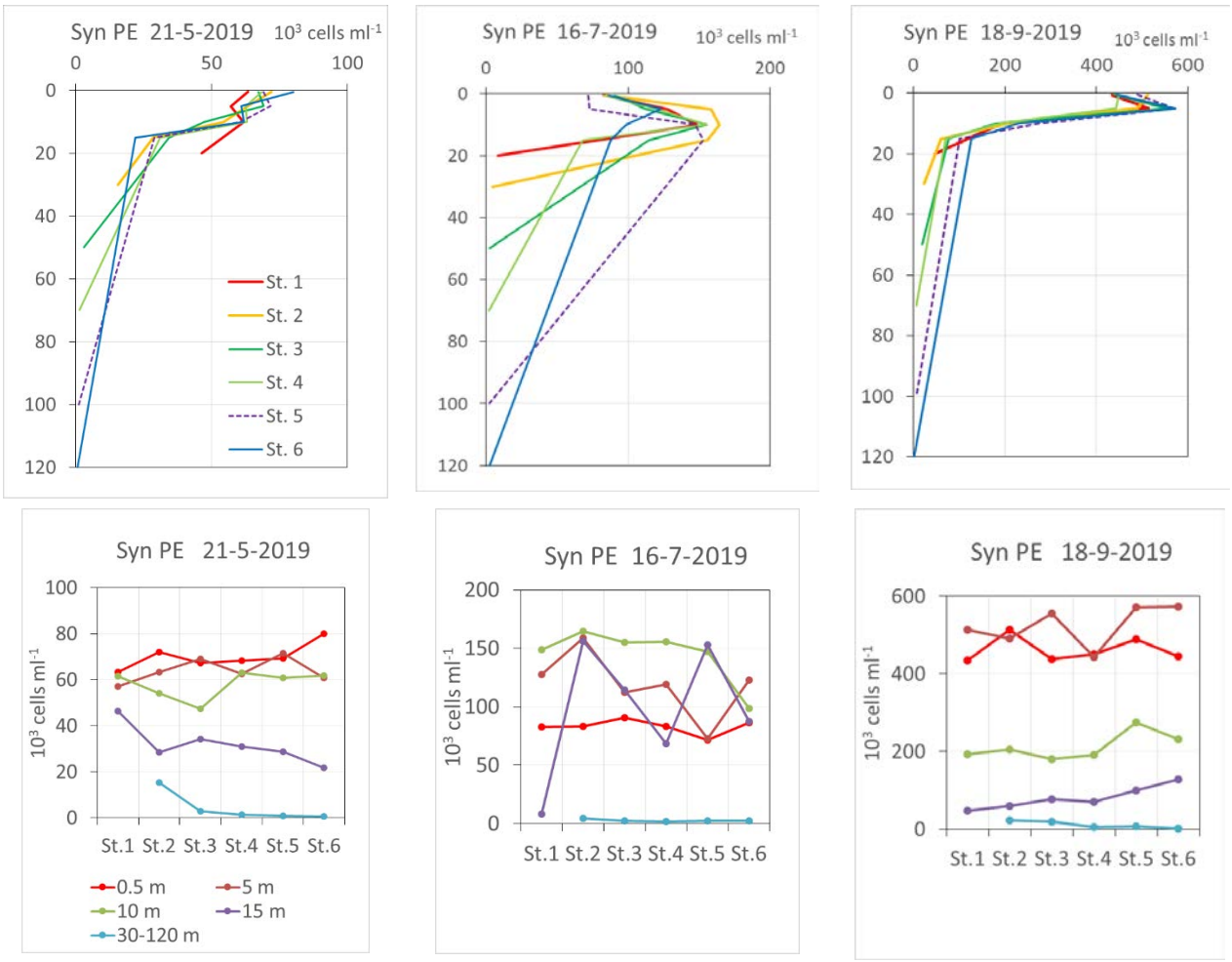

Fig. 10S. Vertical and horizontal profiles of the phycoerythrin-rich picocyanobacteria (PE) number along the transect of Lake Maggiore in May, July and September 2019.

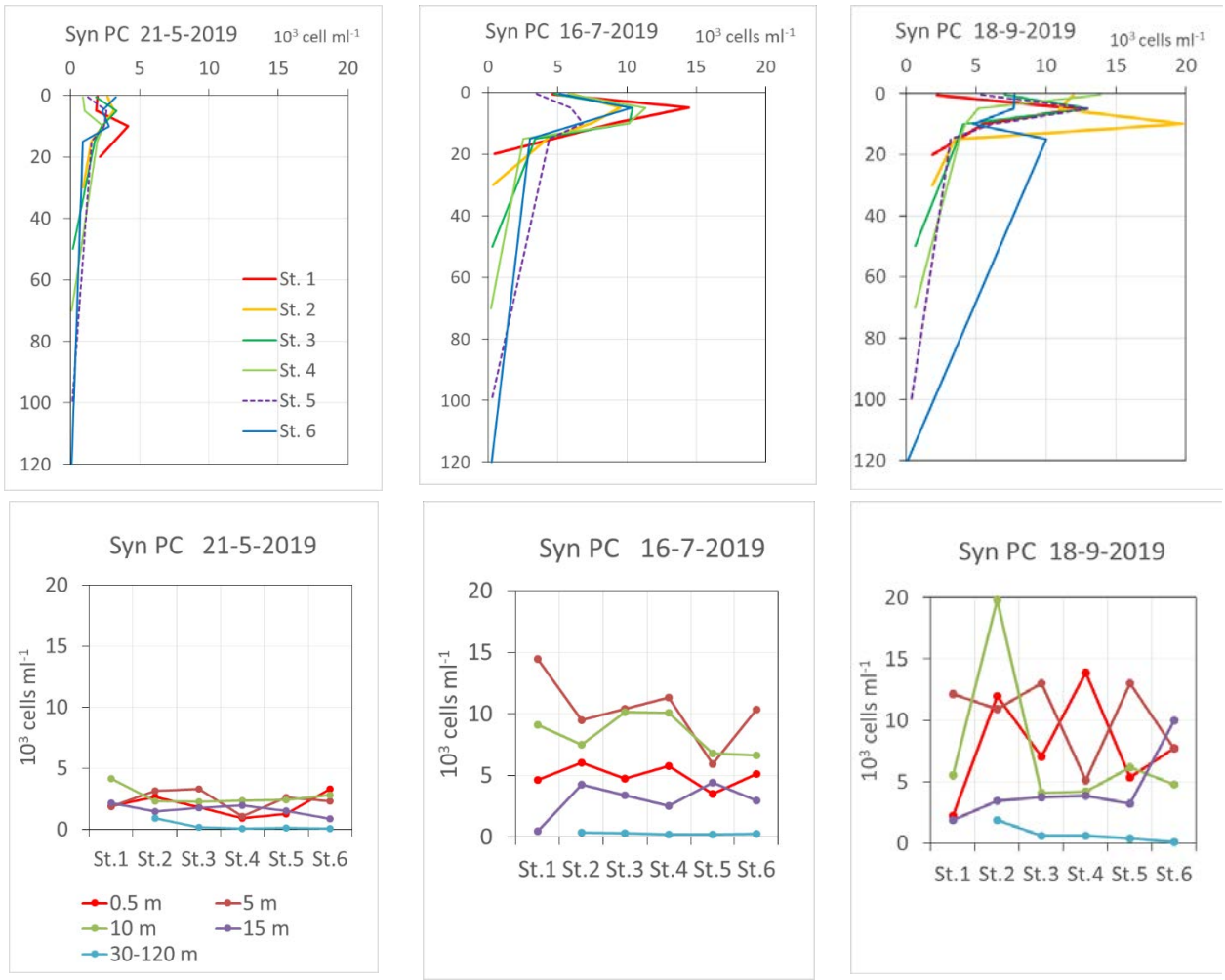

Fig. 11S. Vertical and horizontal profiles of the phycocyanin-rich picocyanobacteria (PC) number along the transect of Lake Maggiore in May, July and September 2019.

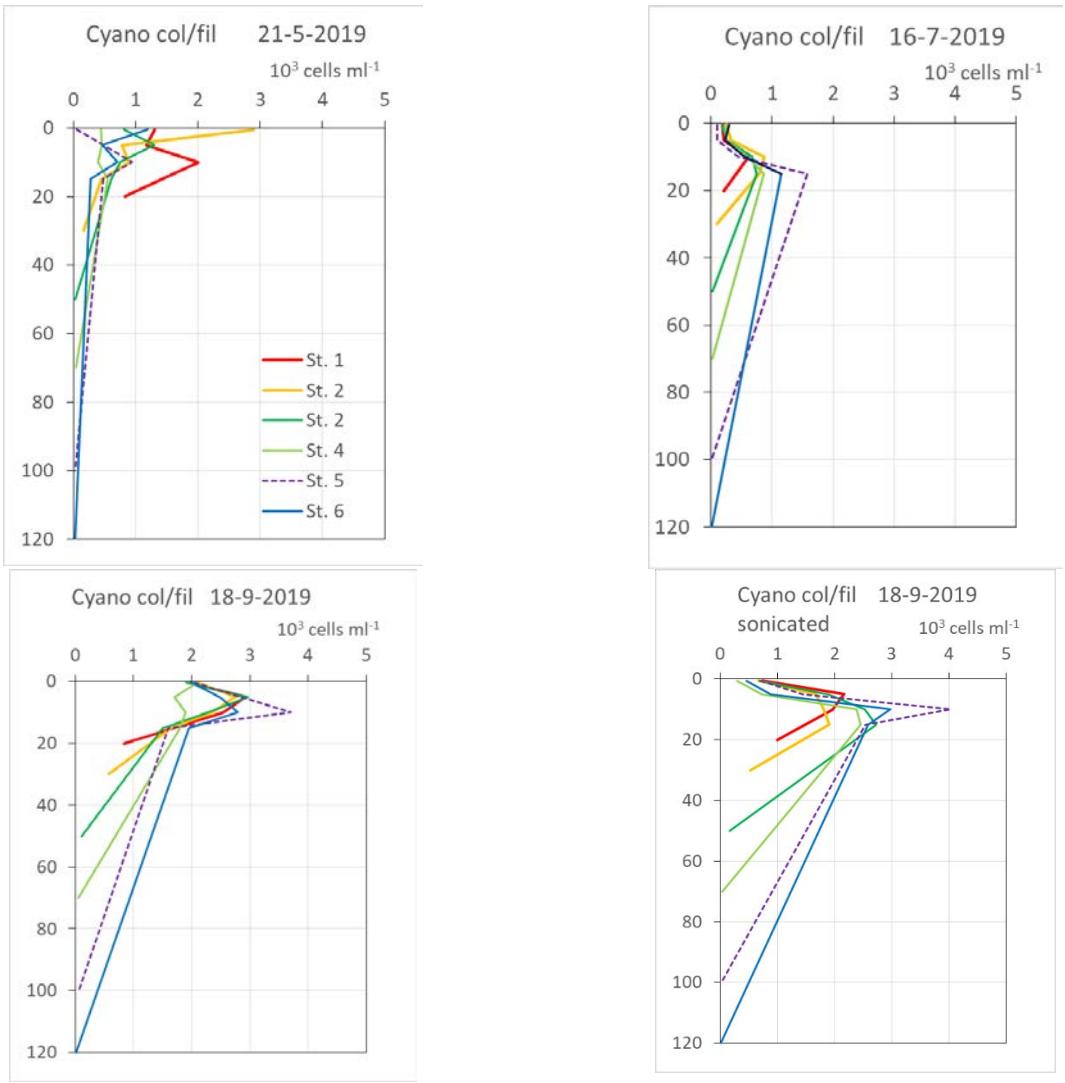

Fig. 12S. Profiles of the number of colonial and filamentous Cyanobacteria as counted by flow cytometry along the transect of Lake Maggiore in May, July and September 2019. In September, in presence of many colonies, the samples were counted before and after sonication.

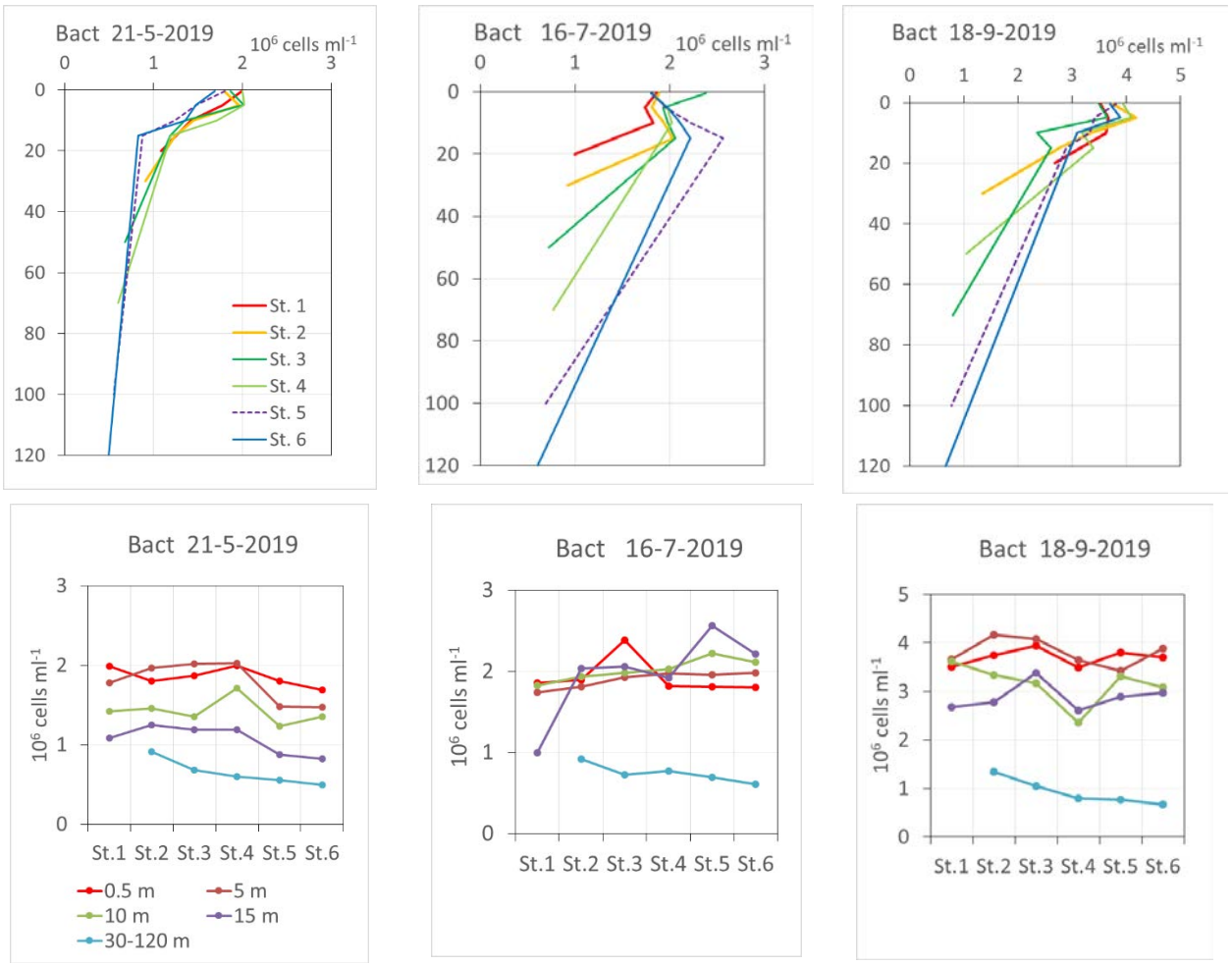

Fig. 13S. Vertical and horizontal profiles of the heterotrophic bacteria (Bact) number along the transect of Lake Maggiore in May, July and September 2019.

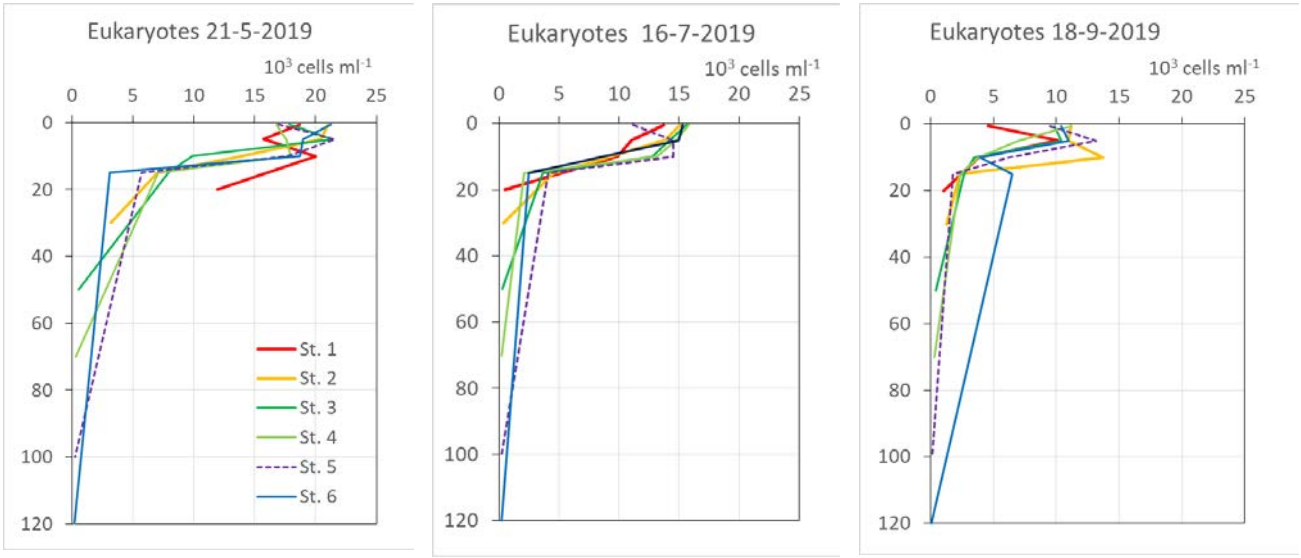

Fig. 14S. Vertical profiles of the number of eukaryotic pico and nano-phytoplankton as counted by flow cytometry along the transect of Lake Maggiore in May, July and September 2019.

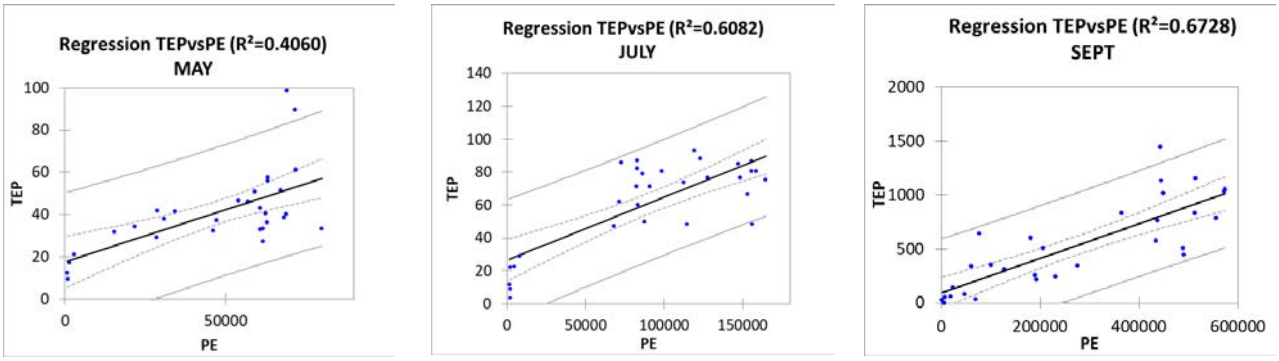

Fig. 15S. Regression of TEP versus PE number in the three months. Confidence interval of 95% of the mean is marked in dashed line.