# Calcification depths of planktic foraminifers constrained by geochemical signatures from sediment-trap samples from the Bay of Bengal

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#### Abstract

The Mg/Ca and oxygen isotope ratios ( $\delta$ 18O) of multiple species of planktic foraminifers provide information on the hydrological conditions between the surface and the thermocline. Knowledge of the apparent calcification depth (ACD) of planktic foraminifers is key to reconstructing paleoenvironments; however, ACDs exhibit seasonal variations and differ over regional scales. We obtained the ACDs of Globigerinoides ruber, Trilobatus sacculifer, and Neogloboquadrina dutertrei in the Bay of Bengal using multiyear sediment-trap samples collected at approximately 900 m depth. The sediment traps were moored in the southwestern Bay of Bengal, with sampling intervals of 17-42 days. The temperature estimates obtained from the  $\delta$ 18O and Mg/Ca patterns of G. ruber, T. sacculifer, and N. dutertrei indicate that G. ruber reflects the temperature within the mixed layer, whereas N. dutertrei precipitates its test in the upper thermocline and T. sacculifer calcifies between these depths. The rapidly attenuating photosynthetically active radiation constrains the living depths of these symbiont-bearing species to within the upper 60 m of the euphotic zone in the southwestern Bay of Bengal. Although G. ruber and N. dutertrei calcify at different depths, as demonstrated by the different  $\delta$ 18O values of the two species ( $\Delta$ 18Or-d), large  $\Delta$ 18Or-d values were not obtained just in spring and summer when stratification is developed. The flux-weighted  $\delta$ 18O value of a species corresponds to the mean annual  $\delta$ 18O value of that species. Seasonal variations in species-specific test fluxes can be averaged out because of recurring flux peaks during the northeast and southwest monsoon seasons.

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22	Key Points:
23 24	• The apparent calcification depths were obtained using planktic foraminifers from sediment trap samples moored in the Bay of Bengal.
25 26	• <i>G. ruber</i> reflects the temperature within the mixed layer, whereas <i>N. dutertrei</i> in the upper thermocline and <i>T. sacculifer</i> between them.
27 28 29	• The flux-weighted values of $\delta^{18}$ O of a species correspond to the mean annual $\delta^{18}$ O value of that species.

### 30 Abstract

The Mg/Ca and oxygen isotope ratios ( $\delta^{18}$ O) of multiple species of planktic foraminifers provide information on the hydrological conditions between the surface and the thermocline. Knowledge of the apparent calcification depth (ACD) of planktic foraminifers is key to reconstructing

- paleoenvironments; however, ACDs exhibit seasonal variations and differ over regional scales.
- We obtained the ACDs of *Globigerinoides ruber*, *Trilobatus sacculifer*, and *Neogloboquadrina*
- *dutertrei* in the Bay of Bengal using multiyear sediment-trap samples collected at approximately
- 900 m depth. The sediment traps were moored in the southwestern Bay of Bengal, with sampling
- intervals of 17–42 days. The temperature estimates obtained from the  $\delta^{18}$ O and Mg/Ca patterns
- 39 of G. ruber, T. sacculifer, and N. dutertrei indicate that G. ruber reflects the temperature within
- 40 the mixed layer, whereas *N. dutertrei* precipitates its test in the upper thermocline and *T*.
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- 43 of the euphotic zone in the southwestern Bay of Bengal. Although *G. ruber* and *N. dutertrei*
- 44 calcify at different depths, as demonstrated by the different  $\delta^{18}$ O values of the two species
- 45  $(\Delta^{18}O_{r-d})$ , large  $\Delta^{18}O_{r-d}$  values were not obtained just in spring and summer when stratification is
- 46 developed . The flux-weighted  $\delta^{18}$ O value of a species corresponds to the mean annual  $\delta^{18}$ O
- 47 value of that species. Seasonal variations in species-specific test fluxes can be averaged out
- 48 because of recurring flux peaks during the northeast and southwest monsoon seasons.

## 49 **1 Introduction**

50 Over the last few decades, geochemical proxies based on the chemical properties of 51 foraminiferal tests have contributed to the elucidation of past oceanographic conditions (e.g., Liu

- et al., 2021; Naik et al., 2015; Nürnberg et al., 2000; Piotrowski et al., 2009; Tripati et al., 2003).
- Paleothermometers based on the oxygen isotope ratio ( $\delta^{18}$ O) (Bemis et al., 1998; Marchitto et al.,
- 54 2014) and Mg/Ca ratio (Evans and Müller, 2012; Nürnberg et al., 1996) of calcareous
- for a for a miniferal tests have been widely applied. The  $\delta^{18}$ O signatures of for a minifers ( $\delta^{18}$ O<sub>c</sub>) mainly
- <sup>56</sup> reflect ambient seawater temperature and  $\delta^{18}$ O of seawater ( $\delta^{18}$ O<sub>w</sub>), and they can be combined

57 with independent temperature estimations obtained using the Mg/Ca thermometer in

- for for a minifers, biomarkers, and modern satellite or in situ data to obtain salinity data (e.g., Grauel
- et al., 2013; Horikawa et al., 2015; Mohtadi et al., 2011; Sijinkumar et al., 2016). However, the
- 60 ecological characteristics of organisms (e.g., habitat depth) and seasonal variations in production
- can generate errors in data obtained from single or combined proxies derived from organisms,

62 with implications for paleoenvironmental reconstructions.

To decode the geochemical signatures of foraminiferal tests, it is essential to decipher the depth ranges over which planktic foraminifers calcify, because the chemical properties of the tests reflect the environmental conditions at those depths (Rebotim et al., 2017; Stainbank et al., 2019; Venancio et al., 2017). Although the habitat depth and calcification depth can overlap, they are different. The habitat depth of a species is the depth range at which that species dwells and concentrated populations are observed using a vertically stratified plankton tow. Planktic

- 69 foraminifers exhibit species-specific habitat depths and seasonal patterns as adaptations to their
- optimal conditions (Jonkers and Kučera, 2017; Kretschmer et al., 2018; Rebotim et al., 2017).
- 71 Moreover, the habitat depth can change as a result of descent during ontogeny and can vary at a
- regional scale (Jonkers and Kučera, 2017; Meilland et al., 2019; Pracht et al., 2019). The
- calcification depth is the depth range at which the calcite of the test is precipitated, which can be

estimated by using the geochemical signatures of tests. The calculated calcification depth range

is called the apparent calcification depth (ACD), which is the mean value of ascendance during

76 neanic stages of the life cycle and descent during gametogenesis. Discrepancies between the

habitat depth (estimated from plankton tows) and the ACD (inferred from geochemical

result from the unevenness of the calcification rate and timing within the life cycle of  $10^{-1}$  signatures) result from the unevenness of the calcification rate and timing within the life cycle of

a planktic foraminifer (Blanc and Bé, 1981; Lombard et al., 2010).

Constraining the range of habitat and calcification depth of planktic foraminifers is 80 crucial. Vertically stratified plankton tow studies provide snapshots of both types of planktic 81 foraminiferal depth range; however, continuously tracking both depths is difficult, and basic 82 information on the vertical distribution of planktic foraminifers is limited in many regions 83 (Jonkers and Kučera, 2017). Sediment-trap samples are an effective alternative for reconstructing 84 85 seasonal ACD variations of planktic foraminifers (Venancio et al., 2017; Wejnert et al., 2013). In addition, species-specific trends in ACD can reveal hydrological conditions in different layers of 86 the upper water column, because each species consistently calcifies in a particular layer (Birch et 87 al., 2013; Steph et al., 2009; Williams and Healy-Williams, 1980). 88

The Bay of Bengal is characterized by low salinity due to freshwater input during the 89 summer (southwest) monsoon (SWM) and prolonged oligotrophic conditions (Kumar et al., 90 2002; Muraleedharan et al., 2007). The semi-annually reversing Asian monsoon wind system is 91 the dominant factor controlling the seasonal oceanographic variations in the Bay of Bengal. The 92 Asian monsoon system, which exerts enormous influences by means of regional physical forces, 93 94 constrains plankton community structures and production (e.g., Sarma et al., 2020a; Singh et al., 2015). The planktic foraminiferal fluxes exhibit clear seasonal patterns with peaks during the 95 SWM and winter and lowest values in spring, influenced by plankton production as species-96 specific food sources in the Bay of Bengal (Maeda et al., 2022). In this tropical region, in which 97 annual sea-surface temperatures vary by less than 4°C (Locarnini et al., 2018), nutrient 98 replenishment through eddies and river systems greatly influences size-fractionated plankton 99 100 productivity and alters the optimal conditions for foraminifers. Therefore, the habitat depth and ACD of planktic foraminifers may differ from those in the open ocean. Understanding the ACD 101 in the Bay of Bengal will be useful for producing accurate paleoceanographic reconstructions in 102 the area using planktic foraminifers 103

We investigated the chemical composition of planktic foraminifers collected in sediment traps moored in the southwestern Bay of Bengal. The purpose of this study was to obtain species-specific ACD values of planktic foraminifers using  $\delta^{18}$ O and Mg/Ca as temperature proxies. Previously obtained data on settling particle fluxes (Rixen et al., 2017) and datasets of planktic foraminiferal fluxes and assemblages for the same sample series (Maeda et al., 2022) were also used in this study.

## 110 2 Materials and Methods

111 2.1 Regional setting of the Bay of Bengal

The Bay of Bengal is affected by annual reversing monsoon winds and several mesoscale and basin-scale physical processes (Vinayachandran, 2009). During the SWM (June–September), the southwest wind speed ( $\sim 10 \text{ m s}^{-1}$ ) is generally higher than the northeast wind speed of the

northeast monsoon (NEM; December–February) (~6 m s<sup>-1</sup>). The SWM winds provide large

amounts of precipitation to central and northern India; as a result, a huge amount of freshwater is

supplied to the bay as rainfall and through large rivers such as the Ganges–Brahmaputra and

- Godavari (Rao and Sivakumar, 2003). The large freshwater input and excess precipitation over evaporation ( $\sim 2 \text{ m yr}^{-1}$ ; Prasad, 1997) reinforces the stratification with high temperature (>28°C)
- in SWM seasons and forms a barrier layer, the base of the mixed layer, and the top of the
- thermocline (Lukas and Lindstrom, 1991; Thadathil et al., 2007). Although seasonal variability
- has been observed in barrier-layer thickness in the Bay of Bengal (Kumari et al., 2018), even
- 123 strong SWM winds do not break the barrier layer to transport cooler thermocline water into the
- 124 mixed layer (Shenoi et al., 2002). However, physical processes, including tropical cyclones (fall
- intermonsoon: FIM), coastal upwelling (SWM), the East India Coastal Current (spring
- intermonsoon: SIM), Ekman pumping (NEM), and cyclonic eddies (FIM–NEM), promote
- biological productivity in the euphotic zone, as does terrestrial nutrient input through river
- 128 plumes (mainly in coastal regions) (Gomes et al., 2000; Kumar et al., 2002, 2007; Jyothibabu et
- al., 2015; Sarma et al., 2013; Vinayachandran, 2009; Vinayachandran and Mathew, 2003;
- 130 Vinayachandran et al., 2005). The low light level (e.g., 417–605  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> at the surface in
- the SWM; Madhu et al., 2006; Jyothibabu et al., 2018) in the Bay of Bengal restricts the size
- distribution of phytoplankton (Sarma et al., 2020a). The depth of the photic zone varies
- seasonally: 48–65 m in June (Sarma et al., 2020b); 60 m during the FIM (Kumar et al., 2007);
- 134 60-100 m in the SIM (Kumar et al., 2007);  $65 \pm 8$  m during the SWM (Lotliker et al., 2016); and
- 135 68–82 m in July (Jyothibabu et al., 2018). In addition, less than 20% of surface
- photosynthetically active radiation (PAR) reaches 40 m depth in the Bay of Bengal (Sarma et al.,2020a).



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Figure 1. Map of the Bay of Bengal and the typical oceanographic settings. The red dot represents the CBBT sediment-trap mooring site in the southwestern bay, where the studied foraminifer samples were collected. The bold black arrows represent southwest monsoon (SWM) wind and northeast monsoon (NEM) wind. The black arrows representing East India Coastal

143 Current (EICC) indicate the strong EICC in each term.

### 144 2.2 Sediment-trap sample treatments

The sediment-trap experiments were conducted as collaborative research programs 145 between the University of Hamburg, Germany, and the National Institute of Oceanography, 146 India. Details of the deployment and treatment of sediment traps are provided in Unger et al. 147 (2003). PARFLUX Mark V and VI and sediment traps (Honjo and Doherty, 1988) with 0.5-m<sup>2</sup> 148 collecting areas were deployed in the southwestern Bay of Bengal (Fig. 1; site CBBT). Our 149 samples were obtained from shallow traps (862-950 m), which were labeled CBBT03 150 (November 1988 to October 1989), CBBT05 (December 1990 to October 1991), CBBT07 151 (January 1993 to October 1993), and CBBT09 (July 1995 to August 1996). The 13 (CBBT03, 152 05, 07) and 7 (CBBT09) time-series samples provided different 4-yr records of settling particles. 153 The sampling intervals were 27 (CBBT03), 25 (CBBT05), 17–23 (CBBT07), and 41–42 days 154

155 (CBBT09). Prior to sediment-trap deployment, the sample cups were filled with seawater with

- mercuric chloride ( $HgCl_2$ ) to hinder organic matter decomposition. After trap recovery, the
- samples were wet-sieved through a 1-mm nylon sieve and split into fractions using a precision

rotary splitter. Then, the samples were dried at 40°C, and the samples were stored at the
 University of Hamburg. Planktic foraminifers were picked from the aliquots using fine brushes

University of Hamburg. Planktic foraminifers were picked from the aliquots using fine brushes and sieved into four size fractions (<150, 150–250, 250–500, and 500–1000 µm). Foraminifers

were identified to species level following Schiebel and Hemleben (2017) and Saito et al. (1981),

as generally accepted at the time of observation. Neanic individuals and malformed specimens

163 were not used in geochemical measurements.

We inferred that the planktic foraminifers in the sediment-trap samples were well preserved because many unbroken pteropods with thin aragonite tests were also present in the samples, the spines of glassy foraminiferal tests were preserved well; furthermore, tests of fragile species such as *Hastigerina pelagica* and *Beella* digitata were unbroken.

168 2.3 Measurement of isotopic signatures and trace-element concentrations

169 We selected three species—*Globigerinoides ruber*, *Trilobatus sacculifer*, and

170 Neogloboquadrina dutertrei—for isotopic analyses and measurement of Mg/Ca ratios. Isotopic

signatures were obtained from CBBT05 and CBBT09; Mg/Ca ratios were measured from

172 CBBT03 and CBBT07. *Globigerinoides ruber* sensu stricto (*G. ruber* s.s.) and *G. ruber* sensu

173 lato (*G. ruber* s.l.) were distinguished because the two morphospecies yielded different  $\delta^{18}$ O

values in a previous study (Carter et al., 2017). However, because of the limited number of
 specimens, Mg/Ca ratios were measured for either *G. ruber* s.s or *G. ruber* s.l. (only in CBBT07-

175 specificity, higher ratios were measured for efficiency 3.5 or 0.74007 s.s. (only in CDD to 176 12) in each sample. The target size ranges were 280–500 µm for *G. ruber*, 440–700 µm for *T*.

177 sacculifer, and 460–650  $\mu$ m for *N. dutertrei*. The major axes of unbroken tests were measured

under a microscope because the sieve fraction method does not restrict test size sufficiently

compared to measurements of individual test size (Beer et al., 2010). There was a lack of

180 geochemical data for the SIM seasons, due to the low foraminiferal fluxes.

Prior to isotopic measurements, selected specimens were cleaned with ethanol in an 181 ultrasonic cleaner to remove fine particles adhering to the tests. After it was confirmed that 182 deposits had been completely removed, isotope measurements of oxygen and carbon were 183 obtained using an isotope mass spectrometer (IsoPrime, Stockport, UK) at the Geological Survey 184 of Japan, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan. 185 Anhydrous phosphoric acid was added to foraminifers at 25°C and Techn2 gas was introduced to 186 the IsoPrime. The external precision was better than  $\pm 0.1\%$ . The values of  $\delta^{18}O$  and  $\delta^{13}C$  are 187 reported relative to the Vienna Pee Dee Belemnite (VPDB) scale using NBS-19 standards. 188

The cleaning protocol for Mg/Ca ratios followed the oxidative method (e.g., Barker et al., 189 2003). Because the sediment-trap samples were preserved well, and lacked Fe-Mn coatings, we 190 omitted leaching with HNO<sub>3</sub> to avoid excessive cleaning. We partly followed the protocol of 191 Cheng et al. (2000) for cleaning of fossil samples for trace-metal analysis, except that molar 192 concentrations of solutions of chemical reagents were diluted twice and the period of ultrasonic 193 cleaning was shortened owing to the fragility of the foraminiferal tests. In short, foraminiferal 194 tests were gently crushed between glass plates and particles were removed with a fine brush. 195 Subsequently, the samples were absorbed in methanol and MQ water in an ultrasonic bath for 15 196 s twice. After rinsing, organic matter was removed using an oxidizing agent (a mixture of equal 197 198 amounts of 30% H<sub>2</sub>O<sub>2</sub> and 0.1 M KOH, TAMAPURE AA-100 from Tama Chemicals, Ltd. and

199 ultrapure grade from Kanto Chemical Co. Inc., respectively) in an ultrasonic bath for 15 s. 200 Samples were further cleaned ultrasonically in a mixture of equal parts by volume of 15% H<sub>2</sub>O<sub>2</sub> 201 and 0.5% HClO<sub>4</sub> (TAMAPURE AA-100), then repeatedly rinsed with methanol and ultrapure 202 water. The samples were dried at 65°C, and the dried samples were dissolved in dilute ultrapure 203 nitric acid solution (2% HNO<sub>3</sub>) to obtain Ca concentrations of 10 µg g<sup>-1</sup>. To control for 204 instrumental drift, internal standards (Be, Sc, Y, and In) were added to HNO<sub>3</sub>. We measured 205  $^{24}$ Mg,  $^{25}$ Mg,  $^{43}$ Ca, and  $^{44}$ Ca during each analysis. Mg/Ca ratios were analyzed using a Thermo

- Scientific iCAP-Qc inductively coupled plasma mass spectrometer (Thermo Fischer Scientific,
   Massachusetts, USA) at the Japan Agency for Marine-Earth Science and Technology operated in
- helium kinetic discrimination mode. Element counts were converted into molar ratios by an intensity ratio method based on a series of matrix-matched standard solutions. Both sample and
- standard solutions were prepared to ensure identical Ca concentrations of 10  $\mu$ g g<sup>-1</sup> (within 5%).
- 211 Repeated analyses of trace elements in the standard samples (JCp-1) exhibited good agreement
- with the consensus Mg/Ca ratio (Hathorne et al., 2013), with a standard deviation of 0.097 and
- an RSD (standard deviation) value of 2.3%.

## 214 2.4 Data Collection

The Global Ocean Data Assimilation System (NCEP/GODAS, Behringer et al., 1998) 215 dataset was downloaded through ERDDAP at the Asia-Pacific Research Data Center. Because 216 the Bay of Bengal is generally a data-poor region compared to other parts of the Indian Ocean, 217 vertical profiles for all the experimental periods within a  $1^{\circ} \times 1^{\circ}$  grid at the CBBT site were not 218 available and there are little vertical salinity profile data. The missing CTD data were not able to 219 be supplemented directly. Therefore, assimilated data for vertical temperature and salinity 220 profile, GODAS, were second best way. To obtain the vertical density profile, we used the 221 GODAS dataset of monthly vertical profiles of salinity and potential temperature within 100 km 222 of the CBBT site. These dataset of vertical profiles were interpolated through spline polynomial 223 function. In this study, the mixed layer depth is defined as the depth where the density ( $\sigma$ t) is 224 more than 0.2 kg m<sup>-3</sup> greater than the surface density (Narvekar and Kumar, 2006, 2014). The 225 top of the thermocline (isothermal layer depth) is defined as the depth at which the temperature is 226 1°C lower than the sea-surface temperature when the temperature profile is not inverted. If the 227 temperature profiles exhibit inversion, the top of the thermocline is defined as the depth at which 228 the temperatures at the top and base of the inversion layer are equal (Thadathil et al., 2007). 229 Although the thermocline is referred to as the depth where the maximum vertical temperature 230 gradient is displayed, in practice, regionally representative isotherms are used because of the 231 insufficient resolution of observation instruments (Yang and Wang, 2009). According to a 232 233 calculation based on buoy data from the Bay of Bengal, the 23°C isotherm is appropriate for describing the thermocline depth in the Bay of Bengal (Girishkumar et al., 2013); thus, we 234 adopted the 23°C isotherm as the bottom of the thermocline. 235

- The euphotic zone (EZ) is defined as the depth at which PAR has been attenuated to 1% of the level at the surface, and can be calculated as follows:
- 238
- 239  $Ez = E0 \exp(-kPAR^*z)(1)$
- 240

where Ez denotes the light intensity at depth z, E0 is the light intensity at the surface, and kPAR is the attenuation coefficient for PAR. In the present study, the mean monthly PAR profile (January 2003–June 2007) was obtained from PAR data observed by MODIS aqua within  $1^{\circ} \times$ 1° grids at the CBBT site. The kPAR range in the southwestern Bay of Bengal was 0.075–0.1 m<sup>-1</sup> (Lotliker et al., 2016). The calculated EZ depth ranges are illustrated in Fig. 2, and the EZ

depth range was confirmed by in situ observational data from the Bay of Bengal (Jyothibabu et

247 al., 2018; Kumar et al., 2007; Lotliker et al. 2016; Sarma et al., 2020a, b).



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**Figure 2**. The monthly euphotic zone depth ranges calculated from satellite data in the southwestern Bay of Bengal. The kPAR value for calculation was 0.0875 m<sup>-1</sup>, which is the average of kPAR in the southwestern Bay of Bengal (Lotliker et al., 2016). The ranges of high (>380  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), medium (26–380  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), and low light (<26  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) columns represent the ranges of respective light levels in Bemis et al. (1998) and sub-compensation light level (foraminiferal respiration > photosynthesis) for *Trilobatus sacculifer* (Jørgensen et al., 1985).

The  $\delta^{18}O_w$ -S relationships in the Bay of Bengal are complicated because of mixing of different riverine inputs with various  $\delta^{18}O$  values at the regional scale; therefore, there is no uniform overall  $\delta^{18}O_w$ -S relationship for the Bay of Bengal (Achyuthan et al., 2013). We adopted  $\delta^{18}O_w$ -S relationships for each season for the central and the southwestern Bay of Bengal from Achyuthan et al. (2013) for June-October (Eq. 2), and Kumar et al. (2018) for winter (Eq. 3) and spring (Eq. 4).

- 262
- 263  $\delta^{18}O_w = 0.14S 4.7(2)$
- 264  $\delta^{18}O_w = 0.14S 4.58$  (3)
- 265  $\delta^{18}O_w = 0.28S 9.1$  (4)
- 266

Previous studies have proposed  $\delta^{18}$ O-temperature calibrations based on inorganic calcite 267 (Kim and O'Neil, 1997), cultured specimens (Bemis et al., 1998; Bouvier-Soumagnac and 268 Duplessy, 1985; Erez and Luz, 1983), plankton-tow studies (Mulitza et al., 2003), and surface 269 sediments (Farmer et al., 2007). Equations based on cultured specimens are preferable when 270 selecting  $\delta^{18}$ O-temperature equations; thus, we used equations obtained by culture studies as far 271 272 as possible. However, the equation for N. dutertrei (Bouvier-Soumagnac and Duplessy, 1985) has been reported to yield lower temperatures (Wejnert et al., 2013) than actual values, and so 273 we did not use it. Equations for *Table*cultured under high light (>380  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and low light 274 conditions (20–30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) are appropriate to our dataset for G. ruber, T. sacculifer, and N. 275 dutertrei (Bemis et al., 1998). We followed the traditional step in foraminiferal paleotemperature 276 equations to convert  $\delta^{18}O_{sw}$  on the Vienna Standard Mean Ocean Water (VSMOW) scale into the 277 VPDB scale by subtracting 0.27‰. We included this step because the scale conversion of Kim et 278 al. (2015) could not be used, because of the lack of  $\delta^{18}$ O data in the study of Bernis et al. (1998). 279

Mg/Ca-temperature equations have been proposed in several studies (e.g., Anand et al., 280 2003; Gray et al., 2018) using both multi-species calibrations and species-specific calibrations. 281 We selected the Mg/Ca-temperature equation for G. ruber proposed in Gray et al. (2018), 282 because this equation is based on sediment-trap/plankton-tow samples including CBBT samples 283 (CBBT06). Although Gray et al. (2018) included the influence of pH or  $[CO_3^{2-}]$ , carbonate 284 system data in the Bay of Bengal are sparse and some data were collected in the coastal region 285 that is influenced by riverine input (Land et al., 2019). Thus, we adopted the Mg/Ca-temperature 286 equation without a carbonate system term (Gray et al., 2018). For T. sacculifer and N. dutertrei, 287 the equations proposed by Anand et al. (2003) for spinose and non-spinose species, respectively, 288 were used because the equations were obtained using sediment-trap samples. 289

### 290 **3 Results**

291 3.1 Foraminiferal Assemblage of CBBT09

The foraminiferal assemblages in CBBT09 are described in Fig. 3. The fluxes of planktic 292 for a minifers varied between 52 and 1093 individuals  $m^{-2} d^{-1}$ . The highest values occurred in the 293 NEM season, the fluxes were lower in the SWM season, and the lowest flux was in spring. The 294 295 dominant species were G. ruber, T. sacculifer, Globigerinella siphonifera, N. dutertrei, Globigerinita glutinata, Globigerina bulloides, and Globorotalia menardii, consistent with 296 CBBT samples from other sampling periods (see Figs. 3–5 in Maeda et al., 2022). The fluxes of 297 G. glutinata, G. bulloides, G. siphonifera, N. dutertrei, and G. ruber were higher in the NEM 298 season than in SWM periods. The fluxes of G. menardii and T. sacculifer were bimodal, with 299 comparable fluxes during the SWM and NEM periods. 300



301

Figure 3. The result of planktic foraminiferal fluxes and assemblages for dominant seven species in CBBT09.

304 3.2 Oxygen and carbon isotope ratios

The  $\delta^{18}$ O and  $\delta^{13}$ C results for *G. ruber*, *T. sacculifer*, and *N. dutertrei* are provided in Fig. 305 4. The  $\delta^{18}$ O ranges were different for each species. The  $\delta^{18}$ O values of G. ruber s.l. varied 306 between -3.36% and -2.71%, and those of G. ruber s.s. were -3.32% to -2.48%. In most 307 samples, the  $\delta^{18}$ O values of both morphotypes of G. ruber were within two standard deviations. 308 The  $\delta^{18}$ O values of T. sacculifer and N. dutertrei were heavier than those of G. ruber, ranging 309 from -2.82% to -1.96% and -2.68% to -1.78%, respectively. The  $\delta^{18}$ O of G. ruber was 310 lightest during SWM seasons in both CBBT05 and CBBT09. In contrast, the  $\delta^{18}$ O values of T. 311 sacculifer gradually decreased from the FIM to NEM seasons, with markedly low values in 312 January–February. The seasonality of  $\delta^{18}$ O values in *N. dutertrei* differed from those of the other 313 species, with peaks in May (CBBT05) and July-August (CBBT09) and lower values in NEM 314 seasons. The  $\delta^{13}$ C values of G. ruber s.s. and G. ruber s.l. were 0.59%–1.18% and 0.60%– 315 0.88%, respectively. Trilobatus sacculifer and N. dutertrei exhibited wider  $\delta^{13}$ C ranges of 316 0.49‰-1.27‰ and 0.63‰-1.66‰, respectively. 317



Figure 4. The results of oxygen (upper panels) and carbon isotope ratios (lower panels). Green rhombi mean *Neogloboquadrina dutertrei*, red ones mean *Trilobatus sacculifer* and black ones represent *Globigerinoides ruber*. The gray shades in CBBT05 represent the lack of data.

322 3.3 Mg/Ca ratios

318

The Mg/Ca ratios of *G. ruber* were  $5.41-7.04 \text{ mmol mol}^{-1}$  and those of *T. sacculifer* were 324  $3.75-4.95 \text{ mmol mol}^{-1}$  (Fig. 5). The Mg/Ca range of *N. dutertrei* was lower, 2.85-4.61 mmol 325 mol<sup>-1</sup>.



Figure 5. The Mg/Ca ratios in CBBT03 (left) and CBBT07 (right). Green rhombi mean



#### 330 4 Discussion

- 4.1 Species-specific temperature records calibrated from signatures and calcificationdepths
- 333 The temperatures calculated from the  $\delta^{18}$ O data for each species are listed in Table S5.
- The  $\delta^{18}$ O-temperature calibrations obtained from cultured *O. universa* (Bemis et al., 1998) were
- applied to infer the temperature records for each species. Summaries of the water-column
- 336 structure based on the GODAS dataset with ACD ranges for each species during the four
- 337 observational periods are provided in Fig. 6.



Figure 6. The water column structure (blue: mixed layer, yellow: between the mixed layer and
thermocline, gray: thermocline) with apparent calcification depth ranges for three planktic
foraminifers in CBBT03 (upper left), CBBT05 (upper right), CBBT07 (lower left), and CBBT09

341 (lower right). The x-axis represent month.

### 342 4.1.1 *Globigerinoides ruber*

The  $\delta^{18}$ O-temperature ranges of *G. ruber* s.s. and *G. ruber* s.l. were 26.2–29.1°C and 26.8–29.5°C, respectively, in CBBT05, and 26.1–28.4°C and 26.9–29.6°C, respectively, in CBBT09. The SWM temperature was 27.5–29.1°C in CBBT03 and 28.3–32.5°C in CBBT07. Both morphotypes of *G. ruber* showed comparable  $\delta^{18}$ O-temperature ranges in each sample.

The  $\delta^{18}$ O- and Mg/Ca-temperature patterns of G. *ruber* are primarily consistent with the 347 mixed-layer temperature records of the Bay of Bengal in all sample series (Fig. 6). The ACD 348 ranges for G. ruber were 0-51 m in CBBT03, 0-78 m in CBBT05, 0-41 m in CBBT07, and 0-349 113 m in CBBT09. The symbiont-bearing species G. ruber is considered to inhabit the EZ. 350 Because the monthly EZ in the Bay of Bengal reaches  $\sim 60$  m, G. ruber dwells in the upper 60 m. 351 Moreover, *T. sacculifer* exhibited a functional absorption cross-section of photosystem II ( $\sigma$ PSII) 352 comparable to that of G. ruber (white) (Takagi et al., 2019), suggesting that G. ruber requires a 353 sub-compensation light level (i.e., for a miniferal respiration > photosynthesis) similar to that of *T*. 354 sacculifer (26–30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>; Jørgensen et al., 1985). Even if the fertile states in seawater 355 affect the growth of G. ruber, in culture experiments with a light level of 50–60  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, 356

this species achieved sizes of more than 400 µm (Bijma et al., 1992). Light levels of 26–30 µmol 357  $m^{-2} s^{-1}$  occur at 35–40 m depth in the Bay of Bengal. The depth ranges of the mixed layer were 358 15-55 m, including the part of the EZ with a sufficient light intensity for G. ruber, consistent 359 with the estimated ACD range of this species. In summary, accurate reconstructions of ACD 360 were hindered by the lack of in situ CTD data and complicated  $\delta^{18}O_w$  mixing in the Bay of 361 Bengal; however, the light conditions suggest that G. ruber calcifies within the mixed layer. 362 Mixed layer calcification of G. ruber is consistent with previous studies reporting that the  $\delta^{18}O$ -363 temperature relationship of G. ruber reflects the mixed layer temperature, because G. ruber 364 occurs in the surface mixed layer (Duplessy et al., 1981; Stoll et al., 2007), as has been 365 demonstrated for concentrated populations in multi-plankton net studies (Peeters and Brummer, 366 367 2002).

#### 368 4.1.2 Neogloboquadrina dutertrei

369 The calculated temperature ranges for N. dutertrei were lower than those of G. ruber. The temperature range was 25.6–34.2°C in CBBT03, 23.8–28.2°C in CBBT05, 25.8–27.7°C in 370 CBBT07, and 24.5–27.2°C in CBBT09. The  $\delta^{18}$ O values of *N. dutertrei* were heavier than those 371 of G. ruber, and vielded ACD estimates of 59-81 m in CBBT03, 0-90 m in CBBT05, 44-93 m 372 in CBBT05, and 47-92 m in CBBT09. The most frequent ACD range was 50-75 m, consistent 373 with the thermocline depth in the Bay of Bengal. Neogloboquadrina dutertrei is a symbiont-374 facultative species (pelagophyte-symbiont; Bird et al., 2018), and previous field observations 375 have reported that up to 94% of individuals possess functional chlorophyll a (chl-a; Takagi et 376 377 al., 2019). The dominance of symbiont-facultative individuals suggests that N. dutertrei can dwell in the EZ, and the higher  $\sigma$ PSII of this species implies that it is adapted to a low light level 378 (Takagi et al., 2019). Neogloboquadrina dutertrei has been observed to prefer an herbivorous 379 diet, but a recent study reported that the species assimilates substantial amounts of protists from 380 particulate organic matter (Bird et al., 2018). The development of the subsurface microbial loop 381 with the subsurface chl-a maximum (>60 m) in spring and the high flux of N. dutertrei in May 382 383 and June in the southwestern Bay of Bengal are consistent with the hypothesis that N. dutertrei inhabits the thermocline and is reliant on subsurface primary production and remineralized 384 385 organic carbon (Kumar et al., 2007; Subha Anand et al., 2017). Experiments on laboratory cultures of N. dutertrei under diurnal light/dark cycles have indicated that this species tends to 386 precipitate its entire test as a thin calcite layer, then thickens the test by adding an outer calcite 387 layer with Mg-banding (Fehrenbacher et al., 2017). Thus, the ACD may reflect the living depth 388 in the final stage of the life cycle for N. dutertrei. The heavier  $\delta^{13}$ C ranges of N. dutertrei than of 389 G. ruber and T. sacculifer agree with the results from plankton net samples (Fairbanks et al., 390 391 1982), which suggest that the habitat depth of N. dutertrei can overlap the habitat depth ranges of

- 392 *G. ruber* and *T. sacculifer*.
- 393 4.1.3 Trilobatus sacculifer

Trilobatus sacculifer exhibited a temperature range of 26.8–28.6°C in CBBT03, 25.1– 27.6°C in CBBT05, 24.9–26.4°C in CBBT07, and 23.9–26.0°C in CBBT09. The ACD ranges were 0–82 m in CBBT03, 0–81 m in CBBT05, 69–85 m in CBBT07, and 35–93 m in CBBT09. The species descends later in its ontogeny with the formation of a sac-like chamber and gametogenic calcite (Bé, 1980; Takagi et al., 2015); in this study, *T. sacculifer* without sacs exhibited substantially heavier  $\delta^{18}$ O values and deeper ACD ranges than those of *G. ruber*. Because *T. sacculifer* possesses dinoflagellates as symbiont algae and symbiotic photosynthesis

helps to regulate host calcification (Bé et al., 1982; Caron et al., 1982), we infer that this species 401 also calcifies within the EZ. An early culture study indicated that the sub-compensation light 402 level for the species is 26–30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> (Jørgensen et al., 1985): this light level occurs at 35– 403 40 m depth in the southwestern Bay of Bengal. In addition, Lombard et al. (2010) reported that 404 T. sacculifer calcified 30% less under 35  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> than under 335  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and the 405 σPSII values of dinoflagellate-symbiont species indicate a high-light-adapted photophysiology 406 (Jørgensen et al., 1985; Spero and Parker, 1985; Takagi et al., 2019). Under higher light levels, 407 T. sacculifer forms heavier and larger tests and individuals need a light level greater than 8–10 408  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> to grow up to 500–600  $\mu$ m in size: PAR is attenuated to that level at 50 m in the 409 southwestern Bay of Bengal (Spero and Lea, 1993). Therefore, the estimated ACD of T. 410 sacculifer may be deeper than the actual habitat depth in the EZ (<60 m). The discrepancy 411 between the ACD and the EZ is attributed to the sparse dataset, limited equations for calibration, 412 and vital effects such as rapid addition of gametogenic calcite with 1.0%–1.4% heavier  $\delta^{18}$ O in 413 the last stage of the lifecycle (Blanc and Bé, 1981; Wycech et al., 2018). Therefore, T. sacculifer 414 can apparently reflect the temperature between the lower mixed layer and upper thermocline, 415 although this species may calcify in the lower mixed layer. 416

417 4.2 Implications for paleoceanographic reconstruction

Vertical hydrological gradients have been reconstructed using species-specific depth 418 habitats of planktic foraminifers; for example, the  $\delta^{18}$ O difference between *T. sacculifer* and *G.* 419 *truncatulinoides* or G. *ruber* and N. *dutertrei* ( $\Delta^{18}O_{r-d}$ ) has been applied as an indicator of the 420 strength of stratification (Mulitza et al., 1997; Nilsson-Kerr et al., 2022; Ota et al., 2019). The 421 energy required for mixing (ERM) of water column indicates the difference between the 422 potential energy of a stratified and unstratified column in a certain depth (Shenoi et al., 2002), 423 which often used to evaluate water column stratification. According to Fousiya et al. (2016), 424 Monthly ERM within the EZ (50 m depth) calculated from GODAS in the Bay of Bengal reaches 425 the maxima in the FIM and the minima in the NEM. Although ERM values using GODAS are 426 often underestimated compared to ERM with observation data (Fousiya et al., 2016), the 427 consistent trends exhibit.  $\Delta^{18}O_{r-d}$  peaks occurred prior to the SWM in CBBT05, but during the 428 NEM in CBBT09 (Fig. 7). Thus, the  $\Delta^{18}O_{r-d}$  signatures do not reflect the ERM trends. 429

The  $\Delta^{18}O_{r-d}$  as a stratification proxy requires an assumption that both target species habit 430 in the same depth range; mixed layer and thermocline. However, the  $\delta^{18}$ O signatures of G. ruber 431 did not always indicate within the mixed layer, which decreased the  $\Delta^{18}O_{r-d}$  (Fig. 6). 432 Furthermore, oligotrophic condition leading a short effective food web in the Bay of Bengal 433 (Jyothibabu et al., 2008; Fernandes and Ramaiah, 2014; Arunpandi et al., 2022) provide large 434 variations in standing stocks and timing of increase for planktic foraminifers (Maeda et al., 435 2022). The plankton production including planktic foraminifers is strongly influenced by 436 sporadic mesoscale eddies in addition to seasonal monsoon winds and currents (Jyothibabu et al., 437 2015; Sarma et al., 2020a; Vinayachandran et al., 2009). Thus, the optimum feeding conditions 438 for G. ruber and N. dutertrei could change vertically and temporally every year, it is hard to hold 439 the requirements for  $\Delta^{18}O_{r-d}$  in the Bay of Bengal. 440



441

Figure 7. The difference of  $\delta^{18}$ O between *Globigerinoides ruber* and *Neogloboquadrina dutertrei* ( $\Delta^{18}$ O<sub>r-d</sub>) in CBBT05 (left) and CBBT09 (right).

444 Annual flux-weighted mean  $\delta^{18}$ O values have been applied to understand the influence of 445 seasonal flux patterns on  $\delta^{18}$ O signatures recorded in marine sediments (e.g., Tedesco et al., 446 2007). The flux-weighted  $\delta^{18}$ O was calculated as given in Venancio et al. (2017) based on the 447 fundamental model of Mix (1987) and discussions in Mulitza et al. (1998) as follows:

(5)

448

450

451 where  $\delta^{18}O_{Fw}$  represents the flux-weighted  $\delta^{18}O$  value, and flux<sub>i</sub> and  $\delta c_i$  denote the 452 species-specific planktic foraminiferal flux and the  $\delta^{18}O$  value of tests in each sample, 453 respectively.

454 Table 1. The comparison of annual mean  $\delta^{18}$ O and flux weighted  $\delta^{18}$ O for each species

 $\delta^{18} \boldsymbol{O}_{FW} = \sum_{i=1}^{n} \frac{(flux_i \times \delta_{ci})}{total flux}$ 

Species	flux-weighted $\delta^{18}O$ (‰)	Annual mean $\delta^{18}$ O (‰)
Globigerinoid ruber	-2.91	-2.86
Trilobatus sacculifer	-2.55	-2.55
Neogloboquadrina dutertrei	-2.20	-2.21

455 Unfortunately, the low fluxes of all species in the SIM and the restricted time intervals of 456 sample collection influenced the annual flux-weighted  $\delta^{18}$ O values for each species.

457 *Neogloboquadrina dutertrei* showed the maximum peak in May/June in all observational years.

458 However, the limited  $\delta^{18}O_r$  data prohibit accurate calculation of flux-weighted  $\delta^{18}O_r$ . Even

though there is a lack of  $\delta^{18}$ O data in March/April and April/May for *T. sacculifer*, the fluxes in

both samples accounted for approximately 1.4%. Therefore, the appropriate  $\delta^{18}$ O dataset for *G*.

*ruber* was CBBT09 and for *T. sacculifer* and *N. dutertrei* were CBBT05. In calculating the flux-

462 weighted  $\delta^{18}$ O<sub>r</sub>, we adopted mean  $\delta^{18}$ O values for *G. ruber* s.s. and *G. ruber* s.l. The flux-

463 weighted  $\delta^{18}$ O value for each species was -2.91‰ for *G. ruber*, -2.55‰ for *T. sacculifer*, and

464 –2.20‰ for *N. dutertrei* (Table 1). The flux-weighted  $\delta^{18}$ O-temperatures for *T. sacculifer* 

465 (28.9°C) and *N. dutertrei* (27.2°C) were apparently biased to be similar to the SIM to SWM high

- temperatures in the upper thermocline and the mixed layer. For *G. ruber* (27.7°C), the flux-
- 467 weighted  $\delta^{18}$ O-temperature appears to be biased towards the Tml in the NEM. However, the 468 mean  $\delta^{18}$ O values of the three species were in good agreement with the flux-weighted  $\delta^{18}$ O
- 469 values (Table 1); therefore, although species-specific seasonal patterns of test fluxes were
- 470 observed in CBBT sediment-trap samples, the influence of the seasonal biases in foraminiferal
- 471 fluxes was averaged out because of the recurring flux peaks in the SWM and the NEM. In
- 472 addition, the flux-weighted  $\delta^{18}$ O value of *G. ruber* was consistent with previously obtained  $\delta^{18}$ O<sub>r</sub>
- 473 values of  $-2.80\% \pm 0.08\%$  (698 y; Da Silva et al., 2017),  $-2.65\% \pm 0.08\%$  (1.18 ka BP; Liu et
- 474 al., 2021), and  $-2.82\% \pm 0.1\%$  (0 y; Ponton et al., 2012) from core-top samples collected in the
- 475 Bay of Bengal. This finding that the measured  $\delta^{18}$ O values of planktic species in sediment
- 476 assemblages reflect the mean annual  $\delta^{18}$ O values will provide strong support for future 477 paleoceanographic reconstructions in the Bay of Bengal.

## 478 **5 Conclusions**

The  $\delta^{18}$ O and Mg/Ca signatures of planktic foraminifers in sediment-trap samples collected from the southwestern Bay of Bengal were investigated to reveal the ACD for each species.

1. The temperature ranges estimated from  $\delta^{18}$ O and Mg/Ca data indicated species-specific ACDs: *G. ruber* in the mixed layer; *T. sacculifer* between the lower mixed layer and the upper thermocline; and *N. dutertrei* in the upper thermocline.

2. The low light level in the Bay of Bengal strongly constrains the ACD of each species. The ACDs of *G. ruber* and *N. dutertrei* are consistent with the light conditions and their photosynthetic strategies. The ACD range of *T. sacculifer* was biased, being apparently too deep for sufficient growth, because of the higher  $\delta^{18}$ O values of gametogenic calcite.

489 3.  $\Delta^{18}O_{r-d}$  may not be appropriate as a proxy of surface stratification in the Bay of Bengal. 490 However, the flux-weighted  $\delta^{18}O$  of each species was consistent with the mean  $\delta^{18}O$  values, and 491 the interannual variation in seasonal trends in fluxes and assemblages of planktic foraminifers 492 can be averaged out.

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499

## 500 Open Research

501 The foraminiferal fluxes of sediment-trap materials in CBBT03, 05, and 07 are available in the paper

and its supporting information of Maeda et al. (2022) and in PANGAEA Data Publisher for Earth &

503 Environmental Science at Rixen et al. (2017). The dataset for The foraminiferal fluxes of in CBBT09

- and chemical property of foraminifers will be available at Zenodo. GODAS dataset (Behringer et al.,
- 505 1998, https://psl.noaa.gov/data/gridded/data.godas.html) can be available through ERDDAP at the

506 Asia-Pacific Research Data Center (http://apdrc.soest.hawaii.edu/index.php). Monthly PAR profile

507 dataset can be available in MODIS aqua (https://oceancolor.gsfc.nasa.gov/data/aqua/).

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