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Precise and Accurate Determination by Infrared Photometry of CO_2 Dynamics in Marine Ecosystems

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ABSTRACT: Preliminary studies with an ampule analyzing unit and infrared (IR) detector showed that procedures for standardization and determination of total carbon dioxide (ΣCO_2), while often precise, lacked the accuracy required to estimate the net productivity and respiration of aquatic ecosystems during studies in which sampling over diel cycles was used. Scaling down sample and standard volumes to the µl range and the use of a commercial sodium carbonate standard without dilution before and after replicate sample injections gave accurate results as shown by comparison with indirect (pH-alkalinity) ΣCO_2 determinations with a standard error of ± 3 µmoles in the laboratory and ± 6 µmoles at sea for 8 to 10 replicates. This was sufficient to detect a diurnal consumption and nocturnal production of CO_2 which were inversely correlated with O_2 variation in a salt marsh, an estuarine mesocosm, and the Caribbean Sea.

INTRODUCTION

Total carbon dioxide (ΣCO_2) changes used to determine net production and respiration in aquatic ecosystems are usually calculated from pH and alkalinity measurements (Park et al., 1958; Smith, 1973; Johnson et al., 1979). Their accuracy and precision are limited by the sensitivity and stability of the pH electrodes, imprecise apparent dissociation constants and the presence of noncarbonate buffers. Although Park (1965) suggested infrared (IR) ΣCO_2 determination as a measure of biological activity, and although the literature contains many discrete measurements of ΣCO_2 by IR, there is not much information on biological productivity and respiration from IR determined CO_2 variation. Seasonal patterns of ΣCO_2 by IR analyzers in lakes (Rich, 1979) and of CO_2 partial pressure in marine waters (Teal and Kanwisher, 1966) have been reported. Schindler and Fee (1973) monitored diel variation in ΣCO_2 in a Canadian experimental lake, but by gas chromatography. The sparsity of IR ΣCO_2 estimates of system metabolism may result from methods too elaborate for use in diel studies (Wong, 1970), and the failure of first generation carbon analyzers to provide data of sufficient accuracy or precision. Furthermore, attempts at such measurements were discouraged by the ^{14}C literature which indicated primary productivity too low to be measured by IR photometry.

With the advent of second generation IR analyzers, Salonen and Holopainen (1979) estimated productivity in freshwater environments by following the consumption of inorganic carbon in bottles suspended at depth. They acidified the sample, stripped the resultant CO_2 from solution in a homemade bubble chamber (Salonen, 1981) and measured its IR absorbance. We have employed this rapid and precise technique using commercially available equipment to measure ΣCO_2 at 2 or 3 h intervals over diel cycles to determine net apparent total ecosystem metabolism by integrating the area under the ΣCO_2 rate of change curves (Odum and Hoskin, 1958). In this paper we summarize our experiences with the ΣCO_2 analysis and describe improvements in technique which yielded good analytical precision and agreement between parallel pH-alkalinity ΣCO_2 determinations in the open ocean. ΣCO_2 variation measured in diverse marine ecosystems by IR was inversely correlated with O_2 variation (Winkler

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method) and indicated that inorganic carbon dynamics may be accurately measured by IR photometry.

MATERIALS AND METHODS

Between 1977 and 1979, ΣCO_2 measurements were made in the Bissel Cove salt marsh embayment in Wickford, RI (Nixon et al., 1976), in a simulated estuarine ecosystem tank of the Marine Ecosystem Research Laboratory (MERL) (Pilson and Nixon, 1980), and in the northwestern Caribbean Sea (R/V Endeavor cruise 033, Burney et al., 1982). The ΣCO_2 measurements were made on the Oceanography International (OI) Total Carbon System (TCS) (model 0524B, College Station, TX) equipped with the Horiba (Irving, CA) PIR-2000 nondispersive IR analyzer (200 mm cells). To analyze ΣCO_2 2.0 ml of phosphoric acid (30 % V/V) was purged with nitrogen to remove ambient CO_2, the sample or standard injected through a septum into the vial containing the acid and then the resultant CO_2 was stripped with nitrogen at 200 ml min^{-1} until a strip chart recorder showed that the CO_2 had passed through the IR detector. The output of the detector was integrated on a model CRS-208 digital integrator (Columbia Scientific, Austin, TX), and a known N_2-CO_2 mixture (span gas) was used to monitor and correct IR amplifier drift. All ΣCO_2 samples were collected in 125-ml serum bottles in the same manner as oxygen samples (Grasshoff, 1976), sealed with serum stoppers and withdrawn by syringe. Analysis was begun within half an hour of collection. For standardization and analysis, a micro-analytical technique and a commercial 0.1 % solution made with anhydrous sodium carbonate (Harleco # 1484A, Harleco Division of American Hospital Supply, Gibbstown, NJ, available from American Scientific Products, Bedford, MA) were employed. Volumes of 35 to 45 µl of the undiluted Harleco standard and 200 µl natural seawater samples containing 3.95 to 5.08 µg C were injected into the purging vial with microliter syringes (# 710, Hamilton, Reno, NE) equipped with Chaney adaptors and a Hamilton constant rate syringe (CR700-200), respectively. Their accuracy was checked gravimetrically by a procedure similar to Kritchevsky et al. (1975). Following injection, 5 s were allowed to elapse to insure equilibrium before stripping the solution of CO_2 with nitrogen. The volume of standard solution in µl was chosen to yield levels of carbon which tightly bracketed the *in-situ* dissolved inorganic carbon, and during the diel studies each replicate sample determination was always preceded and followed by a standard determination to give interreplicate standards for each sample analyzed. The standard was stored at a controlled temperature (18 to 20 °C) in the dark and dispensed fresh daily.

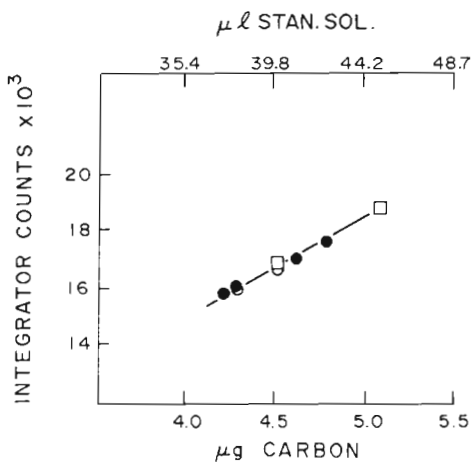

Fig. 1. Response and stability of dissolved inorganic carbon standards (0.113 µg C $µl^{-1}$) analyzed on the Total Carbon System and detected on the Horiba PIR 2000 IR analyzer at a constant carrier gas flow rate of 200 ml min^{-1} and constant amplifier response to a calibration gas. Results represent means of multiple determinations ($n \geq 10$); Jan.–Feb. 1979 ( ), June 1979 (○), Sept. 1980 (●)

Dissolved oxygen determinations were made by the procedure of Carritt and Carpenter (1966) which was adapted to 60-ml BOD bottles for nearshore samples. In the open ocean, ΣCO_2 was calculated from pH and alkalinity using the apparent carbonate dissociation constants of Mehrbach et al. (1973) and the equation of Edmond and Gieskes (1970) for the borate system. Alkalinity and pH were determined at the *in-situ* temperature on a Corning Model 101 digital electrometer (Corning Scientific Instruments, Medfield, MA). Salinity was measured with an induction salinometer (Plessy Model 6230N, San Diego, CA).

RESULTS

Fig. 1 shows the linear response of the total carbon system (TCS) and the Horiba IR to increasing microlevels of inorganic carbon obtained from injections of progressively larger volumes of the standard into the TCS. The regression coefficient (r) for the 8 points shown is 0.997, and in our configuration the regression line has a slope of approximately 3300 integrator counts $µg^{-1}$ C. Fig. 1 also illustrates the batch to batch reproducibility of the standard on the TCS because measurements made over a year and a half (Jan. 1979-Sept. 1980) from 3 different lots of commercial standard did not differ. The day-to-day stability of the standard was tested by filling a serum bottle in the same fashion as a sample, and repeatedly restandardizing with 40 to 45 µl from this bottle. The inorganic carbon level respresented by a constant peak area (integrator count)

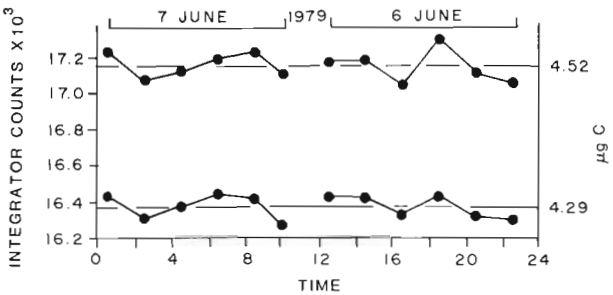

Fig. 2. Temporal response of the Total Carbon System on 6–7 June 1979, at 2 h intervals as shown by variation in mean peak area obtained from 2 precisely known amounts of inorganic carbon (38 and 40 µl of a 0.1 % sodium carbonate solution containing 0.113 µg C μl^{-1}). Horizontal lines indicate mean peak area for combined results. Points shown are means of multiple determinations ($n \geq 3$)

determined from 6 different standard curves had a very satisfactory coefficient of variation (% CV) of 0.33 %. When standards were run over a 2-wk period, the % CV increased to 1.08 %, presumably due to CO_2 loss from the standard solution.

Fig. 2 plots the response of the TCS over 6–7 June 1979, at 2 h intervals to 2 precisely known amounts of inorganic carbon. It shows that the microstandardization procedure and TCS were sensitive enough to track an apparent drift in system response because the peak area variation for the 2 standards was positively correlated over time ($r = 0.82$, $n = 12$, $p < .01$) even though the 24 h % CV for each standard was less than 0.5 % and many of the changes in response were not significantly different from preceding or succeeding results. These data were obtained during a diel study in the estuarine mesocosm (MERL) and as a practical consequence ΣCO_2 results obtained with them yielded a correlation coefficient with parallel O_2 determinations of -0.91 ($n = 12$, $p < .01$) while the comparable result with the composite (average) curve computed from them was -0.80 ($n = 12$, $p < .01$). Net system production (mg C m^{-3} d^{-1}) calculated from the diurnal curve method was 30 % higher using the ΣCO_2 results based on the composite curve and the production to respiration ratio (P/R) was 1.81. For ΣCO_2 results based on the time-dependent standards (Fig. 2) and from the O_2 data the P/R ratios were 1.36 and 1.27, respectively (Johnson et al., 1981). During this diel study the precision (± 1 standard error) for samples was 3 µmoles (error < 0.5 %).

Table 1 is a comparison of simultaneous indirect (pH-alkalinity) and direct (IR) ΣCO_2 measurements in the mixed layer of the northwestern Caribbean Sea during February-March 1979. It shows that the observed means differed by only 4 µmoles. The average absolute difference between individual indirect and direct analyses were compared to results calculated from data given by Park et al. (1964) for a gas chromatography (GC) pH-alkalinity comparison. On average IR determinations showed slightly better agreement with calculated ΣCO_2 than the GC determinations. The shipboard precision (± 1 standard error) for samples determined by IR was 6 µmoles (error < 1.0 %).

Table 2 shows the diel variation of chlorinity, total alkalinity, calculated ΣCO_2, IR ΣCO_2 and O_2 resulting from averaging the temporal mixed layer analyses from the 4 locations given in Table 1. An approximately equal number of analyses per parameter (n, Table 2) were concluded at each sampling time, but the number of replicates per analysis differed. For total alkalinity and calculated ΣCO_2, only a single replicate

Table 1. Comparison of indirect (calculated from pH-alkalinity) and direct (IR) ΣCO_2 analyses from the mixed layer at adjacent locations in the northwestern Caribbean Sea. Also shown are results calculated from data given by Park et al. (1964) for an indirect and direct (GC) comparison of ΣCO_2 analyses

Date	Location latitude	Location longitude	Depth* (m)	Number compared	Mean ΣCO_2 µmoles l^{-1} indirect	Mean ΣCO_2 µmoles l^{-1} direct	Differences (indirect–direct) µmoles l^{-1} observed	Differences (indirect–direct) µmoles l^{-1} average**
01–03 March 79	18°31.7'N	80°32.7'W	15	11	1967	1964	+ 3	25
06–07 March 79	18°58.6'N	81°15.2'W	10	8	1978	1982	− 4	9
09–11 March 79	18°38.6'N	81°38.7'W	70	6	1975	1982	− 7	16
14–16 March 79	18°01.2'N	80°52.8'W	70	13	1990	1967	+23	23
Means					1977	1973	+ 4	18
Park et al. (1964)				9	1360	1350	+10	23

* Average depth of mixed layer was 108 m as determined by repeated drops of expendable bathythermographs and salinity determinations

** $\sum_{x=1}^{n} |x_i - x_d| \div n$

Table 2. Comparison of pooled diel data from the 4 locations listed in Table 1 showing the apparent temporal homogeneity in chlorinity in the mixed layer of the Caribbean Sea in contrast to the significant variation in O_2 and IR determined ΣCO_2. Underscored means are significantly different (t-test, one-sided alternative, $p < .05$) from means denoted by asterisk

			ΣCO_2 (μmoles l^{-1})									
Time	Chlorinity (‰)	Total alkalinity (μeq l^{-1})	Indirect (pH-alk.)	n	se	Direct (IR)	n	se	Diff.	O_2 μmoles l^{-1}	n	se
0000	19.951	2298	1977*	6	6	1977	5	7	0	216.4	7	1.1
0300	19.940	2268	1962	5	3	1985	5	8	−23	215.9	7	2.0
0600	19.945	2272	1970	7	11	1978	6	4	− 8	216.8	9	1.6
0900	19.948	2272	1969	6	10	1971	5	3	− 2	218.6	8	1.1
1200	19.942	2276	1972	7	10	1965	5	9	+ 7	216.8	7	1.9
1500	19.944	2277	1974	6	7	1958*	5	10	+16	220.4*	7	1.2
1800	19.947	2287	1978	7	9	1976	5	7	+ 2	217.7	7	0.9
2100	19.951	2275	1966	7	12	1968	5	9	− 2	218.2	7	1.5
Means	19.946	2278	1971			1972			− 1	217.6		
% CV	0.02 %	0.42 %	0.27 %			0.43 %				0.66 %		
r*			+ 0.22 (n.s.)			− 0.80 ($p < .05$)						

* Correlation between ΣCO_2 and O_2 variation. Significance in ( )

was run per analysis; for O_2 and chlorinity, there were 2; and for IR ΣCO_2, there were 8 to 10 replicates. The means show the agreement between ΣCO_2 analyses and indicate that a significant diel variation of O_2 and IR determined ΣCO_2 were superimposed upon an apparently constant chlorinity (% CV = 0.02 %) and diurnally constant total alkalinity. Mean IR ΣCO_2, but not pH-alkalinity ΣCO_2 variation, was correlated (-0.80, $p < .05$; $+.22$, n.s., respectively) with O_2 variation, with the minimum IR ΣCO_2 and maximum O_2 concentrations coinciding at 15:00 and the inverse at 03:00 h. Net CO_2 uptake during the photoperiod at constant alkalinity averaged 2.2 μmoles l^{-1} h^{-1}, however, for the same period net O_2 production was only 0.4 μmoles l^{-1} h^{-1}.

DISCUSSION

Standardization of the ΣCO_2 analysis was difficult during our initial attempts. The analytical precision for natural water samples was often satisfactory, but their accuracy was questionable because dilute inhouse inorganic carbon standard solutions were irreproducible (unstable) even when buffered to pH values between 7.5 and 8.0. Indeed, Salonen (1981) also found that carbonate solutions had to be analyzed immediately after preparation. Fig. 1 shows that these problems were overcome with the use of the concentrated commercial standard and microanalytical procedure. Furthermore, this stability was achieved with considerable savings of time and work, and a high sensitivity to inorganic carbon. Fig. 2 illustrates another aspect of the accuracy problem because in addition to the quality of the standard, and normal random analytical error, it shows that ΣCO_2 measurements are influenced by changes in system response.

As further verification, we adapted the work of Grubbs (1973), who studied the problem of estimating the precision or variability between instruments vs. the variability of the item or product measured. We related our data to his work by treating the simultaneous measurements at 2 concentrations as measurements by 2 separate instruments, and hours (time) as item or product variability. Following Grubbs (1973), the estimates of variability over time and the random variation of measurements at two concentrations are given by:

$$S^2 = \frac{\Sigma(x_{i_1} - \bar{x}_1)(x_{i_2} - \bar{x}_2)}{n-1}$$

$$S_1^2 = \frac{\Sigma(x_{i_1} - \bar{x}_1)^2}{n-1} - S^2$$

$$S_2^2 = \frac{\Sigma(x_{i_2} - \bar{x}_2)^2}{n-1} - S^2$$

where x_{ij} ($i = 1, --n$, $j = 1,2$) = measurement at i^{th} time and the j^{th} concentration. The integrator count variability over time due to fluctuations in analyzer response (S^2) was computed to be 4017 and the variation of measurements at the two concentrations (S_1^2, S_2^2) were 0 and 2226, respectively. Because S^2 is much larger than the random variation at both concentrations we conclude that drift in analytical system response is detectable over the noise of the system, and that simultaneous or interreplicate standardization is necessary for accurate IR measurements of ΣCO_2 during diel studies. The differences in parameters calculated from ΣCO_2 variation on 6–7 June, 1979, in the

estuarine mesocosm also shows that the method of standardization can significantly influence the calculations. Presumably the best procedure would yield the greater correlation and agreement with parallel O_2 data. Interreplicate standardization gave the most accurate results.

It was not until the microanalytical technique, the commercial standard, and interreplicate standardization were adopted that we obtained significant correlations with other independently measured variables. Diel studies carried out in the marsh on August 30 and October 11, 1978, in addition to the MERL mesocosm on June 6, 1979, showed that ΣCO_2 variation was inversely and significantly ($p < .01$) correlated with O_2 variation ($r = -0.80$, -0.80, and -0.91, respectively). Dissolved organic carbon (DOC) variation, also measured, was inversely correlated with CO_2 uptake in the saltmarsh, and DOC release accounted for approximately 18 % of the apparent net production (Johnson et al., 1981). These data, as well as the agreement between indirect and direct ΣCO_2 determinations in the open ocean (Tables 1 and 2) indicate that the procedures employed were valid.

We have not experimented further with inhouse standards because the commercial standard has proven to be simple to use, stable, precise, and accurate. A fritted inorganic carbon chamber (# 525IC) which can be adapted for use with the 0524B TCS is now available. This device essentially relocates the purging vial much closer to the IR detector, and the frit disperses the carrier gas throughout the acid solution for rapid removal of CO_2 without peak tailing. We have found that the fritted chamber reduces analysis time about 50 %, but without an increase in precision.

In the open ocean a statistically significant CO_2 uptake during the photoperiod was observed at 3 of the 4 locations in Table 1. Pooling these data and averaging over time (Table 2) yielded a significant inverse correlation between diel O_2 and IR ΣCO_2, but neither was correlated with chlorinity. In contrast to ΣCO_2 and O_2, chlorinity variation was apparently reduced by averaging, suggesting that it was random or primarily random analytical error. Temperature never changed by more than 0.2 C° throughout the investigation. While the chlorinity data appear to rule out gross changes in the water mass studied, the effect of small scale spatial variation on ΣCO_2 and O_2 dynamics is not known. Nevertheless, Table 2 shows that lower ΣCO_2's during the photoperiod coincided with O_2 production in the mixed layer and that this occurred at constant alkalinity suggesting photosynthesis but not changes in carbonate minerals as a factor in ΣCO_2 dynamics (Smith and Key, 1975). Higher alkalinities were observed at 24:00 and possibly 18:00 h. Resolution and precipitation of inorganic carbonate salts would affect total alkalinity (TA) and IR ΣCO_2 so that changes in TA may signal physical processes or spatial variation and therefore error in estimates of biological metabolism derived from IR ΣCO_2 measurements. However, changes within the same water mass could indicate nocturnal (Table 2) biological processes involving carbonate minerals, and in this case delta ΣCO_2 could be consistent with net biological activity.

Careful measurements of ΣCO_2 by IR photometry in 3 marine ecosystems, including the open ocean, yielded a similar pattern of diurnal consumption and nocturnal production of CO_2. The open ocean results require verification. To date the North Sea is the only other offshore occurrence of a diel ΣCO_2 variation that we know of (Weichart, 1980). Variation in ΣCO_2 is well documented over coral reef communities (Smith and Kroopnick, 1981) and seaweed beds (Smith, 1981). Unfortunately, the exhaustive GEOSECS (Bainbridge, 1981) evaluation of oceanic ΣCO_2 did not encompass diel studies. From the data of Weichart (1980), we have estimated net CO_2 uptake during the photoperiod to be approximately 0.4 μmoles l^{-1} h^{-1} for the North Sea, while that over a coral reef community (Smith and Kroopnick, 1981) was 9 μmoles l^{-1} h^{-1}. Our result of 2.2 μmoles l^{-1} h^{-1} for the Caribbean Sea lies within this range. However, our findings must be tempered with the knowledge that there was a 6 fold difference in O_2 and ΣCO_2 based estimates of total net system metabolism, a photosynthetic quotient of 0.2, and a wide departure from ^{14}C estimates of carbon fixation which require clarification. For example, the ΣCO_2 and O_2 based estimates of net production in non-contained water exceed by up to 100 and 10 fold, respectively, previous ^{14}C results for the Caribbean Sea (Koblentz-Mishke et al., 1970). The $CO_2 - O_2$ discrepancy in net metabolism was 2 fold in the nearshore environments ($PQ = 0.5$), but both parameters continued to show larger total system metabolism than ^{14}C bottle assays would predict (Johnson et al., 1981). Part of this difference may be due to microbial inhibition by trace metals leaching from glass bottles (Carpenter and Lively, 1980; Knauer and Martin, 1981). Considering the implications of our results for aquatic ecosystem dynamics, more work is needed to refine observations on ΣCO_2 variation as a means of measuring true total system metabolism in both free and contained waters.

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