Responses to All Reviewer Comments
Biogeosciences Discussion Forum
"Factors Influencing Test Porosity in Planktonic Foraminifera"

Authors: Janet E. Burke, Willem Renema, Michael J. Henehan, Leanne E. Elder, Catherine V. Davis, Amy E. Maas, Gavin L. Foster, Ralf Schiebel, Pincelli M. Hull

Reviewer #1: Anthony Rathburn

Reviewer Comment: The term "porosity" has different meanings in geoscience. I think it might be best to use "surface area of pores" at least in the abstract, if not the title, to make it clearer what you are referring to.

Author Response: We agree that the general term porosity can be confusing, and will be more specific in the abstract and the title. We will change the title to "Factors Influencing Test Porosity in Planktonic Foraminifera" and introduce the term porosity as ". . . porosity (the total percentage of a test wall that is open pore space) . . ." on line 20 in the abstract.

RC: What role does food availability play in the size and metabolism of planktonic foraminifera?

AR: This is a good question, as food availability has been shown to effect terminal sizes and morphologies in laboratory culture (i.e. Hemleben et al., 1989). There are two ways in which we might worry about the influence of food availability in our results: i) what is the influence of food availability on the individuals in the core top samples; ii) what is the influence of food availability on the culturing results? We begin with the second, as it helps inform our consideration of the first.

In culture, we fed individuals at what might be the upper end of their natural food intake: one large artemia per day. It is notable in this context that the individuals cultured at near ambient temperatures (25°C), had culture porosities that were statistically indistinguishable from pre-culture values. This might suggest that food intake has a relatively modest effect on porosity.

However, perhaps we just happened to be feeding cultured individuals at comparable rates, amounts, and dietary compositions as their natural environment! We certainly did not directly test for an effect of food available on culture porosity.

Differences in major dietary factors (food type, symbiont status) may provide some additional insight into the influence of this food availability on porosity. In this context, it is notable that our morphogroups do roughly correspond with major food groups. For example, species in the globorotalid group are asymbiotic and species in the globigerinoid group have dinoflagellate symbionts. Also, we do find an effect of morphogroup on pore density, the number of pores in a unit of area.

However, this doesn't account for the difference in feeding frequency during an individual life span or in different spatially or temporally disparate populations of the same species. Given this, we will mention food availability as an important unknown and target for future research in the discussion. At Line 309 we will insert a sentence that says:
"Another environmental factor that may influence terminal sizes and metabolic function is the availability of food sources. Feeding frequency has been shown to influence terminal size and morphology (Bé, 1982; Hemleben et al., 1989), and may thus be expected to influence porosity as well. This factor is difficult to estimate for core top assemblages, but can be tested with simple culture experiments and subsequent imaging."

RC: Technical Corrections 373 Should be ". . .Scripps Institution. . ."

AR: This correction will be made and reflected in future versions of the manuscript.

Reviewer #2: Anonymous

Reviewer Comment: Unit of "pore size" In Figure 2, "average pore size" seems to have a unit of μm, so I thought it means pore diameter. However, when I carefully checked the dataset presented in the Supplementary Table 4 (I also downloaded some SEM images from YPM collections, and measured the pore diameter by myself), I found that the "pore size" values in the table seem to have a unit of μm2. Am I right? If so, I think "size" is not an appropriate term to represent an area of a pore (when we say test size, for instance, it usually indicates test diameter, not area). I also found that in Supplemental Figure 3b, "average pore size" is associated with a unit of μm, but in Figure 4 and in Supplementary Figure 6, "Pore size" is with μm2. Which is correct? Please clarify the definition of the parameter together with its unit. It is the same for "pore density". Perhaps it has a unit of "number μm-2", but please specify it as well.

Author Response: The reviewer is correct that the term "pore size" is indeed more accurately described as "average pore area" with a unit of "um^2". All references to "pore size" and its units in the manuscript have been updated to correct this.

RC: Interpretation of Q10 of porosity: In the discussion part, Q10 of porosity is used to test if porosity increases with temperature at the same rate as respiration. The authors concluded that the Q10 of porosity ranging from 1.3 - 2.4 is close to that of respiration of 3.18 (Lombard et al. 2009), and it indicates the relation in respiration and porosity. In my understanding, however, these values can be said different. Since a Q10 value is a rate of change, 2-fold increase and 3-fold increase eventually cause a large difference. I agree that the porosity increases as temperature increases since the Q10 values are larger than 1 (except for G. inflata). However, the difference in Q10 of respiration and that of porosity is rather large. So, I would say the rate of respiration increase due to temperature rise is faster than the increase of porosity. If the porosity and respiratory gas-exchange are related, it means that the gas-exchange becomes less efficient at a higher temperature (it might indicate that the porosity increase alone cannot meet the

increasing respiratory gas-exchange). Maybe, for example, the presence of symbionts is involved with the efficient scavenging of respiratory gas. . . Anyway, please consider this point (i.e., the difference in Q10 of respiration and porosity) and add a bit more discussion in this part. In addition, according to the values shown in Table 3, the correct range of Q10 of porosity is "1.3 to 2.8", I suppose. Please reconfirm it.

AR: The Reviewer raises some important qualms with our discussion of respiratory Q_{10} and porosity. Although we were using the metric to aid comparisons, it is better to simply compare the respiratory Q_{10} obtained from laboratory study described in Lombard et al., 2009 to the porosity Q_{10}, which we have for a more taxonomically and ecologically diverse set. The conclusion would be that if porosity and respiration were indeed linked to some extent, the level of variation in respiratory Q_{10} and porosity Q_{10} between the same species groups might look similar. Framing the discussion this way does not necessitate that respiration rate is the only driver of porosity or that the two have a 1:1 correlation, as the current discussion did. This allows for some rough assessment of the potential role of symbionts as well, an excellent suggestion by the reviewer, by comparing the porosity Q_{10} of symbiotic and asymbiotic species. A column listing the symbiont ecology of each species has been added to Table 3 (below) and the section starting at Line 276, the section now reads:

"If porosity reflects metabolic rate, both should respond to temperature to a similar degree. To compare the temperature sensitivity of porosity with the respiratory and photosynthetic Q_{10} values (from Lombard et al., 2009), we calculated the change in size-normalized porosity with a ten-degree change in estimated ambient temperature (dubbed the Q_{10} of porosity; Table 3; Supplemental Figure 6). We found an increase in porosity with ambient temperature for six of the eight species found at more than one site (i.e., all species in Table 3 except Globorotalia inflata *and* Globorotalia truncatulinoides*; Supplementary Figure 6). For those species, the Q_{10} of porosity varied from 1.3 to 2.3.*

These porosity Q_{10} values are lower than the respiratory Q_{10} of 3.18 and the photosynthetic Q_{10} of 2.69 reported in Lombard et al., 2009. If porosity and respiratory gas-exchange are related, this means that either gas-exchange becomes less efficient at a higher temperature (suggesting that the porosity increase alone cannot meet the increasing respiratory gas-exchange demand), or that, since porosity is a physical property and not constrained by the same thermodynamic properties as the chemical reactions of photosynthesis and respiration, a 1 to 2 fold change is sufficient to reduce the diffusion limitation and meet the increase respiratory needs of the cells at higher temperatures. Alternatively, this discrepancy could be due to the fact that the measurements of Lombard et al. (2009) were taken from specimens exposed to sudden changes in temperature, which, as the authors noted, may result in higher sensitivity than that present in wild populations.

Furthermore, although we have hypothesized that respiratory demand for O2 is linked to pore size, it must be acknowledged that for symbiont bearing species, foraminifera metabolism is a complex interplay between photosynthesis and respiration. In some cases, where photosynthesis outpaces respiration, symbionts might provide O2 internally, reducing diffusion limitation. Alternatively the substrate demands (both O2 and CO2) and temperature sensitivity of

the symbionts may be driving some of the observed porosity changes. On Table 3, species are sorted by Q_{10} of porosity from highest to lowest, with the symbiont ecologies of each group noted. Here, we can see that the species with the highest Q_{10} is a surface dweller with dinoflagellate symbionts (Globigerinoides conglobatus). The species with the lowest Q_{10} (Globorotalia truncatulinoides) is symbiont-barren with porosity that actually decreases with temperature. Additionally, the other species with a Q_{10} of less than one is Globorotalia inflata, a thermocline dweller with chrysophyte symbionts. These very low porosity Q10s might be due to the fact that the ambient temperatures are approximated from yearly averages of temperature at estimated depth habitats, or they may be a true reflection of a difference in porosity due to symbiont ecology. While Lombard et al., 2009 found that, after normalizing for cell size, the respiratory and photosynthetic Q_{10} of their specimens was consistent among the three species examined (Globigerinella siphonifera, Globigerinoides ruber, and Orbulina universa), what did differ between the species was the net photosynthesis to respiration ratio (P:R). Specifically, this ratio was much lower in the chrysophyte-bearing Globigerinella siphonifera than the dinoflagellate bearers Orbulina universa and Globigerinoides ruber. While we cannot conclude the extent of the relationship with our available data, the general trend of variation in Q_{10} of porosity roughly coinciding with symbiont ecology indicates that there may be some influence of photosynthesis or photosynthesis to respiration ratio on porosity."

Additionally, the range of porosity in Line 281 will be corrected to read "1.3 to 2.3," the correct range.

Species	Porosity at 10°C	Porosity at 20°C	Q_{10} Porosity	Symbiont Type
Globigerinoides conglobatus	-0.1757	-0.0657	2.674	Dinoflagellate[1]
Neogloboquadrina dutertrei	-0.1017	-0.0397	2.562	Pelagophtyes[2]
Orbulina universa	-0.195	-0.084	2.321	Dinoflagellate[1]
Globigerinoides sacculifer	-0.1698	-0.0858	1.979	Dinoflagellate[1]
Globigerinella siphonifera	-0.1625	-0.0965	1.684	Chrysophytes[1]
Globigerinoides ruber	-0.1104	-0.0834	1.324	Dinoflagellate[1]
Globorotalia inflata	-0.0628	-0.0898	0.699	Chrysophytes[1]
Globorotalia truncatulinoides	-0.0499	-0.0869	0.574	Asymbiotic[1]

[1] Ezard, T. H., et al. (2015). Environmental and biological controls on size-specific δ13C and δ18O in recent planktonic foraminifera. *Paleoceanography*, *30*(3), 151-173.

[2] Bird et al., (2018). 16S rRNA gene metabarcoding and TEM reveals different ecological strategies within the genus Neogloboquadrina (planktonic foraminifer). *PloS one*, 13:1.

RC: Q10 calculation based on SST I failed to understand why the authors chose SST to calculate Q10 of porosity instead of ambient temperature that directly affects physiological rates. SST can be an indicator of overall categorization of foraminiferal biomes, but it seems inappropriate to use it to calculate temperature sensitivity (i.e., Q10) of species. Especially, respiration of G. truncatulinoides that lives in deeper water mass won't be affected by SST. Would you please clarify this point, or is it possible to recalculate the Q10 of porosity based on the ambient temperature?

AR: In response to this valid concern, especially as it relates to deep dwellers like *G. truncatulinoides,* I have recalculated the Q_{10} values for the updated manuscript using ambient temperature instead of sea surface temperature. I have also added a column to Table 3 for symbiont type to aid in discussion of the possible role of photosynthesis (shown in the response to comment #2).

RC: Use of the term Q10 In the first place, I wonder if it is appropriate to use the term Q10 for the case like porosity which is not a physiological or chemical reaction rate. In general, Q10 is used to show temperature sensitivity of biological (physiological) or chemical reaction rate. Q10 of porosity is understandable to me, but may not be a suitable terminology, simply because porosity is not a physiological rate. Please check the general usage of this terminology carefully

AR: Temperature sensitivity of porosity is a major theme in this manuscript, and the Q_{10} term is a useful way to describe and compare this sensitivity. However, given that the exact physiological function of pores is unknown, the distinction between respiratory Q_{10} and porosity Q_{10} is communicated more explicitly in the updated version of the manuscript as shown in the response to Comment #2, specifically in the opening sentences:

"If porosity is reflecting metabolic rates, both should respond to temperature to a similar degree. To compare the temperature sensitivity of porosity with the respiratory and photosynthetic Q_{10} values (from Lombard et al., 2009), we calculated the change in porosity with a ten-degree change in estimated ambient temperature (dubbed the Q_{10} of porosity; Table 3; Supplemental Figure 6)."

RC: "Size" of cultured specimens: The authors often mention on "body size" in Section 3.2 (e.g., L236, L420), but what this term indicates is not clear without very careful reading (I could understand that it means the area, not the body mass or the test diameter, only after I reached L234). In the method part, please define the term. I recommend not to use "size" to indicate "area".

AR: "Size" and "body size" are used generally in the manuscript in reference to a number of different size-related parameters (cross-sectional area, surface area, volume, length, sieve size fraction). General terms have been replaced with specific terms in all references to size. For example, the final sentence in the discussion, formerly on Line 348, has been changed from:

"However, when combined, the resulting porosity of an individual is more related to size and temperature, than it is to evolutionary history."

To:

"However, when combined, the resulting porosity of an individual is more related to test surface area, test volume, and temperature, than it is to evolutionary history."

Also, the following sentence has been added to Line 101 of the Methods section:

"Size is an important factor in studies of planktonic foraminiferal ecology and biology, but it can refer to many different test parameters, like major axis length, aspect ratio, sieve size class, or three dimensional volume and surface area measurements. Here, we included two-dimensional area, major axis length, top-half surface area, top-half volume, elliptical estimate surface area, and elliptical estimate volume in the initial analyses to determine which set of size parameters was the most highly correlated with porosity. We include measurements of both surface area and . . ."

RC: Size-normalized porosity I failed to understand how the size-normalized porosity is calculated. Why the values with a unit of % have negative values (e.g., as represented in Figure 5b)? Would you please explain these values and how you calculated them in the method section or the supplementary text?

AR: To control for differences in size, residuals from the porosity to surface area regression were used. These residuals are the values reported in the figures (Figure 5b; Supplemental Figures 4a-c and 6). In core top specimens the size variable is surface area, and in cultured specimens it is the two dimensional area (silhouette). The following sentence has been added to the methods section, starting at Line 111, to reflect this.

"Size-normalized porosity (i.e., the residuals from the porosity to surface area regression) was used in several analyses, where the aim was to explore the relationship between environmental variables and porosity regardless of the organism's size. To do this, residual porosity values from a regression of porosity and surface area (for core top specimens) or two-dimensional area (for cultured specimens) were used in lieu of direct porosity measurements."

RC: L43, L45: Hemleben et al., 2012 —> Isn't it "Hemleben et al., 1989"? The book was firstly published in 1989, and later released as an e-book in 2012, I suppose.

AR: This reference has been corrected to reflect the original release date of the text.

RC: L81: . . .including respiration and photosynthesis —> I did not see any discussion on porosity and photosynthesis in the text. If so, please delete "and photosynthesis". Meanwhile, I think it is good to add discussion on photosynthesis and porosity, if possible. Please see the abovementioned comment on Q10 of porosity.

AR: We agree with the suggestion that photosynthesis be discussed and have now incorporated symbiont ecology as it relates to Q10 of porosity into the discussion (in the response to Comment #2) and as a column in Table 3 (in the response to Comment #3).

RC: L95-96: Supplemental Discussion —> I could not find "Supplemental Discussion" in supplementary materials. Perhaps you mean "Supplemental Text"?

AR: This line has been corrected to read “Supplemental Text” instead of “Supplemental discussion.”

RC: L143: 32.35942N —> ◦ is missing.

AR: This line has been corrected.

RC: L166: Random Forest —> Random forest

AR: This line has been corrected.

RC: L245–247: “The groups were all statistically . . ..., but . . ...” —> The wording sounds strange. Since one-way ANOVA is a method that evaluates whether the group means are drawn from populations with the same mean values or not, your one-way ANOVA result just shows there is a significant difference somewhere. It does not tell you that “the groups were all statistically different”. Then, the post-hoc Tukey’s HSD, a test to check where the difference exists, revealed that the significant difference exists between high- and low-temperature groups. So, the sentence should be “The groups were statistically different . . ., and a pairwise Tukey’s . . ..”.

AR: This sentence has been changed as per the Reviewer’s suggestion and now reads:

“The groups were all statistically different according to a one-way ANOVA (F= 93.57, p-value <0.001). A pairwise Tukey’s HSD post-hoc test showed that only the high and low temperature groups were significantly different (p=0.03 pairwise comparison).”

RC: L248: p>0.335 —> p=0.335? L261: . . .test size (specifically surface area) —> How about just saying “surface area” since “test size” usually represents test diameter.

AR: General references to “size”, “test size”, and “body size” have been replaced with the specific names for the measurements referenced.

RC: L624: Buma, J. —> Bijma, J.

AR: This reference has been corrected.

RC: Through the text: The number of decimal places is sometimes inconsistent among the same parameters (e.g., L216: 71.81% —> 71.8%, L228: p=0.52, 0.171, 1 —> 0.52, 0.71, 1.00(?), Table 3).

AR: The measurement values reported in the text have been carefully reviewed for consistency in significant figures.

RC: Through the text: “Supplemental Figure XX” or “Supplementary Figure XX”? Please use a consistent term.

AR: All references to figures, tables and text has been changed to “Supplemental …” in the next draft.

RC: Through the text: It seems that the term “porosity” is sometimes used in an expanded sense, not for the specific variable indicating the total percent area occupied by pores. In such cases, how about using “pore characteristics” instead? Otherwise, it is quite confusing.

JEB: The text has been reviewed carefully to correct all instances where porosity is used vaguely to refer to any pore variables, and these instances will be changed to “pore characteristics” as suggested.

RC: Through the text: morphogroups or morphotype: In the text, both are used. If both represent the same categorization, please unify them to either one. In addition, the authors say “morphogroups were . . . as per Bé (1968)” in L136, but on the other hand, in the caption of Supplemental 4, they say “. . .morphotype as described in Bé (1960)”. Perhaps the latter should be Bé (1968)? Another concern relating to this is that morphogroups by Bé (1968) are based on test microstructure of species, including characteristics of perforation. Therefore, using this categorization to examine the effect of morphogroups on porosity seems to have a problem (maybe a kind of circular reasoning). Considering this point, the categorization of species should be solely based on, for example, genetic phylogeny (which is constructed independently from pore characteristics) in order to take into account for the evolutionary relationship. In fact, it will not be a big problem because the categorization of morphogroups in this manuscript (i.e., globigeinoid, globigerinid, globoquadrinid, and globorotalid) are usually consistent to the other species categorization which is independent from pore characteristics.

AR: We have edited the manuscript to consistently refer to “morphogroups” and corrected the reference to Bé (1968). The exception is Line 190-194 the Results section where we describe identifying specimens to the morphospecies level.

RC: Table 3: ΔPorosity —> Does it mean Q10 of porosity? Please use the consistent term as appears in the text.

AR: This does mean Q10 of porosity and has been changed to reflect this.

RC: Table 3: Please use consistent genus names. If you use the naming convention in Schiebel and Hemleben (2017) as you declared in the text, Trilobatus should be Globigerinoides, Truncorotaria and Globoconella should be Globorotalia. It is the same for Supplemental Figure 4a, 4b, and 4c.

AR: This table has been corrected to be consistent with the taxonomy used in the rest of the manuscript.

RC: Figure 1: Please indicate longitude and latitude at least at the four end of the represented area.

AR: I believe this comment is requesting that longitude and latitude markers be added to the far ends of the map. These have been added to the figure.

RC: Figure 4: The symbol for Globorotalia in the legend is not identical to the ones in the plot, strictly speaking. In addition, um2 should be μm2.

AR: The symbol for Globorotalia in the legend has been un-bolded and the unit has been corrected in Figure 4.

RC: Figure 5, caption: Body size and porosity of. . .. —> Does "body size" mean "ΔArea (mm2)" in Figure 5a? If so, I think the term is misleading, and needs to be corrected. In addition, more detailed explanation is needed in the caption as this is the only figure showing the results of cultured specimens except for supplementary figures.

AR: In this figure, "Body Size" is indeed two-dimensional area. The axis label now reflects this. The caption has been expanded to better describe the data as follows:

"Figure 5. Total test area and final chamber porosity of each cultured specimen of Globigerinoides ruber grouped by treatment temperature for (a) the total change in silhouette area before and after the experiment, and (b) size-normalized porosity of the final chamber. "

RC: Supplemental Figure 5: The colored bars are not easy to read especially in (b), and they are not so informative. I think it's okay without them. Alternatively, how about rearrange the panels to align each treatment group as a column (transpose columns and rows)? It will make it easy to compare different temperature treatments.

AR: This figure has been re-drafted as per the Reviewer's suggestion.

RC: Supplemental Figure 6: What does the vertical axis mean? The caption says "size normalized porosity (%)", but in the figure, the axis is "Porosity residual".

AR: The vertical axis in this figure is the residual porosity value from a linear regression of porosity and surface area. I changed the axis label to “Size-normalized Porosity” and edited the caption to read as follows:

“Distribution of size-normalized porosity (%) values in each locality, arranged by latitude from lowest to highest. Porosity values shown are the residuals from a linear regression of surface area and porosity measurements.”

Factors Influencing Test Porosity in Planktonic Foraminifera

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Abstract. The clustering of mitochondria near pores in the test walls of foraminifera suggests that these perforations play a critical role in metabolic gas exchange. As such, pore measurements could provide a novel means of tracking changes in metabolic rate in the fossil record. However, in planktonic foraminifera, variation in average pore area, density, and porosity (the total percentage of a test wall that is open pore space) have been variously attributed to environmental, biological, and taxonomic drivers, complicating such an interpretation. Here we examine the environmental, biological, and evolutionary determinants of pore characteristics in 718 individuals representing 17 morphospecies of planktonic foraminifera from 6 core tops in the North Atlantic. Using random forest models, we find that porosity is primarily correlated to test surface area, test volume, and habitat temperature, two key factors in determining metabolic rates. In order to test if this correlation arose spuriously through the association of cryptic species with distinct biomes, we cultured *Globigerinoides ruber* in three different temperature conditions, and found that porosity increased with temperature. Crucially, these results show that porosity can be plastic: changing in response to environmental drivers within the lifetime of an individual foraminifer. This demonstrates the potential of porosity as a proxy for foraminiferal metabolic rates, with significance for interpreting geochemical data and the physiology of foraminifera in non-analog environments. It also highlights the importance of phenotypic plasticity (i.e., ecophenotypy) in accounting for some aspects of morphological variation in the modern and fossil record.

1 Introduction

Geochemical data from foraminiferal calcite often differs among species living in the same habitat due to biological factors collectively known as 'vital effects' (Erez, 1983; Spero et al. 1991; Ezard et al., 2015). Vital effects are often attributed, at least in part, to differences in metabolic processes such as respiration and photosynthesis (e.g. Wolf-Gladrow et al., 1999). Importantly though, these factors have not been directly measured in the vast majority of species, leaving this idea largely untested (e.g. Ravelo & Fairbanks, 1995). A robust metabolic proxy could provide an independent constraint on the impact of vital effects on geochemical proxy signals such as $\delta^{13}C$ and $\delta^{11}B$ recorded in fossil foraminifera, thus impacting estimates of past atmospheric CO_2 concentrations (e.g. Anagnostou et al., 2016) and carbon cycling processes (e.g. Birch et al., 2016). Various aspects of foraminiferal test morphology have been observed to respond directly and measurably to metabolically-

relevant conditions in laboratory culture. For example, food quality and abundance can affect the terminal test size of an adult foraminifer and the shape of its final chambers (Bé, 1982; Hemleben et al., 1989) and varying light levels have been related to changes in the size and shape of foraminiferal chambers in species that house photosynthetic symbionts (Bé, 1982; Spero, 1988; Bijma et al., 1992; Hemleben et al., 1989).

A particularly promising morphological characteristic that could provide insights into metabolic processes is porosity. Porosity is the total percent area of the test that is occupied by pores — small perforations in the tests of all planktonic foraminifera. The exact function of pores in foraminifera is not fully understood. Photosynthetic symbionts and mitochondria have been observed clustering near pores of benthic foraminifera (Hottinger & Dreher, 1974), and dissolved substances can be absorbed through pores (Berthold, 1978). These observations suggest that pores may be involved in the physiological processes of osmoregulation and gas exchange. Porosity increases with the overall size of the test during ontogenetic development, potentially as a result of changes in depth ecology accompanying maturation, to accommodate increased movement of gas and solutes with increasing size, or to regulate buoyancy as the shell size increases (Bolli et al., 1994; Bé, 1968; Bé, et al 1973; Brummer et al., 1986; Marszalek et al., 1982; Huber et al., 1997; Schmidt et al., 2013).

Regardless of the exact function of pores, variation in porosity within and across species has frequently been attributed to environmental factors. A linear relationship exists between porosity and latitude, with higher porosities of >10% of the measured test wall associated with low latitudes and low porosities of <5% associated with high latitudes (Bé 1968; Frerichs et al., 1972). This pattern is commonly attributed to habitat temperature and has been used to track water masses during glacial-interglacial cycles in fossil and sub-fossil foraminiferal assemblages (Wiles, 1965; Bé, 1968; Frerichs et al., 1972; Bé & Duplessy, 1976; Malmgren & Healy-Williams, 1978; Colombo & Cita, 1988; Fisher et al., 2003). Other environmental factors have also been hypothesized as drivers of morphological variation in porosity, including water density, salinity, oxygenation, and nitrogen concentration (Bé, 1968; Bé et al., 1973; Hottinger & Dreher, 1975; Berthold, 1978; Leutenegger & Hansen, 1979; Bé et al, 1980; Caron, 1987a,b; Hemleben et al., 1989; Bijma, et al., 1990; Moodley and Hess, 1992; Gupta & Machain-Castillo, 1993; Fisher, et al., 2003; Glock, 2011; Kuroyanagi, et al, 2013; Kuhnt, et al., 2014).

Pore variation across species and populations is also associated with evolutionary history. Average pore area is the basis for a fundamental taxonomic division that distinguishes two major groups of planktonic foraminifera: the macroperforate (pores larger than 1µm in diameter) and microperforate (pores of 1µm or less) planktonic foraminifera (Bé et al., 1980; Kennett & Srinivasan, 1983; Qianyu & Radford, 1991). Within macroperforate planktonic foraminifera, there is a wide range of pore sizes and distribution patterns, some of which are characteristic of particular lineages. Globorotalid foraminifera, such as *Globorotalia tumida* and *Globorotalia menardii*, can be distinguished from globigerinoid foraminifera like *Globigerinoides ruber* based on the shape, size and distribution of their pores (Bé et al., 1980). Porosity has also been used to distinguish between pseudo-cryptic species in modern foraminifera (Huber et al, 1997; Morard et al., 2009; Marshall et al., 2015; Weiner et al., 2015; Schiebel & Hemleben, 2017).

In summary, previous studies generally identify three different categories of factors influencing porosity: biological, environmental, and phylogenetic. However, these factors are not independent of one another, and no previous study has attempted to detangle these various potential influences on porosity. Here we use core top samples from across the Atlantic Ocean to explore how porosity varies within and between populations, species, communities, size classes, and environments in order to identify the major determinants of porosity in modern macroperforate planktonic foraminifera. As an independent test

of the findings based on core tops, we also present cultured *Globigerinoides ruber* specimens grown in different temperature conditions. These analyses are used together to consider the relationship between planktonic foraminiferal porosity and metabolic processes including respiration and photosynthesis.

2 Methods

2.1 Core Top Sample Selection and Processing

Planktonic foraminifera from six Atlantic core-top localities spanning the major planktonic foraminifera biomes were sampled from six sieve size fractions ranging from 150µm - 850µm (Figure 1, Table 1; biomes from Darling and Wade, 2008). At four sites (KC78, CH82-21, VM20-248, and EW93-03-04; Figure 1), a random split of 50-100 individuals from each size fraction was picked. At two additional sites, AII-60-10 and AII-42-15-14, target species were specifically picked to increase the taxonomic and environmental range of our analyses (Table 1; Figure 1). Species were identified on the basis of the naming conventions in Schiebel & Hemleben (2017). Specimens were mounted on microfossil slides and imaged at multiple focal heights (z-stacks) from the spiral and umbilical side at a 10x magnification using a 5-megapixel Leica DFC450 digital camera mounted on a Leica Microsystems DM6000M compound transmitted-light microscope with an automated x-y stepping stage and drive focus. Umbilical views were used in the analysis of test size (see Supplemental Figures 1 and 2 and Supplemental Text). Using *AutoMorph* (Hsiang et al., 2016; Hsiang et al, 2017), two-and three-dimensional shape and size information was extracted from the z-stacked photographs of each individual, including surface area and volume (Supplemental Figure 1). Two-dimensional measurements included cross-sectional area, major axis length, minor axis length, and perimeter length (Supplemental Figure 1). Three-dimensional measurements included multiple estimates of volume and surface area using the top (i.e., visible) half and a combination of visible top-halves with hypothetical backsides (see Hsiang et al., 2016; Supplemental Figure 1).

Size is an important factor in studies of planktonic foraminiferal ecology and biology, but it can refer to many different test parameters, like major axis length, aspect ratio, sieve size class, or three dimensional volume and surface area measurements. Here, we included two-dimensional area, major axis length, top-half surface area, top-half volume, elliptical estimate surface area, and elliptical estimate volume in the initial analyses to determine which set of size parameters was the most highly correlated with porosity. We include measures of both surface area and volume in our analysis due to their interactive effect on potential gas exchange. Planktonic foraminifera with a flattened test shape (such as *Globorotalia menardii*) have a high surface area to volume ratio, essentially maximizing the diffusive surface for their overall size. Conversely, spherical morphologies, like the adult form of *Orbulina universa*, have the lowest possible surface-area to volume ratio for a given diameter, minimizing the diffusive surface for their overall size. We focused on top-half estimates for this study because they are directly measured and correlated with other estimates of surface area and volume (see Supplemental Figure 2). We were also interested in elliptical estimates, as it has been suggested that, in vivo, spines and/or pseudopods would extend radially, making elliptical estimates more representative of where respiration and photosynthesis take place (Zeebe et al., 1999). Elliptical estimates of surface area and volume were calculated using height, length and width measurements assuming an elliptical solid. Because the two measurements (top-half and elliptical) potentially represent different diffusive states that may be experienced by the living

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organism, both were considered for the final analysis. Additionally, size-normalized porosity was used in several analyses where the aim was to explore the relationship between environmental variables and porosity regardless of the organism's size. To do this, residual porosity values from a regression of porosity and surface area (for core top specimens) or two-dimensional area (for cultured specimens) were used in lieu of direct porosity measurements.

After whole-specimen imaging, tests were dissected to remove the final and penultimate chamber and expose its inner wall for porosity measurements (Figure 2). We quantified porosity from the inner wall of the penultimate chamber in order to avoid known irregularities in the porosity of the final chambers (Bé et al, 1980; Constandache et al., 2017). In *Orbulina universa*, the only exception, we measured the final chamber, as preceding chambers are typically dissolved in sedimentary remains of this species. Chamber fragments were then mounted on scanning electron microscope pins, coated in gold or platinum and carbon, and imaged in a scanning electron microscope (SEM) at a magnification of 300-600x to obtain the widest views of the inner chamber wall that were undistorted by the curvature of the chamber (Figure 2). SEM images were processed in ImageJ (Schneider et al., 2012) to select an undistorted section of the chamber wall. The cropped image was converted to black and white and analyzed for the percent area occupied by pores (i.e., relative proportion of black pixels), average pore area, and total pore number. The total cropped area was used to convert pore number into a pore density estimate (i.e., number of pores/area). Images were cleaned if necessary to prevent debris from obscuring the pore measurements (Figure 2). Light photographic, SEM, and processed ImageJ images are provided through the Yale Peabody Museum collections portal (http://collections.peabody.yale.edu/search/), using the Yale Peabody catalog numbers provided in Supplemental Table 1. Supplemental Tables 2-4 include all measurements collected for this study.

2.2 Explanatory Variables

We tested two-dimensional area, major axis length, top-half surface area, top-half volume, elliptical estimate surface area, elliptical estimate volume, sea surface temperature (SST), latitude, ambient temperature and oxygen concentration at habitat depth, and morphogroup for their effect on porosity. Depth habitats were determined based on estimates from Schiebel & Hemleben (2017) and are given in Supplemental Table 7. Annual average sea surface temperature (SST, using temperature data from World Ocean Atlas for 10 meters depth), ambient temperature and oxygenation at depth habitat of each species were obtained from World Ocean Atlas 2013 database (Locarnini et al., 2013 for temperature; Garcia et al., 2013 for oxygen) for each site and species (Supplemental Table 7). Morphogroups were globigerinid, globigerinoid, globorotalid and globoquadrinid as per Bé (1968)(Table 1).

2.3 Cultured Samples

Specimens of *Globigerinoides ruber* were cultured under controlled temperature conditions at the Bermuda Institute of Ocean Sciences in St. Georges, Bermuda in September 2016 in order to quantify the response of individual foraminiferal porosity to temperature. Specimens were live-caught 15-20 km off the coast of St. Georges, Bermuda (between 32.35012°N and 32.35942°N, -64.59673°W and -64.68807°W) from the top 15 meters of the water column using a 150µm mesh Reeve net. All specimens were in the adult life stage at the time of the experiments. Specimens were picked from the towed material and placed in recovery baths at 25°C until they showed signs of good health (spines, streaming cytoplasm, presence of symbionts,

successful feeding) at which time they were moved to isolated culture jars and placed in a water bath held at a treatment temperature of 23°C, 25°C or 28°C. Both temperature and pH of the treatment water was monitored and kept stable throughout the experiments. Culture vial oxygen concentrations were checked for all temperature treatments with an oxygen optode attached to a Pyroscience FireSting optical oxygen meter to assure that concentrations did not fall below half saturation. Specimens were fed single *Artemia* spp. nauplii and measured every other day to document growth. Specimens were kept in culture until they underwent gametogenesis or died (identified by the loss of cytoplasm within the test).

Specimens that accumulated 1 or more chambers in culture were imaged at a voxel size of 0.5-0.85µm using a Zeiss Xradia microXCT 400 at the University of Texas at Austin and a Zeiss Xradia 520 Versa micro-CT at Naturalis Biodiversity Center in Leiden, The Netherlands. Scanned specimens were reconstructed and extracted for inner wall porosity in VG StudioMax 3.0 using clipping planes and the ImageJ procedure explained above (Figure 2). In order to capture pre- and post-culture pore measurements for comparison pores were measured on the final 3-4 chambers.

2.4 Statistical methods

Random forest models were used to build predictive models and identify the major determinants for each pore characteristic (porosity, pore density, and pore area) using the *rpart*, *randomForest*, and *party* packages in R. Random forest models are supervised learning procedures that work by identifying the variables with the most explanatory power from a suite of theoretical decision trees (500 in this case) constructed from random samples of the data and predictor variables (Evans et al., 2011). The strength of each predictor variable is assessed by the reduction in model fit when that variable is excluded. In other words, the higher the percentage of incremental mean standard error associated with the removal of a variable, the higher that variable is ranked in terms of importance. They are robust to colinearity, nonlinearity, and deviations from normality in the data. Random forest models are useful for data sets with some missing data, and are applicable in situations without a strong a priori hypothesis (Cutler et al., 2007; Davidson et al., 2009; Boyer, 2010). Even so, the variable importance rankings output by the standard random forest algorithm can be misleading if several explanatory variables covary and if the variables are of different types. In this study, the environmental variables are strongly covariant and the model contains more than one variable type (all continuous except for morphogroup, which is categorical). To account for this and aid in interpretation of the rankings, an unbiased, conditional variable importance ranking method was incorporated via the *party* package in R, which disentangles the most important variable from the model (Strobl et al, 2008). This method examines whether a correlation between the response variable and a predictor is conditional on another variable proceeding it in the tree, thereby identifying the most influential variable and demoting others (Strobl et al, 2008).

2.5 Testing for Phylogenetic Signal

Porosity, pore density, and average pore area were examined for a phylogenetic signal by estimating Pagel's lambda using average porosity for each species and the Cenozoic planktonic foraminiferal phylogeny of Aze et al. (2011). Pagel's lambda is a test designed to identify statistically significant grouping of trait values in phylogenetic clades as compared to the random distribution expected in the absence of a phylogenetic signal (Pagel, 1999). Pore measurement values were normalized using model residuals from random forests run without morphogroup. A matrix of the average residual pore values for each species

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was created and analyzed using the "phylosig" function in the *phytools* package in R. The tree was trimmed of all branches lacking pore data.

3 Results

1,278 foraminifera were picked, imaged and identified to the morphospecies level for this study (Supplemental Figure 1). 718 specimens representing 17 morphospecies were successfully extracted for both two- and three-dimensional size metrics (i.e., surface area and volume), and are the focus of the statistical analyses presented here. Of the 17 morphospecies, 7 species occur in 3 or more localities and 10 occur in 2 or more localities, allowing us to examine variation within morphospecies across environments.

3.1 Factors influencing porosity in core top samples

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In the original exploratory analyses (Supplemental Figure 3), six different, highly correlated measurements of test size were examined. Using all of them in the random forest models would be redundant, so we ran iterations of the models with three different sets of size variables—two-dimensional area and major axis length, top-half surface area and volume, and elliptical surface area and volume—and chose the set which produced the model which explained the most variance in the porosity data. We found that measurements of elliptical or top half surface area paired with volume always produced better-fitting models than the two-dimensional measurements. These metrics better account for the surface area and volume disparities between different morphologies that is lost in two-dimensional measurements. The elliptical and top-half measurement sets performed comparably, but the top-half set produced a slightly stronger model for the porosity data set, so we used those measurements in all three models for consistency. Random forest models were then built with the following seven variables: Top-half surface area, top-half volume, sea surface temperature, morphogroup, ambient oxygen concentration, ambient temperature, and latitude.

The random forest model for the porosity data set explained 75.50% of the data. The most important variable was top-half surface area, which caused a 34.10% increase in error when omitted from the model, followed by top-half volume and sea surface temperature (26.60% and 23.80% increase in error, respectively; Figure 3; Table 2). The conditional variable analysis also identified surface area as the most important variable. The random forest model for pore area explained 81.50% of the variance in the data and was the strongest model built for the three different measures of pores (i.e., porosity, pore area, and pore density). For pore area, ambient temperature was the strongest predictor (15.90% increase in error when absent) followed by sea-surface temperature and surface area (14.50% and 12.80% increase in error when omitted from the model; Figure 3). In contrast to the random forest model, the conditional variable analysis identified sea surface temperature as the most important variable in explaining pore area, followed by latitude and ambient temperature (Table 2). The random forest for pore density explained 71.81% of the variance in the data. Here, morphogroup was the most important factor (resulting in a 15.30% increase in model error if omitted), followed by ambient temperature (11.30%; Figure 3). However, the conditional variable analysis (which is not biased toward factors as random forests are) identified sea surface temperature and ambient temperature as the most important variables.

Pore variables were compared against each other to consider their covariance. Within the pore characteristics, more of the variation in porosity is explained by variation in pore area (r^2=0.64) than by pore density (r^2=0.17, Figure 4). Pairwise relationships among porosity, pore area, and pore density were often non-linear and clustered by morphogroup (Figure 4). Although globigerinoid foraminifera have a similar range of overall porosities to other morphogroups, they have the widest range in pore areas, and a narrow range of consistently low pore densities. These patterns in pore density and area, and other characteristics not measured in this study like pore shape and rim type, are what makes the pore structures of these morphogroups distinguishable (Bé, 1980).

Model residuals for all three pore characteristics were analyzed for phylogenetic signal using Pagel's Lambda. The lambda value was 0.25 for porosity (p-value=0.52), 1.09 for pore density (p-value=0.17), and >0.01 for pore area (p-value=1). This means that there was no significant phylogenetic signal detected for any of the three pore characteristics at 95% confidence level. Even so, the lambda value of 1.09 for pore density indicates the presence of a phylogenetic signal at an 80% confidence level for pore density.

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3.2 Temperature effect on cultured *Globigerinoides ruber*

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The temperature experiments resulted in statistically significant differences in terminal porosity (Figure 5, Supplemental Figure 5) and test size. Average terminal porosity in low, medium and high temperature were 4.37% (1 standard deviation [s.d.] = 0.88%), 8.21% (1 s. d. = 1.33%), and 11.49% (1 s.d. = 0.91%). The groups were all statistically different according to a one-way ANOVA (F=57.10, p-value <0.01) and a pairwise Tukey's HSD post-hoc test (p<0.001 in all pairwise comparisons). Measurements of pre- and post-culture porosity from CT scans show a trend toward the treatment-average porosity as chambers are accumulated (Supplemental Figure 5). In the high temperature treatment, pre-culture chambers all have porosities below 6%, but final cultured chamber porosities of above 10% by the end of the experiment. The specimens in the high temperature treatment also grew more chambers during their time in culture, with the high-temperature group accumulating an average of 0.45 chambers per day versus 0.38 and 0.24 for the low and medium temperature groups respectively. Average terminal test size in low, medium and high temperature were 55334 μm^2 (1 s. d. = 17500.6 μm^2), 88430 μm^2 (1 s.d. = 32268.3 μm^2), and 103394 μm^2 (1 s.d. = 36340.2 μm^2) respectively. The groups were all statistically different according to a one-way ANOVA (F= 93.57, p-value <0.001), and a pairwise Tukey's HSD post-hoc test showed that only the high and low temperature groups were significantly different (p=0.03 pairwise comparison). Pre-culture measurements of test area and porosity were not significantly different between treatments (Figure 5: F=1.18 and p>0.33 for test size, F=3.70 and p=0.06 for porosity), the high-temperature treatment foraminifera accumulated more chambers and achieved larger terminal test sizes than the low temperature group, and the size-normalized porosity was still significantly higher in the high-temperature group (Figure 5).

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4 Discussion

Previous work on the pore characteristics of planktonic foraminifera identified a number of environmental and biological correlates which often co-vary in time and space (Bé, 1968; Bé et al., 1976; Hottinger & Dreher, 1974; Berthold, 1978; Leutenegger & Hansen, 1979; Bé et al, 1980; Caron, 1987a,b; Hemleben et al., 1989; Bijma, et al., 1990; Moodley & Hess,

1992; Gupta & Machain-Castillo, 1992; Fisher, et al., 2003; Glock et al., 2011; Kuroyanagi et al., 2013). Our study builds on existing work by simultaneously investigating the three major types of drivers that may account for pore variation: biology, environment, and evolutionary history. Two key conclusions emerge from the models and experiments: that the main predictors on the porosity of planktonic foraminifera are test surface area, test volume and temperature (Figure 3), and that both porosity and test size can be affected by changes in temperature during the life of an individual (Figure 5).

Both size and temperature are known to have important effects on metabolism (Schmidt-Nielsen, 1984; Hochachka & Somero, 2002). Although there is variability among and within species, on average metabolic rate scales with body mass to the power of 3/4 in multicellular organisms (Kleiber 1961; Schmidt-Nielson, 1984; Brown et al., 2004), and 2/3 to 1 in protozoa (Caron et al., 1990; Agutter & Wheatley, 2004; Glazier, 2009). Overall size in planktonic foraminifera, similar to porosity, is smaller at high latitudes (Hecht, 1976; Schmidt et al., 2013). Size variation, including changes in size throughout ontogeny, has been linked to variation in stable isotope values and the incorporation of trace metals into test calcite, possibly relating to variation in metabolic rate (e.g. Schmidt et al., 2008). Similarly, temperature has a powerful effect on metabolism that can be characterized by the respiratory Q_{10} relationship—the factor by which an organism's respiration rate increases with a ten-degree increase in temperature. Estimates for the respiratory Q_{10} of symbiont-bearing planktonic foraminifera (specifically *Globigerinoides ruber, Globigerinella siphonifera* and *Orbulina universa*) are approximately 3.18 (Lombard et al., 2009).

For single celled organisms like planktonic foraminifera, the metabolisms of large individuals are diffusion limited compared to small individuals, as volume increases to the third power, but surface area to the second. This is supported by our findings, which suggest that surface area was by far the most important factor in the porosity model (Fig. 3). If porosity is reflecting metabolic rates, both should respond to temperature to a similar degree. To compare the temperature sensitivity of porosity with the respiratory and photosynthetic Q_{10} values (from Lombard et al., 2009), we calculated the change in size-normalized porosity with a ten-degree change in estimated ambient temperature (dubbed the Q_{10} of porosity; Table 3; Supplemental Figure 6). We found an increase in porosity with ambient temperature for six of the eight species found at more than one site (i.e., all species in Table 3 except *Globorotalia inflata* and *Globorotalia truncatulinoides*; Supplemental Figure 6). For those species, the Q_{10} of porosity varied from 1.3 to 2.3.

These porosity Q_{10} values are lower than the respiratory Q_{10} of 3.18 and the photosynthetic Q_{10} of 2.69 reported in Lombard et al. (2009). One reason for this might be that those measurements were taken from specimens exposed to sudden changes in temperature, which, as the authors noted, may result in higher sensitivity than that present in wild populations. Also, variation in the ratio of photosynthesis to respiration could play a role in the variation we see in the Q_{10} of porosity. While Lombard et al. (2009) found that, after normalizing for cell size, the respiratory and photosynthetic Q_{10} of their specimens was consistent among the three species examined (*Globigerinella siphonifera*, *Globigerinoides ruber*, and *Orbulina universa*). What did differ between the species was the net photosynthesis to respiration ratio (P:R). Specifically, this ratio was much lower in the chrysophyte-bearing *Globigerinella siphonifera* than the dinoflagellate bearers *Orbulina universa* and *Globigerinoides ruber*.

On Table 3, species are sorted by Q_{10} of porosity from highest to lowest, with the symbiont ecologies of each group noted. Here, we can see that the species with the highest Q_{10} is a surface dweller with dinoflagellate symbionts (*Globigerinoides conglobatus*). The species with the lowest Q_{10} (*Globorotalia truncatulinoides*) is asymbiotic with porosity that actually decreases with temperature. Additionally, the other species with a Q_{10} of less than one is *Globorotalia inflata*, which has

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chrysophyte symbionts. These deviations from expectation might be due to the fact that the ambient temperatures are approximated from yearly averages of temperature at estimated depth habitats. While we cannot conclude the extent of the relationship with our available data, the general trend of variation in Q_{10} of porosity roughly coinciding with symbiont ecology indicates that there may be some influence of photosynthesis or photosynthesis to respiration ratio on porosity.

Warmer water temperatures could lead to higher porosity for two reasons: warmer temperatures drive up metabolic rate and/or oxygen solubility and concentrations are lower in warmer water, necessitating higher rates of diffusion into the cell. For this reason, it was important to disentangle the effects of oxygen and temperature on porosity, and random forest models are specifically suited to dealing with such collinear variables. In all cases, oxygen was deemed less important than temperature and nearly all other variables considered. Our observations demonstrate that temperature is the underlying factor that drives the latitudinal trend in porosity observed by Bé (1968) at the species-level. Indeed, our results show a similar trend at the assemblage level and the morphospecies level: a decrease in average porosity with increasing latitude once normalized for size (i.e. top-half surface area, see Figure 6; Supplemental Figure 6). However, planktonic foraminifera species are known to inhabit characteristic biomes, and an alternative explanation for the apparent relationship between temperature and porosity could be that the change in porosity is driven by the turnover in species rather than temperature –in other words, by their shared evolutionary history. Three results argue against this alternative hypothesis. First, a phylogenetic signal was not found for porosity using Pagel's lambda. Second, morphogroup (a coarse, categorical approximation for evolutionary relationship) explained relatively little of the variance in porosity in our random forest models and conditional variable analysis. Third, a two-way ANOVA to test for independent and interactive effects of species identity and temperature on the porosities of foraminifera, showed a much stronger effect of temperature ($F=594.42$, $p<0.001$), than the effect of species ($F=7.28$, $p<0.001$). There was a significant interaction effect between the two factors, indicating that the two are not independent ($F=7.3$, $p<0.001$), and that species with higher porosities do occur at lower latitudes, and vice versa.

A second alternative explanation for the relationship between porosity and temperature is the presence of different cryptic species across localities. Differences in porosity have been observed among genetic species within two morphospecies complexes: *Orbulina universa* and *Globigerinella siphonifera* (Huber et al., 1997; de Vargas et al., 1999; Morard et al., 2009; Morard et al., 2013; Marshall et al., 2015; Weiner et al., 2015). In fact, it is the sole characteristic by which two cryptic species of *Globigerinella siphonifera* can be identified in empty tests (Huber et al., 1997). In *Orbulina universa*, variation in areal aperture density and placement distinguish among the three cryptic species, along with variation in wall-thickness in *Orbulina universa* (Morard et al., 2009; Marshall et al., 2015). We examined this, by culturing individuals of *Globigerinoides ruber* to test whether, and to what extent, porosity could vary based on environmental conditions at the time of chamber formation. We observed that individuals grown in the high temperature treatment became more porous, larger, and accumulated more chambers in culture as compared to those individuals grown in the low temperature treatment (similar to the findings of Bijma et al., 1990) (Figure 5, Supplemental Figure 6). The average porosity of the high temperature group is approximately three times higher than that of the lower temperature group. Our culturing results indicate that porosity is highly plastic and varies rapidly in response to temperature changes in *Globigerinoides ruber*. Similarly, *Orbulina universa* cultured under different oxygen concentrations showed variation in areal aperture size as large as that observed across genetic species (Kuroyanagi et al., 2013). Another environmental factor that may influence terminal sizes and metabolic function is the availability of food sources. Feeding frequency has been shown to influence terminal size and morphology (Bé, 1982; Hemleben et al., 1989), and

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may thus be expected to influence porosity as well. This factor is difficult to estimate for core top assemblages, but can be tested with simple culture experiments and subsequent imaging.

Both culturing experiments point to the importance of environment in shaping the porosity of individuals, or ecophenotypy. Ecophenotypy in planktonic foraminifera has largely fallen out of favor as an explanation for variation in morphology, with the observations that ecophenotypes often align with different genetic complexes (Huber et al., 1997; de Vargas et al., 1999; de Vargas et al., 2001; Morard et al., 2009; Quillévéré et al., 2011; Morard et al., 2013; Marshall et al., 2015; Weiner et al., 2015). However, it is well-established that the expression of any phenotypic trait is a product of both its genes and its environment (e.g. Visscher et al., 2008), with the heritability of a trait measuring the relative influence of genetics. In planktonic foraminifera, heritability has yet to be measured for any morphological trait, although it is likely to vary amongst traits as it does in all other organisms studied to date (Visscher et al., 2008). In this context, it is interesting to note that genetic-species of planktonic foraminifera are often found in distinct environments (i.e., different biomes or different depth habitats) (Huber et al., 1997; de Vargas et al., 2001; Darling & Wade, 2008; Morard et al., 2009; Quillévéré et al., 2011; Morard et al., 2013; Morard et al., 2016). While evidence for high heritability of wall thickness and porosity is lacking, both porosity and wall thickness have been observed to vary with environmental conditions in culture and across environments gradients (this study; Colombo & Cita, 1980; Caron, 1987a-b; Bijma et al., 1990; Lea et al., 1999; Spero et al., 1997; Bijma et al., 1999; Russell et al., 2004; Lombard et al., 2009; Kuroyanagi et al., 2013; Spero et al., 2015; Henehan et al., 2017). This raises the interesting possibility that some of the morphological differences between different genetic species are driven primarily by differences in the environment in which they occur, rather than by heritable genetic differences. While explanations of ecophenotypy have been dismissed in the past (Huber et al., 1997; Morard et al., 2009), our results suggest it should be seriously considered, at least for some traits like porosity, going forward.

Our results do show an evolutionary signal in some pore characteristics, but it is not the dominant factor in determining porosity. We find evidence for the importance of evolutionary history in determining pore density— one of the two factors that together determine porosity (the other being pore area). Random forest models found morphogroup to be the most important explanatory variable of pore density, although the conditional variance analysis attributed much of this explanatory power to a dependence on temperature (SST and ambient temperature). A Pagel's lambda of 1.09 for pore density on the model residuals likewise indicates a phylogenetic signal in the pore density data. Although this analysis was insignificant with alpha=0.1, we consider this finding important given the small sample size. For all three pore characteristics examined, pore density, pore area, and the resultant porosity, morphogroup does explain 12%-20% of the observed variation, so it is unsurprising that pore area has been such a useful trait for taxonomy. Similarly, the pairwise comparison of all three pore characteristics (Fig. 4) emphasizes the non-linear relationship between pore density and pore area, and the role of morphogroup in driving the bifurcating relationship between the two factors underlying porosity. However, when combined, the resulting porosity of an individual is more related to test surface area, test volume, and temperature, than it is to evolutionary history.

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5 Conclusion

Test porosity in planktonic foraminifera from core top samples is primarily explained by test size and temperature. These two factors are key determinants of respiration rate, and therefore suggest that porosity could be closely linked to metabolic rate –

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likely through a role of porosity in allowing gas-exchange across the test wall. Experimental manipulations of *G. ruber* in cultures show that both test size and chamber porosity are sensitive to temperature, and that porosity is a plastic trait that responds to conditions experienced at the time of chamber formation. These results suggest that porosity has the potential to be a metabolic proxy that could aid in the interpretation of geochemical data and paleoecological reconstructions.

Data Availability

The data used in this study are available in the Supplemental Tables associated with this article.

Competing Interests

The authors declare that they have no conflict of interest.

Author Contributions

JEB and PMH designed the study and drafted the manuscript. JEB and WR conducted the CT scanning of cultured specimens. MJH, LEE, JEB, CVD, AEM, PMH, and GLF cultured foraminifera used in this study, and JEB, PMH, and RS identified the species. JEB extracted and analyzed the data. All coauthors contributed to writing the manuscript.

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Table 1. Locality and sieve size fraction for all core-top species sampled. Marker sizes correspond with sieve size fractions (•=250-300µm, o=300-425µm, O=425-600µm, O=600-710µm, O=710-850µm).

Species	Morphogroup	AII-42-15-14	AII-60-10	CH82	EW9303	KC78	VM20
Globigerina bulloides	Globigerina			o	•o		
Globigerina falconensis	Globigerina				•		
Globigerinella siphonifera	Globigerina	o				•o	•o
Globigerinoides conglobatus	Globigerinoid		O	o		oOO	•o
Globigerinoides ruber	Globigerinoid	o	•	o		•oO	•o
Globorotalia inflata	Globorotalid		•	oO			•o
Globorotalia crassaformis	Globorotalid			oO			
Globorotalia tumida	Globorotalid	oO				oO	
Globorotalia hirsuta	Globorotalid			oO			•o
Globorotalia menardii	Globorotalid					oOOO	
Neogloboquadrina dutertrei	Globoquadrinid		o			•o	
Neogloboquadrina incompta	Globoquadrinid				•		
Orbulina universa	Globigerinoid	o	o			oOOO	
Pulleniatina obliquiloculata	Globoquadrinid					oO	
Sphaeroidinella dehiscens	Globigerinoid					oO	
Globigerinoides sacculifer	Globigerinoid	o	oo			•oOO	•o
Globorotalia truncatulinoides	Globorotalid	oO		oO			•o

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Table 2. Variable importance rankings from random forest models and conditional variable importance analysis. RF variable importance values are based on the percent increase in error when the variable is removed from the model. Conditional variable importance values reflect a re-assessment of relative variable importance rankings without bias toward factorial or highly correlated variables.

Variable	RF Variable Importance			Conditional Variable Importance		
	Porosity	Pore Density	Pore Area	Porosity	Pore Density	Pore Area
Surface Area	34.98	11.54	10.39	0.53	0.03	0.02
Volume	21.4	1.95	8.29	0.14	0.01	0.02
Sea Surface Temperature	19.39	8.81	13.91	0.13	0.39	0.50
Oxygen	17.15	13.23	8.96	0.03	0.07	0.01
Morphotype	15.86	16.3	11.39	0.04	0.12	0.09
Latitude	15.68	9.54	11.31	0.07	0.16	0.20
Ambient Temperature	15.14	12.74	17.2	0.05	0.21	0.16

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Table 3. Magnitude of porosity increase with a ten-degree temperature increase, as inferred from regressions of average size-normalized porosity and sea-surface temperature for core-top species that occurred at more than 2 localities. The size-normalized porosity average at ten degrees and twenty degrees is listed, along with the factor by which porosity increases over this interval (Q_{10}) are shown. See Supplemental Figure 6 for plots.

Species	Porosity at 10°C	Porosity at 20°C	Q_{10} Porosity	Symbiont Type
Globigerinoides conglobatus	-0.18	-0.07	2.67	Dinoflagellate[1]
Neogloboquadrina dutertrei	-0.10	-0.04	2.56	Pelagophtyes[2]
Orbulina universa	-0.20	-0.08	2.32	Dinoflagellate[1]
Globigerinoides sacculifer	-0.17	-0.09	1.98	Dinoflagellate[1]
Globigerinella siphonifera	-0.16	-0.10	1.68	Chrysophytes[1]
Globigerinoides ruber	-0.11	-0.08	1.32	Dinoflagellate[1]
Globorotalia inflata	-0.06	-0.09	0.70	Chrysophytes[1]
Globorotalia truncatulinoides	-0.05	-0.09	0.57	Asymbiotic[1]

[1] Ezard, T. H., et al. (2015).
[2] Bird et al., (2018).

Figure Captions

Figure 1. Map of core-top sample localities (modified from Darling & Wade, 2008): a) EW9303-04: 64.71°N, -28.91°E, Sub-Polar; b) CH82-21: 43.288°N, -29.83°E, Transitional; c) VM20-248: 33.5°N, -64.4°E, Sub-Tropical/Tropical; d) AII-42-15-14: 19.567°N, -44.95°E, Tropical; e) KC78: 5.267°N, -44.133°E, Tropical; f) AII-60-10: -29.6°N, -34.667°E, Sub-Tropical.

Figure 2. Workflow diagrams for porosity and CT scan analyses. Pore characteristics for this study were measured on the internal test wall from SEM images of dissected foraminifera (pathway illustrated on the left side) or from CT scans (as shown on the right side). The method for extracting volume and surface area measurements is also shown on the far right.

Figure 3. Variable importance plots for the random forest models for each pore characteristic. Importance rankings are based on the increase in error produced when the variable in removed (% incremental mean squared error). Marker size refers to the ranking in the conditional variable importance analyses, with the largest markers denoting the most important variables.

Figure 4. Scatter plots of pore variables (with results of pairwise linear regressions) to visualize the relationship between pore variables.

Figure 5. Total test area and final chamber porosity of each cultured specimen of *Globigerinoides ruber* grouped by treatment temperature for (a) the total change in area before and after the experiment, and (b) size-normalized porosity of the final chamber.

Figure 6. Distribution of size-normalized porosity (%) values in each locality, arranged by latitude from lowest to highest. Grey boxes are samples from which a random split was taken, white boxes were picked for specific species.

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Figure 1.

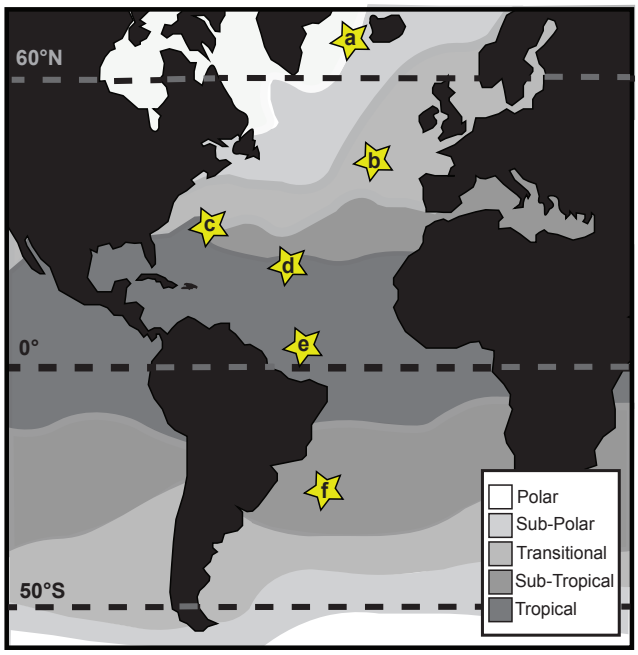

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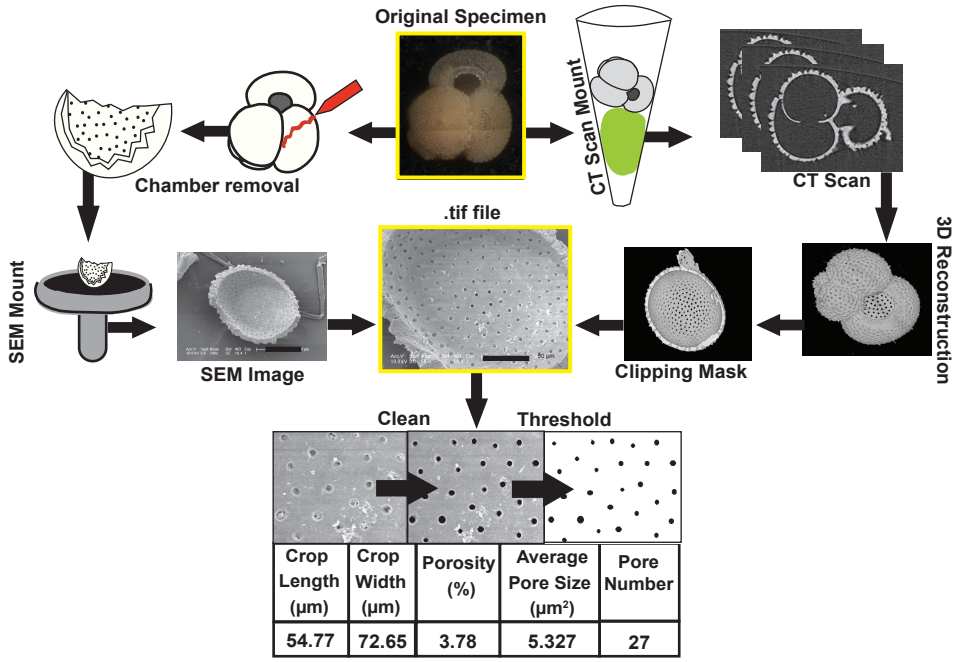
Figure 2.



Crop Length (μm)	Crop Width (μm)	Porosity (%)	Average Pore Size (μm²)	Pore Number
54.77	72.65	3.78	5.327	27

600

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Figure 3.

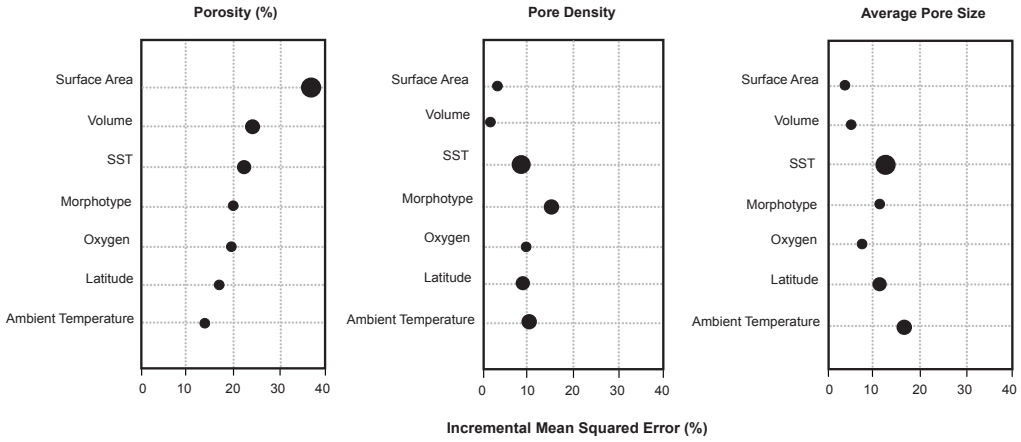
Porosity (%)
Pore Density
Average Pore Size
Surface Area
Volume
SST
Morphotype
Oxygen
Latitude
Ambient Temperature
0
10
20
30
40
Incremental Mean Squared Error (%)

Figure 4.

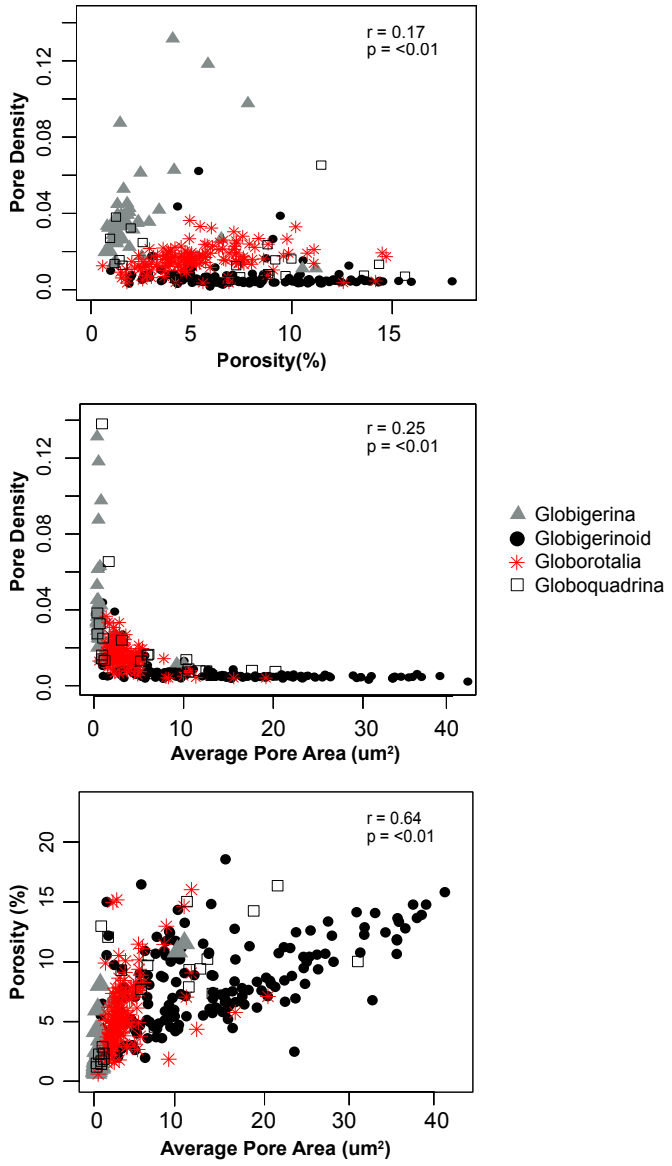
r = 0.17
p = <0.01
Pore Density
0.0
0.04
0.08
0.12
0
5
10
15
Porosity(%)
r = 0.25
p = <0.01
Pore Density
0.0
0.04
0.08
0.12
0
10
20
30
40
Average Pore Area (um²)
r = 0.64
p = <0.01
Porosity (%)
0
5
10
15
20
0
10
20
30
40
Average Pore Area (um²)
Globigerina
Globigerinoid
Globorotalia
Globoquadrina

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Figure 5.

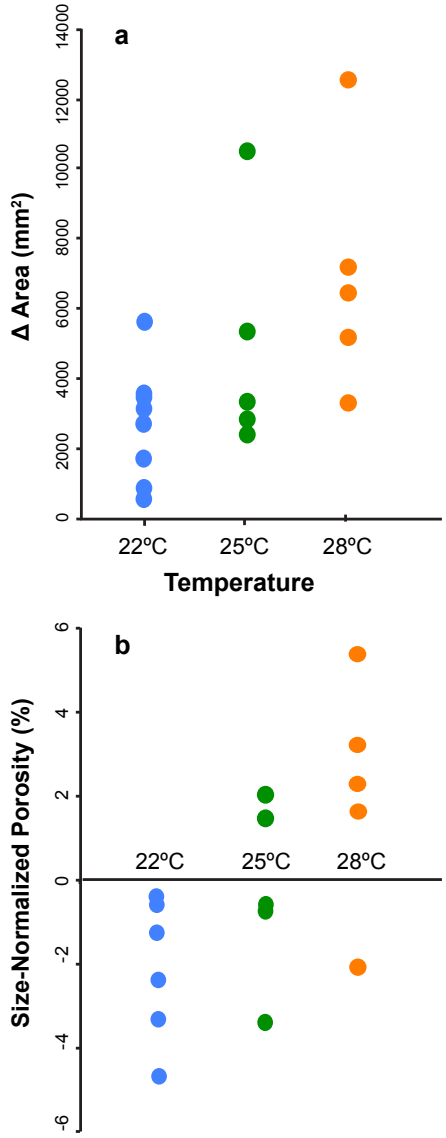
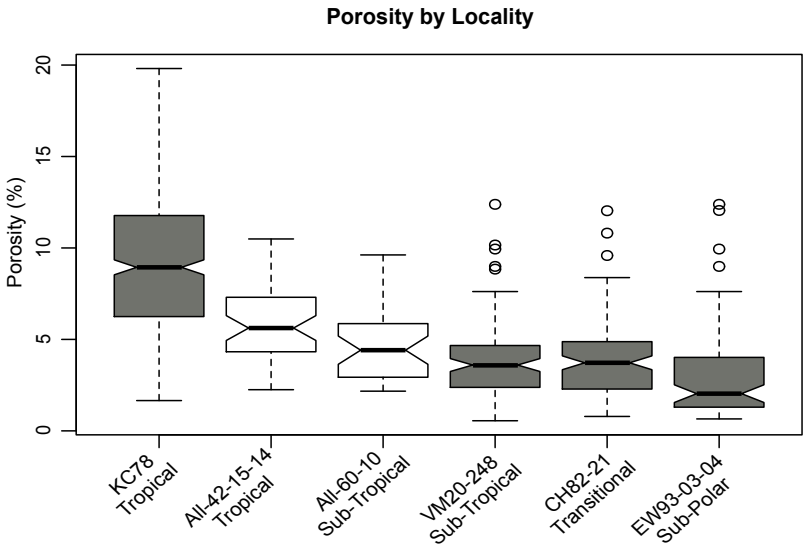

a
Δ Area (mm²)
0
2000
4000
6000
8000
10000
12000
14000
22ºC
25ºC
28ºC
Temperature
b
Size-Normalized Porosity (%)
6
4
2
0
-2
-4
-6
22ºC
25ºC
28ºC

Figure 6.


Porosity by Locality
Porosity (%)
0
5
10
15
20
KC78
Tropical
All-42-15-14
Tropical
All-60-10
Sub-Tropical
VM20-248
Sub-Tropical
CH82-21
Transitional
EW93-03-04
Sub-Polar