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CALCULATION OF THE PHOTOSYNTHETIC QUOTIENT (PQ) IN THE GULF OF GDAŃSK (SOUTHERN BALTIC)

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Abstract

The photosynthetic quotient (PQ) is defined as the molar ratio of oxygen released to the carbon dioxide assimilated during photosynthesis. Calculation of correct PQ values of natural phytoplankton populations is crucial for understanding of carbon budgets but often result in values that differ highly from the expected stoichiometric proportions of photosynthetic products. In the present study, measurements of primary production, that is, CO_2 assimilation, performed with the standard isotopic method (^{14}C) and oxygen release estimated during photosynthesis with the light-and-dark bottle method were compared to calculate PQ in the southern Baltic (Gulf of Gdańsk, Poland). The PQ average was 1.28 (ANOVA; $F = 872$; $R^2 = 0.92$; $n = 77$; $p < 0.001$). Neither nitrogen source nor phytoplankton composition was noted to impact PQ values. Very high PQ values in the lower range of production rates (that cannot be explained by the stoichiometric proportion of photosynthesis products) were interpreted as artifacts related to the lower sensitivity of the oxygen method compared to that of the ^{14}C method.

Key words: photosynthetic quotient, primary production, Gulf of Gdańsk, Baltic Sea, conversion, methods

INTRODUCTION

Phytoplankton photosynthesis is a basic process affecting carbon fluxes within an ecosystem and its correct measurement is crucial to understanding of local environmental processes, and global carbon budgets of any water body (e.g. Nędzarek et al.

2013, Arst et al. 2008). However, it is known that estimates derived from different methods used are not directly comparable (Laws 1991, Marra 2009, Regaudie-de-Gioux et al. 2014, Williams and Robertson 1991). Here, we present comparison of two most frequently used methods: a standard isotopic method (^{14}C) and oxygen production estimation with light-and-dark bottles. We search for a proper conversion factor (a photosynthetic quotient) applicable for the local environment and an explanation to the differences in estimations derived from different methods.

The photosynthetic quotient (PQ) is defined as the molar ratio of oxygen released to carbon dioxide assimilated via photosynthesis. Over longer time periods, it is assumed that PQ falls within the range expected from the stoichiometric proportions of photosynthetic products.

PQ depends primarily on the composition of the organic matter produced. PQ ranges from 1.09 to 1.48, averaging about 1.20, when the relative stoichiometric composition of the photosynthetic products of carbon, hydrogen, and oxygen atoms is observed (Ryther 1956). The lowest PQ values are 0.625 (glycolate production). Glucose production results in an O_2/C ratio of 1, and maximum PQ is 1.78 with nucleic acid production (Williams and Robertson 1991).

PQ is also known to vary as a function of the N substrate utilized. If the plants obtain their nitrogen from ammonia, the PQ can be lower since the nitrogen is already reduced. If nitrate is the main source of nitrogen, then PQ will be higher because nitrate reduction releases two oxygen molecules per atom of nitrogen assimilated (Ryther 1956, Williams and Robertson 1991). Assuming "typical" photosynthetic production (for "typical" cell composition), Williams and Robertson (1991) calculated a PQ value of 1.08 for ammonium as a nitrogen source and 1.34 for nitrate. Laws (1991) calculated similar values: 1.1 ±0.1 for ammonium as the nitrogen source, and 1.4 ±0.1 for nitrate. The discrepancies between the oxygen and ^{14}C primary production measurement approach still raises questions about the uncertainty of each of the techniques used in primary production estimations (Geider and MacIntyre 2002, Marra 2009).

Calculations of the correct PQ value of natural phytoplankton populations often result in values that differ from the expected stoichiometric proportions of photosynthetic products because PQ probably depends on many physiological and environmental factors. In the present study, we calculated the PQ values applicable to the carbon and oxygen budget in the region of the southern Baltic. The aim of this attempt was to quantify the applicable PQ values and to identify possible factors that might influence PQ calculations. Some possible methodological problems with measurements are discussed.

MATERIAL AND METHODS

In the present work, we analyzed parallel measurements of primary production. Carbon uptake was measured with ^{14}C isotope simultaneously with measurements of oxygen release during photosynthesis with the light-and-dark bottles method. Research was conducted during two cruises of the r/v *Baltica* in the Gdańsk Basin

(southern Baltic Sea, Fig. 1). The first cruise took place between June 17-28, 2002 (stations: P63, G2, P110, E64, E52a) and the second one was between June 14-24, 2005 (stations: P63, G2, E60, M1). The stations stretched from the area close to the Vistula River mouth (at distances of several km, e.g., E52a, M1) toward open waters of the Gulf of Gdańsk (at distances of up to 120 km, e.g., P63 (Fig. 1)). Primary production was measured *in situ* at different depths.

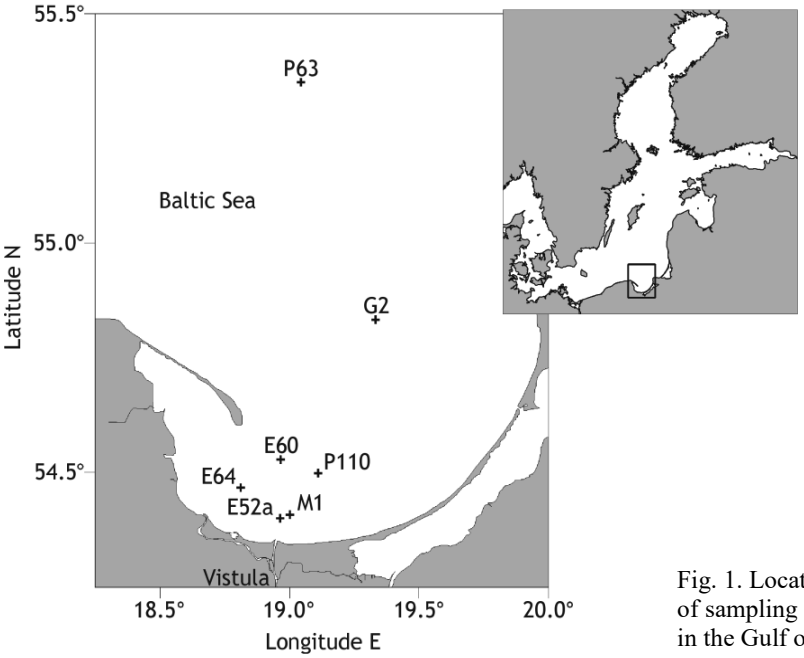

Fig. 1. Location of sampling stations in the Gulf of Gdańsk

Samples were collected with a 5 dm^3 water sampler (General Oceanic Multi Water Sampler) combined with a Niels Brown CTD. Every time, the water was sampled at respective depths, and primary production was simultaneously measured *in situ* with two methods: (i) CO_2 assimilation was assessed with the standard isotopic method (^{14}C) (Steeman Nielsen 1952) and (ii) oxygen production was estimated with the light-and-dark bottles method. All incubation was performed *in situ* using two or three experimental sets comprised of buoys and bottle holders. Frame-shaped, rectangular buoys were used to minimize the shading of the incubated bottles. Production was measured at six discrete levels: 0 m (surface), 2.5 m, 5 m, 10 m, 15 m, and 20 m; the sampled water from the respective depths was incubated. Measurements were performed from dawn to dusk for approximately 17 hours (daytime) and for four hours (4 h) around noon with the ^{14}C method according to Ærtebjerg Nielsen and Bresta (1984). In 2002, additional 24-hour-long (24 h) incubations were performed. It should be mentioned that in 2002 water from the same samples was incubated for 4 h and 24 h (the water was sampled at approximately 10:00 and the samples were incubated around midday for the ^{14}C method and for 24 h until 10:00 the following day for the oxygen method), whereas for daytime measurements, conducted from dawn to dusk, separate water sampling was performed.

Incubation for the ^{14}C method was performed in 100 cm^3 glass bottles (transparent bottles in duplicate and one dark bottle as the control). Sample activity was measured with a Beckman LS 6000 IC scintillation counter. Following BMB recommendations (Ærtebjerg Nielsen and Bresta 1984), the production value was multiplied by a correction factor of 1.06 to compensate for organic ^{14}C losses from phytoplankton respiration during incubation. A correction factor of 1.05 was used to compensate for the effect of ^{14}C discrimination (5% slower uptake of ^{14}C-isotope than that of ^{12}C-isotope). Daily production was calculated by multiplying the production obtained during the entire *in situ* incubation time by the ratio of a full day's dose of light to the dose of light during the incubation period.
Incubation for the oxygen measurements was performed in 1,000 cm^3 glass bottles (transparent and dark bottles in duplicate). Before and after incubation, the water from the bottles was siphoned through gas-tight tubing into three Winkler flasks where oxygen concentrations were measured with the Winkler method. All oxygen measurements were conducted using Metrohm Titrino 702SM piston titrators combined with a potentiometric electrode (Metrohm Pt Titrode), which permits automatically detecting the titration end-point. Gross production was calculated as a sum of respiration (oxygen consumption in dark bottles) and net ecosystem production (oxygen production in light bottles) assuming that the respiration rate is the same in darkness as it is in light. Daily and 24 h values of gross production measured with oxygen method were considered equivalent.
Measured downward irradiance was used to calculate the vertical irradiance attenuation coefficient, and, thus, the euphotic zone depth (depth of the compensation point). Earlier experiments conducted in the Gulf of Gdańsk revealed that primary production was negligible below 20 m (e.g., Renk 2000, Renk et al. 2000). Additionally, measurements of temperature, salinity, oxygen content, chlorophyll *a*, and nutrient (nitrogen and phosphorus) concentrations were made, and phytoplankton composition was analyzed (see e.g., Wielgat-Rychert et al. 2013).

Statistical analyses were performed with Statistica 10.0 Statsoft.

RESULTS

Environmental conditions at the study site

In June of 2002 and 2005, the environmental conditions were typical of early summer (Wielgat-Rychert et al. 2013). Conditions were comparable at all measurement sites with the exception of station M1 (sampled in 2005), where the strong impact of water from the Vistula River was noted in the 0-5 m layer as indicated by differences in temperature and much higher chlorophyll *a* concentrations, among other factors (Fig. 2).On the basis of the temperature profile, surface mixing zone can be estimated at about 0-10 m in 2002 and about 0-5 m in 2005 (Fig. 2A). More information on environmental parameters measured along with primary production used for PQ calculations is given in Table 1.

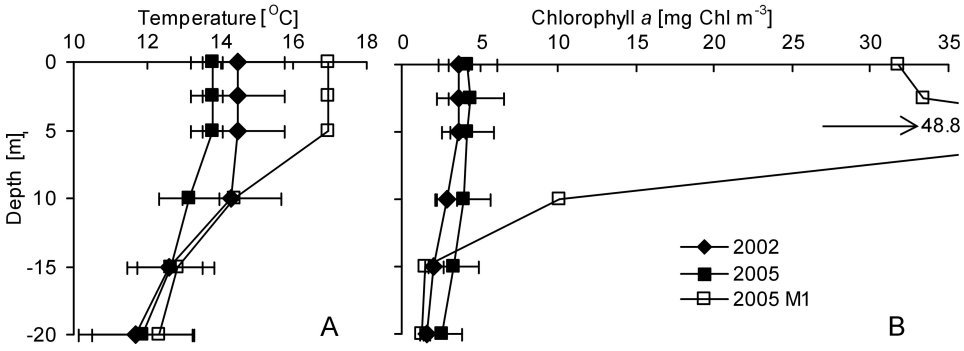

Fig. 2. Vertical profiles of temperature (A) and chlorophyll *a* concentrations (B); mean vales (±SD) calculated from all stations down to 20 m. Station M1 is presented separately

Table 1

Environmental parameters measured along with primary production used for PQ calculations, mean values for the 0-20 m. Primary production rates measured down to 20 m with oxygen and ^{14}C methods and different periods of incubation

Date	Station	Salinity	P-PO4	N-NO3	P-tot	N-tot	PAR	GPP (^{14}C)	GPP (O_2)	GPP (O_2)
		PSU	mmol m^{-3}	mmol m^{-3}	mmol m^{-3}	mmol m^{-3}	kJ m^{-2} d^{-1}	mgC m^{-2} 24 h (from 4 h)	mlO_2 m^{-2} 24 h	mlO_2 m^{-2} daily
2002-06-18	P63	7.0	0.10	0.15	0.77	24.5	14,053	1,214	2,829	3,536
2002-06-20	G2	6.9	0.04	0.09	0.84	24.9	7,675	829	2,926	2,564
2002-06-22	P110	6.8	0.01	0.07	0.77	25.1	9,027	2,140	2,498	3,526
2002-06-24	E52A	6.7	0.06	0.09	0.88	28.9	12,002	2,452	4,364	4,548
2002-06-26	E64	7.1	0.10	0.07	0.69	21.8	7,065	1,037	2,136	2,031
2005-06-17	P63	7.3	0.07	0.13	0.68	21.8	12,506	676	-	2,408
2005-06-19	E60	6.7	0.05	0.10	0.65	25.0	22,583	1,896	-	4,694
2005-06-21	G2	7.1	0.05	0.14	0.58	23.7	26,377	1,241	-	3,101
2005-06-23	M1	5.4	0.08	6.84	1.45	40.6	28,789	3,368	-	8,204

Primary production

The values of primary production observed in this study were within the range of those observed during early summer in this part of the Baltic Sea (Renk 2000, Renk et al. 2000). Daily primary production values ranged from 676 to 2,452 mgC $m^{-2}d^{-1}$

with one much higher value of 3,368 mgC m^{-2} d^{-1}at station M1 (Table 1), as compared to the mean monthly GPP of 15 gC m^{-2} per month (528 mgC $m^{-2}d^{-1}$) calculated for the 1966-1995 period by Renk (2000). The euphotic zone depth was delimited by 1% of surface PAR penetrating down to about 3 m at station M1 and E52A; to 11.5 m at stations E60, E64, and P110; and about 16 m at the outer-most stations P63 and G2 (Fig. 1). Generally, below the depth of 10 m, production rates were very low at an average less than about 10% of areal production.

Primary production and incubation time

The oxygen method measures gross primary production (calculation is described in the Material and Methods section), whereas it is believed that the ^{14}C method measures gross production during short incubation times (of a few hours) and that it shifts toward net production as incubation time increases (e.g., Marra 2002, Moigis 2000, Ryther 1956). In the present study production values measured with the ^{14}C method during longer incubation periods (daytime and 24 h incubation periods) were lower than those measured during 4 h incubation periods and then recalculated to 24 h. These also differed significantly from the values obtained with the oxygen method for the same incubation periods (Table 1). The production values measured with the ^{14}C method for a 4 h incubation period and then recalculated to 24 h values were assumed to represented gross primary production, as these did not differ from the values measured with the oxygen method (Table 2). Therefore only these measurements were used for calculations of PQ.

Table 2

Differences (diff) in volumetric [mmol m^{-3} d^{-1}] primary production rates measured down to 20 m with oxygen and ^{14}C methods and different periods of incubation; *t*-test for dependent samples ($p < 0.05$). Data pooled for 2002 and 2005

	^{14}C 24 h	^{14}C daytime	^{14}C 24 h (from 4 h)
Oxygen 24 h	diff (n = 29)	---	no diff (n = 29)
Oxygen daytime	---	diff (n = 43)	no diff (n = 48)

PQ values

Figure 3 presents primary production values measured down to 20 m and down to the compensation point delimited by 1% of surface PAR penetration. In the present paper PQ was calculated from rates considered as gross production, that is: 4 h incubation periods with the ^{14}C method and daily and 24 h oxygen measurements. PQ averaged 1.28 (ANOVA; $F = 872$; $R^2 = 0.92$; $n = 77$; $p < 0.001$). PQ calculated within the euphotic zone was 1.29 (ANOVA; $F = 590$; $R^2 = 0.91$; $n = 54$; $p < 0.001$).

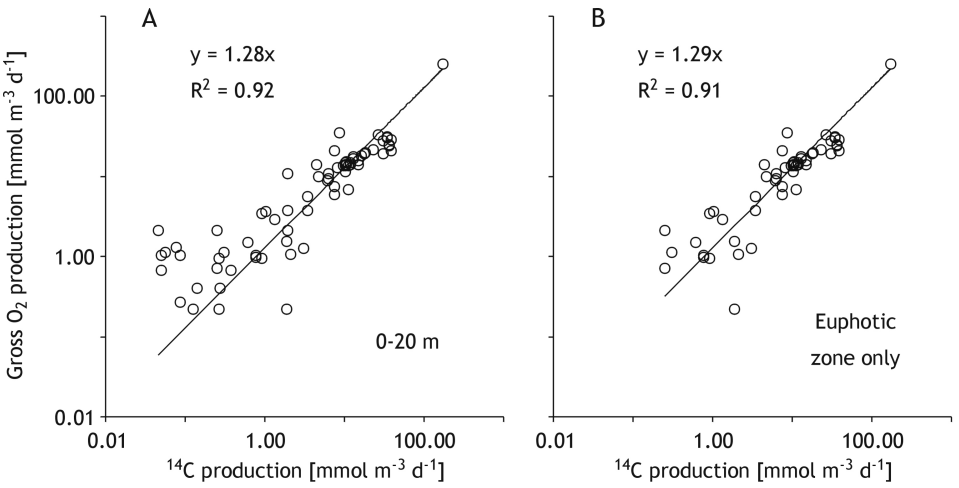

Fig. 3. Photosynthetic quotient (PQ) calculated from volumetric production rates down to 20 m (n = 77) and in the euphotic zone determined by 1% of surface PAR values (n = 54). The 4 h incubation periods with the ^{14}C method (recalculated to 24 h period) and daily and 24 h oxygen measurements are compared

DISCUSSION

Many studies have compared rates of primary production obtained with different methods but even large data sets reveal considerable variations in results (e.g. Regaudie-de-Gioux et al. 2014). In the present study, a PQ of 1.28 was calculated. This average value was close to values observed in other regions (e.g., Geider and MacIntyre 2002, Marra 2002, Moigis 2000, Robinson and Williams 1999), and it was close to those that are typically recommended at 1.2 (e.g., Wetzel and Likens 1991).

Nitrogen substrate utilization, phytoplankton composition

PQ is known to vary as a function of the N substrate utilized, and PQ can be smaller when ammonia is used because nitrogen is already reduced (Williams and Robertson 1991). The impact of the Vistula River plume, with its high nutrient concentrations, within the study area was detectable only at station M1 in the 0-5 m layer. Otherwise, concentrations of inorganic nitrogen (NO_3 and NH_4) in the euphotic zone were usually below or close to the detection limit (Wielgat-Rychert et al. 2013). Small differences among stations (Table 1) meant that the impact of nitrogen source on PQ values was difficult to identify, and no clear trend was observed.

In June 2002, the phytoplankton communities were dominated by dinoflagellates (mainly *Gymnodinium* cf. *simplex* and *Heterocapsa triquetra*) and cyanobacteria (mainly *Aphanizomenon* sp.) (Gromisz unpublished). At the outermost sea stations in June 2005, dinoflagellates were numerous (*Heterocapsa triquetra*, *Dinophysis norvegica,* and *Glenodinium* spp.) as were cyanobacteria (mainly *Aphanizomenon flos-aquae*) and autotrophic nanoflagellates from different taxonomic groups. Diatoms

dominated (mainly *Cyclotella* sp.) in the vicinity of the Vistula River mouth (Wielgat-Rychert et al. 2013). Phytoplankton composition did not correspond with PQ and no further statistical analysis was performed.

Methodological difficulties

Variation in PQ values can stem from various methodological limitations. One of them is sampling water at different times for ^{14}C and the oxygen measurements. Here, for daytime measurements, conducted from dawn to dusk, separate water sampling was performed than for 4 h measurements conducted around noon (as a consequence, incubations of different water masses were compared). The relatively small number of measurements did not allow testing significance of this methodological problem.

Variation in PQ was also noted when different production rates were compared (Fahnenstiel and Carrick 1988, Ostrom et al. 2005, Regaudie-de-Gioux et al. 2014). Specifically, very high PQ values were calculated in deeper layers, similarly to observation of other authors, who reported increase of PQ values with depth. Figure 3 illustrates that within the range of low primary production values (approximately 1 mmol m^{-3} d^{-1}) the rates of production measured with the oxygen method are higher than those measured with the ^{14}C method (recalculated from 4 h incubation periods that are considered to be gross production). This effect is especially evident when the data obtained from the euphotic zone (Fig. 3B) are compared with those obtained from the entire 0-20 m layer (Fig. 3A). Assuming that the present PQ value was calculated from gross production measurements, no increase in PQ values should be noted with depth. If values obtained with the ^{14}C method represented net production, PQ should progressively increase with depth (as discussed by Williams and Robertson 1991), but no such regular increase was observed (Fig. 3). Instead, these high PQ values could have resulted from methodological difficulties in measuring very low rates with the oxygen method, because the ^{14}C method is much more sensitive than is the oxygen method (e.g., HELCOM 2014, Marra 2002). In this study, 10% of the lowest production rates measured with the ^{14}C method (4 h recalculated to 24 h) ranged from 0.046 to 0.077 mmol C m^{-3} d^{-1}, whereas for the oxygen method ranged from 0.223 to 0.714 mmol O_2 m^{-3} d^{-1} (measurements down to 20 m depth). Consequently, extremely high PQ values have arisen when comparing the smaller rates measured with the ^{14}C method with the much higher rates measured with the less sensitive oxygen method. Some PQ values were much higher than any realistic values resulting from the comparison of stoichiometric oxygen and carbon proportions in phytoplankton cells (e.g., PQ in the range of 5.6-21). However, removing these extreme values did not affect the PQ values obtained from the regression line (Figs. 3A vs. 3B).

Methodological differences might not be the only explanation for greater variation in PQ within the low range of production rates. Ostrom et al. (2005) discuss how high PQ at low production rates could reflect differences in physiological rates at which oxygen and carbon are processed. If carbon and oxygen flux equilibrium is obtained during photosynthesis over time periods similar to the generation time of phytoplankton (Ostrom et al. 2005), lower production rates would increase the imbalance

in oxygen and carbon fluxes. The overall phytoplankton biomass turnover (calculated as the doubling time) in the surface layer of the Gulf of Gdańsk measured during the 2005 cruise (Wielgat-Rychert et al. 2013) ranged from 2.6 to 4.9 days, which was shorter at the inner stations than at the outer ones. This means that the turnover time of phytoplankton populations in this region was indeed much lower than the primary production measurement time in our study (which was a maximum of 24 h). In the deeper layers at low irradiance the turnover time was certainly even longer than that in the surface waters were it was measured in 2005.

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OBLICZENIA WSPÓŁCZYNNIKA FOTOSYNTETYCZNEGO (PQ) DLA ZATOKI GDAŃSKIEJ (BAŁTYK POŁUDNIOWY)

Streszczenie

Współczynnik fotosyntetyczny (PQ) definiowany jest jako molowy stosunek uwolnionego tlenu do węgla związanego w procesie fotosyntezy. Obliczenie poprawnej wartości PQ dla populacji fitoplanktonu występujących w środowisku jest zasadnicze dla sporządzenia poprawnego bilansu węgla, ale często obliczone z pomiarów wartości PQ różnią się znacznie od proporcji stechiometrycznych tlenu i węgla w produktach fotosyntezy. W niniejszej pracy przeprowadzono pomiary asymilacji węgla, czyli produkcji pierwotnej, przy użyciu standardowej metody izotopowej (z izotopem węgla ^{14}C) oraz pomiary uwolnionego tlenu za pomocą metody jasnych i ciemnych butelek i na tej podstawie obliczono współczynnik fotosyntetyczny dla Bałtyku południowego (Zatoka Gdańska, Polska). Obliczona średnia wartość PQ wynosiła 1,28 (ANOVA; $F = 872$; $R^2 = 0,92$; $n = 77$; $p < 0,001$). Nie odnotowano, aby wartości PQ zależały od rodzaju azotu wykorzystanego w procesie asymilacji (azot azotanowy czy amonowy) lub od składu fitoplanktonu. Bardzo wysokie wartości PQ obliczone w zakresie niskich wartości produkcji pierwotnej (wartości, które nie zgadzają się zupełnie z proporcjami stechiometrycznymi produktów fotosyntezy) zostały zinterpretowane jako artefakty wynikające z metodyki pomiarowej, ponieważ metoda tlenowa ma znacznie mniejszą czułość niż metoda izotopowa.