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Precise and Accurate Determination by Infrared Photometry of CO₂ 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 (ΣCO₂), 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 ~1 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) ΣCO₂ 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 CO₂ which were inversely correlated with O₂ variation in a salt marsh, an estuarine mesocosm, and the Caribbean Sea.

INTRODUCTION

Total carbon dioxide (ΣCO₂) 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) ΣCO₂ determination as a measure of biological activity, and although the literature contains many discrete measurements of ΣCO₂ by IR, there is not much information on biological productivity and respiration from IR determined CO₂ variation. Seasonal patterns of ΣCO₂ by IR analyzers in lakes (Rich, 1979) and of CO₂ partial pressure in marine waters (Teal and Kanwisher, 1966) have been reported. Schindler and Fee (1973) monitored diel variation in ΣCO₂ in a Canadian experimental lake, but by gas chromatography. The sparsity of IR ΣCO₂ estimates of system metabolism may result from methods too elabo-

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rate 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 ¹⁴C 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 CO₂ 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 ΣCO₂ at 2 or 3 h intervals over diel cycles to determine net apparent total ecosystem metabolism by integrating the area under the ΣCO₂ rate of change curves (Odum and Hoskin, 1958). In this paper we summarize our experiences with the ΣCO₂ analysis and describe improvements in technique which yielded good analytical precision and agreement between parallel pH-alkalinity ΣCO₂ determinations in the open ocean. ΣCO₂ variation measured in diverse marine ecosystems by IR was inversely correlated with O₂ variation (Winkler...
METHODS AND METHODS

Between 1977 and 1979, $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ measurements were made on the Oceanography International (01) 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 $\Sigma$CO$_2$ 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 (Columbus Scientific, Austin, TX), and a known N$_2$-CO$_2$ mixture (span gas) was used to monitor and correct IR amplifier drift. All $\Sigma$CO$_2$ 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 ml of the undiluted Harleco standard and 200 ml natural seawater samples containing 3.95 to 5.08 $\mu$g C were injected into the purging vial with microliter syringes ($\approx 7$ ml, 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 ml 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.

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, $\Sigma$CO$_2$ 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 $\mu$g$^{-1}$ 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 ml from this bottle. The inorganic carbon level represented 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 ≥ 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 CO₂ 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 ΣCO₂ results obtained with them yielded a correlation coefficient with parallel O₂ 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 ΣCO₂ results based on the composite curve and the production to respiration ratio (P/R) was 1.81. For ΣCO₂ results based on the time-dependent standards (Fig. 2) and from the O₂ 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) ΣCO₂ 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 ΣCO₂ 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 ΣCO₂, IR ΣCO₂ and O₂ 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 ΣCO₂, only a single replicate

Table 1. Comparison of indirect (calculated from pH-alkalinity) and direct (IR) ΣCO₂ 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 ΣCO₂ analyses

<table>
<thead>
<tr>
<th>Date</th>
<th>Location latitude longitude</th>
<th>Depth * (m)</th>
<th>Number compared</th>
<th>Mean ΣCO₂ µmoles l⁻¹</th>
<th>Differences (indirect–direct) µmoles l⁻¹ observed average**</th>
</tr>
</thead>
<tbody>
<tr>
<td>01–03 March 79</td>
<td>18°31.7' N 80°32.7' W</td>
<td>15</td>
<td>11</td>
<td>1967</td>
<td>1964</td>
</tr>
<tr>
<td>06–07 March 79</td>
<td>18°38.6' N 81°15.2' W</td>
<td>10</td>
<td>8</td>
<td>1978</td>
<td>1982</td>
</tr>
<tr>
<td>09–11 March 79</td>
<td>18°38.6' N 81°38.7' W</td>
<td>70</td>
<td>6</td>
<td>1975</td>
<td>1982</td>
</tr>
<tr>
<td>14–16 March 79</td>
<td>18°01.2' N 80°52.8' W</td>
<td>70</td>
<td>13</td>
<td>1990</td>
<td>1967</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>9</td>
<td>1360</td>
<td>1350</td>
<td></td>
</tr>
</tbody>
</table>

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

** n  \sum_{i=1}^{n} |x_i - \bar{x}_d| + n
was run per analysis; for O₂ and chlorinity, there were 2; and for IR ΣCO₂, there were 8 to 10 replicates. The means show the agreement between ΣCO₂ analyses and indicate that a significant diel variation of O₂ and IR determined ΣCO₂ were superimposed upon an apparently constant chlorinity (% CV = 0.02 %) and diurnally constant total alkalinity. Mean IR ΣCO₂, but not pH-alkalinity ΣCO₂, variation, was correlated (−0.80, p < 0.05; +0.22, n.s., respectively) with O₂ variation, with the minimum IR ΣCO₂ and maximum O₂ concentrations coinciding at 15:00 and the inverse at 03:00 h. Net CO₂ uptake during the photoperiod at constant alkalinity averaged 2.2 μmoles l⁻¹ h⁻¹, however, for the same period net O₂ production was only 0.4 μmoles l⁻¹ h⁻¹.

**DISCUSSION**

Standardization of the ΣCO₂ 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 ΣCO₂ 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:

\[ S_i^2 = \frac{\Sigma(x_{ij} - \bar{x}_i)(x_{ij} - \bar{x}_2)}{n - 1} \]

\[ S_j^2 = \frac{\Sigma(x_{ij} - \bar{x}_j)^2}{n - 1} - S_i^2 \]

\[ S_k^2 = \frac{\Sigma(x_{ij} - \bar{x}_j)^2}{n - 1} - S_i^2 \]

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 (\( S_i^2 \)) was computed to be 4017 and the variation of measurements at the two concentrations (\( S_j^2, S_k^2 \)) were 0 and 2226, respectively. Because \( S_i^2 \) 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 ΣCO₂ during diel studies. The differences in parameters calculated from ΣCO₂ 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 O₂ 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 ΣCO₂ variation was inversely and significantly (p < .01) correlated with O₂ variation (r = -0.80, -0.80, and -0.91, respectively). Dissolved organic carbon (DOC) variation, also measured, was inversely correlated with CO₂ 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 IR CCO₂ 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 CO₂ 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 CO₂ 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 O₂ and IR ΣCO₂, but neither was correlated with chlorinity. In contrast to ΣCO₂ and O₂, 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 ΣCO₂ and O₂ dynamics is not known. Nevertheless, Table 2 shows that lower ΣCO₂'s during the photoperiod coincided with O₂ production in the mixed layer and that this occurred at constant alkalinity suggesting photosynthesis but not changes in carbonate minerals as a factor in ΣCO₂ dynamics (Smith and Key, 1975). Higher alkalinites were observed at 24:00 and possibly 18:00 h. Resolution and precipitation of inorganic carbonate salts would affect total alkalinity (TA) and IR ΣCO₂ so that changes in TA may signal physical processes or spatial variation and therefore error in estimates of biological metabolism derived from IR ΣCO₂ measurements. However, changes within the same water mass could indicate nocturnal (Table 2) biological processes involving carbonate minerals, and in this case delta ΣCO₂ could be consistent with net biological activity.

Careful measurements of ΣCO₂ by IR photometry in 3 marine ecosystems, including the open ocean, yielded a similar pattern of diurnal consumption and nocturnal production of CO₂. The open ocean results require verification. To date the North Sea is the only offshore occurrence of a diel ΣCO₂ variation that we know of (Weichart, 1980). Variation in ΣCO₂ is well documented over coral reef communities (Smith and Kroopnick, 1981) and seaweed beds (Smith, 1981). Unfortunately, the exhaustive GEOSCECS (Bainbridge, 1981) evaluation of oceanic ΣCO₂ did not encompass diel studies. From the data of Weichart (1980), we have estimated net CO₂ uptake during the photoperiod to be approximately 0.4 μmol l⁻¹ h⁻¹ for the North Sea, while that over a coral reef community (Smith and Kroopnick, 1981) was 9 μmol l⁻¹ h⁻¹. Our result of 2.2 μmol 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 O₂ and ΣCO₂ based estimates of total net system metabolism, a photosynthetic quotient of 0.2, and a wide departure from ¹⁴C estimates of carbon fixation which require clarification. For example, the ΣCO₂ and O₂ based estimates of net production in non-contained water exceed by up to 100 and 10 fold, respectively, previous ¹⁴C results for the Caribbean Sea (Koblentz-Mishke et al., 1970). The CO₂ - O₂ 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 ¹⁴C 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 Marti, 1981). Considering the implications of our results for aquatic ecosystem dynamics, more work is needed to refine observations on ΣCO₂ variation as a means of measuring true total system metabolism in both free and contained waters.

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LITERATURE CITED


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