EXPERIMENTAL DETERMINATION OF THE CARBON BIOMASS OF NATURAL PHYTOPLANKTON

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A method for experimental determination of carbon biomass of natural phytoplankton is described. The procedure entails comparison between functional parameters of phytoplankton – measured by the traditional methods carbon and chlorophyll production. The proposed approach permits to evaluate carbon/chlorophyll ratio and hence to compute carbon biomass of phytoplankton through chlorophyll content estimates.

Introduction. Investigations of functioning of pelagic ecosystems require reliable and easy–to–apply methods for fast determination of phytoplankton biomass and production. However, unlike primary production (PP) easily assessed by ^{14}C [1], measuring phytoplankton biomass presents difficulties. Usually, carbon biomass is computed from the number and dimensions of phytoplankton cells in fixed samples through time–consuming determinations under a microscope. The alternative is costly and therefore often inaccessible measuring by a flow–through fluorometer.

Attempts to apply ^{14}C–method to experimentally determine phytoplankton biomass have been repeatedly made [2, 3]. However, the proposed procedures are too complicated to have been a common practice. We propose to assess phytoplankton biomass through carbon–to–chlorophyll *a* ratio (C/Chl *a*) obtained from comparison of functional parameters of phytoplankton: synchronously measured carbon (C) and chlorophyll *a* (chl *a*) production. Knowing C/Chl *a* estimates, it is easy to compute the biomass of phytoplankton from chl *a* concentrations. Classic methods can provide the methodological platform. In particular, carbon production can be measured by ^{14}C–method for determination of PP [1] and chlorophyll production – by some procedures of dilution method [4].

V.D. Tchmyr first proposed the idea of the method described below at the sessions held in the department of algal ecophysiology, Institute of Biology of the Southern Seas (IBSS) during 2003–2004. The experiments with ^{14}C which are presented in this publication were conducted in September 2005. Preliminary results of the investigation were reported at the international conference held in Sevastopol in September, 2006 [5].

Materials and methods. Samples of sea water were collected from the sea surface in the mouth of Sevastopol bay on September 7, 2005. The temperature of the sea water was 22°C and the salinity 17.58‰. Initial concentration of chlorophyll *a* in the samples was 0.922 mg/m^3.

For the series of dilution experiments sea water was filtered onto *Sartorius* membrane filters. Before use the filters were thrice boiled to remove bactericidal filling. Sea water was filtered at first through the filters with 3– and then 0.45–mcm pore size. The portion of unfiltered sea water used in the experimental series made up 0; 0.05; 0.10; 0.18; 0.25; 0.50; 0.75 and 1.0 of the total sample volume.

Experiments were performed in 3 *l* flasks exposed close to the eastern glass wall of the laboratory during September 8–13 at near–*in situ* temperature. On September 8, the solution of $Na_2C^{14}O_3$ (10 μCi/*l*) was injected into six of 8 experimental flasks. In our computations we used the estimates of chl *a* concentration and PP measured on September 9 and 12. On September 12, the samples were exposed to dark for 24 hours; after that period of time chlorophyll elimination was measured.

Chlorophyll *a* concentrations in acetone extracts were determined on a laboratory fluorometer assembled from a Specol fluorometric accessory [6]. In assessing PP the levels of radioactivity were measured with a *Rack–Beta Spectral* radiometer. *Synpore* filters (0.3–mcm pore size) were used in measuring PP and chl *a* concentrations.

To compute the phytoplankton biomass under the natural abundance the water was first investigated in a "living drop" and then in a non–fixed samples condensed through reverse filtration.

The autotrophic cells were counted in blue–and–violet rays under a luminescent microscope ML–2 [7]. Only the cells with characteristic red or orange fluorescence were counted. Heterotrophic chlorophyll–free cells produced bright–green fluorescence.

Carbon biomass of the phytoplankton was computed from Strathmann equations [8].

Results. Experimental data are presented at the tables 1 – 3. Table 1 gives chl *a* concentration measured on September 9, 12 and 13 in 8 samples with different dilutions of sea water, the corresponding estimates of the apparent growth rate and elimination rate, and the actual growth rates. Though the apparent growth and elimination estimates considerably fluctuated in different samples, the actual growth rates of phytoplankton were relatively similar as the relevant mean and statistical characteristics point out. Estimates of the growth and elimination rates observed during the experiment allow to compute the mean chl *a* concentrations (X_M) for the 3-day exposition in accord with the equation given in [9]:

Table 1 — Chlorophyll *a* (X, mcg·m^{-3}) content during 3–day normal dark/light exposition and chlorophyll *a* elimination rate (m) during 1 – day dark exposition in experiments with different dilutions. Sevastopol Bay mouth, September 2005

Unfiltered/filtered sea water	X_0, 09.IX	X_t, 12.IX	X'_t, 13.IX	μ', $Ln(X_t/X_0)\cdot t^{-1}$, day^{-1}	m, $Ln(X'_t/X_t)\cdot t^{-1}$, day^{-1}	μ, day^{-1}
0	26	79	73	0.371	–0.071	0.442
0.05	88	155	140	0.188	–0.102	0.290
0.10	139	161	101	0.049	–0.463	0.512
0.18	125	314	205	0.306	–0.427	0.733
0.25	280	408	304	0.125	–0.293	0.418
0.50	366	781	548	0.252	–0.355	0.607
0.75	588	1339	1186	0.279	–0.121	0.400
1.00	882	2410	1988	0.334	–0.192	0.526
Mean						0.491
n						8
s						0.136
σ						0.128

X_0 – initial chlorophyll *a* concentration, X_t – final chlorophyll *a* concentration, X'_t – chlorophyll *a* concentration after dark exposition, μ' – apparent chlorophyll *a* growth rate, μ – actual chlorophyll *a* growth rate

Table 2 — Carbon/Chlorophyll *a* ratio calculation as a result of the comparison of the parallel values of phytoplankton carbon and chlorophyll *a* production in the same sample (the same bottle). X_M – mean chlorophyll *a* concentration during exposition

Unfiltered/filtered sea water	X_M, mcg/m^3	Production, mcg/m$^3\cdot$ day^{-1}		C/Chl *a*
		Chl *a*	C	
0	48	21	709	33.8
0.05	119	34	1686	49.6
0.10	152	78	1786	22.9
0.18	206	151	4870	32.3
0.25	339	142	8631	60.8
0.50	554	333	15141	45.5
0.75	907	363	–	–
1.00	1511	801	–	–
Mean				40.82
n				6
s				13.72
σ				12.52

Table 3 — Comparison of the parallel values of phytoplankton carbon production (ΔC) and chlorophyll *a* apparent growth (ΔX) at the end of 3-day exposition. Other symbols look at the table 1

Unfiltered/filtered sea water	$X_t - X_0$, ΔX, mcg/m^3	PP, ΔC, mcgC/m^3	y	x
			ΔC/ΔX	$\Delta X/X_t$
0	53	2126	40	0.67
0.05	67	5059	76	0.43
0.10	22	5358	244	0.14
0.18	189	14611	77	0.60
0.25	128	25893	202	0.31
0.50	415	45423	109	0.53

$$X_M = X_0 \cdot [e^{(\mu - m)t} - 1] / (\mu - m) \cdot t, \quad (1)$$

where μ is the chl *a* growth rate (d^{-1}), m – the chl *a* elimination rate (d^{-1}), t – exposition time (d), X_0 – initial concentration of chl *a*. Knowing X_M, one can compute daily production of chlorophyll (X): $P_X = X_M \cdot \mu$. Comparing the

resulting estimate with that of carbon production, we have calculated C/Chl *a* ratio for six of the 8 performed experiments. Table 2 summarizes results of the calculations including the mean of C/Chl *a* ratio evaluated 40.82 mg C/mg chl *a*.

Results of the experiment provide another path for computing C/Chl *a* ratio – through comparison between apparent estimates of the ratio and relative estimates of the chl *a* apparent growth rates in the experiments. Table 3 summarizes the data used for the computations: measured in six experiments apparent chlorophyll growth for 3-days ($\Delta X = X_t - X_0$) and carbon production for the same period of time (ΔC) equal to the PP yielded for the 3-day exposition. Relative estimates of the apparent growth of chl *a* concentrations represented as $\Delta X/X_t$ and the apparent estimates of C/Chl *a* ratio represented as $\Delta C/\Delta X$ are also given in Table 3. Relative estimates of the apparent growth of chlorophyll content ($\Delta X/X_t$) observed in the experiments are related to the rate of phytoplankton elimination. The less grazing impact in the experiment, the higher value of $\Delta X/X_t$, that under maximum dilution and in the absence of grazing impact approximates 1 ($\Delta X = X_t$).

Estimates of the apparent ratios $\Delta C/\Delta X$ make inverse relationship ($R^2 = 0{,}746$) with estimates of $\Delta X/X_t$. The latter increasing from 0.14 to 0.67, the values of $\Delta C/\Delta X$ drop from 244 to 40 mcg C/mcg chl *a*. Applying logarithmic scale and placing the estimates of $\Delta X/X_t$ on the axis *X* and those of $\Delta C/\Delta X$ on the axis *Y*, one infers the power function $Y \cdot X$:

$$Y = 41.197 \cdot X^{-1.004}. \qquad (2)$$

Table 4 — Phytoplankton species composition and biomass at the mouth of Sevastopol Bay. September, 2005

Species composition	N	V	B	C
Autotrophs				
Dactilyosolen fragilissima	114231	3634	415.1	22.00
Pseudonitzschia delicatissima	96657	750	72.5	5.626
Chaetoceros compressus	58580	1057	61.9	4.426
Pseudosolenia calcaravis	609	83251	50.7	1.273
Cyclotella caspica	14645	2526	37	2.146
Chaetoceros affinis	17574	240	21.8	1.759
Chaetoceros insignis	93728	95	8.9	1.135
Proboscia alata	144	47126	8.2	0.235
Thalassionema nitzschiodes	5858	597	3.5	0.287
Nitzschia tenuirostris	8787	205	1.8	0.191
Another (3 species)	175	743	1.3	0.101
Total Bacillariophyta				39.179
Emiliania huxleyi	8816	544	4.8	0.742
Small Flagellatae	8786	273	2.4	0.405
Hermesinum adriaticum	29	10345	0.3	0.032
Distephanus speculum	29	3448	0.1	0.012
Ceratium tripos	29	200000	5.8	0.415
Cysts (Dinophyta)	50	25862	1.5	0.14
Total autotrophs				40.925
Heterotrophs				
Dinophyta				
Peridinium breve	6032	9101	54.9	5.874
Hatodinium lenticula	377	23342	8.8	0.833
Protoperidinium diversus	58	86207	5	0.4
Glenodinium paululum	2929	819	2.4	0.35
Prorocentrum compressus	29	17246	0.5	0.049
Total heterotrophs				7.506
Total phytoplankton				48.431

N — phytoplankton quantity, cells/*l*; V – cell volume, mcm^3; B – raw biomass, mg/m^3; C – carbon biomass, mg/m^3

Interpolating the regression line to $\Delta X/X_t = 1$, one deduces the value of $\Delta C/\Delta X$, which corresponds to the actual carbon–to–chlorophyll *a* ratio of the phytoplankton. The plot (Fig.1) evaluates this ratio 41.197 mg C/mg chl *a* that is close to the average 40.82 mg C/mg chl *a* in Table 2.

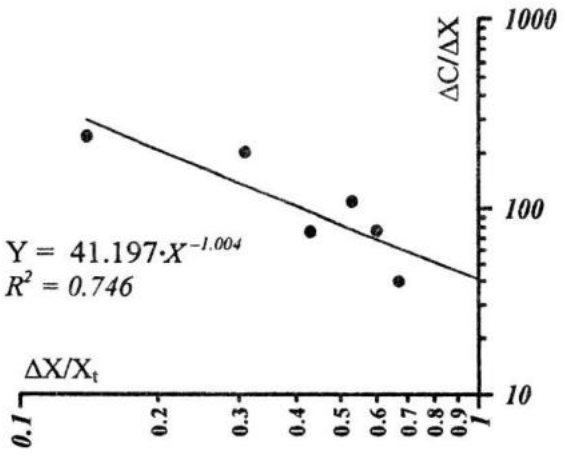

Figure 1 — Relationship between carbon production - to - chlorophyll apparent growth ratio ($\Delta C/\Delta X$) and chlorophyll apparent growth - to - final chlorophyll concentration ratio ($\Delta X/X_t$), in experiments with different dilutions

Table 4 shows the species composition and biomass of phytoplankton. Examination under a luminescent microscope points out that autotrophic phytoplakton are predominantly Bacillariophyta (13 species) with only a few Chlorophyta, Chrysophyta and Prymnesiophyta. Biomass is mostly owing to large diatoms – primarily, *Dactilyosolen fragilissima* – and some other. Of six Dinophyta only one – large *Ceratium tripos* – is autotrophic. Total carbon biomass of the autotrophic phytoplankton makes up 40.92 mg/m^3 and the ratio C/Chl *a* – 44.39.

Conclusion. In accord with the C/Chl *a* ratio estimated in the experiment as 40.82 – 41.20, the corresponding values of carbon biomass are 37.64 – 37.98 mg/m^3, or 92–93% of that instrumentally measured, that is a reliable correspondence for present comparison. Results of the experiment suggest that when modified, the proposed technique can be used as an express–method for simultaneous determination of not only carbon biomass and production of natural phytplankton but also of the rates of its growth and elimination.

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