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collected at depths with the CTD/carousel sampler, it has been recommended that the seawater samples be directly withdrawn from Niskin bottles into the optical cells (DOE, 1994).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 HgCl₂.

In our study, ca. 500 cm³ of water sample was drawn with a silicone rubber tube, which was connected to the outlet cock of a Niskin bottle, into a 250 cm³ borosilicate glass bottle (250 cm³ was overflowed). After the temperature of the sample was adjusted to $25.0_0 \pm 0.05$ °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 (1 σ , *n* = 32). Problems associated with the comparability and precision of pH_T measurements of bottle samples

include a) CO_2 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 $HgCl_2$ 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 CO_2 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 (1 σ , 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 pCO_2 perturbation in bottle samples, we calculated the change in pH_T caused by CO₂ 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 pCO_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 pCO_2 value of 350 µatm (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 cm³ in a 250 cm³ sample bottle. a) Quantity of CO₂ (µmol) exchanged between sample and headspace and b) pH_T change caused by CO₂ exchange between a seawater sample and air in a bottle.



Fig. 8 Observed pH_T change caused by the addition of 0.2 cm³ of saturated HgCl₂ solution to 250 cm³ seawater (closed circles). The broken line shows the linear regression of observed values, $pH_T(0.4) - pH_T(0.2) = (-0.00094 \pm 0.00059) pH_T(0.2) + (0.00616 \pm 0.00465), \gamma^2 = 0.17, p = 0.14$. Calculated values (solid line) were obtained from TCO₂ and TA using stability constants β of Hg²⁺ complexes at ionic strength 0.7. Dotted lines indicate the uncertainty ($\pm 2\sigma$) of calculation derived from the variability of inputted TCO₂ and TA.

4.2 Perturbation of pH_T caused by addition of saturated HgCl₂ 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 TCO₂ could change owing to biological activity occurring prior to measurement. Therefore, bottle samples must be sterilized with saturated HgCl₂ solution. However, the addition of saturated HgCl₂ solution may affect pH_T because of hydrolysis of HgCl₂ and dilution of the sample. To evaluate the perturbation of pH_T caused by the addition of HgCl₂ 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 HgCl₂ solution (0.2 and 0.4 cm³) were added to each of a given pair of samples.

The injection of additional 0.2 cm³ saturated HgCl₂ solution to each sample already containing 0.2 cm³ saturated HgCl₂ caused pH_T changes (pH_T(0.4) – 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 HgCl₂ addition by adding a constant value of 0.0012₃ (average of pH_T(0.4) – 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 litters of surface water at 4° 10' N, 156° 40' E during the cruise MR02-K06. This large sample was subsampled into 30 borosilicate glass bottles 250 cm³ each and then sterilized with 0.2 cm³ of saturated HgCl₂ 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 (1 σ , *n* = 21; Fig. 9). There was no significant temporal change (γ^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 HgCl₂ 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 (= $pH_T(stored) - pH_T(asap)$ and $pH_T(asap)$ (Fig. 10b) and the positive correlation between the change in pH_T and pCO_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 TCO₂ of $-4 \mu mol$ kg⁻¹ at constant TA. However, TCO₂ values previously have been observed to remain unchanged when samples are stored by the method described here (Ishii et al., 2000). Since pCO_2 is high in deep-water samples with lower pH_T and higher TCO_2 than those in near-surface seawater, CO2 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 HgCl₂. 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, $pH_T(asap)$, and after 50-day storage, $pH_T(stored)$, versus a) depth, b) $pH_T(asap)$ and c) pCO_2 calculated from TCO₂ and $pH_T(asap)$. 0.2 cm³ of saturated HgCl₂ 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 TCO₂ 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 TCO₂ and TA. Although the CRMs were not certified for pH_T , their pH_T values could be calculated from the certified TCO₂ and TA values because these values are stable. We compared our measured pH_T values with the values calculated from the certified TCO₂ 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 (fCO_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 fCO_2 calculated from pH_T and TCO₂ using dissociation constants based on the data of Mehrbach et al. (1973) agreed well with measured fCO_2 values.

The pH_T values of CRMs measured during several cruises agreed well with pH_T values calculated from the certified values of TCO₂ 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 TCO₂ 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.