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 pCO_2 values in the atmosphere and in surface waters in the western subtropical North Pacific Ocean increased at rates of 1.60 ± 0.03 μatm yr^{-1} and 1.6 ± 0.2 μatm yr^{-1}, respectively between 1984 and 2003. This rate of increase in oceanic pCO_2 corresponds to an annual change in pH of –0.002 yr^{-1} at a constant TA of 2300 μmol 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)):

$$HI^- \rightleftarrows H^+ + I^{2-}. \quad (1)$$

[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

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

where $[H^+]_T$ denotes the concentration of hydrogen ions (mol kg^{-1} seawater) expressed by the total hydrogen ion concentration scale: $[H^+]_T = [H^+]_F(1 + [SO_4]_T/K_{HSO4})$, where $[H^+]_F$ is the concentration of free hydrogen ions, $[SO_4]_T$ is the total concentration of sulphate ions and K_{HSO4} is the acid dissociation constant of hydrogensulphate ion (Dickson, 1990). Seawater pH_T is then expressed as Eq. (3),

$$pH_T = -\log[H^+]_T = pK_2 + \log\frac{[I^{2-}]}{[HI^-]}. \quad (3)$$

The dye absorption spectrum in the visible wavelength region is used to estimate $[I^{2-}]/[HI^-]$. The maximum absorbance is observed at 578 nm for I^{2-} and at 434 nm for HI^-. The $[I^{2-}]/[HI^-]$ 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 $\varepsilon_{578}(HI^-)/\varepsilon_{434}(HI^-)$, $\varepsilon_{578}(I^{2-})/\varepsilon_{434}(HI^-)$ and $\varepsilon_{434}(I^{2-})/\varepsilon_{434}(HI^-)$, respectively ($\varepsilon_\lambda(X)$ denotes the molar extinction coefficient of ion X at wavelength λ). Then, pH_T of seawater is calculated from Eq. (4):

$$pH_T = pK_2 + \log\frac{[I^{2-}]}{[HI^-]} = pK_2 + \log\left(\frac{R - e_1}{e_2 - Re_3}\right). \quad (4)$$

The ratios of extinction coefficients e_1, e_2 and e_3 have been determined by Clayton and Byrne (1993). The value of pK_2 (= $-\log K_2/k^0$, k^0 = 1 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):

$$\begin{aligned} pK_2 &= pK_2\,(\text{Clayton \& Byrne, 1993}) + 0.0047 \\ &= 1245.69/T + 3.8322 + 0.00211\,(35 - S). \\ &293\ \text{K} \le T \le 303\ \text{K};\ 30 \le S \le 37 \\ e_1 &= 0.0069_1;\ e_2 = 2.222_0;\ e_3 = 0.133_1. \end{aligned} \quad (5)$$

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):

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

where A_λ(SW) is absorbance of seawater and A_λ(SW+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}(SW), A_{578}(SW), A_{488}(SW) and A_{434}(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 cm^3/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 μmol/kg and varied within ±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 $25.0_0 \pm 0.05$ °C by circulating temperature-controlled water, and the temperatures were monitored just outside the cell with a Pt-100Ω resistance thermometer calibrated with a standard resistance thermometer (Pt-25Ω). 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.

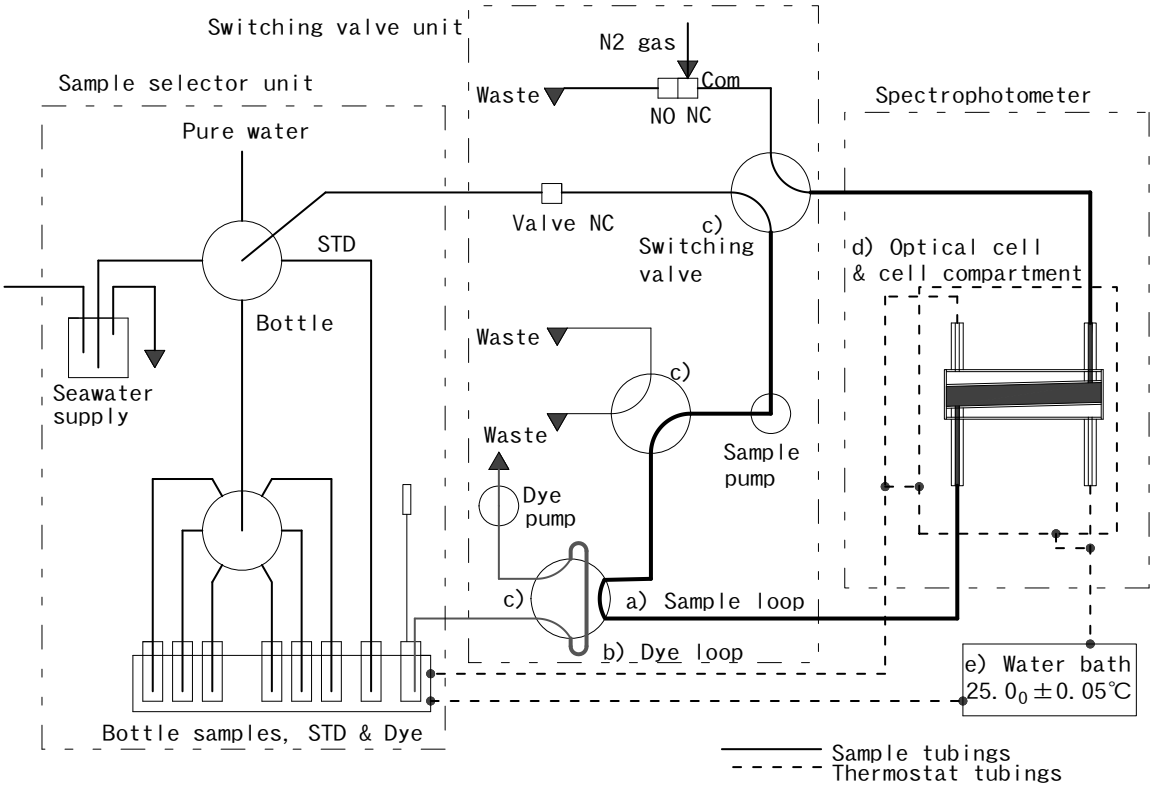

Fig. 1 Schematic diagram of pH_T measurement system. a) Sample loop (13.1_6 cm^3), b) dye loop (0.051 cm^3), c) automated switching valve, d) optical cell and e) water bath. NO, NC and Com indicate the valve position to be normal open (when switched off), normal close, and common (open at all time), 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 ($C_{21}H_{17}O_5NaS$) (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, $R_{dye} = A_{578}/A_{434}$, 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 ($\Delta R_{2\text{-}1} = R_2 - R_1$) assuming $\Delta R_{2\text{-}1}$ to be identical to the change effected by the addition of first aliquot ($\Delta R_1 = R_1 - R$) (Eq. (7)):

$$\Delta R = R_2 - R_1 = R_1 - R \quad \text{(Assumption)}, \qquad (7)$$
$$R = R_1 - \Delta R.$$

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:

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

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 μmol kg^{-1}).

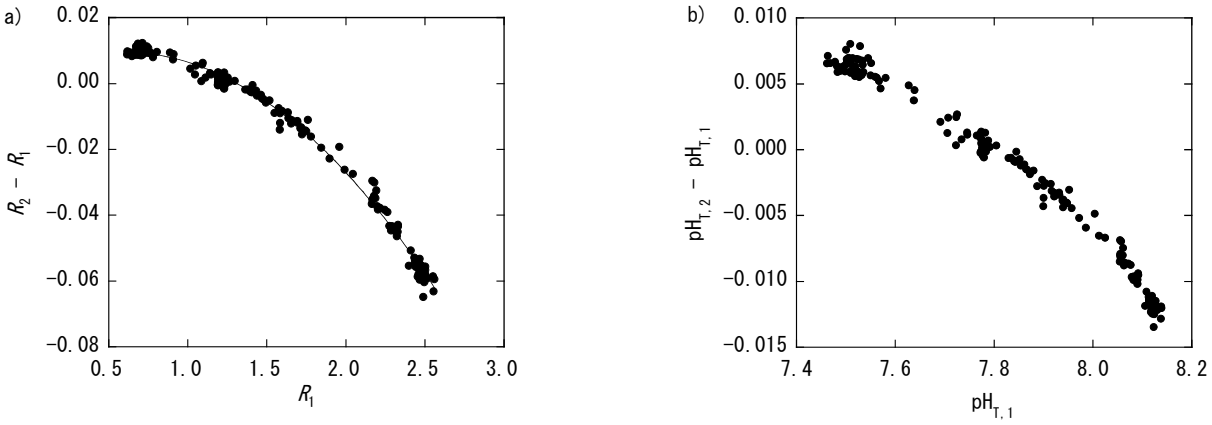

Fig. 2 Example of pH_T perturbation caused by the addition of dye solution. The difference between the addition of the second and first aliquots of dye solution for batch 784C was expressed as a) absorbance ratio R and b) pH_T. Such plots were constructed for each batch of the dye solution used in the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

2.5 Correction for temperature deviations

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

$$(pH_T(t) - pH_T(25)) / (t - 25.00)$$
$$= (2.00170 - 0.735594\ pH_T(25) + 0.0896112\ pH_T(25)^2 - 0.00364656\ pH_T(25)^3). \quad (9)$$

This equation was determined as follows. Values of $pH_T(t)$, 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 $pH_T(t)$, 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 ($25.0_0 \pm 0.05$ ºC) were used for calculation. Finally, pH_T at temperature t' in the range from 24.7 to 25.3 ºC was calculated from TCO_2, salinity and calculated TA values. The slope of the linear regression of $pH_T(t') - pH_T(25)$ versus $t' - 25$ was expressed as a cubic function of $pH_T(25)$ with a correlation coefficient of 0.99993 ($p < 10^{-8}$, Fig 3b).

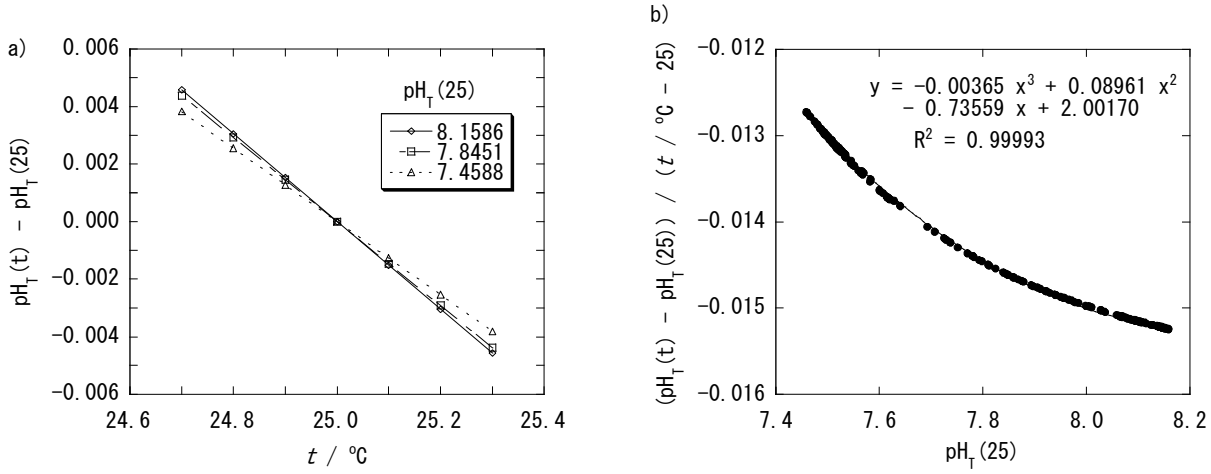

Fig. 3 Effect of sample temperature on the measured pH_T at temperatures from 24.7 °C to 25.3 °C. The difference between $pH_T(t)$ and $pH_T(25)$ was proportional to the sample temperature (a). The slope of the plot $pH_T(t) - pH_T(25)$ vs. $t - 25$ was expressed by a cubic function of $pH_T(25)$ with 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 dm^3 min^{-1}), which was a branch of seawater pumped up continuously from the sea-chest (ca. 20 dm^3 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 pCO_2 measurements.

Bottle samples were collected with Niskin bottles (30 dm^3 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 cm^3). A small portion (2 cm^3) of sample was removed to allow thermal expansion of the seawater, and 0.2 cm^3 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 cm^3 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 dm^3). 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