1986

A comparison of system (O_2 and CO_2) and C-14 measurements of metabolism in estuarine mesocosms

Candace A. Oviatt
University of Rhode Island, coviatt@uri.edu

David T. Rudnick
University of Rhode Island

See next page for additional authors

Citation/Publisher Attribution
Oviatt, C. A., Rudnick, S. T., Keller, A. A., Sampou, P. A., & Almquist, G. T. (1986). A comparison of system (O_2 and CO_2) and C-14 measurements of metabolism in estuarine mesocosms. *Marine Ecology Progress Series, 28*, 57-67. Retrieved from https://www.int-res.com/articles/meps/28/m028p057.pdf
Available at: https://www.int-res.com/articles/meps/28/m028p057.pdf

Vol. 28: 57–67, 1986 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published January 9

A comparison of system (O_2 and CO_2) and C-14 measurements of metabolism in estuarine mesocosms

Candace A. Oviatt, David T. Rudnick, Aimee A. Keller, Peter A. Sampou & Glen T. Almquist

Marine Ecosystems Research Laboratory, Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02882-1197, USA

ABSTRACT: Metabolism in estuarine mesocosms was measured by total system oxygen and carbon dioxide and by C-14 bottle incubations to determine the effects of nutrient enrichment (6 levels) over a 9 mo period. These data provided an unprecedented opportunity for calculating metabolic ratios (photosynthetic quotient [P.Q.] and respiratory quotient [R.Q.]) based on the 3 measures of metabolism and determining the impact of other system processes. System metabolism ratios based on daily data varied from 0 to 5.0. System metabolism ratios of P.Q. and R.Q. based on integrated data were highly correlated ($r = 0.95$ to 0.96) and similar to traditional ratios obtained in phytoplankton studies. A system photosynthetic quotient of 1.2 and a system respiratory quotient of 1.1 were calculated from the integrated data. These ratios were only slightly affected by carbon dioxide diffusion and 3 benthic processes: denitrification, sulfur metabolism and calcium carbonate dissolution. There was no trend for system metabolism ratios up the nutrient gradient. The C-14 estimations of productivity appeared nitrogen limited in the lower nutrient treatments and provided lower estimates than the 2 system measures of production.

INTRODUCTION

Every measure of primary production has its own complexities. Metabolism measurements by oxygen changes in dark and light bottles began in the 1920's. In the 1950's this technique was largely replaced by more sensitive C-14 changes in bottles. However, uncertainties in the C-14 technique have led to the practice of measuring primary production by oxygen and converting to carbon using metabolic ratios to calculate carbon flow from primary producers to higher trophic levels. Similar conversions of oxygen to carbon have commonly been made for respiration estimates. These metabolic ratios, the photosynthic quotient (P.Q.) and the respiratory quotient (R.Q.) need to be assessed for their variability over short and long term time scales.

Both oxygen and C-14 bottle incubations have long histories (Carpenter & Lively 1980, Peterson 1980, Harris 1978). Decisions must be made on trace metal clean techniques, incubation period, depths of incubation, length of incubation (Ryther & Vaccaro 1954), size and composition of bottles for incubation (Gieskes et al. 1979), whether and how to filter at the end of incubation (O'Reilly & Thomas 1982), whether to use complex light models for calculations of integrated day and depth values (e.g. Fee 1973, 1980) or to use empirical values derived by others (e.g. Vollenweider 1966, Oviatt et al. 1981). After 30 yr it is still uncertain how the use of C-14 measurement compares to gross and net productivity as determined by changes in oxygen (Davies & Williams 1984).

Even less well understood is the relation between bottles techniques and total system measures of metabolism using oxygen and/or total CO_2 (e.g. Odum & Hoskin 1958, Park et al. 1958, Nixon & Oviatt 1973, Johnson et al. 1981). Since total system measurements are usually made in a field situation, horizontal advection, turbulence of water masses and diffusion across the air-sea interface confuse the interpretation of data. Even though diffusion of gases is usually taken into account (Nixon & Oviatt 1973, Day 1983), these proces-

0171-8630/86/0028/0057/$ 05.00

ses are complex and quantified only with considerable uncertainty (Bower & McCorkle 1980, Emerson 1975a, b). Several other processes may affect system concentrations of oxygen and carbon dioxide which do not affect bottle concentrations. These are usually benthic process, and include denitrification, sulfur metabolism and calcium carbonate precipitation or dissolution.

For these reasons it was useful to compare measures of metabolism in mesocosms which did not have the problem of an erratically advected water mass and in which separate estimates of benthic metabolism and other processes which impact oxygen and carbon dioxide concentrations could be made. From June 1981 to September 1983 a nutrient addition experiment was conducted in 9 mesocosms at the Marine Ecosystems Research Laboratory (MERL) (Nixon et al. 1984). During the last 9 m of the experiment, weekly total system metabolism was estimated by measuring dawn-dusk-dawn changes in oxygen and carbon dioxide and by biweekly, midday, bottle C-14 incubations. In this paper, system and C-14 bottle measures of metabolism will be compared at different levels of nutrients in both daily and integrated data sets and some of the processes affecting metabolism ratios will be examined.

The experiment

For 28 m, from June 1981 to October 1983 inorganic nutrients in ratios similar to those of sewage were added daily to 6 mesocosms (Nixon et al. 1984). The sewage input to Narragansett Bay (1×) on an areal basis is 2.88 mmol N m^{-2} d^{-1}, 0.22 mmol P m^{-2} d^{-1} and 0.21 mmol Si m^{-2} d^{-1}. The 6 nutrient addition treatments were geometric multiples of 1×, 2×, 4×, 8×, 16× and 32×, spanning the known range of nutrient inputs to estuaries. Three other mesocosms with no nutrient additions served as controls for the gradient experiment.

The mesocosms

The mesocosms (13 m^3) are scaled to the natural system in terms of sunlight, mixing, flushing, temperature, sediment resuspension and bottom communities. The tanks (1.83 m dia., 5.49 m height) are constructed of fiberglass-reinforced polyester resin and have white interior walls. The water column is 5 m deep to prevent sunlight from reaching the bottom, and the sediments, a soft bottom community obtained from central Narragansett Bay, are 37 cm deep. Sea water is fed in a pulsed period every 6 h providing a 27 d turnover time. Temperature control (± 2 °C) is accomplished with glass heat exchangers which can both heat and cool. Mixing is accomplished with a plunger, 50 cm in diameter. It moves in a vertical excursion of 60 cm at a rate of 5 cycles min^{-1} for one 2 h period each 6 h and resuspends bottom sediments to the same levels that are observed in Narragansett Bay.

METHODS

Total system oxygen. Daily production and night respiration were estimated from consecutive dawn-dusk-dawn oxygen measurements (Odum & Hoskin 1958). Oxygen concentrations were determined from Winkler titrations as modified by Carritt & Carpenter (1966) with a precision of ± 0.05 mg O_2 l^{-1}. The bottles (60 ml glass B.O.D.) were filled from the bottom with a siphon and allowed to overflow to eliminate air bubbles. Samples were fixed immediately and titrated within 24 h. The oxygen concentration (mg l^{-1}) at dawn was subtracted from the dusk oxygen concentration to calculate daytime production. This value in mg l^{-1} was multiplied by the depth of the tank (5 m) to convert to g m^{-2}, then divided by the number of hours between the dawn dusk sampling times. The resulting value (g O_2 m^{-2} h^{-1}) was corrected for oxygen diffusion from or to the atmosphere.

The diffusion flux was calculated from an empirical linear regression derived from measurements in the Marine Ecosystems Research Laboratory (MERL) tanks (Roques 1985). A dome floating at the tank surface was flushed with N_2 and the increase in O_2 was monitored. Diffusion was calculated using the equation:

$$\text{diffusion flux} = f(0.1) \cdot (\text{tank } O_2 - O_2 \text{ saturation})$$

where f = a temperature dependent gas exchange coefficient determined empirically. The diffusion flux (+ or −) was added to the uncorrected production values and multiplied by the number of daylight hours (sunrise to sunset) to estimate daily oxygen production. The nighttime respiration was calculated in a similar manner using dusk-dawn samples.

Trapezoidal integration was used to calculate tank production and respiration per week, per season and per year. In 3 control tanks over 2 annual cycles the coefficient of variation (c.v.) in production was 10 to 19 %; the c.v for night respiration was 5 to 10 %.

Total system carbon dioxide. Dawn-dusk-dawn estimates of TCO_2 concentration were derived from measurements of pH, alkalinity, salinity and temperature using the method of Strickland & Parsons (1972), but utilizing a more accurate determination of salinity effects (Table 1). Water samples were siphoned from the tanks, taking care not to trap air in the bottles, and transferred to a temperature control bath set for *in situ* tank temperature. The pH meter was standardized

Table 1. Procedure for calculating total carbon dioxide (TCO_2) from pH, total alkalinity, temperature and salinity (M. Pilson, pers. comm.)

$$TCO_2 = \left[TA\ (equiv) - \frac{K_w}{a_H} - \frac{K'_B * salinity * 1.243 \times 10^{-5}}{a_H + K'_B}\right] * \left[\frac{a^2_H}{K'_1 (a_H + 2K'_2)} + \frac{a_H + K'_2}{a_H + 2K'_2}\right]$$

Derived from Culberson et al. (1970):

$$K^1_w = a_H + c_{OH} = (K^{sw}_w \text{ of C \& P})\ \gamma_{H^+}$$

$$Y = -0.80046393 + (0.08708525 * °C) \text{ for Sal} = 30‰$$

$$K_w = \exp(Y) * (10. ** (-14.1)) \text{ molar}$$

where

$$a_H = 10^{-(pH)}$$

$$K'_B = 10^{-(pK'_B)}$$

$$pK'_B = \frac{2291.9}{°K} + (°K)\ 0.01756 - 3.3850 - 0.26316\ (Salinity)^{1/3}$$

$$K'_1 = 10^{-(pK'_1)}$$

$$K'_2 = 10^{-(pK'_2)}$$

Mehrback constants (Mehrback et al. 1973):

$$pK'_1 = -13.7201 + 0.031334(°K) + \frac{3235.76}{°K} + 1.300 \times 10^{-5}\ (Sal)\ (°K) - 0.1032\ (Sal)^{1/2}$$

$$pK'_2 = 5371.9645 + 1.671221\ (°K) + 0.22913\ (Sal) + 18.3802 \log(Sal) - 128375.28/°K - 2194.3055 \log(°K) - 8.0944 \times 10^{-4}\ (Sal)\ (°K) - 5617.11 \log(Sal)/°K + 2.136\ (Sal)/°K$$

monthly with fresh buffers (pH 9, pH 7 and pH 4) and was rechecked with a pH 7 buffer solution prior to sample analysis. Samples were read within 1 h after collection. Precision was ± 0.02 pH units.

Total alkalinity was calculated by the method of Culberson et al. 1970. For alkalinity measurements 100 ml of sample were pipeted into a clean jar with 25.0 ml of 0.010M HCl. Samples were read within 2d at 25 °C with a pH electrode after calibration with a pH 4 buffer. Precision was ± 0.006 pH units. Salinity measurements were determined the same day by $AgNO_3$ titration. Day production and night respiration were calculated in a manner similar to oxygen except that no diffusion corrections were made. Precision of controls was ± 4.2 mmol C m^{-2} per day or night or 1 mol C m^{-2} over a 9 mo period.

Production by C-14. Primary production measured by the fixation of C-14 CO_2 was determined every 2 wk using the method of Strickland & Parsons (1972) with some modifications (Almquist 1983). Pooled samples were siphoned from 0.1, 2.5 and 4.5 m during the morning mixing cycle into 2 l amber polyethylene bottles and taken to a darkened laboratory. Polycarbonate centrifuge bottles (85 ml) with polypropylene screw closures were used for incubations to avoid metal contamination. The bottles had been initially cleaned by soaking in warm (50 °C) HCl (high purity) for at least 3 d. Between incubations these bottles were rinsed with quartz-redistilled, deionized water with 3 drops Ultrex-N HCl added to prevent bacterial growth and to remove trace metals that may have accumulated during prior incubations. For each tank 5 light and 1 dark bottles were filled with 80 ml samples and inoculated with 1 µCi $NaH^{14}CO_3$ solution (New England Nuclear) with an automatic pipet. Incubation bottles were transferred to the mesocosms in a dark box, and suspended cap down in their respective tanks at 0.1, 0.5, 1.0, 2.5 and 4.5 m. The dark bottle was suspended at 4.5 m. Bottles were incubated from 1000 h to 1400 h. After incubation, the bottles were transferred to the laboratory in a dark box and filtered immediately through 25 mm Gelman A/E glass fiber filters (retaining 1 µm size particles) at 125 mm Hg maximum vacuum. Filters were rinsed with 2 successive filtered sea water rinses of 20 ml each to remove any residual inorganic C-14. The filters were not allowed to dry. The edge of the filter was also rinsed 3 times. The filters were placed in scintillation vials with forceps and 10 ml or 3.5 ml of scintillation cocktail (Aquasol II, New England Nuclear) were added depending on the size of the vials. The samples were left uncapped for 5 min to allow degassing of any residual inorganic ^{14}C. The samples were shaken for 3 h to aid penetration of the filters by the cocktail and to degas inorganic ^{14}C. The samples were then placed in the scintillation counter and remained in the dark for at least 8 h prior to counting.

Counts per minute (cpm) were converted to disintegrations per minute (dpm) using the external standard channels ratio method of calibration against a quench curve. Primary production was calculated using the equations of Strickland & Parsons (1972) and depth integrated by trapizoidal integration. Reproducibility of replicate bottle values was ± 10 %.

Photosynthetic and respiration quotients. Total system O_2 changes and total system CO_2 changes were used to calculate photosynthetic quotients:

$$P.Q. = \frac{+\Delta O_2}{-\Delta CO_2}$$

and respiratory quotients:

$$R.Q. = \frac{+\Delta CO_2}{-\Delta O_2}$$

For whole system P.Q. calculations, daytime apparent production by O_2 was divided by daytime apparent system production by CO_2. No attempt was made to convert these values to gross production by adding night respiration.

The comparison of C-14 production with systems O_2 and CO_2 changes is less direct than the comparison of O_2 with CO_2. The O_2 and CO_2 estimations include wall production and benthic respiration whereas C-14 measures water column production. Furthermore, the C-14 incubations, made for 4 h during midday must be extrapolated to a whole day.

Rates from diel system O_2 measurements were manipulated to be comparable to water column midday bottle measurements. Benthic O_2 consumption was added to system O_2 production to estimate water column processes. Benthic respiration was measured using a chamber that covered the entire sediment surface by taking initial and final water samples over a period of several hours. The entire benthos was capped with a 1.76 m diameter, 13 cm high clear plastic chamber designed to fit over the sediment tray. Initial and final samples were withdrawn by siphon after mixing with a hand operated stirring bar. A control bottle was incubated on top of the chamber to correct for changes induced by the water within the chamber. Incubation time varied inversely with temperature and ranged from 9 to 1 h over the course of the experiment. Intercepts of individual treatment regressions of daily C-14 values versus systems O_2 plus benthic respiration were interpreted as wall production. No manipulations were made for CO_2 systems production since no measures of CO_2 flux from the sediments were made.

Comparison of C-14 and O_2 production was made using a functional regression (Ricker 1973) of hourly C-14 versus daily O_2 system production plus benthic O_2 consumption. The regression slopes of hourly C-14 production versus daily O_2 and CO_2 production were compared to the Vollenweider (1966) factor. Vollenweider (1966) has estimated that 55 to 60 % of the production occurs during the midday period (the hourly rate times factors of 6.67 to 7.27).

RESULTS

Daily variability in P.Q. and R.Q.

The metabolic ratios for daily data, collected weekly from January through September 1983 in all 9 mesocosms, range from 0 to 5 and can occasionally be greater (Fig. 1). The central tendency, or median value, for P.Q. was 0.9, but there was also a peak at 1.2. The

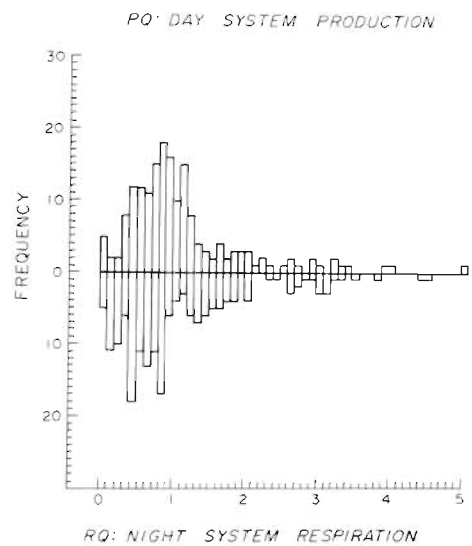

Fig. 1 Frequency of values for P.Q. and R.Q. from system O_2 and TCO_2 measures of production and respiration taken weekly from Jan to Sep 1983

central tendency for R.Q. was 0.5, and there was also a peak at 0.9.

A source of error could arise from uncertainties in the calculation of CO_2 concentrations. Concentrations of CO_2, calculated based on pH, alkalinity, temperature and salinity, were compared with measurements using the coulometric titration of samples for the control, 8× and 32× treatment levels (K. M. Johnson et al. 1985) (Table 2). Differences ranged from +8.2 to −36.3 µmol. Overall titrated CO_2 values tended to predict higher production ($\overline{X}$ = 5.0 µmol) and slightly lower respiration ($\overline{X}$ = − 1.2 µmol) (Fig. 2). If the worst case dawn-dusk control mesocosm is examined, the production from titrated CO_2 was 16.7 µmol and the production from calculated CO_2 was 6.2 µmol (Fig. 2). The

Table 2. A comparison of CO_2 concentrations by direct titration and calculated from pH, alkalinity and salinity. From samples taken July 5–6, 1983

Time	Treatment	CO_2^1 µmol	CO_2^2 µmol	Difference
Dawn	8×	1884.5	1875.0	− 9.5
	Control	1995.1	1858.8	−36.3
	32×	1530.8	1525.8	− 5.0
Dusk	8×	1862.3	1854.6	− 7.7
	Control	1878.4	1852.6	−25.8
	32×	1526.5	1523.8	− 2.7
Dawn	8×	1874.5	1865.6	− 8.9
	Control	1893.8	1861.8	−32.0
	32×	1561.8	1570.0	+ 8.2

[1] Coulometric titration (Johnson et al. 1985)
[2] Calculated from pH, alkalinity (Culberson et al. 1970), temperature and salinity

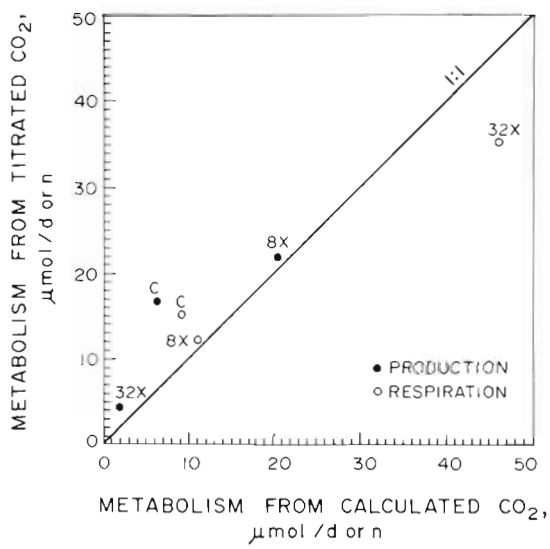

Fig. 2. The difference in production and respiration as determined by calculated TCO_2 compared with coulometric titration values of TCO_2

other 2 treatments (8× and 32×) had productivity values that agreed within 2 µmol by the 2 methods of measurement. The reason for these differences is not known. In a small data set, the differences were large; more comparative measures are needed to evaluate a large data set.

P.Q. and R.Q. from integrated metabolism – Jan to Sep 1983

The integrated values for production and respiration from total system changes in O_2 and CO_2 up the nutrient gradient show a high correlation contrasting with highly variable daily values. The ratios for P.Q. and R.Q., derived from functional regressions (Ricker 1973, 1975) on the integrated values, had correlations between 95 and 96 % (Table 3). Absolute values for production and respiration increased up the nutrient gradient but P.Q. and R.Q. showed no trend related to the gradient. For all treatments P.Q. was 1.24 and R.Q. was 1.16 (the slopes of the regression lines). The gain in correlation by using integrated data means a sacrifice in degrees of freedom. Thus 95 % confidence intervals on these ratios were large (Table 3).

Comparison with integrated C-14 production

The comparison of C-14 to O_2 + benthic respiration was in good agreement with Vollenweider's factors (Table 4). A slope of 6.70 was obtained for the comparison of C-14 with O_2 + benthic respiration. A lower slope of 4.92 was obtained for the comparison of C-14 with uncorrected CO_2 (Table 4). An intercept of 57 mmol was obtained for the oxygen regression which may be partially attributable to wall production. At first, this value appeared unacceptably high. However, individual treatment regressions of daily C-14 values versus O_2 + benthic respiration (in which the intercepts were interpreted as wall production), on all days when concurrent measurements were made, indicated that a mean of 10 % total production occurred on the walls (Table 5). A low of 2 % was found in the 16× treatment and a high of 20 % was found in the 32× treatment. Wall growth (mainly benthic diatoms) was noticeably greater in the 32× treatment and even twice-weekly wall cleanings did not totally eliminate its vigor.

Photosynthetic quotient calculations were made between C-14 production and both net and gross oxygen production in the water column (Table 5). Daily values for C-14 were calculated by multiplying by a rounded factor of 7 and integrating over the 9 mo period. Benthic respiration was added and wall production was subtracted from total system oxygen production to provide estimations of net water column production. Night respiration was used to calculate values of water column respiration and added to net water column production to estimate gross production although no estimate was available to make corrections for wall respiration (Table 5). The functional regressions using net and gross water column productions resulted in P.Q.s of 0.81 and 1.45 (Table 5). The mean of these (1.13) was slightly less than the system P.Q. (Table 3). The intercepts in these regressions cannot be interpreted as wall production and might reflect the irregular trend in the C-14 values up the nutrient gradient (Table 5). Control, 1× and 8× treat-

Table 3. Integrated values for production and respiration from dawn-dusk-dawn measurements of O_2 and CO_2 changes. Metabolism calculations for O_2 are diffusion corrected; those from CO_2 are not diffusion corrected. Period of measurement from Jan to Sep 1983. Values in mol m^{-2} 9mo^{-1}

Treatment	Production			Respiration					
	X CO_2	Y O_2	CO_2/O_2	X O_2	Y CO_2	P/R O_2	P/R CO_2	P−R O_2	P−R CO_2
Control	9	14	0.6	13	6	1.1	1.5	1	3
Control	8	14	0.6	16	8	0.9	1.0	−2	0
Control	6	11	0.5	15	6	0.7	1.0	−4	0
1×	13	16	0.8	18	12	0.9	1.1	−2	1
2×	24	30	0.8	27	18	1.1	1.3	3	6
4×	28	33	0.8	30	16	1.1	1.8	3	12
8×	27	25	1.1	21	15	1.2	1.8	4	12
16×	29	40	0.7	34	31	1.2	0.9	6	−2
32×	37	52	0.7	44	42	1.2	0.9	8	−5

P.Q. from a functional regression: $Y = 1.24X + 1.02$; $r = 0.95$
P.Q. = 1.24 95% confidence interval: 0.31–4.87
R.Q. from a functional regression: $Y = 1.16X - 10.9$; $r = 0.96$
R.Q. = 1.16 95% confidence interval: 0.33–4.22

ments showed particularly poor agreement between corrected O_2 and C-14 which was not evident between O_2 and CO_2 (Table 3).

The nutrient gradient

No obvious trends in system P.Q. and R.Q. occurred up the nutrient gradient suggesting that the processes which affect P.Q. and R.Q. are independent of absolute values of metabolic activity (Table 3). Production to respiration (P/R) ratios do show some irregular differences up the gradient with values ranging from 0.7 to 1.8 (Table 3). Despite similar proportionality in metabolic ratios, the magnitudes of O_2 metabolism and to a less regular extent CO_2 metabolism do increase up the treatment gradient. Thus net ecosystem production (P−R), based on O_2 was less than 1 mol O_2 m^{-2} 9mo^{-1} in control mesocosms, and 8 mol O_2 m^{-2} 9mo^{-1} in the 32× treatment.

Table 4. A comparison of total system apparent production by O_2 and CO_2 changes with midday hourly C-14 production in the water column from Jan to Sep 1983 (9 mo or 273 d)

Treatment	Benthic respiration	Production		Water column production
		Y_1	Y_2	X
	O_2 mol m^{-2} 9mo^{-1}	O_2 + benthic respiration mol m^{-2} 9mo^{-1}	CO_2 mol m^{-2} 9mo^{-1}	C-14 mol m^{-2} h^{-1} × 9mo
Control	5.0	19.0	9	0.89
Control	4.9	18.9	8	1.27
Control	5.3	16.3	6	1.05
1×	6.9	22.9	13	0.86
2×	4.3	34.3	24	3.41
4×	2.9	35.9	28	3.11
8×	8.0	33.0	27	1.24
16×	5.9	45.9	29	5.12
32×	11.2	63.2	37	7.34

Functional regressions:
C-14 *vs* O_2 + benthic: mmol m^{-2} d^{-1} = 6.70 (C-14) + 57 mmol
r = 0.95; 95% confidence interval: 1.7–26.3
C-14 *vs* CO_2: mmol m^{-2} d^{-1} = 4.92 (C-14) + 82 mmol
r = 0.83; 95% confidence interval: 0.7–32.6

Table 5. A comparison of water column net and gross production from Jan through Sep 1983. Hourly C-14 values have been multiplied by a rounded factor of 7 and integrated (from Table 4)

Treatment	1 Net production (S + BR) − (Walls)		2 Day respiration (1.078 NR) − (BR)		1 + 2 Gross	C-14
	mol O_2 m^{-2} $9mo^{-1}$				Y	mol C m^{-2} $9mo^{-1}$ X
Control	19.9	2.0	14.5	5.0	27	6.2
Control	18.9	3.0	17.2	4.9	28	8.9
Control	16.3	1.9	16.2	5.3	25	7.4
1×	22.9	1.5	19.4	6.9	34	6.0
2×	34.3	3.5	29.1	4.3	56	23.8
4×	35.9	2.7	32.3	2.9	63	21.8
8×	33.0	8.5	22.6	8.0	40	8.7
16×	45.9	1.0	36.7	5.9	76	35.9
32×	63.2	12.6	47.4	11.2	87	51.4

P.Q. from functional regressions:
Based on net water column production
$Y = 0.81X + 14.3$; $r = 0.96$
P.Q. = 0.81
Based on gross water column production
$Y = 1.45X + 20.9$; $r = 0.96$
P.Q. = 1.45

S = System daytime production
BR = Benthic respiration
Walls = Wall production from treatment intercepts
NR = System night respiration; the factor 1.078 corrects for day length

Table 6. Metabolic ratios derived in other studies and processes potentially impacting these ratios

Location	Method	P.Q.	R.Q.	Influencing factors	Source
Texas coastal bays	System O_2 & CO_2	0.3–1.0	0.5–3.0*	Microbial activity, water mass changes	Park et al. 1958
Aquatic microcosms	System O_2 & CO_2	1.1–2.5*	0.5–3.3	Light	Beyers 1963
Estuarine & coastal	Bottle O_2 & C-14	1.1–2.3	—	Nitrate utilization	Williams et al 1979
Marine culture	Bottle O_2 & CO_2	0.1–1.8	—	O_2 concentration	Burris 1981
Marine ecosystems	System O_2 & CO_2	0.2–0.7	—	Microbial activity	Johnson et al. 1981
Enclosed ecosystems	O_2 & C-14	1.3–1.6	—	Nitrate utilization, DOC production	Davis & Williams 1983
Mesocosms: daily values	System O_2 & CO_2	0–5.0	0–5.0	—	This study
Mesocosms: integrated values	System O_2 & CO_2	1.2	1.2	—	This study
	System O_2 & C-14	0.8–1.5	—	—	

* Inverse of reported values

DISCUSSION

System metabolism ratios

The system P.Q. value of 1.24 was within the range of traditional water column estimates of 1.1 to 1.3 (Ryther 1965); although the system R.Q. value of 1.16 was slightly high (Parsons et al. 1977). In the daily data set any P.Q. and R.Q. between 0 and 5 might occur (Fig. 1). Many processes in the water column are thought to affect metabolic ratios (Table 6). High P.Q. values may be caused by nitrate utilization (Burris 1981, Williams et al. 1979) and by low light (Anderson & Sand-Jensen 1980); low P.Q. values may be caused

by photorespiration (Burris 1981), photooxidation and microbial activities (Johnson et al. 1983). However, in the integrated data set (9 mesocosms measured weekly for 9 mo) these processes appear to cancel out and more traditional ratios were obtained (Table 3).

Processes affecting metabolism ratios

A number of processes change P.Q. and R.Q. values (Table 6), including carbon dioxide diffusion, nitrification-denitrification, sulfate reduction-sulfide oxidation and calcium carbonate precipitation/dissolution. We calculated the effects of these processes on the integrated P.Q. and R.Q. values. These calculations were made only for the control mesocosms, but the net effects were assumed to be proportional up the nutrient gradient (Table 3). The lack of long term trends does not rule out large changes in some processes over the short term, particularly in the highly enriched treatments. For example, shell formation in bivalves in the 8× treatment may have affected CO_2 concentrations during the first year of the experiment (Donaghay unpubl.); nitrification and sulfur metabolism rates were high periodically and could have affected metabolic ratios (Berounsky & Nixon in press, Sampou unpubl.). While these short term rate changes apparently did not affect long term metabolic ratios, they probably contribute to variability observed in daily ratios (Fig. 1).

Corrections for oxygen exchange with the atmosphere were applied to oxygen metabolism values but carbon metabolism values were not corrected for the slower exchange of CO_2 with the atmosphere. Rough CO_2 diffusion corrections were calculated assuming a previously determined film thickness at the air-water interface of 0.07 cm (Bopp et al. 1981). (We have ignored potential chemical enhancement of CO_2 flux suggested by Broecker & Peng [1974] and wind effects calculated by Teal & Kanwisher [1966]). The changes calculated were generally in fractions of a mole for day or night corrections over a 9 mo period (Table 7). Skirrow (1975) noted that it is not possible to calculate the rate of transfer of CO_2 across the air-sea interface in either direction from first principles and these values were at best approximate. The main point, however, is that the values were small and would not change the metabolism estimate when rounded to the nearest mole (Table 3). The error caused by lack of CO_2 diffusion correction appears too small to have affected P.Q. and R.Q.

Nitrate is formed in the water column and the sediments from ammonia and oxygen (Fenchel & Blackburn 1979):

$$NH_3 + 2O_2 \rightarrow HNO_3 + H_2O \quad (1)$$

Table 7. Net CO_2 diffusion calculated assuming (1) a film thickness previously determined to be 0.07 cm, (2) $P_{CO_2\ sat}$ equal to 330 µatm (3) diffusivity equal to 2×10^{-5} cm^2 s^{-1} and (4) equations from Skirrow (1975)

Treatment	Diffusion (mol C m^{-2} 9mo^{-1}) Day	Night
Control	−0.4	−0.3
Control	−0.2	−0.1
Control	−0.4	−0.2
1×	−0.4	−0.3
2×	+0.1	+0.1
4×	+0.1	+0.1
8×	−0.9	−0.6
16×	−0.2	−0.1
32×	−0.5	−0.4

When water column nitrate is utilized by phytoplankton, the oxygen used in formation is returned. The formula is given by Williams et al. (1979):

$$NO_3 + 2H_2O \rightarrow NH_3 + OH + 2O_5 \quad (2)$$

While in short-term data sets the latter reaction may increase P.Q. (Williams et al. 1979), in a long-term data set where the principal nitrogen form is ammonia, the 2 reactions should be roughly equivalent and P.Q. should not be affected. However, most of the nitrate formed in the sediments appears to be denitrified (Seitzinger & Nixon in press). Denitrification proceeds in the sediment utilizing organic matter and nitrate (Fenchel & Blackburn 1979):

$$C_6H_{12}O_6 + 24/5\ NO_3 + 24/5\ H \rightarrow 6CO_2 + 12/5\ N_2 + 42/5\ H_2O \quad (3)$$

For each cycle of a N atom in the nitrification-denitrification reaction in sediments, 4 oxygen atoms are used in nitrification and only 2.5 atoms reappear as carbon dioxide in denitrification for a deficit of 1.5 oxygen atoms as H_2O. These reactions thus result in the net consumption of 0.75 mol O_2/mol N with no corresponding CO_2 reduction. Denitrification proceeded at a rate of 7.20 m mol N m^{-2} 24 h or 1.86 mol N m^{-2} 9 mo^{-1} in control systems (Seitzinger & Nixon in press). If nitrification is assumed equal to denitrification, then 0.90 mol O_2 m^{-2} 9 mo^{-1} by day and 0.77 by night can be calculated as deficit amounts (Table 8).

Anaerobic sulfur metabolism in the sediments can contribute to higher metabolic ratios. Organic matter can be utilized by bacteria using sulphate as an oxygen source; the sulphide produced is then reoxygenated by molecular oxygen (Fenchel & Blackburn 1979):

$$CH_3COO + SO_4 \rightarrow 2CO_2 + 2H_2O + HS \quad (4)$$

$$HS + 2O_2 \rightarrow SO_4 + H \quad (5)$$

Table 8. Effect of 3 benthic processes on system P.Q. and system R.Q. in control mesocosms

	Source
A. Denitrification	
Rate: 1.86 mol N m^{-2} 9mo^{-1} 1.67 mol O_2 m^{-2} 9mo^{-1}	Seitzinger & Nixon in press
O_2: + △ system Apparant daytime production 6.9% (.90 mol) − △ system apparent nighttime respiration 5.1% (.77 mol)	
B. Sulfate reduction – sulfide oxidation	
Rate: Oxidation 5.8 mol O_2 m^{-2} 9mo^{-1} Reduction 6.4 mol CO_2 m^{-2} 9mo^{-1}	Sampou unpubl.
O_2: + △ system apparent daytime production 2.6 % (.34 mol) − △ system apparent nighttime respiration 2.1 % (.31 mol)	
C. Calcium carbonate dissolution	
Rate: + 2.08 mol Ca + CO_2 m^{-2} 9mo^{-1} •	Smith & Key 1975
CO_2: + △ system apparent daytime production 14 % (1.1 mol) − △ system apparent nighttime respiration 14 % (0.9 mol)	
D. Combined effect on system P.Q. and system R.Q. (Table 3)	
System P.Q.: $1.24 \frac{\triangle O_2}{\triangle CO_2} \left(\frac{N\ 1.069 \quad S\ 1.026}{CaCO_3\ 1.14} \right) = 1.19$	
System R.Q.: $1.16 \frac{\triangle CO_2}{\triangle O_2} \left(\frac{.86}{.949 \quad .979} \right) = 1.08$	

• Corrected for sulfide storage (0.64 equiv. m^{-2})

These equations are balanced but Jorgensen (1977) found that 10 % of the sulfide produced remains in the sediment on a yearly basis. For each mole of stored S, 2 moles of CO_2 had been produced for which no O_2 had been consumed. Several measurements in control mesocosms integrated over the year indicated that 4.27 mol SO_4 were reduced m^{-2} yr^{-1} in control mesocosms to a depth of 20 cm (Sampou unpubl.). If the 10 % deficit is assumed, and the amount is made proportional to a 9 mo period, then 0.34 mol O_2 m^{-2} 9 mo^{-1} by day and 0.31 by night must be added to production and subtracted from respiration, respectively (Table 8).

Calcium carbonate precipitation or dissolution can affect CO_2 concentration and therefore P.Q. and R.Q. The processes can be detected by changes in alkalinity. Calcification lowers the total CO_2 content of sea water 1 mol for each mole of $CaCO_3$ precipitated and the total CO_2 content of seawater is, therefore, lowered by 0.5 mol for each equivalent of total alkalinity reduction or the reverse reaction for dissolution (Smith & Key 1975). Each mole of sulfide stored in the sediment yields 2 equivalents of alkalinity production for the water column. If 0.32 mol of sulfide were buried over 9 mo, 0.64 equivalents were produced. Thus the change in alkalinity was partially due to sulfide storage. The changes in alkalinity due to $CaCO_3$ dissolution and sulfide storage were corrected to yield the change due to $CaCO_3$ dissolution. The daytime average increase in 3 control mesocosms in alkalinity due to $CaCO_3$ in control mesocosms over the 9 mo period results in an increase of 1.1 mol CO_2 m^{-2} by day or 0.9 mol CO_2 m^{-2} by night (Table 8). This dissolution would have the effect of lowering both P.Q. and R.Q. slightly (Table 8).

The combined effect of these 3 benthic processes was to lower P.Q. from 1.24 to 1.19 and to lower R.Q. from 1.16 to 1.08 (Table 8). Given the uncertainty of these calculations, values have been rounded to a P.Q. of 1.2 and an R.Q. of 1.1. It takes a certain lack of caution to take the rates from several investigators and extrapolate them to a 9 mo period even when the rates are measured in the same mesocosms. The benthic processes examined show evidence of impacting P.Q. and R.Q. slightly; whether the exact impact has been correctly calculated is unknown.

Water column production

Since the C-14 method measures primary production rates that are between gross and net production, P.Q.'s were calculated using both measures of water column

O_2 production. Neither calculation provided a value of 1.2 and the ratios were not consistent up the treatment gradient (Table 5). Treatments with lower phytoplankton biomass (controls, 1× and 8× (which had a high abundance of filter feeders)) had higher P.Q. values and treatments with high phytoplankton biomass had lower P.Q. values. Of the 3 methods of measurements, C-14 appears to be least consistent.

One reason for differences in controls and 1× treatment for production measured by C-14 appears to be nitrogen limitation in the C-14 bottles (Table 9). Nitrogen limitation was calculated by comparing the C-14

Table 9. Calculation of nitrogen limitation based on the calculated demand using the Redfield ratio (C:N of 106:16) from the C-14 production and comparing the demand to standing stock concentrations

	Ammonia limiting % time	Inorganic N limiting % time
Control	65	48
Control	71	59
Control	70	30
1× treatment	70	50
8× treatment	0	0

primary production demand using the Redfield ratio (C106:N16:P1) and comparing the demand over a 4 h incubation period to the standing stock from January to September 1983. These calculations indicate N limitation from 30 to 50 % of the time. System measures of production would likely experience more nitrogen availability because of movement through the water column and because of benthic nitrogen regeneration which the bottle incubations would not receive. This explanation does not explain the discrepancy for 8× treatment which experienced no nitrogen limitation (Table 9). The large number of filter feeders in the 8× may have reduced the phytoplankton biomass to a level which precluded initiation of a bloom during the incubation period. A lower production and respiration in the 8× treatment also was found using total system measures (Table 3).

CONCLUSION

The measurements of metabolism by 3 methods in controlled ecosystems have provided a holistic evaluation of metabolic ratios and processes affecting them. Integrated long term metabolic values were highly correlated and only slightly affected by the processes examined. By contrast, daily ratios were variable and little information emerged that can explain this variability (Fig. 1). It is likely that benthic and water column processes may have a significant short term influence on metabolic ratios. In taking 3 benthic processes into account a P.Q. of 1.2 and a R.Q. of 1.1 was calculated for integrated system measures of production and respiration by oxygen and carbon dioxide. These ratios do not appear to be affected by a wide range of nutrient treatments suggesting the processes occur proportionally up the nutrient gradient. A comparison of C-14 production to net and gross oxygen production yielded P.Q.'s of 0.8 and 1.5, respectively. Despite the manipulations of total system oxygen to make these comparisons, the C-14 values appear less comparable than the 2 system methods of measurement. These results provide evidence supporting traditional corrections of oxygen to carbon for system measures of metabolism for long term integrated data sets, but not for short term data sets.

Acknowledgements. This paper is but one small part of the nutrient gradient experiment in which over 25 MERL scientists, students and technicians participated. We thank Lynn Laffin-Beatty for her help in dawn-dusk-dawn measurements. The paper was much improved by discussions and comments by Peter Doering and Percy Donaghay. This study was supported by the EPA Centers Program, Cooperative Agreements 807795-02 and 810265-01.

LITERATURE CITED

Almquist, G. T (1983). ^{14}C estimation of primary production. In: Lambert, C. E., Oviatt, C. A., (ed.) Manual of biological and geochemical techniques in coastal areas. MERL Series Report No. 1, Univ. of Rhode Island, Kingston, p. 14–19

Anderson, H. M., Sand-Jensen, K. (1980). Discrepancies between O_2 and ^{14}C methods for measuring phytoplankton gross photosynthesis at low light levels. Oikos 35: 359–364

Berounsky, V. M., Nixon, S. W. (1985). Eutrophication and the rate of net nitrification in a coastal marine ecostytem. Estuar coast. Shelf Sci. 20: 773–781

Beyers, R. J. (1963). The metabolism of twelve aquatic laboratory microecosystems. Ecol. Monogr 33 (4): 282–306

Bopp, R. F., Sanschi, P. H., Li, Y.-H., Deck, B. L. (1981). Biodegradation and gas exchange of gaseous alkanes in model estuarine ecosystems. Org. Geochem. 3: 9–14

Bower, P., McCorkle, D. (1980). Gas exchange, photosynthetic uptake and carbon budget for a radiocarbon addition to a small enclosure in a stratified lake. Can. J. Fish. Aquat. Sci. 37: 464–471

Broecker, W S., Peng, T.-H. (1974). Gas exchange rates between air and sea. Tellus 26: 21–35

Burris, J. E. (1981). Effects of oxygen and inorganic carbon concentrations of the photosynthetic quotients of marine algae. Mar Biol. 65: 215–219

Carpenter, E. J., Lively, J. S. (1980). Review of estimates of algal growth using ^{14}C tracer techniques. In: Falkowski, P. G. (ed.) Primary productivity in the sea. Plenum Press, New York, p. 161–178

Carritt, D. E., Carpenter, J. H. (1966). Comparisons and evaluation of currently employed modifications of the Winkler Method for determining oxygen in seawater J. mar Res. 24 (3): 286–318

Culberson, C., Pytkowicz, R. M., Hawley, J. E. (1970). Seawater alkalinity determination by the pH method. J. mar Res. 28: 15–21

Davies, J. M., Williams, P. J. leB. (1984). Verification of ^{14}C and O_2 derived primary organic production measurements using an enclosed ecosystem. J. Plankton Res. 6 (3): 457–474

Day, J. W., Jr. (1983). Carbon dynamics of estuarine ponds receiving treated sewage wastes. Estuaries 6 (1): 10–19

Emerson, S. (1975a). Chemically enhanced CO_2 gas exchange in a eutrophic lake: a general model. Limnol. Oceanogr. 20 (5): 743–753

Emerson, S. (1975b). Gas exchange rates in small Canadian shield lakes. Limnol. Oceanogr 20 (5): 754–761

Fee, E. J. (1973). Modelling primary production in water bodies, a numerical approach that allows vertical inhomogenities. J. Fish. Res. Bd Can. 30: 1469–1473

Fee, E. J. (1980). Important factors for estimating annual phytoplankton production in the experimental lakes area. Can. J. Fish. Aquat. Sci. 37: 513–522

Fenchel, T., Blackburn, T. H. (1979). Bacteria and mineral cycling. Academic Press, New York

Gieskes, W. W. C., Kraay, G. W., Baars, M. A. (1979). Current ^{14}C methods for measuring primary production: gross underestimations in oceanic waters. Neth. J. Sea Res. 13 (1): 58–78

Harris, G. P. (1978). Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. Arch. Hydrobiol. Beih. Ergeb. Limnol. 10 (I–IV): 1–171

Johnson, K. M., Burney, C. M., Sieburth, J. McN. (1981). Enigmatic marine exosystem metabolism measured by direct diel CO_2 and O_2 flux in conjunction with DOC release and uptake. Mar. Biol. 65: 49–60

Johnson, K. M., Davis, P. G., Sieburth, J. McN. (1983). Diel variation of TCO_2 in the upper layer of oceanic waters reflects microbial composition, variation and possibly methane cycling. Mar Biol. 77: 1–10

Johnson, K. M., King, A. E., Sieburth, J. McN. (1985). Coulometric TCO_2 analyses for marine studies; an introduction. Mar. Chem. 16: 61–82

Jorgensen, B. B. (1977). The sulfur cycle of a coastal marine sediment (Linfjorden, Denmark). Limnol. Oceanogr. 22 (5): 814–832

Mehrback, C., Culberson, C. H., Hawley, J. E., Pytkowicz, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18 (6): 897–907

Nixon, S., Oivatt, C. (1973). Ecology of a New England Saltmarsh. Ecol. Monogr. 43: 463–498

Nixon, S. W., Pilson, M. E. Q., Oviatt, C. A., Donaghay, P., Sullivan, B., Seitzinger, S., Rudnick, D., Firthsen, J. (1984). Eutrophication of a coastal marine ecosystem – an experimental study using MERL microcosms. In: Fasham, M. J. R. (ed.) Flows of energy and materials in marine ecosystems: theory and practice. Plenum Press, London, p. 105–135

Odum, H. T., Hoskin, C. M. (1958). Comparative studies of the metabolism of marine waters. Publs Inst. mar Sci. Univ. Tex. 5: 16–46

O'Reilly, J. E., Thomas, J. P. (1982). A manual for the measurement of total daily primary productivity using ^{14}C simulated in situ sunlight incubation. Biomass Handbook No. 10 NOAA, NMFS, Sandy Hook, New York, p. 1–123

Oviatt, C., Buckley, B., Nixon, S. (1981). Annual phytoplankton metabolism in Narragansett Bay calculated from survey field measurements and microcosm observations. Estuaries 4 (3): 167–175

Park, K., Hood, D. W., Odum, H. T (1958). Diurnal pH variability in Texas bays and its application to primary production estimation. Publs Inst. mar. Sci. Univ. Tex. 5: 47–64

Parsons, T R., Takahashi, M., Hargrave, B. (1977). Biological oceanographic processes, 2nd edn. Pergamon Press, New York

Peterson, B. J. (1980). Aquatic primary productivity and the ^{14}C-CO_2 method: a history of the productivity problem. In: Johnston, R. F., Frank, P. W., Michener, C. D. (ed.) Annual review of ecology and systematics, Vol. II. Palo Alto, California, p. 359–386

Ricker, W. E. (1973). Linear regressions in fishery research. J. Fish. Res. Bd Can. 30: 409–434

Ricker, W. E. (1975). A note concerning Professor Jolicoeur's comments. J. Fish. Res. Bd Can. 32: 1944–1998

Roques, P. (1985). Anoxic nutrient regeneration and the eutrophication of estuarine waters. Ph. D. thesis, Univ. of Rhode Island

Ryther, J. H., Vaccaro, R. F. (1954). A comparison of the oxygen and ^{14}C methods of measuring marine photosynthesis. J. du Conseil 20 (1): 25–34

Seitzinger, S. P., Nixon, S. W. (1985). Eutrophication and the rate of denitrification and N_2O production in coastal marine sediments. Limnol. Oceanogr.30 (6): 1332–1339

Skirrow, G. (1975). The dissolved gases – carbon dioxide. In: Riley, J. P., Skirrow, G. (ed.) Chemical oceanography, Vol. 2, 2nd edn. Academic Press, New York, p. 1–92

Smith, S. V., Key, G. S. (1975). Carbon dioxide and metabolism in marine environments. Limnol. Oceanogr 20 (3): 493–495

Strickland, J. D. H., Parsons, T R. (1972). A practical handbook of seawater analysis. Bull Fish. Res. Bd Can. 167

Teal, J. M., Kanwisher, J. (1966). The use of P CO_2 for the calculation of biological production with examples from the waters off Massachusetts. J. mar Res. 24: 4–14

Vollenweider, R. P. (1966). Calculation models of photosynthesis depth curves and some implications regarding day rate estimates in primary production measurements. In: Goldman, C. R. (ed.) Primary production in aquatic environments. Univ. of California, Berkeley, p. 427–457

Williams, P. J. leB., Raine, R. C. T., Bryan, J. R. (1979). Agreement between ^{14}C and oxygen methods of measuring phytoplankton production: reassessment of the photosynthetic quotient. Oceanologica Acta 2 (4): 411–416

This paper was submitted to the editor; it was accepted for printing on September 23, 1985