

**Precise Spectrophotometric Measurement of Seawater pH_T with
an Automated Apparatus using a Flow Cell in a Closed Circuit**

by

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閉流路中のフローセルを用いた分光光度法自動分析装置による

海水の高精度 pH_T 測定

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Preface

Since the Industrial Revolution, huge amounts of carbon dioxide have been released into the atmosphere as a result of human activities. The increase of anthropogenic CO₂ in the atmosphere is thought to cause global warming that gives rise to climate change. About one-third of the anthropogenic CO₂ has been absorbed by the oceans. When dissolved in seawater, CO₂ exists in the form of carbonic acid. Therefore, the uptake of anthropogenic CO₂ by the world's oceans leads to acidification of the oceans. Ocean acidification is of great concern because it can seriously affect marine ecosystems. Therefore, it is necessary to monitor the pH of seawater, which is a measure of ocean acidification.

Measurements of pH have long been used in the field of chemistry. However, a universally accepted method of measurement, which includes the standards for measurements of seawater samples, has not yet been established. Consequently, comparison of pH data obtained from different sources is problematic. Furthermore, pH measurements collected from different cruises or other locations must be directly comparable so that ocean acidification may be detected.

For these reasons, the present study was initiated with financial support from the research programme “Global Carbon Cycle and Related Mapping based on Satellite Imaginary Programme” of the Research Promotion Fund from the Ministry of Education, Culture, Sports, Science and Technology, Japan, as well as the research programmes “Observational Study on the Seasonal and Interannual Variability and its Mechanisms of the Carbon Dioxide System at the Air-Sea Interface and in the Upper Layer of the Ocean” and “Observational Study on the Variability of Carbon Cycle in the Ocean, I and II” of the Meteorological Research Institute, Japan Meteorological Agency's research fund.

In this technical report, a new spectrophotometric technique for the measurement of p*H*_T, p*H* as measured on the total hydrogen ion concentration scale, is described. This technique provides p*H*_T values for seawater with repeatability better than 0.002 and with comparability of 0.003. By applying the technique to oceanic waters, we will be able to detect seawater acidification as well as obtain new information on ocean carbon cycles.

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序 文

産業革命以来の人類活動の結果、膨大な量の二酸化炭素が大気中に放出されてきた。大気中における人類起源の二酸化炭素の増加は気候変動を引き起こす地球温暖化の原因と考えられている。大気中に放出された人類起源二酸化炭素のかなりの量が海洋に吸収される。即ち、人類起源の二酸化炭素のおよそ三分の一は海洋に吸収されている。一方、一旦溶液（海水）に溶解した二酸化炭素は酸（炭酸）として挙動する。海洋へ多量の人類起源の二酸化炭素の溶入は、海洋の酸性化をもたらす。さらに、海洋の酸性化は海洋生態系に重大な影響が及ぶのではないかと懸念されている。従って、酸性化の尺度である海水の pH を監視することは緊急の課題である。

化学の分野では古くから pH の測定が実施されてきた。しかし、pH 測定分野で高精度のトレーサビリティの確保された pH 測定システムはまだ確立されていない。海水の酸性化を検出するためには、高精度であるばかりでなく異なった航海や異なった観測機関で pH の測定値のコンパラビリティ（比較可能性）が確保されることが重要である。その理由の一つは、海洋の二酸化炭素吸収により引き起こされる pH 変化が小さく長期に及ぶ観測が必要なためである。

以上のことを背景に、本研究は「炭素循環に関するグローバルマッピングとその高度化に関する国際共同研究（科学技術庁振興調整費）」のサブテーマである「海洋表層における炭素フラックスと一次生産に関する研究」の一環として始まり、続いて気象研究所の経常研究「大気・海洋間及び海洋表層における炭酸系の季節・経年変動とそのメカニズムの解明に関する観測的研究」、「海洋における炭素循環の変動に関する観測的研究 I、II」の一環として行われた。

本報告では、 pH_T （全海水水素イオン濃度）を定義すると共に、新しい分光学的方法による pH_T の測定が紹介される。この方法では、pH 単位で 0.002 以下の繰り返し精度で、また 0.003 のコンパラビリティで高精度の pH_T の測定を実施することができる。海水の pH に対してこの分析法を適用すれば、海洋の炭素循環に新しい知見を加えるばかりでなく海洋の酸性化に対する直接の証拠を提供することができるであろう。

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Abstract

We constructed a new automated apparatus for the precise spectrophotometric measurement of the pH of seawater samples. This method uses *m*-cresol purple as an indicator dye. The new apparatus was capable of continual onboard measurement at intervals of 10 minutes as well as bottle sample measurement. The measured pH values (on a total hydrogen ion concentration scale, pH_T) for certified reference materials (CRMs) used as standards for total dissolved inorganic carbon (TCO₂) and total alkalinity (TA) analyses agreed with the pH_T values calculated from the certified TCO₂ and TA values within ±0.003 when the carbonic acid dissociation constants proposed by Lueker et al. (2000) were used in the calculations. The pH_T values of surface seawater measured at sea during various cruises coincided with the values calculated from the partial pressure of carbon dioxide (*p*CO₂) and TCO₂ within ±0.020. The repeatability of pH_T measurements for a single cruise was ±0.0002 (*n* = 32) for onboard measurements and ±0.002 for bottle sample measurements. The larger value for bottle sample measurements resulted from an uncertainty of ±0.0011 (*n* = 32) caused by bottling of seawater samples and from a perturbation of 0.0012 caused by the addition of a saturated HgCl₂ solution as a bactericide. Data for onboard measurements and for bottle sample measurements agreed within the error limits. The reproducibility of several sets of measurements taken during different cruises over several years as examined by the measurement of CRMs using different batches of dye solutions was ±0.003 (1σ, *n* = 34). We obtained stable pH_T values (±0.002, *n* = 24) for samples of surface seawater that had been sterilized with HgCl₂, sealed in borosilicate glass bottles, and stored. The stability of the values enabled us to analyse the pH_T of seawater samples at a laboratory on land after collecting the samples during cruises.

概 要

指示薬色素として *m*-クレゾールパープルを使用する分光光度法により海水の pH を高精度に自動分析する装置を我々は新たに開発した。新装置は洋上での連続測定と共に容器試料の測定を 10 分間隔で行うことが可能である。全炭酸濃度 (TCO₂) と全アルカリ度 (TA) 分析用の認証参照物質 (CRM) の pH (全海水水素イオン濃度、pH_T) 分析結果は、Lueker ら (2000) によって提唱された炭酸の酸解離定数を用いて TCO₂ と TA の認証値から計算した pH_T 値と ±0.003 の範囲で一致した。洋上観測においては、表面海水の pH_T 分析値は海水中の二酸化炭素分圧 (*p*CO₂) と TCO₂ から計算した pH_T 値と ±0.020 の範囲で一致した。単一航海における pH_T 測定の繰り返し性 (repeatability) は、連続測定については ±0.0002 (n = 32)、容器試料測定については ±0.002 だった。容器試料測定のばらつきがより大きいのは、海水の瓶詰めで生じる不確かさ (±0.0011、n = 32) と殺菌のために添加された飽和塩化水銀 (II) 溶液による摂動 (0.0012) の結果である。連続測定と容器試料測定の結果は測定誤差範囲内で一致した。異なるバッチの色素溶液を使用して CRM 測定によって見積もった数年間の異なる航海間の測定の再現性 (reproducibility) は、標準偏差 0.003 (n = 34) だった。海水試料を塩化水銀 (II) で殺菌してホウケイ酸ガラスびんに密封した場合、表面海水試料を保存してもその pH_T は安定していた。これによって、航海後に陸上実験室で海水の pH_T を分析することが可能になった。

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1. Introduction

The concentration of carbon dioxide (CO₂), a major greenhouse gas, has been increasing in the atmosphere because of anthropogenic activities such as fossil fuel burning (IPCC, 2007b; WDCGG, 2007). The current concentration of CO₂ in the atmosphere is approximately 100 ppm higher than that during the preindustrial era, i.e. early 18th century (280 ppm; IPCC, 2007a). To predict future atmospheric CO₂ concentrations on the basis of emission scenarios and the potential alteration of carbon cycles caused by climate change, the processes that control the flux of CO₂ and its temporal variability among global carbon reservoirs, including the atmosphere, the terrestrial biosphere and the oceans, must be understood.

The oceans are the important sinks for anthropogenic CO₂. The latest estimate of ocean CO₂ uptake is 2.2 ± 0.5 GtC yr⁻¹ (IPCC, 2007b). Recent model simulation revealed that uptake of anthropogenic CO₂ by the oceans will lower the average pH of surface seawater by approximately 0.1 compared with the pH in 1750 (Caldeira and Wickett, 2003). The increase in CO₂ concentration in the ocean's surface layer affects the chemical equilibrium in seawater. As CO₂ concentrations increase, surface waters become more acidic, and the concentration of carbonate ion consequently decreases. This change in chemical equilibrium reduces the capacity of the ocean to take up additional CO₂ (Sabine et al., 2004). Moreover, model simulations have indicated that anthropogenic ocean acidification might impact calcifying organisms at high latitudes, particularly in the Southern Ocean, within the next few decades, not centuries as suggested previously (Orr et al., 2005). Therefore, monitoring ocean acidification and its influence on biogeochemical processes by means of field observations is highly important.

At the hydrogen ion concentration range of seawater observed in the oceans, CO₂ predominantly exists as hydrogencarbonate (HCO₃⁻) or carbonate (CO₃²⁻) ions. The concentrations of these ions are determined by mass balance, charge balance and the acid–base equilibrium within the carbonate system in seawater. This carbonate system can be described by four measurable parameters: CO₂ partial pressure (*p*CO₂), total inorganic carbon (TCO₂), hydrogen ion concentration ([H⁺]) and total alkalinity (TA). The hydrogen ion concentration, usually reported as pH (= -log [H⁺]), determines the ratio of carbonate to TCO₂ and the ratio of hydrogencarbonate to TCO₂. Given two of these four parameters, one can calculate the remaining two from the thermodynamic relationships between the parameters (e.g., Lee and Millero, 1995).

We have been observing *p*CO₂ in the atmosphere and in near-surface seawater (i.e., air in which CO₂ gas exchange between a great excess of near-surface water has attained the equilibrium) for more than two decades and TCO₂ in seawater for more than a decade, mainly in the western Pacific Ocean and the equatorial Pacific Ocean (Inoue, 1995; Inoue, 2000; Ishii et al., 2001; Midorikawa et al., 2005). We aimed

to describe the temporal and spatial variations of these parameters and to understand the factors that control them. In addition, we started to measure pH in seawater with high comparability and precision in the late 1990s to describe the variability in the carbonate system not only in surface waters but also at greater depths. We continually improve our methods to measure these parameters precisely.

Midorikawa et al. (2005) reported that $p\text{CO}_2$ values in the atmosphere and in surface waters in the western subtropical North Pacific Ocean increased at rates of $1.60 \pm 0.03 \mu\text{atm yr}^{-1}$ and $1.6 \pm 0.2 \mu\text{atm yr}^{-1}$, respectively between 1984 and 2003. This rate of increase in oceanic $p\text{CO}_2$ corresponds to an annual change in pH of -0.002 yr^{-1} at a constant TA of $2300 \mu\text{mol kg}^{-1}$. Therefore, a pH measurement precision of 0.002 or better is required to monitor long-term oceanic acidification (DOE, 1994).

Seawater pH was formerly measured by a potentiometric method using a pair of glass and Ag/AgCl reference electrodes (e.g., Grasshoff, 1976) based on the operational definition of pH in the absence of seawater-based reference material. However, because of experimental problems such as the fluctuation in electrode sensitivity and residual liquid junction potential, a precision better than ± 0.01 was rarely achieved, even with very careful measurements (Dickson, 1993). For more precise measurements, a spectrophotometric method has been developed (Byrne et al., 1988; Dickson, 1993). In this study, we constructed an automated apparatus with a flow-type spectrophotometric cell in a closed circuit. Using this apparatus, we achieved the precision of ± 0.0002 for on board measurements. The reproducibility of measurements and the effects of sample storage were also examined.

2. Methods and materials

2.1 Principles

In the spectrophotometric method, pH is determined by measuring the visible absorption spectra of seawater samples containing indicator. Our method was based on that described by Clayton and Byrne (1993), who used *m*-cresol purple as an indicator dye.

At the pH range of seawater (7.4–8.2), the predominant pair of acid (HI^-)¹ and base (I^{2-})¹ forms of *m*-cresol purple are in equilibrium with each other (Eq. (1)):



¹ Though the net charges of the acid form and the base form of *m*-cresol purple are 0 and -1 , respectively, we express these ions as HI^- and I^{2-} for the sake of convenience, as such notation is conventional for dibasic acids.

The dissociation constant of HI^- (K_2) is expressed as

$$K_2 = \frac{[\text{H}^+]_{\text{T}}[\text{I}^{2-}]}{[\text{HI}^-]}, \quad (2)$$

where $[\text{H}^+]_{\text{T}}$ denotes the concentration of hydrogen ions (mol kg^{-1} seawater) expressed by the total hydrogen ion concentration scale: $[\text{H}^+]_{\text{T}} = [\text{H}^+]_{\text{F}}(1 + [\text{SO}_4]_{\text{T}}/K_{\text{HSO}_4})$, where $[\text{H}^+]_{\text{F}}$ is the concentration of free hydrogen ions, $[\text{SO}_4]_{\text{T}}$ is the total concentration of sulphate ions and K_{HSO_4} is the acid dissociation constant of hydrogensulphate ion (Dickson, 1990). Seawater pH_{T} is then expressed as Eq. (3),

$$\text{pH}_{\text{T}} = -\log[\text{H}^+]_{\text{T}} = \text{p}K_2 + \log \frac{[\text{I}^{2-}]}{[\text{HI}^-]}. \quad (3)$$

The dye absorption spectrum in the visible wavelength region is used to estimate $[\text{I}^{2-}]/[\text{HI}^-]$. The maximum absorbance is observed at 578 nm for I^{2-} and at 434 nm for HI^- . The $[\text{I}^{2-}]/[\text{HI}^-]$ ratio is calculated from the ratio $R = A_{578}/A_{434}$ and the ratios of the molar extinction coefficients e_1 , e_2 and e_3 , which are expressed as $\varepsilon_{578}(\text{HI}^-)/\varepsilon_{434}(\text{HI}^-)$, $\varepsilon_{578}(\text{I}^{2-})/\varepsilon_{434}(\text{HI}^-)$ and $\varepsilon_{434}(\text{I}^{2-})/\varepsilon_{434}(\text{HI}^-)$, respectively ($\varepsilon_{\lambda}(\text{X})$ denotes the molar extinction coefficient of ion X at wavelength λ). Then, pH_{T} of seawater is calculated from Eq. (4):

$$\text{pH}_{\text{T}} = \text{p}K_2 + \log \frac{[\text{I}^{2-}]}{[\text{HI}^-]} = \text{p}K_2 + \log \left(\frac{R - e_1}{e_2 - Re_3} \right). \quad (4)$$

The ratios of extinction coefficients e_1 , e_2 and e_3 have been determined by Clayton and Byrne (1993). The value of $\text{p}K_2$ ($= -\log K_2/k^0$, $k^0 = 1 \text{ mol kg}^{-1}$) has also been expressed as a function of temperature T (in Kelvin) and salinity S (in practical salinity unit, psu) by the same authors, but the calculated value has been subsequently corrected by 0.0047 on the basis of a revised pH_{T} value accounting for “tris” buffer (DelValls and Dickson, 1998):

$$\begin{aligned} \text{p}K_2 &= \text{p}K_2(\text{Clayton \& Byrne, 1993}) + 0.0047 \\ &= 1245.69/T + 3.8322 + 0.00211(35 - S). \end{aligned} \quad (5)$$

$293 \text{ K} \leq T \leq 303 \text{ K}; 30 \leq S \leq 37$

$e_1 = 0.0069_1; e_2 = 2.222_0; e_3 = 0.133_1.$

For practical calculation of R , measured absorbances are corrected for shifts in baseline absorbance and for the absorbance of the sample itself (background absorbance) by means of Eq. (6):

$$R = \frac{A_{578}(\text{SW} + \text{Dye}) - A_{578}(\text{SW}) - A_{730}(\text{SW} + \text{Dye}) + A_{730}(\text{SW})}{A_{434}(\text{SW} + \text{Dye}) - A_{434}(\text{SW}) - A_{730}(\text{SW} + \text{Dye}) + A_{730}(\text{SW})}, \quad (6)$$

where $A_{\lambda}(\text{SW})$ is absorbance of seawater and $A_{\lambda}(\text{SW}+\text{Dye})$ is absorbance of seawater that contains dye at wavelength λ .

2.2 Apparatus

We constructed an automated pH_T measurement system (Fig. 1) that consists of an ultraviolet/visible (UV/VIS) spectrophotometer (Varian Instruments, Cary 50), a sample and dye solution circuit with an optical flow cell, a temperature-controlled water bath (ADVANTEC[®], LP-3110) and an auto-sampling unit. This apparatus was used for onboard pH_T analysis of surface seawater and bottle samples. Details of sample collection are described in Section 2.6. All tubing was made of fluorinated ethylene-perfluoroalkoxyethylene copolymer (Teflon[®] PFA) except the peristaltic pump (Pharmed[®] tube) and the inlet for the dye solution (polyether-etherketone, PEEK). All commands and sample temperature data were sent to a personal computer (PC) through an I/O controller. Absorbance data were acquired through the PC board, which was directly connected to spectrophotometer.

To achieve precise analysis, a constant dye/sample mixing ratio, precise temperature control and precise absorbance measurement are critical. To keep the dye/sample mixing ratio constant, fixed volume loops (shown as bold lines in Fig. 1) for dye solution and for samples connected to a 6-port switching valve (2 positions) were introduced to the apparatus. First, the sample loop that included the optical cell of the spectrophotometer was filled with a portion of sample (13.1_6 cm^3), and absorbances of sample at wavelength 730 nm, 578 nm, 488 nm and 434 nm ($A_{730}(\text{SW})$, $A_{578}(\text{SW})$, $A_{488}(\text{SW})$ and $A_{434}(\text{SW})$, respectively) were measured. Then, a small amount of dye solution (0.051_2 cm^3) was introduced into another loop. After switching the 6-port valve to its second position, the sample and dye solutions were mixed together by circulation at a flow rate of ca. $75 \text{ cm}^3/\text{min}$. To accelerate mixing, a capillary made of PFA tubing (0.5 mm inner diameter) was placed at the inlet of the cell. Reproducibility and completeness of mixing were confirmed by monitoring the absorbance at the isosbestic point of *m*-cresol purple (488 nm).

Approximately 1.5 minutes (ca. 9 cycles) was required to homogenize the dye concentration by circulation through the combined loop. The final concentration of dye in the sample solutions was $6.2 \mu\text{mol/kg}$ and varied within $\pm 1\%$.

The optical cell is a specially designed water-jacketed cylindrical quartz cell with a path length of 8 cm. The inner tube of the cell was tilted to facilitate ejection of bubbles in the sample. The temperatures in the water jacket (outer tube) and cell holder were regulated within $25.0_0 \pm 0.05 \text{ }^\circ\text{C}$ by circulating temperature-controlled water, and the temperatures were monitored just outside the cell with a Pt-100 Ω resistance thermometer calibrated with a standard resistance thermometer (Pt-25 Ω). Using a flow cell in a closed circuit, seawater sample was not drained from the optical cell before the dye solution was injected. As the result, we reduced the shift in baseline absorbance (± 0.0005) as well as the sample volume required for analysis. To regulate the sample temperature after the seawater sample and dye solutions were mixed, 2.5 minutes was required. This time was also reduced by circulating seawater sample through the temperature controlled closed circuit for approximately 9 cycles. Each complete sample analysis procedure took 10 minutes.

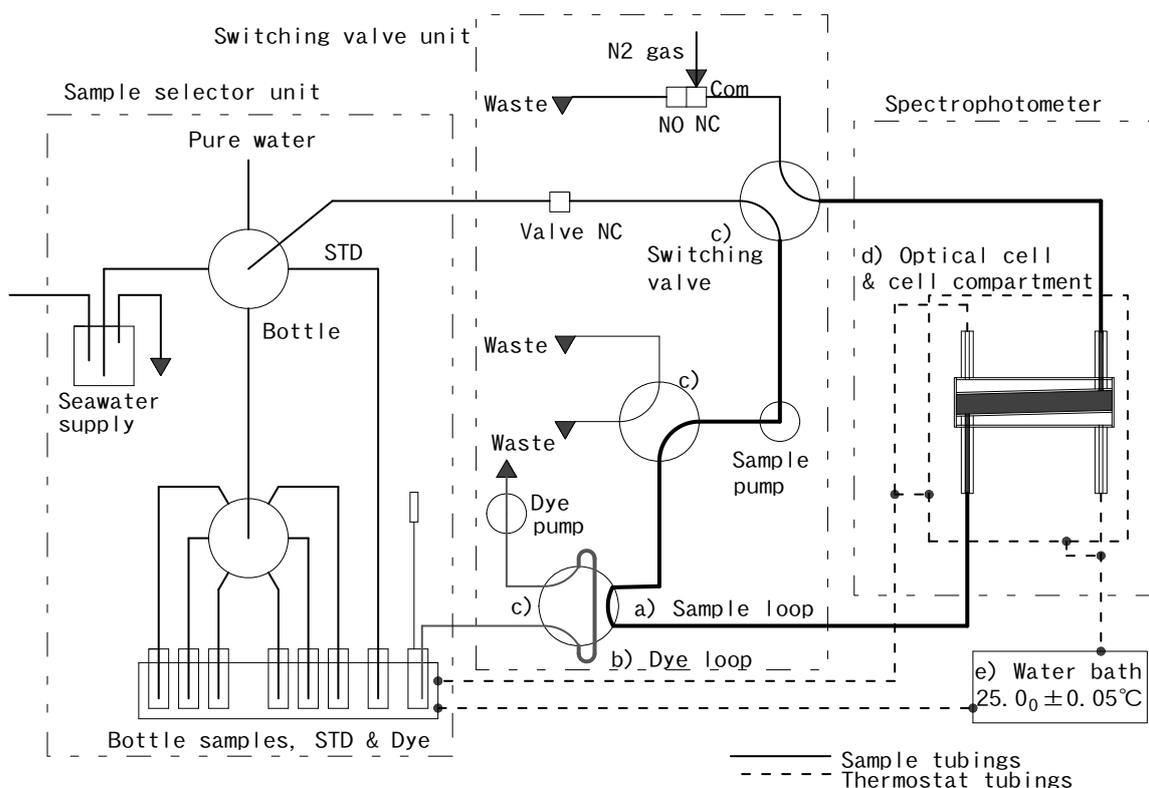


Fig. 1 Schematic diagram of pH_T measurement system. a) Sample loop (13.1_6 cm^3), b) dye loop (0.051 cm^3), c) automated switching valve, d) optical cell and e) water bath. NO, NC and Com indicate the valve position to be normal open (when switched off), normal close, and common (open at all time), respectively.

2.3 Preparation of dye solution

We prepared the indicator dye solution at a concentration of 1.6 mmol kg^{-1} : approximately 1.3 g of *m*-cresol purple sodium salt ($\text{C}_{21}\text{H}_{17}\text{O}_5\text{NaS}$) (Acros Organics, water soluble) was dissolved in 2.0 kg of purified water obtained with a Milli-Q SP TOC system (Millipore). Approximately 0.4 cm^3 of ca. 1.0 M NaOH solution was added to this dye solution while monitoring the absorbance of approximately 0.5 cm^3 aliquot in a 0.5-mm path length quartz cell until the absorbance ratio of the dye solution, $R_{\text{dye}} = A_{578}/A_{434}$, reached 1.6 ± 0.1 .

2.4 Perturbation caused by addition of dye solution

The injection of *m*-cresol purple solution affects the pH_T of seawater samples because the acid-base equilibrium of the seawater is disrupted by the addition of the dye acid-base pair (DOE, 1994). There are two approaches to correct for the perturbation caused by the dye solution: a) an empirical method in which a second aliquot of dye solution is added to the seawater sample (DOE, 1994; Clayton and Byrne, 1993) and b) a theoretical method in which the acid-base equilibrium is calculated (Hunter and Macaskill, 1999). We used the empirical method. In this method, the absorbance ratio R extrapolated at zero dye addition was calculated by subtracting the change in absorbance ratio (ΔR) after the addition of the first and second aliquots of dye solution ($\Delta R_{2-1} = R_2 - R_1$) assuming ΔR_{2-1} to be identical to the change effected by the addition of first aliquot ($\Delta R_1 = R_1 - R$) (Eq. (7)):

$$\begin{aligned} \Delta R &= R_2 - R_1 = R_1 - R \quad (\text{Assumption}), & (7) \\ R &= R_1 - \Delta R. \end{aligned}$$

The value of R obtained by subtracting ΔR from R_1 was used to calculate pH_T by means of Eq. (4).

We measured ΔR over a range of seawater pH_T values and determined a calibration curve for ΔR as a function of R_1 for each batch of the dye solution (Fig. 2a), since ΔR is also a function of the difference in pH between the sample solution and the dye solution. As described above, R_{dye} was adjusted to 1.6 ± 0.1 . The pH value corresponding to R_{dye} was 7.9 ± 0.1 . A quadratic regression (Eq. (8)) reproduced the measured values well with a correlation coefficient of 0.9946. The standard error of ΔR was ± 0.0019 :

$$\Delta R = -0.0185 R_1^2 + 0.0221 R_1 + 0.0029 \quad (R_{\text{dye}} = 1.60) \quad (8)$$

The change in pH_T caused by the addition of the indicator dye ranged from -0.013 to $+0.008$ (Fig.

2b) when 0.051 cm^3 of the dye solution (1.6 mmol kg^{-1}) was added to 13 cm^3 of sample (dye concentration in the sample corresponding to this mixing ratio was $6.2 \text{ } \mu\text{mol kg}^{-1}$).

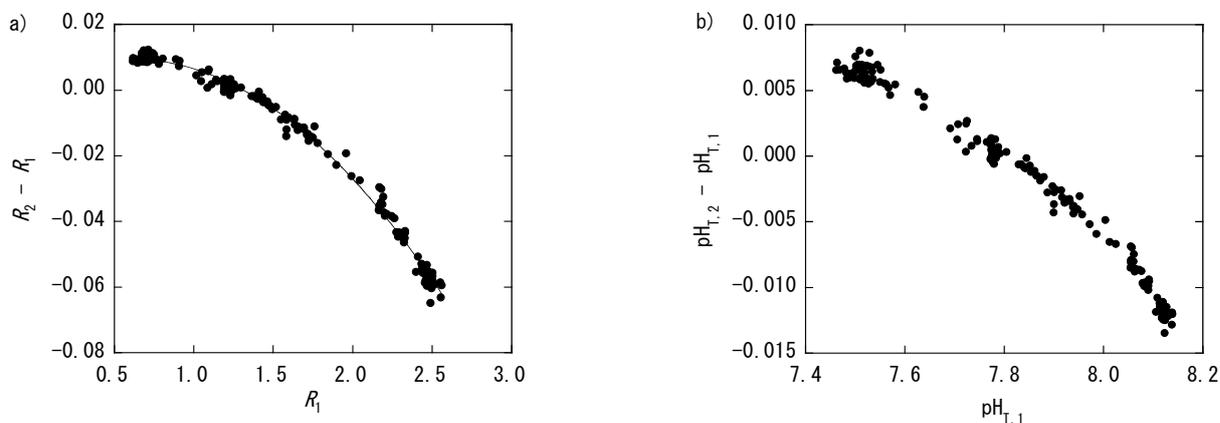


Fig. 2 Example of pH_T perturbation caused by the addition of dye solution. The difference between the addition of the second and first aliquots of dye solution for batch 784C was expressed as a) absorbance ratio R and b) pH_T . Such plots were constructed for each batch of the dye solution used in the measurements.

2.5 Correction for temperature deviations

As described in Section 2.2, the temperature of seawater samples was maintained at $25.0_0 \pm 0.05$ °C and monitored in our pH_T measurement system. However, a change in sample temperature of 0.05 °C could give rise to the change in pH_T of ca. 0.001 (Fig. 3a). Therefore, the pH_T determined at a temperature t ($\text{pH}_T(t)$, with t in °C) was corrected to the pH_T at 25.00 °C ($\text{pH}_T(25)$) with Eq.(9):

$$\begin{aligned} & (\text{pH}_T(t) - \text{pH}_T(25)) / (t - 25.00) \\ & = (2.00170 - 0.735594 \text{pH}_T(25) + 0.0896112 \text{pH}_T(25)^2 - 0.00364656 \text{pH}_T(25)^3). \quad (9) \end{aligned}$$

This equation was determined as follows. Values of $\text{pH}_T(t)$, TCO_2 and salinity were obtained for all bottle samples collected during cruise MR02-K06 (described below). TA was then calculated from each set of measured $\text{pH}_T(t)$, TCO_2 and salinity values by means of the procedure described in DOE (1994). The equilibrium constants given by Lueker et al. (2000) at temperature t ($25.0_0 \pm 0.05$ °C) were used for calculation. Finally, pH_T at temperature t' in the range from 24.7 to 25.3 °C was calculated from TCO_2 , salinity and calculated TA values. The slope of the linear regression of $\text{pH}_T(t') - \text{pH}_T(25)$ versus $t' - 25$ was expressed as a cubic function of $\text{pH}_T(25)$ with a correlation coefficient of 0.99993 ($p < 10^{-8}$, Fig 3b).

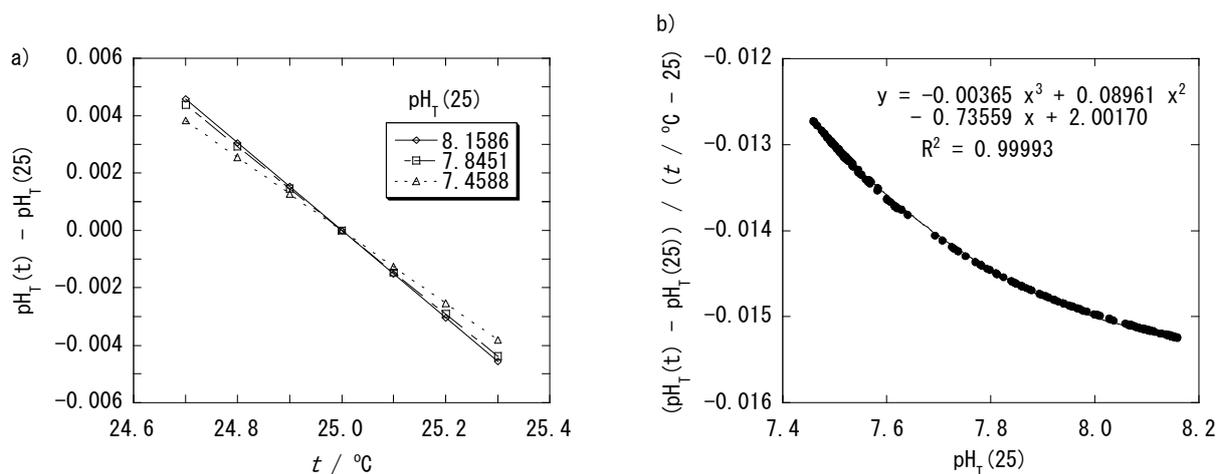


Fig. 3 Effect of sample temperature on the measured pH_T at temperatures from 24.7 °C to 25.3 °C. The difference between $\text{pH}_T(t)$ and $\text{pH}_T(25)$ was proportional to the sample temperature (a). The slope of the plot $\text{pH}_T(t) - \text{pH}_T(25)$ vs. $t - 25$ was expressed by a cubic function of $\text{pH}_T(25)$ with a correlation coefficient 0.99993 (b).

2.6 Seawater samples

Seawater samples were collected during cruises MR00-K08 (January 2001) and MR02-K06 (January 2003) of the research vessel *Mirai*, which belongs to the Japan Agency for Marine-Earth Science and Technology. Samples consisted of near-surface waters, which were pumped continuously from the sea-chest at about 4.5 m below the surface and collected onboard, and of water samples at depth collected with a CTD/carousel sampler.

Onboard measurements for near-surface waters were made along the equator from 160° E to 160° W in the western Pacific warm pool and in the equatorial divergence zone of the Pacific. A portion of water (ca. 0.5 dm³ min⁻¹), which was a branch of seawater pumped up continuously from the sea-chest (ca. 20 dm³ min⁻¹), was used for pH_T measurements. Each sample was introduced into the measurement system through the Teflon[®] PFA tube. Samples were collected twice every 1.5 hours, concurrently with onboard TCO_2 and $p\text{CO}_2$ measurements.

Bottle samples were collected with Niskin bottles (30 dm³ polyvinyl chloride) on the CTD/carousel sampler for deep water and with a bucket for surface water. The samples were transferred to borosilicate glass bottles (Shibata, 250 cm³). A small portion (2 cm³) of sample was removed to allow thermal expansion of the seawater, and 0.2 cm³ of saturated HgCl_2 solution (0.25 mol kg⁻¹) was injected into the larger portion as a bactericide. The bottles were sealed with greased ground-glass stoppers (Apiezon[®] grease, type L). The addition of the bactericide and the sealing of the bottles prevented an increase in CO_2 caused by biological activity in the bottle and prevented CO_2 exchange with ambient air. The effects of bottling and addition of HgCl_2 solution are examined in Section 4.

2.7 Certified reference materials (CRMs) and working standards

We examined the repeatability of the pH_T measurements for a variety of time ranges from a day to a few years using batches of certified reference materials (CRMs; provided by Andrew Dickson; <http://andrew.ucsd.edu/co2qc/>) used as standards for TCO_2 and TA analyses in seawater. We analysed the CRMs at the beginning and the end of each run of pH_T measurements. Since TCO_2 and TA of a CRM are certified, its pH_T is expected to be stable at constant temperature, although its pH_T value has not been certified yet. In addition, we prepared several batches of reference seawaters as working standards, by a method similar to that used to create the CRMs (Dickson, 1991). These reference seawaters were sterilized with HgCl_2 , sealed as described above, and stored in 250 cm³ borosilicate glass bottles.

3. Onboard measurements

We first evaluated the repeatability of onboard pH_T measurements in the laboratory on land by analysing seawater stored in a large-volume polyethylene container (20 dm³). This seawater was a mixture collected at stations along the equator from 145° E to 160° W during cruise MR00-K08 and kept in the dark at room temperature after the addition of HgCl_2 . The average pH_T (with standard deviation) of this test seawater was 7.8598 ± 0.0002 ($n = 32$). The standard deviation was attributable to the baseline fluctuation of the spectrophotometer.

We then examined the repeatability of onboard pH_T measurements at sea from the standard deviations of pH_T data measured when the ship was stopped at several stations during cruise MR02-K06. Although the number of measurements was limited to 5 at most, the standard deviations of pH_T data ranged from 0.0001 to 0.0011 (Table 1). As evident from fluctuations in salinity, seawater samples taken at each station were not as homogeneous as the seawater mixed in a polyethylene container in the laboratory. However, the standard deviations of the pH_T data in onboard measurements suggest that measurement repeatability was comparable to that obtained in the laboratory on land.

Table 1. Repeatability of onboard pH_T measurements of near-surface waters along the equator during cruise MR02-K06.

Date	Longitude	pH_T	Salinity (psu)
2003/1/17	164°29.4'E – 164°33.6'E	8.1345 ± 0.0006 ($n = 5$)	34.434 ± 0.010
2003/1/18	169°59.1'E – 169°59.3'E	8.1396 ± 0.0001 ($n = 4$)	34.337 ± 0.005
2003/1/21	179°17.0'E – 179°18.8'E	8.1472 ± 0.0002 ($n = 5$)	34.101 ± 0.005
2003/1/22	175°38.3'W – 175°37.5'W	8.1557 ± 0.0003 ($n = 4$)	34.143 ± 0.012
2003/1/24	169°59.5'W – 169°57.2'W	8.1001 ± 0.0011 ($n = 3$)	34.170 ± 0.009
2003/1/25	164°48.2'W – 164°47.8'W	8.0934 ± 0.0002 ($n = 4$)	35.321 ± 0.003
2003/1/28	159°58.9'W – 159°58.4'W	8.0689 ± 0.0006 ($n = 3$)	35.265 ± 0.003

Such onboard measurements of pH_T with good repeatability, taken every 45 minutes at an interval of about 12 nautical miles, can be used to find fine structures of pH_T distribution in near-surface seawater. During cruise MR02-K06, a clear boundary was observed near 172° W at the east end of the western Pacific warm pool (Fig. 4). In the warm pool, pH_T was higher than 8.120. It exceeded 8.140 in the region from the international dateline to 172° W. In the divergence zone to the east of 160° W, pH_T decreased towards the east (minimum 8.069 at 160° W). The results of these observations will be described in a future report.

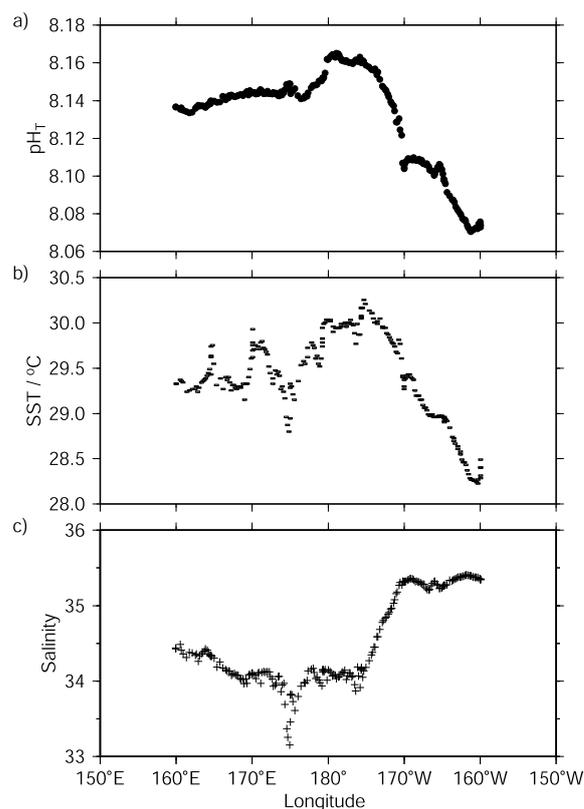


Fig. 4 Horizontal distributions of a) pH_T , b) temperature and c) salinity in near-surface water along the equator observed in January 2003 during the R/V *Mirai*'s MR02-K06 cruise.

4. Measurements of bottle samples taken at depth

For pH_T analysis of bottle samples collected at depths with the CTD/carousel sampler, it has been recommended that the seawater samples be directly withdrawn from Niskin bottles into the optical cells (DOE, 1994). However, it is not convenient to prepare and handle many optical cells during fieldwork. Therefore, we examined the use of sampling bottles and the effect of sterilization with HgCl_2 .

In our study, ca. 500 cm^3 of water sample was drawn with a silicone rubber tube, which was connected to the outlet cock of a Niskin bottle, into a 250 cm^3 borosilicate glass bottle (250 cm^3 was overflowed). After the temperature of the sample was adjusted to $25.0_0 \pm 0.05 \text{ }^\circ\text{C}$ in a water bath, the sample was introduced into the sample loop of the measurement system with a peristaltic pump (see Fig. 1). During cruise MR02-K06, bottle samples were taken in duplicate from a Niskin bottle. The repeatability for the measurement of bottle samples as inferred from duplicate measurements (Fig. 5) was 0.0011 (1σ , $n = 32$). Problems associated with the comparability and precision of pH_T measurements of bottle samples

include a) CO₂ exchange between the sample and ambient air during bottling and during withdrawal of the sample from a rigid glass bottle, b) hydrolysis of HgCl₂ and dilution caused by addition of saturated HgCl₂ solution and c) pH_T change during storage owing to imperfect sterilization or sealing.

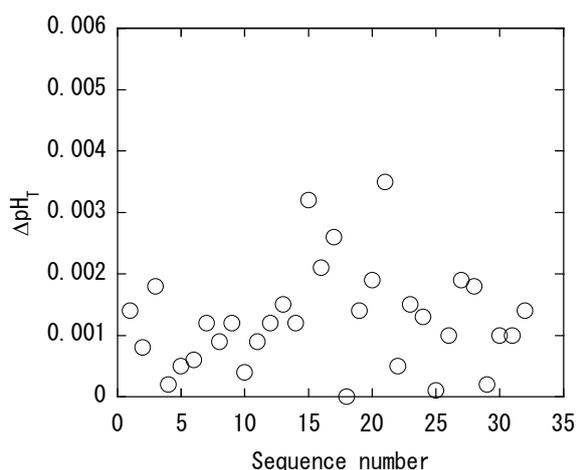


Fig. 5 Differences in pH_T between duplicate measurements, ΔpH_T, of bottle samples without HgCl₂ addition. The short-term standard deviation (repeatability) of the measurements was 0.0011, calculated with Equation 3 in SOP 23 described in DOE (1994).

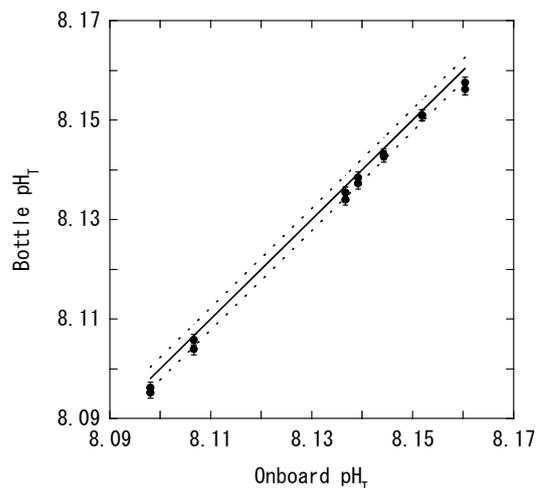


Fig. 6 Comparison of pH_T between bottle and onboard measurements without HgCl₂ addition. Error bars indicate the standard deviation of measurements. The solid line indicates a slope of 1. Broken lines indicate the error limits ($\pm 2\sigma$).

4.1 Effects of bottling and headspace

The effects of CO₂ exchange with ambient air during bottling and exchange with the headspace in a bottle, which arose from withdrawing a seawater sample from a rigid glass bottle, were examined by comparing the analytical results of onboard pH_T measurements with those acquired from bottle sample measurements.

Bottle samples were taken from near-surface water (see Section 2.6) immediately after onboard measurement. No HgCl₂ solution was added to these samples. The difference in pH_T (bottle minus onboard) was -0.0019 ± 0.0010 (1σ , $n = 14$; Fig. 6) and was significant, considering the repeatability of onboard measurements (0.0002) and that of bottle sample measurements (0.0011). However, the uncertainty due to bottling and short-term storage of seawater samples in bottles was as small as the required precision described in the introduction (± 0.002).

Using a method similar to that used to correct for $p\text{CO}_2$ perturbation in bottle samples, we calculated the change in pH_T caused by CO_2 exchange between a sample and headspace in a bottle (DOE, 1994; see Appendix A for details). For surface water (initial pH_T of ~ 8.12), the estimated effect of the headspace on pH_T ranged from -0.0003 to $+0.0000$, depending on the initial headspace $p\text{CO}_2$ value (Fig. 7). These changes were sufficiently smaller than the above-mentioned repeatability of pH_T measurement for bottle samples. Therefore, the effect of headspace, which is less than 1% of the sample volume, on the change in pH_T was negligible for surface water. For deep waters (initial pH_T of 7.48), the estimated effect of the headspace on pH_T reached a maximum of $+0.0012$ for an initial headspace $p\text{CO}_2$ value of $350 \mu\text{atm}$ (Fig. 7). This change was also comparable to the repeatability of pH_T measurements and was hardly detectable. With regard to bottling and headspace, we concluded that pH_T values for bottle samples should reasonably agree with onboard measurements within $\pm 2\sigma$ (0.002) of the repeatability in bottle sample measurements.

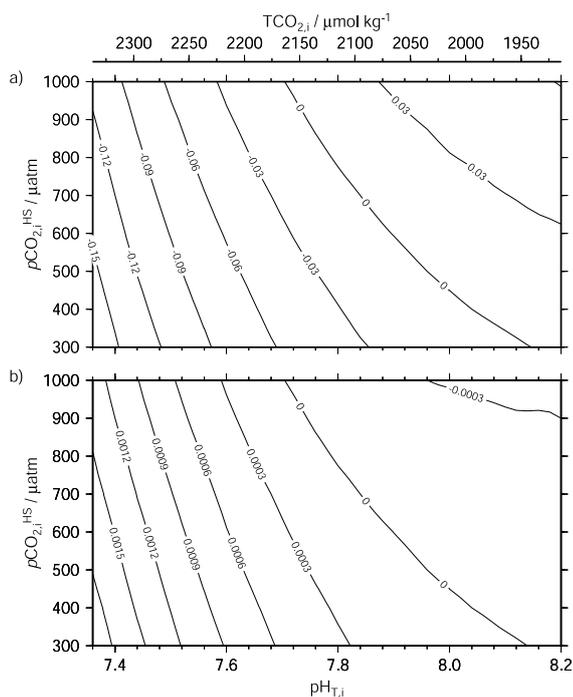


Fig. 7 Effect on pH_T of headspace of 2 cm^3 in a 250 cm^3 sample bottle. a) Quantity of CO_2 (μmol) exchanged between sample and headspace and b) pH_T change caused by CO_2 exchange between a seawater sample and air in a bottle.

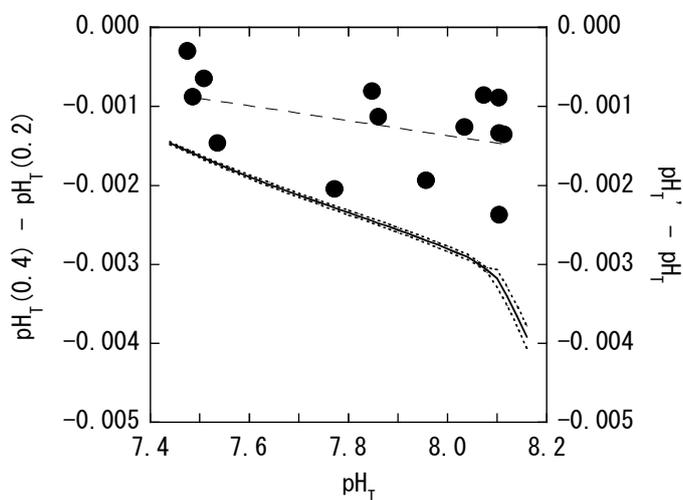


Fig. 8 Observed pH_T change caused by the addition of 0.2 cm^3 of saturated HgCl_2 solution to 250 cm^3 seawater (closed circles). The broken line shows the linear regression of observed values, $\text{pH}_T(0.4) - \text{pH}_T(0.2) = (-0.00094 \pm 0.00059) \text{pH}_T(0.2) + (0.00616 \pm 0.00465)$, $\gamma^2 = 0.17$, $p = 0.14$. Calculated values (solid line) were obtained from TCO_2 and TA using stability constants β of Hg^{2+} complexes at ionic strength 0.7. Dotted lines indicate the uncertainty ($\pm 2\sigma$) of calculation derived from the variability of inputted TCO_2 and TA.

4.2 Perturbation of pH_T caused by addition of saturated HgCl_2 solution

The pH_T measurements of 27 bottle samples collected from various depths at a given station took about 8 hours. If a sample is not sterilized, its pH_T and its TCO_2 could change owing to biological activity occurring prior to measurement. Therefore, bottle samples must be sterilized with saturated HgCl_2 solution. However, the addition of saturated HgCl_2 solution may affect pH_T because of hydrolysis of HgCl_2 and dilution of the sample. To evaluate the perturbation of pH_T caused by the addition of HgCl_2 solution and to correct for this perturbation empirically, we compared the pH_T values of 14 pairs of samples taken from several ocean layers ranging from the surface to a depth of 794 m in the equatorial Pacific Ocean (from 170° W to 165° W) during the cruise MR02-K06. Duplicate samples were collected at each depth, and different volumes of saturated HgCl_2 solution (0.2 and 0.4 cm^3) were added to each of a given pair of samples.

The injection of additional 0.2 cm^3 saturated HgCl_2 solution to each sample already containing 0.2 cm^3 saturated HgCl_2 caused pH_T changes ($\text{pH}_T(0.4) - \text{pH}_T(0.2)$) ranging from -0.0024 to -0.0003 (Fig. 8, closed circles). These changes appeared larger for samples with higher pH_T values, but the correlation was insignificant ($\gamma^2 = 0.17$, $p > 0.1$). Therefore, we corrected for perturbation caused by HgCl_2 addition by adding a constant value of 0.0012₃ (average of $\text{pH}_T(0.4) - \text{pH}_T(0.2)$) to measured pH_T values. The cause of this perturbation is discussed later in Section 7.2.

4.3 Storage of samples

We investigated whether it was possible to store seawater samples for pH_T analysis for a period of a few months. To monitor the change in pH_T of seawater samples stored in borosilicate glass bottles, we collected approximately 20 liters of surface water at $4^\circ 10' \text{ N}$, $156^\circ 40' \text{ E}$ during the cruise MR02-K06. This large sample was subsampled into 30 borosilicate glass bottles 250 cm^3 each and then sterilized with 0.2 cm^3 of saturated HgCl_2 solution before being plugged with a greased ground-glass stopper. The sealed samples were stored at room temperature. The mean pH_T for samples stored for 50 days was 8.1252 ± 0.0011 (1σ , $n = 21$; Fig. 9). There was no significant temporal change ($\gamma^2 = 0.06$, $p > 0.2$). This result suggests that surface water samples can be stored for pH_T analysis with a precision that is comparable to the repeatability of pH_T measurements of samples without HgCl_2 addition (± 0.0011).

For deep-water samples, 42 pairs of duplicate samples were collected at layers ranging from the surface to a depth of 5104 m at 0° , 160° W during the cruise MR02-K06. One of the duplicate samples was

analysed within 15 hours after bottling (referred to as “asap”), and another was analysed 50 days later at a laboratory on land (referred to as “stored”). The changes in pH_T ranged from -0.001 to $+0.007$ after 50 days of storage (Fig. 10). The change in pH_T was larger at 400–1500 m, where pH_T was lower than 7.5. The negative correlation between the change in pH_T ($= \text{pH}_T(\text{stored}) - \text{pH}_T(\text{asap})$) and $\text{pH}_T(\text{asap})$ (Fig. 10b) and the positive correlation between the change in pH_T and $p\text{CO}_2$ (Fig. 10c) suggest the possibility of CO_2 gas exchange between ambient air and samples taken at depth, where pH_T was lower than 7.5. A change in pH_T of $+0.005$ corresponds to a change in TCO_2 of $-4 \mu\text{mol kg}^{-1}$ at constant TA. However, TCO_2 values previously have been observed to remain unchanged when samples are stored by the method described here (Ishii et al., 2000). Since $p\text{CO}_2$ is high in deep-water samples with lower pH_T and higher TCO_2 than those in near-surface seawater, CO_2 could escape from deep-water samples during sampling and measuring processes. Further studies are needed regarding the storage of seawater for pH_T analysis.

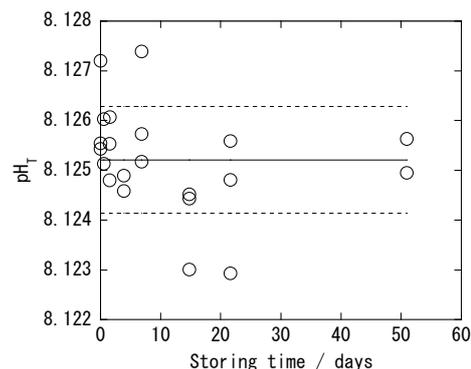


Fig. 9 Time course of pH_T of surface water samples stored in glass bottles with HgCl_2 . The mean (solid line) and the standard deviation (broken line) were 8.1252 and 0.0011, respectively.

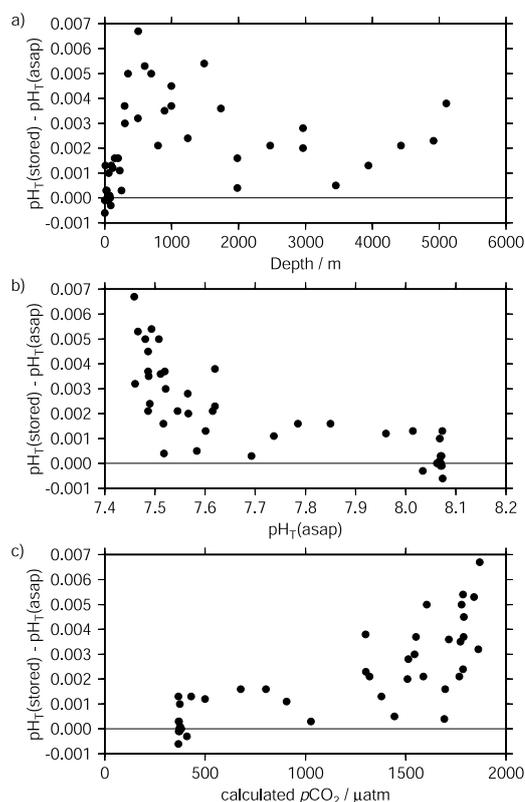


Fig. 10 Difference between the pH_T values measured on board as soon as possible after water samples arrived on deck, $\text{pH}_T(\text{asap})$, and after 50-day storage, $\text{pH}_T(\text{stored})$, versus a) depth, b) $\text{pH}_T(\text{asap})$ and c) $p\text{CO}_2$ calculated from TCO_2 and $\text{pH}_T(\text{asap})$. 0.2 cm^3 of saturated HgCl_2 solution was injected into each sample.

4.4 Uncertainty in pH_T measurements of bottle samples

The pH_T values of bottle samples agreed reasonably with the values measured onboard. The precision of bottle sample measurements as estimated from duplicate sample measurements was 0.0011 (Fig. 5 in Section 4). Taking into account the perturbation due to storage, the uncertainty of bottle sample measurements was 0.001 for surface waters (Fig. 9 in Section 4.3) and a maximum of 0.006 for deep waters (Fig. 10 in Section 4.3) over a period of 50 days.

5. Consistency of measured and calculated pH_T values for CRMs and comparability of pH_T data among several cruises

We measured pH_T values of several batches of CRMs used as standards for TCO_2 and TA analyses to investigate the consistency of pH_T measurements obtained by means of our system with the calculated pH_T values of CRMs certified for TCO_2 and TA. Although the CRMs were not certified for pH_T , their pH_T values could be calculated from the certified TCO_2 and TA values because these values are stable. We compared our measured pH_T values with the values calculated from the certified TCO_2 and TA values of the CRMs using the dissociation constants of carbonic acid in seawater reported by Lueker et al. (2000). We adopted the dissociation constants of Lueker et al. (2000) for the following reasons:

- a) The fugacity of CO_2 ($f\text{CO}_2$) calculated from TCO_2 and TA using these dissociation constants agreed well with measured values in the range up to 500 μatm (Lueker et al., 2000).
- b) The dissociation constants of Lueker et al. (2000) are based on the data of Mehrbach et al. (1973). Lee et al. (2000) reported that $f\text{CO}_2$ calculated from pH_T and TCO_2 using dissociation constants based on the data of Mehrbach et al. (1973) agreed well with measured $f\text{CO}_2$ values.

The pH_T values of CRMs measured during several cruises agreed well with pH_T values calculated from the certified values of TCO_2 and TA (Table 2). Although the difference between the measured and calculated pH_T values for batch 65 was larger than for any other batch, this difference was within the range of experimental error (mean $\pm 2\sigma$). We concluded that the pH_T values obtained by our measurement system were consistent with certified TCO_2 and TA values and that the pH_T measurements we obtained during the different cruises (in which different dye solutions and working standards were used) were comparable.

Table 2 pH_T of CRMs.
Mean \pm standard deviation (bottles, measurements) during individual cruises

Experiment periods (ddmmyy)	Apparatus	Dye soln. batch	WS batch	Batch 58	Batch 62	Batch 65	Batch 72
140103-190303	1	784C	V	7.9118 \pm 0.0007 (2, 4)			
301003-111103	1	826C	W		7.8760 \pm 0.0011 (3, 8)		
170304-210404	1	826C	W		7.8740 \pm 0.0007 (3, 6)		
270804-100904	2	837C	W,X		7.8747 \pm 0.0007 ^a (1, 2)		
241004-271204	1	837C	X		7.8709 \pm 0.0014 (3, 6)		
180805-180805	2	957C	X,Y		7.8747 \pm 0.0005 ^a (1, 2)		
091105-290306	2	957C	Y		7.8712 \pm 0.0016 (3, 6)	7.9155 \pm 0.0006 (2, 4)	7.9009 \pm 0.0001 ^a (1, 2)
130706-140806	2	957C	Y,Z		7.8690 \pm 0.0001 ^a (1, 2)	7.9079 \pm 0.0001 ^a (1, 2)	7.8962 \pm 0.0006 ^a (1, 2)
131206-200107	2	957C, 1058C	27				7.8949 \pm 0.0022 (3, 7)
Whole period				7.9118 \pm 0.0007 (2, 4)	7.8731 \pm 0.0025 (16, 34)	7.9129 \pm 0.0039 (3, 6)	7.8956 \pm 0.0027 (7, 15)
Calculated from TCO_2 and TA ^b				7.9119 \pm 0.0010	7.8735 \pm 0.0014	7.9189 \pm 0.0015	7.8986 \pm 0.0017
Measured – Calculated				-0.0001	-0.0005	-0.0059	-0.0029

^a Mean \pm standard deviation for the difference in analytical results between duplicate measurements.

^b Means \pm standard deviations of pH_T calculated from certified values of TCO_2 and TA using dissociation constants for carbonic acid in seawater reported by Lueker et al. (2000).

6. Procedure to measure pH_T with high comparability and precision

On the basis of our experimental results, we recommend the following experimental procedure for pH_T measurements at sea and on land. CRMs should be measured at the beginning and the end of a cruise or a series of experiments. Working standards should be measured at the beginning and the end of each day of pH_T measurements during a cruise. Prior to data collection during a cruise, we recommend making a property control chart of measured pH_T values (SOP 22 in DOE (1994)) and calculating the mean and standard deviation from at least 12 data points obtained from working standards measurements. If a newly measured value is out of the range of mean $\pm 2\sigma$, an additional bottle of working standard should be analysed. If a couple of measured values are out of the range of mean $\pm 3\sigma$, another batch of working standards or CRMs should be analysed. If the results are out of the range of each mean $\pm 3\sigma$, the apparatus or reagents should be checked to determine the reason for discrepancy. The mean and standard deviation are updated by adding newly accepted data.

The following data processing method is recommended:

- 1) pH_T is calculated from spectrophotometric data by means of Eqs. (4) and (5).
- 2) pH_T should be corrected for the perturbation induced by the addition of dye and saturated HgCl_2 solutions to the sample. Dye correction is expressed by the term ΔR and expressed as a quadratic function of R_1 (Eqs. (7) and (8)). The coefficients in equation (8) should be determined for each batch

of indicator solution. To correct for the perturbation caused by the addition of saturated HgCl_2 solution, the constant value of 0.0012₃ is added to the pH_T data of a sample that has been sterilized with HgCl_2 .

- 3) pH_T measured at temperature t ($^{\circ}\text{C}$) is normalized at temperature of 25.00 $^{\circ}\text{C}$ by means of Eq. (9).

7. Discussion

7.1 Standard deviation of pH_T in reference materials

The averages and standard deviations of the pH_T values of CRMs and working standards for a single cruise and for several cruises are listed in Tables 2 and 3, respectively. The standard deviations for working standards (0.0004–0.0059) during each cruise were on the whole larger than those for CRMs (0.0006–0.0022), except the values from duplicate measurements. The standard deviations were somewhat larger than the repeatability of bottle sample measurements described in Section 4.3 (0.0011). This result indicates that the CRMs and, in particular, working standards were somewhat inhomogeneous. Working standard batch W is likely to be more inhomogeneous than others, because the standard deviation of pH_T for working standard W was larger than that for working standard V and X, which was measured in the same periods of experiments with same apparatus (Table 3). If we exclude the results of working standard batch W, we can conclude that the repeatability of our measurements within a cruise or a series of experiments was better than 0.002.

Table 3 pH_T of working standards.
Mean \pm standard deviation (bottles, measurements) during individual cruises

Experiment periods (ddmmyy)	Apparatus	Dye soln. batch	Batch V	Batch W	Batch X	Batch Y	Batch Z
140103–190303	1,	784C	7.7776 \pm 0.0011 (43, 43)				
171003–221003	1	826C	7.7756 \pm 0.0015 (7, 9)	7.8613 \pm 0.0059 (7, 9)			
301003–111103	1	826C	7.7810 \pm 0.0025 (4, 4)	7.8704 \pm 0.0057 (40, 41)			
170304–210404	1	826C		7.8640 \pm 0.0038 (45, 45)			
270804–100904	2	837C		7.8718 \pm 0.0040 (3, 5)	7.9576 \pm 0.0020 (23, 27)		
300904–011004	1	837C			7.9494 \pm 0.0013 (6, 6)		
041004–081004	2	837C			7.9550 \pm 0.0020 (8, 11)		
241004–271204	1	837C			7.9504 \pm 0.0015 (31, 32)		
180805–180805	2	957C			7.9550 \pm 0.0004 (7, 7)		
091105–290306	2	957C				7.9177 \pm 0.0021 (93, 101)	7.8356 \pm 0.0013 (7, 7)
130706–140806	2	957C				7.9187 \pm 0.0010 (5, 5)	7.8348 \pm 0.0021 (40, 40)
131206–200107	2	957C, 1058C				7.9169 \pm 0.0001 ^a (2, 2)	7.8358 \pm 0.0021 (6, 6)
Whole period			7.7776 \pm 0.0017 (54, 56)	7.8667 \pm 0.0061 (95, 100)	7.9537 \pm 0.0037 (75, 83)	7.9177 \pm 0.0021 (101, 109)	7.8330 \pm 0.0024 (54, 54)

^a Mean \pm standard deviation for the difference in analytical results between duplicate measurements.

In contrast, cruise-to-cruise differences were observed in the pH_T measurements. The standard deviations in pH_T measurements of CRMs for the whole period (0.0007–0.0039) were larger than those for each cruise (0.0006–0.0022) (Table 2). For example, the standard deviations of the pH_T values in CRM batch 62 and in working standard batch X measured during the same cruises were 0.0025 and 0.0037, respectively. When we analysed the CRMs and working standards at the same time, both pH_T values tended to change similarly. This result suggests that the variations of pH_T were caused not by changes in the quality of the CRMs or working standards but by changes in the conditions of apparatus or indicator solutions. If we corrected the pH_T values to reflect the amount of change we commonly observed for the CRM and working standard measurements, the standard deviation of the measured pH_T values of working standard batch X decreased from 0.0037 to 0.0023; therefore we can expect to obtain more precise measurements than present measurements. However, there is no guarantee that we can apply this correction to the whole pH_T range in seawater, because the pH_T range of the CRMs or working standards was limited between 7.8 and 7.9. We would be able to confirm the possibility to correct for the cruise-to-cruise difference by measuring several sets of working standards with different pH_T values.

For precise pH_T measurements, it is important to satisfy the requirements for analyses of reference materials before measuring a series of samples, as described in Section 6. Our experimental results suggest that the procedure described here for analysing reference materials effectively enables us to obtain pH_T measurements with an uncertainty of less than 0.003 during different cruises.

7.2 Origin of the pH_T perturbation caused by the addition of HgCl_2

To determine the reasons for the pH_T decrease of -0.0024 to -0.0003 caused by the addition of HgCl_2 solution (see Section 4.2), we calculated pH_T from TCO_2 and TA before and after addition of the HgCl_2 solution. In this calculation, we took into account the effect of dilution and the effect of complexation of Hg^{2+} with OH^- and with CO_3^{2-} ions (see Appendix B for details).

The result of this calculation supports the experimental results that the addition of HgCl_2 solution reduced the pH_T in seawater. The change in pH_T calculated for the addition of 0.2 cm^3 of saturated HgCl_2 solution to 250 cm^3 of seawater ranged from -0.0039 to -0.0015 , depending on the combination of the values of TCO_2 and TA of seawater (Fig. 8). These calculated results of the negative differences and the negative correlation to pH_T were consistent with experimental results. Although the calculated results differed significantly from the experimental results at a 99% confidence level ($p = 6 \times 10^{-9}$), the magnitude of the calculated change in pH_T was within the same order as that of the experimental data. A change in TA

($-3.8 \mu\text{mol/kg}$) due to hydrolysis of Hg^{2+} and HgCO_3 formation corresponded to a change in pH_T of -0.0025 . Most of the experimentally observed changes in pH_T caused by HgCl_2 addition could be accounted for by the change in TA and dilution. The difference between experimental and calculated values might be derived from uncertainty in the stability constants of Hg^{2+} complexes.

7.3 Internal consistency of CO_2 system parameters

When we measure three of four parameters controlling the CO_2 system (TCO_2 , TA, pH_T and $p\text{CO}_2$), we can examine each data point by comparing measured values and the one parameter calculated from the other two parameters (e.g., Lee and Millero, 1995; McElligott et al., 1998; Lee et al., 2000). We calculated $p\text{CO}_2$ from measured pH_T and TCO_2 and compared the results with measured $p\text{CO}_2$ using the calculation procedure described in DOE (1994). Various dissociation constants for carbonic acid, including those reported by Lueker et al. (2000), Mojica Prieto and Millero (2002), Roy et al. (1993), Dickson and Millero (1987), Goyet and Poisson (1989), Mehrbach et al. (1973) and Hansson (1973), were used for these calculations and were compared on the scale of pH_T .

The pH_T , TCO_2 and $p\text{CO}_2$ values in surface water were measured simultaneously during the cruise MR02-K06. Seawater samples for onboard measurements of these three parameters were obtained from the same onboard pumping system. TCO_2 was determined by the coulometric method described by Ishii et al. (1998) and calibrated with the same CRMs described above. $p\text{CO}_2$ was measured by the method described by Inoue (2000) and was calibrated by the MRI87 scale that is traceable to the WMO scale.

The difference between measured and calculated $p\text{CO}_2$, $\Delta p\text{CO}_2 = p\text{CO}_2^{\text{meas}} - p\text{CO}_2^{\text{calc}}$, ranged from -6 to $+18 \mu\text{atm}$ ($< 4\%$), depending on the dissociation constants used (Fig. 11). The $\Delta p\text{CO}_2$ value calculated with

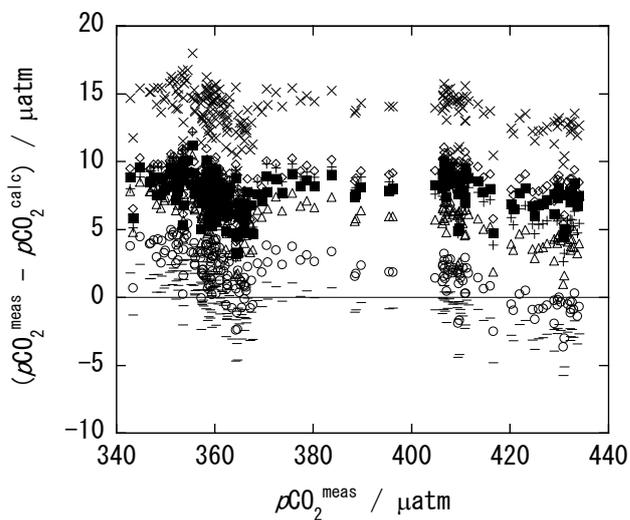


Fig. 11 Comparison of $p\text{CO}_2$ values observed and calculated from measured pH_T and TCO_2 using dissociation constants for carbonic acid given by Mehrbach et al. (1973; diamond), Hansson (1973; bar), Dickson and Millero (1987; plus), Goyet and Poisson (1989; circle), Roy et al. (1973; triangle), Lueker et al. (2000; box) and Mojica Prieto and Millero (2002; cross).

dissociation constants reported by Lueker et al. (2000) was in the middle of these differences. Taking into account the uncertainties of $\pm 2 \mu\text{atm}$ for $p\text{CO}_2^{\text{meas}}$ and $\pm 2 \mu\text{atm}$ for $p\text{CO}_2^{\text{calc}}$ derived from onboard measurements of pH_T (± 0.0002) and TCO_2 ($\pm 2 \mu\text{mol kg}^{-1}$), $p\text{CO}_2^{\text{calc}}$ calculated with dissociation constants reported by Lueker et al. (2000) was approximately equivalent to $p\text{CO}_2^{\text{meas}}$.

Lee et al. (2000) reported that $p\text{CO}_2$ and TA calculated from measured TCO_2 and pH_T showed the best agreement with measured values when the dissociation constants of Mehrbach (1973), which were given as functions of temperature and salinity by Dickson and Millero (1987), were used. In the study of Lee et al. (2000), the difference in fugacity, $\Delta f\text{CO}_2 = f\text{CO}_2^{\text{meas}} - f\text{CO}_2^{\text{calc}}$, was $+1.4 \pm 1.4 (1\sigma) \%$ ($+5.2 \pm 5.2 \mu\text{atm}$ at $370 \mu\text{atm}$) for seawater collected at the 170°W meridian. For our pH_T results, the calculation with dissociation constants of Mehrbach (1973) gave $\Delta p\text{CO}_2$ of $+2.3 \pm 0.4 (1\sigma) \%$ ($+8.7 \pm 1.4 \mu\text{atm}$). This value of $\Delta p\text{CO}_2$ differed insignificantly from the value described by Lee et al. (2000).

Although further consideration may be required to the comparability of pH_T measurements and to the variability of calculation caused by the uncertainty of dissociation constants of carbonic acid, the $p\text{CO}_2$ values measured in the equatorial Pacific Ocean were reproduced within the range of $\pm 11 \mu\text{atm}$ by calculation from our measured pH_T and TCO_2 values.

8. Summary

We developed an automated, precise pH_T measurement system based on a spectrophotometric technique with the indicator dye *m*-cresol purple. We proposed a procedure for precise pH_T measurement involving the analysis of reference materials, and we evaluated the uncertainty of the pH_T values obtained with our analytical procedure. We also examined the effects of bottling and storage, as well as the effect of the addition of HgCl_2 solution, on the pH_T measurements obtained from bottle samples.

The standard deviations of pH_T measurements obtained with our system were 0.0002 for onboard measurements, 0.002 for bottle samples of surface waters and 0.006 for deep waters after 50 days of storage. Our measured pH_T values for CRMs were consistent with those calculated from certified TCO_2 and TA values with the dissociation constants of carbonic acid reported by Lueker et al. (2000). The onboard pH_T measurements were consistent with those derived from TCO_2 and $p\text{CO}_2$ measurements in the field study.

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Appendix A: Calculation of change in pH_T due to CO_2 gas exchange between headspace and seawater sample in a bottle.

Initial pH_T and TCO_2 values ($\text{pH}_{T,i}$ and $\text{TCO}_{2,i}$) in the seawater samples without CO_2 gas exchange were chosen arbitrarily so as to reproduce the variety of seawater conditions that were observed during cruise MR02-K06. Then, initial $p\text{CO}_2$ and TA values in the seawater ($p\text{CO}_{2,i}^{\text{SW}}$ and TA_i) were calculated from $\text{pH}_{T,i}$ and $\text{TCO}_{2,i}$ using the equilibrium relations for carbonates (DOE, 1994). The initial $p\text{CO}_2$ in the headspace of the sealed bottle ($p\text{CO}_{2,i}^{\text{HS}}$) was chosen from the values between 350 μatm (uncontaminated atmosphere) and 1000 μatm (room air). The final $p\text{CO}_2$ in the headspace ($p\text{CO}_{2,f}^{\text{HS}}$) was assumed to be close to $p\text{CO}_{2,i}^{\text{SW}}$. From the initial and final $p\text{CO}_2$ values in the headspace, the change in the number of micromoles of CO_2 (Δn_{CO_2}) in the headspace with a volume of V^{HS} was calculated with the following equation of state (Eq. A.1), assuming that CO_2 behaved as an ideal gas:

$$\Delta n_{\text{CO}_2} = \frac{(p\text{CO}_{2,f}^{\text{HS}} - p\text{CO}_{2,i}^{\text{HS}})f_p \cdot V^{\text{HS}}f_v}{RT} . \quad (\text{A.1})$$

Variables in Eq. A.1 that have not been defined are as follows:

- f_p : Factor to convert pressure unit (101325 Pa atm^{-1}).
- V^{HS} : Volume of the headspace in a bottle (2 cm^3).
- f_v : Factor to convert volume unit (10^{-6} $\text{m}^3 \text{cm}^{-3}$).
- R : Gas constant (8.31451 J $\text{mol}^{-1} \text{K}^{-1}$).
- T : Temperature in K.

The change in TCO_2 in the seawater sample (in $\mu\text{mol kg}^{-1}$) was calculated with the following equation:

$$\Delta\text{TCO}_2 = \text{TCO}_{2,f} - \text{TCO}_{2,i} = -\frac{\Delta n_{\text{CO}_2}}{\rho V^{\text{SW}}} , \quad (\text{A.2})$$

where ρ and V^{SW} are the sample density (in kg dm^{-3}) and volume (0.250 dm^3), respectively. Then, the perturbed pH_T was calculated from the equilibrium relationship for carbonates from perturbed $\text{TCO}_{2,f}$ and initial TA_i , which does not change during CO_2 gas exchange.

Appendix B: Calculation of the pH_T change derived from the addition of saturated HgCl_2 solution.

The Hg^{2+} ion reacts with various inorganic ligands to form metal complexes. The stability constants of major Hg^{2+} complexes are given in Table B.1 (Stumm and Morgan, 1996). The formation of complexes with hydroxide and carbonate affects TA (Eq. B.1) and TCO_2 (Eq. B.2) in seawater and hence perturbs pH_T .

Table B.1 Stability constants for various Hg^{2+} complexes^a.

Ligands Complexes	OH^-	Cl^-	CO_3^{2-}	SO_4^{2-}
HgL	10.6	7.2	16.1	2.5
HgL_2	21.8	14.0		3.6
HgL_3		15.1		
HgL_4		15.4		
HgOHL		18.1		

^a Constants are given by the logarithms of the overall formation constants, β , at 25 °C and an ionic strength of zero. The stability constants were corrected to an ionic strength of 0.7 mol kg^{-1} with activity coefficients ($-\log \gamma$) of 0.146 and 0.584 for charges of ± 1 and ± 2 , respectively.

$$\begin{aligned} \text{TA}^{\text{mix}} = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] - [\text{H}^+]_{\text{F}} - [\text{HSO}_4^-] \\ & + [\text{Hg}(\text{OH})^+] + 2[\text{Hg}(\text{OH})_2] + [\text{Hg}(\text{OH})\text{Cl}] + 2[\text{Hg}(\text{CO}_3)] \end{aligned} \quad (\text{B.1})$$

$$\text{TCO}_2^{\text{mix}} = [\text{CO}_2^*] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] + [\text{Hg}(\text{CO}_3)] \quad (\text{B.2})$$

The basic algorithm for calculating the pH_T change caused by the addition of saturated HgCl_2 solution involves correcting for the consumption of hydroxide and carbonate ions caused by the formation of Hg complexes in the calculation of pH_T from TCO_2 and TA.

To use the stability constants in Table B.1, which are given at an ionic strength $I = 0 \text{ mol kg}^{-1}$, we corrected for the activity coefficient to obtain the constants at the ionic strength of seawater (0.7 mol kg^{-1}). The thermodynamic stability constant β of complex HgL_x is expressed by Eq. B.3:

$$\beta_{\text{HgL}_x} = \frac{a_{\text{HgL}_x}}{a_{\text{Hg}} a_{\text{L}}^x} = \frac{[\text{HgL}_x^{2-x\nu}](m^0)^x}{[\text{Hg}^{2+}][\text{L}^{\nu-}]^x} \cdot \frac{\gamma_{\text{HgL}_x}}{\gamma_{\text{Hg}} \gamma_{\text{L}}^x} \quad (\text{B.3})$$

Variables in Eq. B.3 are as follows:

- a_M : Activity of an ion M (dimensionless).
 $[M]$: Concentration of an ion M in mol kg⁻¹.
 m^0 : Standard value of concentration (1 mol kg⁻¹).
 γ_M : Activity coefficient of ion M (dimensionless).

Eq. B.3 can be rearranged to Eq. B.4 to give the stability constant X_{HgL_x} :

$$\begin{aligned} \log X_{\text{HgL}_x} &= \log \left(\frac{[\text{HgL}_x^{2-xv}](m^0)^x}{[\text{Hg}^{2+}][\text{L}^{v-}]^x} \right) \\ &= \log \beta_{\text{HgL}_x} - \log \gamma_{\text{HgL}_x} + \log \gamma_{\text{Hg}} + x \cdot \log \gamma_{\text{L}} \end{aligned} \quad (\text{B.4})$$

The values of $\log \beta_{\text{HgL}_x}$ in Eq. B.4 are given in Table B.1. The values of $-\log \gamma$ in Eq. B.4 are substituted by 0.584 or 0.146 depending on the charge of the ionic species (Stumm and Morgan, 1996). For example, $\log X_{\text{HgL}_x}$ for HgCl_4^{2-} is calculated as follows: $\log \beta_{\text{HgCl}_4} = 15.4$; $-\log \gamma_{\text{HgCl}_4} = 0.584$; $+\log \gamma_{\text{Hg}} = -0.584$; $+\log \gamma_{\text{Cl}} = -0.146$. Then, $\log X_{\text{HgCl}_4} = 15.4 + 0.584 - 0.584 - 4 \times 0.146 = 14.8$.

We used the following procedure to calculate the pH_T in seawater that contained HgCl_2 :

- a) First, TA was calculated from pH_T , TCO_2 , temperature and salinity measured for seawater obtained in the equatorial Pacific Ocean during the cruise MR02-K06 with the dissociation constants for carbonic acid reported by Lueker et al. (2000). Because the samples were diluted with saturated HgCl_2 solution by the ratio of volume $V^{\text{SW}}:V^{\text{Hg}}$, the diluted values of TCO_2 ($\text{TCO}_2^{\text{mix}}$), TA (TA^{mix}) and salinity (S^{mix}) were calculated with the following equations:

$$\begin{aligned} \text{TCO}_2^{\text{mix}} &= \text{TCO}_2 \cdot V^{\text{SW}} / (V^{\text{SW}} + V^{\text{Hg}}), \\ \text{TA}^{\text{mix}} &= \text{TA} \cdot V^{\text{SW}} / (V^{\text{SW}} + V^{\text{Hg}}), \\ S^{\text{mix}} &= S \cdot V^{\text{SW}} / (V^{\text{SW}} + V^{\text{Hg}}). \end{aligned}$$

- b) The effect of dilution on pH_T was then calculated by iterative methods from $\text{TCO}_2^{\text{mix}}$ and TA^{mix} to give pH_T^{mix} after the addition of HgCl_2 solution without considering the effect of Hg^{2+} complexes.
- c) Concentrations of hydroxide ion $[\text{OH}^-]$ and carbonate ion $[\text{CO}_3^{2-}]$ were calculated from pH_T^{mix} and $\text{TCO}_2^{\text{mix}}$. The concentrations of chloride and sulphate ions were calculated from salinity S^{mix} (DOE, 1994).

- d) The concentration of each Hg^{2+} complex was calculated from Eqs. B.5 and B.6, using the result of procedure c):

$$[\text{HgL}_x^{2-xv}] = X_{\text{HgL}_x} [\text{Hg}^{2+}] [\text{L}^{v-}]^x, \quad (\text{B.5})$$

$$C_{\text{Hg}} = [\text{Hg}^{2+}] + \sum [\text{HgL}_x^{2-xv}] = [\text{Hg}^{2+}] (1 + \sum X_{\text{HgL}_x} [\text{L}^{v-}]^x). \quad (\text{B.6})$$

- e) Perturbations of TA by HgOH^+ , $\text{Hg}(\text{OH})_2$, $\text{Hg}(\text{OH})\text{Cl}$ and HgCO_3 from TA^{mix} in Eq. B.1 were calculated to give a corrected TA value, TA' . This procedure corresponds to the correction for the consumption of OH^- and CO_3^{2-} caused by HgCl_2 addition.
- f) The concentration of HgCO_3 was subtracted from $\text{TCO}_2^{\text{mix}}$ in Eq. B.2 to give a corrected TCO_2 value, TCO_2' . This procedure corresponds to the correction for the consumption of CO_3^{2-} .
- g) A corrected pH_T value, pH_T' , was calculated from TCO_2' and TA' by the same method described in procedure b).
- h) If the difference between pH_T' and pH_T^{mix} was larger than 0.00001, pH_T^{mix} was substituted by pH_T' . Then procedures c) through h) were repeated. The value of pH_T' converged after two or three iterations.

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