This manuscript has been submitted for publication in <u>Nature Communications</u> and is currently under review. Please note this is a <u>non-peer reviewed pre-print</u> and has yet to be accepted for publication. Subsequent versions of this manuscript may have different content. If accepted, the final version of this manuscript will be available via the '*Peer reviewed publication DOI*' link on the right-hand side of this webpage. Please feel free to contact any of the authors (Tobias Agterhuis, <u>t.agterhuis@uu.nl</u>; Martin Ziegler, <u>m.ziegler@uu.nl</u>; Lucas Lourens, <u>l.j.lourens@uu.nl</u>); we welcome feedback.

1 Extreme deep-sea warmth supports high climate sensitivity in the early Eocene

2 hothouse

3

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9 The early Eocene (56-48 Ma) hothouse experienced the highest CO₂ levels of the Cenozoic, as well as the 10 occurrence of multiple transient global warming events, so-called hyperthermals. The deep ocean 11 constitutes a stable and vast heat reservoir in the climate system, and hence compromises a robust setting 12 to estimate past global mean temperatures. However, available deep-sea temperature reconstructions rely 13 on uncertain assumptions of non-thermal influences. Here, we apply for the first time the carbonate 14 clumped isotope paleothermometer (Δ_{47}), a proxy not governed by these uncertainties, on early Eocene 15 benthic foraminifera to evaluate South Atlantic deep-sea temperatures across two hyperthermal events 16 (ETM2 and H2; ~54 Ma). In comparison to the conventional δ^{18} O-based estimates, our new temperature 17 reconstructions indicate two and a half degrees warmer deep water conditions, i.e. 13.2±1.9 °C (95% 18 Confidence Interval) for background state, and average deep-sea warming of 3.3±2.9 °C (95% CI) during 19 these hyperthermal events. These findings imply a reassessment of the assumed isotope composition of 20 the ancient seawater and of a potential pH effect on foraminiferal oxygen isotopes. On a broad scale, our 21 Δ₄₇-based overall warmer deep-sea temperatures provide new evidence for high climate sensitivity during 22 the early Eocene hothouse.

23

24 A potential analogue for the future climate state is the extremely warm climate of the early Eocene (56–48 Ma)^{1–3}, 25 a period in the Cenozoic when ice sheets were absent and CO₂ levels were much higher than today (~600-2500 26 ppm)⁴. While sea surface temperature reconstructions may be biased by local seasonal, latitudinal, and 27 geographical induced variations in temperature⁵⁻⁷, as well as by preservation issues⁸, deep-sea temperature proxy signals are not affected by these processes^{9,10}. Deep-sea temperature reconstructions can therefore be used as a 28 29 reliable indicator of global mean temperatures in the geological past^{11,12}. Currently, much of our understanding of 30 global climate evolution and variability during the early Eocene is based on deep-sea temperature estimates derived 31 from benthic foraminiferal oxygen isotopes (δ¹⁸O) and Mg/Ca ratios^{9,13-16}. For instance, negative excursions in high-32 resolution stable oxygen and carbon isotope records have revealed the periodical occurrence of multiple transient 33 (10-100 kyr) episodes of global warming and ocean acidification (hyperthermal events; e.g. Paleocene-Eocene

Thermal Maximum, ETM2 and ETM3)¹⁶, likely driven by massive release of isotopically light carbon into the ocean atmosphere system¹.

36 Yet, the reliability of foraminiferal δ^{18} O- and Mg/Ca-based temperature reconstructions is hampered by 37 uncertainties in nonthermal factors. Both proxies demand assumptions on the past δ^{18} O and Mg/Ca composition of 38 the seawater and are influenced by the carbonate chemistry of the ocean and species-specific physiological 39 factors^{17–19}. The carbonate clumped isotope proxy (Δ_{47}), which uses the temperature dependence of the bonding 40 of the two heavy rare isotopes (¹³C and ¹⁸O) within the carbonate ion, shows great potential as it is independent of 41 the isotope composition of the seawater and is largely unaffected by pH and biology²⁰⁻²². Recent analytical 42 developments in this technique now allow for analysis of relatively small sample sizes (e.g., foraminifera), and thus 43 enable reconstruction of marine temperatures independent from these non-thermal uncertainties²³⁻²⁵. While 44 application of clumped isotope paleothermometry on the relatively cooler Miocene and middle Eocene have 45 revealed a much warmer deep ocean than traditionally accepted²⁶⁻²⁸, it is currently not known whether warmer 46 deep-sea temperatures than assumed are also explicit during the hothouse world of the early Eocene.

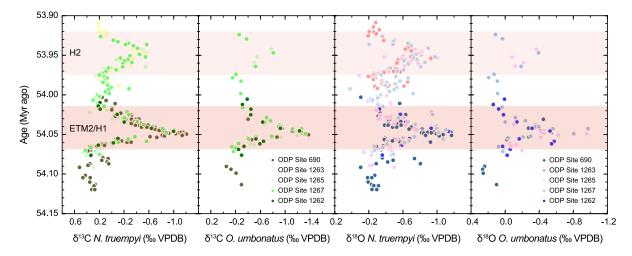
47 Here, we report the first Δ_{47} -based deep-sea temperature estimates of the early Eocene and across two 48 hyperthermal events. Early Eocene hyperthermals ETM2 (or H1) and H2 (~54.1 and 54.0 Ma), that occurred 49 approximately 2 Myrs after the PETM, were discovered for the first time in South Atlantic sediment cores²⁹. High-50 resolution (~1 kyr) benthic foraminiferal carbon and oxygen isotope records were generated from four ODP Sites 51 (1262, 1263, 1265 and 1267) at the Walvis Ridge and one site at Maud Rise in the Weddell Sea (ODP Site 690) 52 with paleowater depths ranging from ~1500 m to ~3600 m (ref 30) (Extended Data Fig. 1). The benthic δ^{18} O records 53 of all these sites show almost identical values, suggesting that they reflect similar deep-sea conditions³⁰. Here, we 54 revisit these sites and apply paired stable and clumped isotope analysis on the benthic foraminiferal species 55 Nuttallides truempyi and Oridorsalis umbonatus for confident reconstruction of deep-sea temperatures across these 56 two hyperthermals.

57

58 Stable and clumped isotope analysis

59 Our new carbon and oxygen isotope measurements (Fig. 1) are consistent with the previously published benthic 60 δ^{13} C and δ^{18} O records³⁰. Prominent negative excursions in δ^{13} C (-1.4‰ and -0.8‰, respectively) and δ^{18} O (-0.8‰ 61 and -0.5‰ respectively) characterize ETM2 and H2, indicating the injection of large amounts of ¹³C-depleted 62 carbon into the climate system and elevated deep-sea temperatures^{29,30}. Limited isotope data is obtained for the 63 peak ETM2 interval at the Walvis Ridge sites, as benthic foraminifera are rare and small-sized in the associated 64 red clay layer, known as the Elmo horizon^{29,30}.

55 Due to the sporadic natural abundance of ${}^{13}C{-}^{18}O$ bonds within carbonate ions, clumped isotope analysis is 66 characterized by a low analytical precision^{20,23–25}. Averaging multiple replicate Δ_{47} measurements is required to 67 obtain precise estimates of temperature, and therefore demands large sample sizes (>5 mg, each replicate ~100





69Fig. 1 | South Atlantic benthic foraminiferal δ13C and δ18O records across ETM2 and H2. This figure shows the δ13C and70 δ^{18} O measurements for benthic foraminifera *N. truempyi* and *O. umbonatus*. The apparent offset in the stable isotopes between71the two species indicates different isotope fractionation34.

73 μ g)^{24,25}. The strategy in how to average Δ ₄₇ measurements for precise reconstruction of small temperature variability 74 becomes important when sample size is limited³¹. In our study, the abundance of *N. truempyi* and *O. umbonatus* is 75 too low in the samples at all sites to undertake enough measurements for the precise reconstruction of deep-sea 76 temperature change across ETM-2 and H2. As a solution to obtain the desired precision (<3°C at 95% confidence 77 interval), we sorted the Δ_{47} measurements from all sites based on their corresponding δ^{18} O values and compiled 78 three average clumped isotope temperatures that represent the average background state (high δ^{18} O), average 79 slope (middle δ^{18} O), and average hyperthermal peak (low δ^{18} O) conditions across the studied interval. So instead 80 of showing deep-sea temperatures in a time series, we plotted three clumped isotope temperature bins that are 81 representative for the different climate states. A Welch t-test was performed between the 'high δ^{18} O' and 'low δ^{18} O' 82 Δ_{47} measurements to obtain the most optimal bin size for the background and hyperthermal temperatures³¹ 83 (Extended Data Table 1 and Extended Data Figures 2 and 3).

84

85 Deep-sea temperatures

So $Our \Delta_{47}$ -based reconstruction indicates very warm deep waters with average background temperatures of 13.2±1.9 °C (95% CI) and average hyperthermal peak temperatures of 16.5±2.2 °C (95% CI) (Fig. 2b). These temperatures represent an average hyperthermal deep-sea warming of 3.3±2.9 °C (95% CI). The peak temperature of ETM2 cannot be estimated due to the averaging of Δ_{47} data and limited measurements derived from the Elmo horizon. However, the ETM-2 peak temperature may be assumed to be warmer than 16.5±2.2 °C (95% CI) based the magnitude of the stable isotope excursion, being almost twice the magnitude of H2 (ref 30).

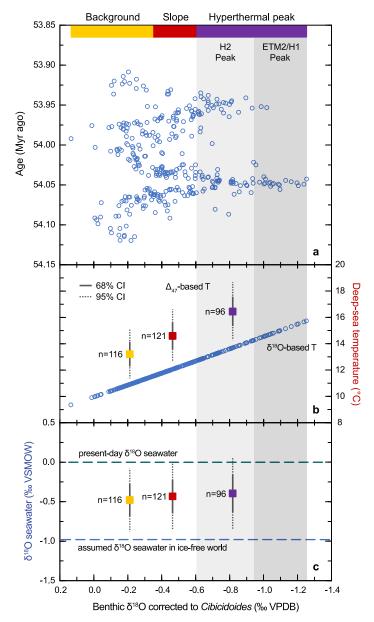




Fig. 2 | Deep-sea temperatures and δ¹⁸O seawater composition. a, Benthic δ¹⁸O values that are corrected towards Cibicidoides 95 (for seawater equilibrium) across ETM2 and H2. **b**, Deep-sea temperatures based on benthic Δ_{47} and δ^{18} O across ETM2 and H2. 96 The yellow, red, and purple (background, slope, hyperthermal peak) bars indicate the range in δ^{18} O values that correspond to the 97 Δ_{47} measurements used for compiling these three bins. The error bars (68% and 95% CI) in the Δ_{47} -based temperatures represent fully propagated analytical and calibration uncertainties. c, $\delta^{18}O_{sw}$ across the ETM2 and H2 interval, which we calculated by using 98 99 the Δ_{47} -based temperatures in combination with the foraminiferal δ^{18} O values in the oxygen isotope-temperature relationship of 100 ref 33 (Methods). The uncertainty in the Δ_{47} -based temperatures is propagated to estimate the precision of the $\delta^{18}O_{sw}$ calculations. 101 The dashed red line is the assumed $\delta^{18}O_{sw}$ value based on ice-free conditions, while the dashed blue line represents the present-102 day icehouse $\delta^{18}O_{sw}$ value.

103

104 Our background temperature reconstruction falls within the broad range of possible Mg/Ca-based deep-sea 105 temperatures for the early Eocene, of ~10 to ~15 °C, associated with significant uncertainty in seawater Mg/Ca 106 ratios prior to the middle Eocene^{18,32}. Regarding hyperthermal deep-sea temperature change based on Mg/Ca,

107 reconstructions are currently only available for the PETM and indicate 5–6 °C warming¹⁴. The deep-sea 108 temperatures we estimate for ETM2 from clumped isotopes suggest that Mg/Ca underestimates deep-sea 109 hyperthermal warming, considering ETM2 has half the magnitude of the PETM based on their stable isotope 110 excursions^{16,29,30}. Generally, it is assumed that reconstruction of temperature change across the short-lived 111 hyperthermal events is not influenced by secular (long-term) changes in seawater Mg/Ca composition¹⁸. Rather, 112 this underestimation of deep-sea temperature variability by Mg/Ca in the Eocene may result from a lowering of the 113 Mg/Ca-temperature sensitivity at low Mg/Ca_{sw} values¹⁹.

114 To compare the clumped isotope-based deep-sea temperatures with those derived from conventional δ^{18} O, we 115 applied the revised δ^{18} O-temperature relationship of ref 33 on our new data (Methods). As in previous 116 palaeoceanographic research¹³, we adopted a fixed value of -0.98‰ VSMOW for the mean oxygen isotopic 117 composition of the seawater ($\delta^{18}O_{sw}$) for the early Eocene global ocean, based on the assumption of absence of 118 continental ice. We adjusted the *N. truempyi* and *O. umbonatus* δ^{18} O values to *Cibicidoides* using well-documented interspecies offset correction factors for the late Paleocene-early Eocene³⁴ (Methods). In contrast to previous 119 120 studies^{13,35}, we consider no specific offset between the $\delta^{18}O_{sw}$ and the foraminiferal tests of *Cibicidoides*, since 121 recently it has been shown that modern *Cibicidoides* δ^{18} O values are in close equilibrium with seawater³³. Note that 122 if we would have applied the generally assumed offset correction of 0.64‰ for this genus³⁵, our δ^{18} O-based 123 temperature estimates would have been 2–3 °C colder. In comparison to the δ^{18} O-based deep-sea temperatures, 124 our clumped isotope reconstructions are on average two and a half degrees warmer, i.e., 13.2±1.9 °C (95% CI) 125 versus 10.9 °C for background, 14.6±2.0 °C (95% CI) versus 12.1 °C for slope, and 16.5±2.2 °C (95% CI) versus 126 13.7 °C for average hyperthermal peak temperatures (Fig. 2b).

127

128 Constraints on Early Eocene seawater oxygen isotope composition

An important consequence of the warmer Δ_{47} -derived deep-sea temperatures for the early Eocene is that they imply a mean value for $\delta^{18}O_{sw}$ of approximately $-0.4\pm0.4\%$ VSMOW (95 % Cl), which is much higher than the value of -0.98 % VSMOW based on the assumption of ice-free conditions (Fig. 2c). A first explanation may be that major continental ice sheets were present during the early Eocene. However, this hypothesis can be refuted with subtropical temperatures and vegetation that prevailed at both poles^{36,37}. Though small ephemeral ice caps may have been present in Antarctica and in the Arctic region, records of ice rafted debris show that significant highlatitude ice formation was not initiated prior to the middle Eocene^{38,39}.

136 In the absence of large ice sheets, a second explanation for our reconstructed higher $\delta^{18}O_{sw}$ value than currently 137 assumed may be that local factors were important for determining the isotopic composition of the deep-water 138 masses in the South Atlantic. As such, the high $\delta^{18}O_{sw}$ value may suggest that saline Atlantic deep waters were 139 more persistent during the early Eocene, perhaps driven by evaporation-dominated deep-water formation at the 140 low-latitudes⁴⁰. However, a complicating factor is that other regions of the deep ocean must have had a much lighter

141 $\delta^{18}O_{sw}$ (fresher waters) to balance the global ocean $\delta^{18}O_{sw}$ towards ice-free conditions. Such large spatial 142 differences in deep-sea $\delta^{18}O_{sw}$ are difficult to reconcile with small isotope gradients between ocean basins⁴¹.

A third explanation for the high $\delta^{18}O_{sw}$ comes from recent studies that hypothesize that groundwater reservoirs are an important factor in causing the apparent large sea level fluctuations (tens of meters) in hothouse periods during the Cretaceous⁴². Whether such aquifers as large reservoirs for light oxygen on land⁴² could explain a high $\delta^{18}O_{sw}$ in the early Eocene hothouse remains an open question.

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148 Potential pH effect on foraminiferal oxygen isotopes and species-specific isotope fractionation

149 Factors other than a different $\delta^{18}O_{sw}$ may also explain the temperature discrepancy between clumped isotopes and 150 δ^{18} O. The assumed δ^{18} O_{sw} value of -0.98 ‰ VSMOW may be correct when a pH effect on foraminifera δ^{18} O is 151 taking into account. A much lower deep-sea pH existed during the early Eocene (~0.4 units lower than modern)⁴³. 152 Both culture experiments and field studies have shown that for aminiferal δ^{18} O is sensitive to pH, characterized by 153 increasing δ^{18} O values with decreasing pH, but with a varying magnitude of this effect among different species⁴⁴. 154 Although this pH effect on δ^{18} O has so far not been demonstrated for benthic foraminifera³³, the underestimated 155 early Eocene deep-sea temperatures indicated by δ^{18} O are consistent with a scenario of highly reduced sea water 156 pH. Though actual proxy deep-sea pH reconstructions are currently not available for the hyperthermals, a further 157 acidification of the deep water is expected from numerical models⁴⁵. The assumed effect of an even lower pH would 158 result in a dampening of the negative benthic foraminiferal δ^{18} O excursion of these warming events (almost half of 159 the original magnitude)⁴⁵, with a consequent underestimation of the deep-sea warming. Based on our proxy 160 reconstruction, it is though not possible to support that δ^{18} O underestimates hyperthermal deep-sea warming with 161 one degree Celsius or more due to the temperature uncertainty in the clumped isotope bins and the maximum 162 hyperthermal peak temperatures that cannot be estimated due the averaging approach.

163 In addition to a potential pH effect, changes in species-specific isotope fractionation over time are also important 164 to consider as a possible explanation for the relatively cold δ^{18} O-based deep-sea temperatures. Whether modern 165 foraminiferal species precipitate their calcite tests with a certain offset from seawater has been determined from 166 culture experiments and core top studes^{33,35}. Yet, differences in interspecies offsets in $\delta^{18}O$ (and $\delta^{13}C$) have been 167 found between the Paleogene and more recent times³⁴. These different offsets may suggest that vital effects were 168 not constant through the Cenozoic, which are however difficult to quantify³⁴, considering the evolutionary and pH 169 changes that may have played a role through time. In conclusion, our study shows that all these uncertainties in 170 the conventional proxies hamper confident deep-sea temperature reconstructions of the Eocene. The reliability of 171 these classic reconstructions can also be questioned for other periods of the Cenozoic, such as the Miocene^{26,27}. 172 These findings clearly warrant a revision of the traditional Cenozoic history of deep-sea temperatures that has 173 provided us with a presumed benchmark of our understanding of global climate evolution over the past 66 Myrs^{13,15}.

175 Implications for climate sensitivity in the early Eocene

176 To incorporate our new constraints on early Eocene deep-sea temperatures based on clumped isotopes in a 177 broader perspective of the Eocene climate state, we assume that the reconstructed South Atlantic deep-sea 178 temperatures are representative for the global deep sea, i.e., a homogeneous global deep ocean. This assumption 179 of globally uniform deep-sea temperatures is supported by the small interbasinal isotopic differences found for the 180 early Paleogene⁴¹, and almost identical clumped isotope-based deep-sea temperatures across the Atlantic for 181 middle Eocene times²⁸. As such, our new constraints on deep-sea temperatures have important implications for 182 estimates for Equilibrium Climate Sensitivity (ECS), defined as the equilibrium global mean temperature (GMT) 183 increase in response to a doubling in atmospheric CO_2 (ref 11), of the early Eocene.

184 In order to estimate ECS from our data, we need to transfer deep-sea temperatures to GMT estimates. Following 185 previous studies^{46,47}, we apply the assumption that in warmer climates prior to the Pliocene deep-sea temperature 186 change scales directly to GMT change (Δ GMT = Δ DT) (ref 12). In the icehouse climate state of the Pleistocene, this 187 relation may be different and therefore deep-sea temperature change could be smaller than the GMT change¹² 188 (Methods). Using this approach with a δ^{18} O-based Pliocene deep-sea temperature of 2 °C (ref 12) based on the 189 assumption of a 2:1 contribution of temperature and ice volume respectively on benthic $\delta^{18}O$ (Methods), we derive 190 a GMT of 28.4±1.9 °C (95% CI) for background early Eocene conditions from our reconstructed deep-sea 191 temperatures. We note that all the assumptions made here introduce uncertainty in the GMT and consequently 192 ECS estimates. Future independent deep-sea temperature reconstructions from clumped isotopes for the entire 193 Cenozoic will provide more constraints on these scaling factors.

194 To characterize radiative forcing, we use the recently published CO₂ level reconstructions for the Eocene at 54 195 Ma from ref 4, showing background CO₂ levels of 900±200 ppmv (95% CI), i.e., >3 times preindustrial (278 ppmv) 196 values. We do not assess ECS during peak conditions of ETM2 (~5 degrees global warming), as the magnitude of 197 the carbon cycle perturbation of this event is not well-constrained yet⁴⁸. To isolate the GMT change purely ascribed 198 to a change in CO₂ forcing, an important step beforehand is to account for the different boundary conditions of the 199 early Eocene, such as the absence of global ice sheets and a different continental configuration. Climate model 200 comparison of the Eocene and preindustrial worlds, both simulated with preindustrial CO2 forcing, shows that GMT 201 are 3-5 degrees warmer in the Eocene case⁴⁹. Therefore, to correct for the different boundary conditions we 202 subtract this 3-5 degrees temperature range from the GMT difference between the early Eocene and preindustrial. 203 Employing the equations used by ref 4 (Methods), we then obtain an early Eocene ECS of 5.9 °C (3.6 to 9.8 °C 204 95% CI) per doubling of CO₂. This estimate is close to ECS inferred by recent climate models for the Eocene in 205 which processes like cloud feedbacks operating in much warmer worlds appear to play a key role in amplifying CO2-206 induced warmth⁵⁰. Our new deep-sea temperature reconstructions of the early Eocene provide new evidence for 207 high ECS in hothouse climates, supporting a climate state dependency of ECS^{4,50}. Such state dependency of ECS

- 208 may have major implications for the IPCC projections of future warming, as these currently follow the typically
- estimated range in present-day ECS (1.5 to 4.5 °C).
- 210

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331

- Author contributions T.A., M.Z., and L.J.L. designed the study. T.A. generated the stable and clumped isotope
 data. All authors contributed to data interpretation and the written manuscript.
- 334
- 335 **Competing interests** The authors declare no competing interests.
- 336
- 337 Materials and correspondence requests should be addressed to T.A.

- 339 METHODS
- Foraminiferal preservation and sampling. Since recently, clumped isotope thermometry has been applied on foraminifera in several paleoclimate studies^{26,28,51–54}. In our study to the early Eocene, we performed stable and clumped isotope measurements on monospecific multispecimen samples of *Nuttallides truempyi* and *Oridorsalis*

343 umbonatus, which were picked from the >212 µm sediment size fractions of the samples of the previous study of 344 ref 30. Grouping of Δ_{47} measurements from different benthic species together to produce one temperature estimate 345 requires the assumption of absence of species-specific effects on Δ_{47} . This assumption is supported by both modern core top and deep time studies that show no indication of these biological factors^{22,26,51,55-58}. Scanning electron 346 347 microscope (SEM) images were previously generated to examine the preservation throughout the studied interval 348 and indicate that the foraminiferal tests are moderately altered by recrystallization/secondary calcite³⁰. 349 Nevertheless, the effect of recrystallization of primary calcite on the Δ_{47} signal in benthic foraminifera has been 350 shown to be largely negligible^{26,28,59}, since this diagenesis usually occurs during early burial^{10,60,61}, and therefore 351 records similar temperatures as the seafloor temperatures. Due to limiting numbers of picked foraminifera per 352 sample, we needed to combine picked foraminifers of neighboring depths to enable sufficient foraminiferal material 353 (~25 specimens per measurement). Prior to isotope analysis, foraminiferal samples were cleaned by removing 354 adherent contaminants, for instance nannofossils, organics and clay particles. The foraminifers were gently cracked 355 between two glass plates to enable any chamber infill to escape, and subsequently sonicated in deionized water 356 two times for 20 seconds. The test fragments were rinsed until the suspended particles were removed (~2 x 2 357 times), and oven-dried at 40 °C for one night.

358 Clumped isotope analysis. In total, 333 successful stable and clumped isotope sample measurements were 359 generated across the ETM2 and H2 interval. Our analytical methods generally follow previous work^{23,24}, and a more 360 detailed description can be found in the Supplementary Information. We performed measurements of ~100 µg 361 foraminiferal material each on a Thermo Scientific Kiel IV carbonate preparation device coupled to a Thermo 362 Scientific MAT 253 Plus mass spectrometer at Utrecht University. Apart from samples, we measured additional 363 carbonate standards in an approximate 1:1 ratio. We employed three standards (ETH-1, 2, and 3), which differ in 364 δ^{13} C, δ^{18} O, and Δ_{47} composition, to correct the results and monitor the long-term reproducibility. Two additional 365 check standards (IAEA-C2 and Merck) were also measured to evaluate this long-term instrument performance. 366 External reproducibility (one standard deviation) in Δ47 of all the carbonate standards after correction was typically 367 0.030–0.040‰. The δ^{13} C and δ^{18} O values (reported relative to the VPDB scale) of the standards show an external 368 reproducibility (one standard deviation) of respectively 0.10 and 0.05‰ on average. In Fig. 1, the stable isotope 369 data is plotted against age following the age models of refs 30,62.

370 Temperature calculations. To determine clumped isotope-based deep-sea temperatures, we employed a recent 371 foraminifer-based calibration⁵⁸ recalculated to the Intercarb-Carbon Dioxide Equilibrium Scale (I-CDES) using the 372 newly accepted values of the carbonate standards of ref 63.

373

374
$$\Delta_{47}(I-CDES90^{\circ}C) = 0.0407(\pm 0.0016) \cdot 10^{6}/T^{2} + 0.1385(\pm 0.0189)$$
 (T in K) (1)

This Δ_{47} -temperature relationship represents a composite of previously published foraminifer based-calibrations^{22,57} with new data⁵⁸. Within uncertainty, this regression is consistent with calibrations based on inorganic calcite^{25,64}. For our data representation and visualization, we fully propagated analytical and calibration uncertainties in the temperature estimates. The combined uncertainty is reported as 68% and 95% confidence intervals (CI) in the temperature bins.

381 Calculation of the δ^{18} O-based deep-sea temperatures follows equation 9 of ref 33 based on the benthic 382 foraminifera *Cibicidoides*:

383

 $384 \qquad \delta^{18}O_{b} [\% VPDB] - \delta^{18}O_{sw} [\% VSMOW] + 0.27 = -0.245T + 0.0011T^{2} + 3.58$ (2)

385

This quadratic regression shows good agreement with the calibration of ref 65, which is based on inorganic calcite precipitated between 10 and 40 °C^{65,66}. Prior to temperature calculation, we adjusted δ^{18} O of *O. umbonatus* and *N. truempyi* towards *Cibicoides* values for assumed seawater equilibrium using the following correction factors from ref 34: (Nutt + 0.10)/0.89 = Cib; and Orid - 0.28 = Cib.

390 Global mean temperatures. Previous studies have used δ^{18} O-based deep-sea temperature to estimate 391 GMT^{11,12,46,47}. We calculated GMT from our reconstructed Δ_{47} -based deep-sea temperatures following the rationale 392 of ref 12,67,68, who suggest that deep-sea temperature change is similar to GMT change when several degrees 393 above the freezing point of ocean water. This approach argues that deep-sea temperatures are closely related to 394 high-latitude (region of deep-water formation) sea surface temperatures, and changes in the latter are amplified 395 relative to the global mean sea surface temperature change. Yet, the air temperature change on the continents is 396 larger than in the mean surface ocean. Ref 12,67,68 suggest that these two factors are offsetting in relatively equal 397 proportions, and therefore they assume that temperature change in the deep-ocean tends to approximate the GMT 398 change. They use surface temperature reconstructions and climate model results for the Eocene, the Pliocene, and 399 the Last Glacial Maximum to determine the exact relationship between deep-sea temperature and GMT change 400 during different periods in Cenozoic. Deep-sea temperature change is proposed to be tied to GMT change in a 1:1 401 ratio for the pre-Pliocene (Δ GMT = Δ DT), though they do not disregard the possibility of a Δ GMT = 1.5 x Δ DT 402 relationship, which shows more consistency with sea surface temperature reconstructions (which in the higher 403 latitudes though may be overestimated due to a potential summer bias)¹². In the Late Cenozoic Pliocene and 404 Pleistocene, however, this scaling changes, as the deep ocean cannot become much colder when it approaches 405 the freezing temperature of ocean water. In other words, in these cooler climates the deep-sea temperature change 406 is smaller than the GMT change^{12,67,68}. Combining the pre-Pliocene relationship (Δ GMT = Δ DT) and the Pliocene 407 relationship (equation 4.2 in ref. 12) results in the following equation to calculate GMT of the early Eocene:

408

409
$$GMT_{Eocene} (^{\circ}C) = (2.5 \times DST_{Plio} + 12.15) [=GMT_{Plio}] + (DST_{Eocene} - DST_{Plio})$$
(3)

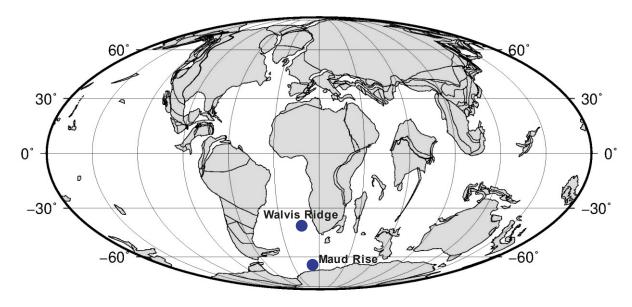
410			
411	where we use a Pliocene and early Eocene deep-sea temperature of respectively 2 °C (ref 12) and 13.2±1.9 °C.		
412	This Pliocene deep-sea temperature of 2 °C is based on benthic δ^{18} O assuming a 2:1 contribution of temperature		
413	and ice volume respectively from sea level reconstructions ¹² .		
414	Climate sensitivity. To determine ECS, we follow the approach in refs 4,46,69. We first calculate the relative		
415	change in climate forcing (Wm ⁻²) attributed to a CO ₂ change in the early Eocene relative to preindustrial using the		
416	equation of ref 70:		
417			
418	$\Delta F_{CO2} = 5.32 \ln(C_{EE}/C_{PI}) + 0.39 [\ln(C_{EE}/C_{PI})]^2 $ (4)		
419			
420	where C_{EE} represents the background atmospheric CO ₂ levels in the early Eocene (at 54 Ma), and C_{PI} refers to the		
421	CO ₂ concentrations during the preindustrial (278 ppm).		
422	ECS of the early Eocene relative to preindustrial is obtained using the following equation ^{4,46,69} :		
423			
424	$ECS = (\Delta GMT_{EE-vs-Pl}/\Delta F_{CO2}) \times 3.87 $ (5)		
425			
426	where $\Delta GMT_{EE-vs-Pl}$ is the GMT change that is attributed to a change in CO ₂ forcing, and $\Delta F_{CO2-vs-Pl}$ indicates the		
427	relative change in climate forcing by CO ₂ . The 3.87 (in Wm ⁻²) expresses the climate forcing of a doubling in CO ₂ .		
428	We account for the contribution of different paleogeography and paleotopography, solar constant, and slow		
429	climate feedbacks associated with ice sheets (absence), vegetation, and aerosols to the GMT difference between		
430	early Eocene and preindustrial (GMT of 28.4±1.9 °C and 14 °C for the early Eocene and preindustrial respectively).		
431	Based on climate model simulations ⁴⁹ , we subtract a value of 3–5 degrees from the early Eocene GMT to obtain		
432	the temperature change that is purely ascribed to different CO ₂ forcing.		
433			
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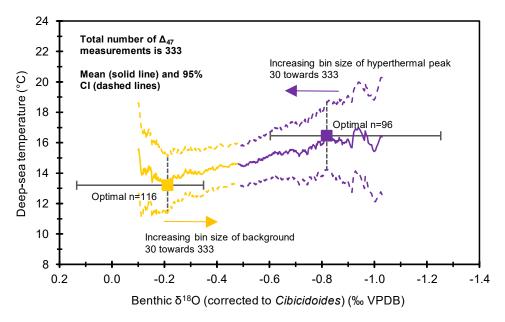
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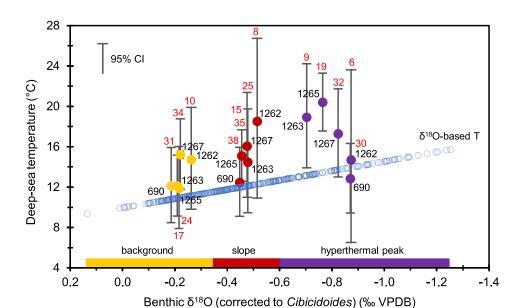


Extended Data Fig. 1 | Paleogeographic reconstruction (54.0 Ma) with location of Walvis Ridge and Maud Rise. This map
is generated from the plate tectonic reconstruction service of the Ocean Drilling Stratigraphic Network (ODSN;
http://www.odsn.de). The ODP Sites from the Walvis Ridge used for benthic foraminiferal stable and clumped isotope analysis
include Sites 1262, 1263, 1265 and 1267. Paleowater depth ranges from ~1500 m (Site 1263) to ~3600 m (Site 1262)⁷¹. Maud
Rise is represented by ODP Site 690 with a paleowater depth of ~2100 m)⁷².

- 491
- 492
- 493

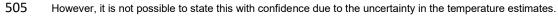


495 Extended Data Fig. 2 | Background and hyperthermal peak temperatures (mean and 95% Cl) as a function of bin size. 496 Adding Δ_{47} measurements with progressively lower and higher δ^{18} O values results in increasing bin sizes of the background 497 (orange) and hyperthermal peak (purple) states respectively. The mean values of these two bins move towards each other with 498 increasing bin size. The 95% Cl decreases with increasing bin size. A size of 116 and 96 Δ_{47} measurements for the background 499 and hyperthermal peak bins respectively yields the most significant difference between the two populations. 500





502 Extended Data Fig. 3 | The compiled Δ_{47} -based temperature bins (background, slope and hyperthermal peak) 503 deconvolved into data per site (mean and 95% Cl). The variability in the temperatures among the sites can attributed to limited 504 measurements. Deep-sea temperatures at Site 690 though seem to be slightly cooler in comparison to the Walvis Ridge sites.



- 506
- 507

- 508 Extended Data Table 1 | Optimal Δ_{47} bin sizes. Overview of the 30 most optimal bin sizes for the background and hyperthermal
- 509 peak Δ₄₇ populations determined using a Welch t-test, i.e., the bin sizes that result in the most significant difference (lowest p-
- 510 values).

Δ₄7 bin size background	Δ₄⁊ bin size hyperthermal peak	Lowest p-values
116	96	0.0191
116	93	0.0199
116	97	0.0204
116	94	0.0204
116	99	0.0214
111	96	0.0215
116	92	0.0216
116	95	0.0218
111	93	0.0223
118	96	0.0229
111	94	0.0229
111	97	0.0230
116	98	0.0230
117	96	0.0237
118	93	0.0238
111	99	0.0241
111	92	0.0242
116	100	0.0243
116	91	0.0243
118	94	0.0244
118	97	0.0244
111	95	0.0244
119	96	0.0245
117	93	0.0246
115	96	0.0247
116	55	0.0249
117	94	0.0253
117	97	0.0253
119	93	0.0254
120	96	0.0255

- 511
- 512

513 Supplementary Information

514

515 Mass spectrometry and clumped isotope data processing

516 In total, we carried out 27 runs with most of them containing 20 samples interspersed with 26 carbonate standards,

517 over a period of one year. We randomized the distribution of the samples over this period to prevent biases in the

518 obtained data due to variability and longer-term trends in the mass spectrometer performance. The relative

519 proportions of the measured standards ETH-1:ETH-2:ETH-3 was 1:1:10, as optimal minimization of uncertainties 520 in the Δ_{47} data is achieved by measuring relatively more ETH-3 (ref 73). In the *Thermo Scientific Kiel IV* device, the 521 carbonate material was digested with phosphoric acid (H₃PO₄) at a reaction temperature of 70 °C (ref 74). Steps 522 were subsequently taken in the instrument to purify the sample gas. First, the gas was transported to a first 523 cryogenic liquid nitrogen (LN₂) trap with a temperature of -196 °C. Here, CO₂ and H₂O amongst others were frozen 524 and trapped while non-condensable gases (originating from impurities in the sample) were removed⁷⁴. The gas was 525 then transferred through a Porapak-Q trap cooled at -40 °C to remove organic contaminants, such as halo-526 /hydrocarbons and reduced sulfur compounds⁷⁴. After a second cold trap a near pure CO₂ gas remained, which 527 was sent into the Thermo Scientific MAT 253 Plus instrument for isotope analysis. The mass spectrometer collected 528 the signals of m/z 44–49 of the gas, which correspond with the different isotopologues of CO₂ (refs 74, 75). The m/z 529 48 and 49 signals were used to detect potential contamination⁷⁶. To obtain the necessary accuracy of the 530 measurements, the sample gas was measured against a reference gas of known isotopic composition^{24,77}. 531 Measurements with the mass spectrometer followed the long-integration dual-inlet (LIDI) method^{24,78}.

532 We applied a pressure sensitive baseline correction (PBL) to correct the obtained data for negative backgrounds 533 using peak scans at m/z 44 intensities of 5, 10, 15, 20, and 25 V, which were performed during run preparation^{23,76,79}. 534 Subsequently, the ETH-1, ETH-2, and ETH-3 standards were used to correct sample A47 data and normalize it into 535 the InterCarp-Carbon Dioxide Equilibrium Scale (I-CDES)^{25,63,80}, to correct for offsets in δ^{13} C and δ^{18} O, and to 536 correct for scale compression/stretching in δ^{18} O. We applied an offset correction based on two neighboring ETH-3 537 standards to correct the Δ_{47} sample measurements for long-term variability in the mass spectrometer performance. 538 Then, the data was transferred into the I-CDES by using an empirical transfer function (ETF) that is based on the 539 raw Δ47 values of the ETH standards of a window of 4 runs and their InterCarp accepted values at 90 °C acid 540 reaction temperature as determined by ref 63 (ETH-1: Δ₄₇ = 0.2052 ±0.0016(1SE)‰; ETH-2: Δ₄₇ = 0.2085 541 ±0.0015(1SE) ‰; ETH-3: Δ_{47} = 0.6132 ±0.0014(1SE)‰. To determine final values for both δ^{13} C and δ^{18} O, an offset 542 correction was applied based on the measured and reported stable isotope values of all three ETH standards in ref 543 25 (ETH-1: δ¹³C_{VPDB} = 2.02±0.03‰, δ¹⁸O_{VPDB} = -2.19±0.04‰; ETH-2: δ¹³C_{VPDB} = -10.17±0.06‰, δ¹⁸O_{VPDB} = -544 18.69±0.11‰; ETH-3: δ^{13} C_{VPDB} = 1.71±0.02‰, δ^{18} O_{VPDB} = -1.78±0.06‰).

The external reproducibility (one standard deviation) of the δ^{13} C, δ^{18} O, and Δ_{47} measurements for each of the standards is listed in the Archive in the Supplementary Data File. Regarding the clumped isotope data, outliers are absent in the successful (no issues with backgrounds, contamination, and/or intensity) sample measurements. The final Δ_{47} values of all these successful measurements are within 4 standard deviations off from the mean. Raw stable and clumped isotope data for all standard and sample measurements are included in the Supplementary Data File.

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