

**Response to reviewers for “What controls planktic foraminiferal calcification?” by Barrett et al., submitted to Biogeosciences.**

We thank the reviewers for their insightful and constructive comments on our manuscript. Please see our response to these comments in the following, which we hope you agree, strengthens our paper. Reviewer comments are shown in bold, our responses below in normal text, and actioned responses in red text.

**Reviewer #1: Brian Huber**

The paper is very well written and it provides excellent observations that demonstrate that planktonic foraminiferal size normalized weight is highly variable among species, in different regions, and in response to different environmental variables. Results clearly demonstrate that size normalized should not be used as a pCO<sub>2</sub> proxy. The paper is very well written, well organized and well-illustrated, and it merits publication and wide distribution among the research community. My edits are only minor grammatical corrections, and I have no concerns about the methodology, results, interpretations or conclusions.

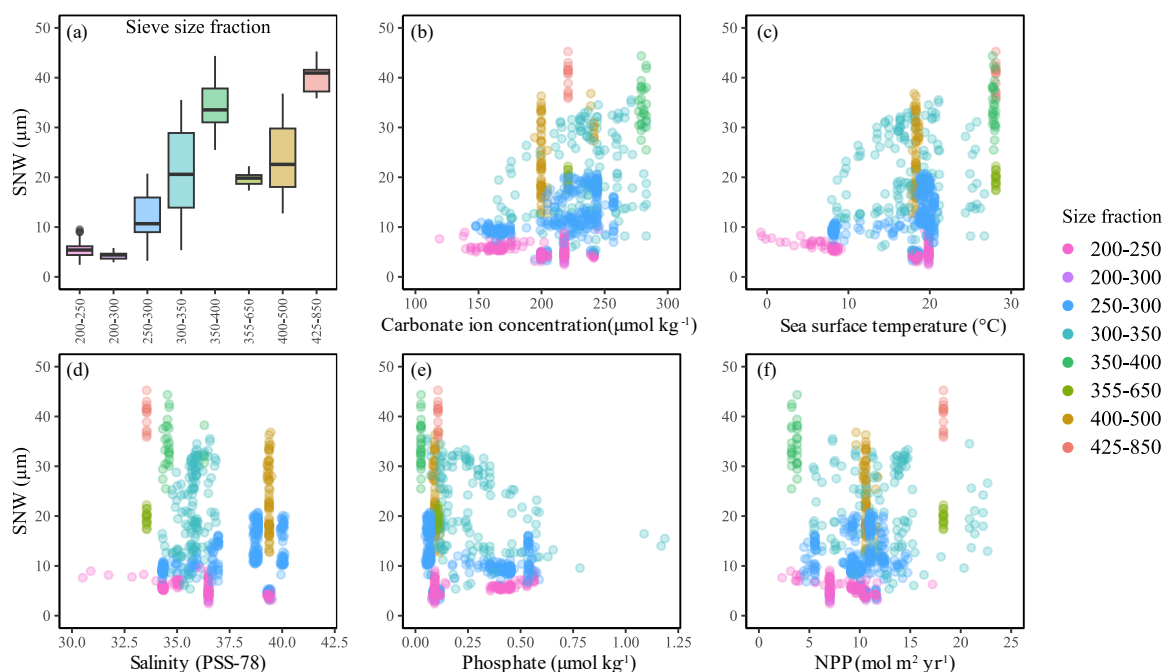
We thank the reviewer for their comments, and have updated the manuscript throughout following their suggestions for improved grammar. Regarding the use of size-normalised weight as a pCO<sub>2</sub> proxy, we would like to clarify that although its use is not as straight forward as initially postulated as there are clearly other factors affecting weight, we do not suggest that carbonate chemistry has no impact.

**Reviewer 1: I'm not sure what is meant by services [at line 34 of the original manuscript: "The unprecedented rise in CO<sub>2</sub> and temperature is altering our oceans and impacting marine ecosystems and their services."]**

As per the reviewer's feedback we have expanded on this by giving an example of an ocean ecosystem service: "...marine ecosystems and their services (such as marine biogeochemical cycles)"

**Reviewer 1: is there supposed to be an x axis label here [figure 2a of original manuscript] or just go by the colours on the legend?**

We have added x axis labels to make it clear to the reader that the boxplots refer to the different size fractions.



**Figure 1** (a) Boxplot showing SNW distribution across sieve size fractions. (b-f) Planktic foraminiferal size-normalised weight (MBW) against environmental variables extracted from the CMIP6 modelling suite (see methods). Colour indicates the size-fraction foraminifera were initially sieved at before being normalised to their length or area. See Fig. S7 for planktic foraminiferal SNW separated by species, with sieve size fraction information.

## Reviewer #2 : Anonymous

This study by Barrett et al. examines the use of size-normalized weight (SNW) of planktic foraminifera shells as a proxy for reconstructing past environmental conditions, particularly seawater CO<sub>2</sub> levels (pCO<sub>2</sub>). Using global data and Bayesian regression modeling, the authors find that no single environmental factor explains SNW variability across species and regions. Instead, species-specific and regionally variable responses suggest that cryptic species and phenotypic plasticity, such as changes in calcite thickness during reproduction, may influence shell weight. The study emphasizes the importance of regional calibration and careful species selection when using SNW as a pCO<sub>2</sub> proxy.

I find this study important and timely, as it effectively reminds the community that foraminifera shell weights are not an absolute indicator of seawater carbonate chemistry in the open ocean's natural conditions. However, I do have two major concerns about the study's methodology. First, although the authors, in their introduction and methodology, consider most of the factors that have been proposed over the years to explain variations in foraminifera shell weight, they do not account for recent studies that suggest changes in seawater density as a potential driver of these variations. I understand that seawater density and salinity may covary (in the surface ocean), but I still believe this factor is worth investigating. Second, the authors compare the SNW of deep-dwelling foraminifera species to surface ocean properties, which raises some concerns.

We thank the reviewer for their feedback and their acknowledgement of the importance and timeliness of our research. First we address their two primary concerns before responding to minor comments.

**1. More specifically, Zarkogiannis et al. (2019) suggest that foraminifera utilize their shells for buoyancy regulation, adjusting their shell weight to maintain their position in the water column. In a subsequent study, Zarkogiannis et al. (2022) also discuss gametogenic calcite as a mechanism for buoyancy regulation. They observe that if foraminifera shell weights were primarily governed by CO<sub>3</sub><sup>2-</sup> concentrations, deep-dwelling species residing in more acidic waters would exhibit the lightest shells. However, this is not the case. Their findings align with both the current study and that of Béjard et al. (2023), in which *G. truncatulinoides*, a deep-dwelling species, is observed to possess the heaviest shells. Moreover, Zarkogiannis et al. (2022) provide an additional 16 core-top samples that could be incorporated into the present study. As both CT and volumetric data are provided, size normalization to a linear dimension should be feasible.**

We agree with Reviewer 2 that we should include reference to a study that suggests a seawater density control on SNW. **As such, we add text to this effect in the introduction** “...and seawater density (Zarkogiannis et al., 2019)”

We stand by our decision not to include seawater density in our Bayesian modelling as although seawater density and shell weight will (in part) control the position of foraminifera in the water column, lipids and the shape of the test also impact buoyancy (Caromel et al., 2014; Schiebel and Hemleben, 2005). While the link to buoyancy is an intriguing suggestion, there are many open questions, such as why an adult would need to add the weight if this is a regulation i.e., a driven process, or could the weight in *G. truncatulinoides* be linked to its unique life cycle. Due to this complexity, it is beyond the scope of this study to investigate seawater density.

We have contacted the author of the suggested paper requesting this additional data but have not received a response. Although some of these data are available online it does not contain all the necessary information to get at SNW using ferret's diameter or area for the individual specimens and hence the data could not be included.

**2. My second concern pertains to the environmental data used for comparison with the SNWs. How surface were they? 0, 2, 5, 5 or 10m? Additionally, do the authors believe that comparing surface ocean conditions with the SNWs of *G. truncatulinoides*, a species typically found at depths below 400 meters, is appropriate? What is the underlying assumption in comparing deep-dwelling individuals with surface ocean conditions? If not at bibliographic species-specific calcification depths, I would have expected to see at least a comparison with the averaged conditions between 0 and 100 meters depth, extracted from the models, to be used for comparison with the SNWs.**

We agree with Reviewer 2 that ideally our environmental data would be from the exact living depth for each species. However, the reason we have not been able to compare SNW with this idealised environment data at depth is in part due to the challenge of estimating exact habitat depth. This changes through a foraminifera's life time hence it would be difficult to know which depth is most suitable (Schiebel and Hemleben, 2017). Even if depth was known, there is uncertainty in how much calcification happens at which depth and high resolution in situ analysis of proxies would be needed, though they would need to be calibrated for ontogenetic signals and not bulk calcite which is the current practice. Ideally, we would dissolve the foraminifera to extract oxygen isotope values and back calculate the correct habitat depth. However, this was beyond the scope of the current analysis. Furthermore, while several taxa are deep dwelling species and live below the thermocline, this depth is different in different parts of the ocean (Mulitza et al., 1997) and varies with the seasons (Waterson et al., 2017). Hence while we appreciate that species-specific calcification depths and their variation across locations would be the ideal approach to understand the multiple, competing drivers of SNW, it was beyond the scope of this manuscript to determine these exact depths for specimens.

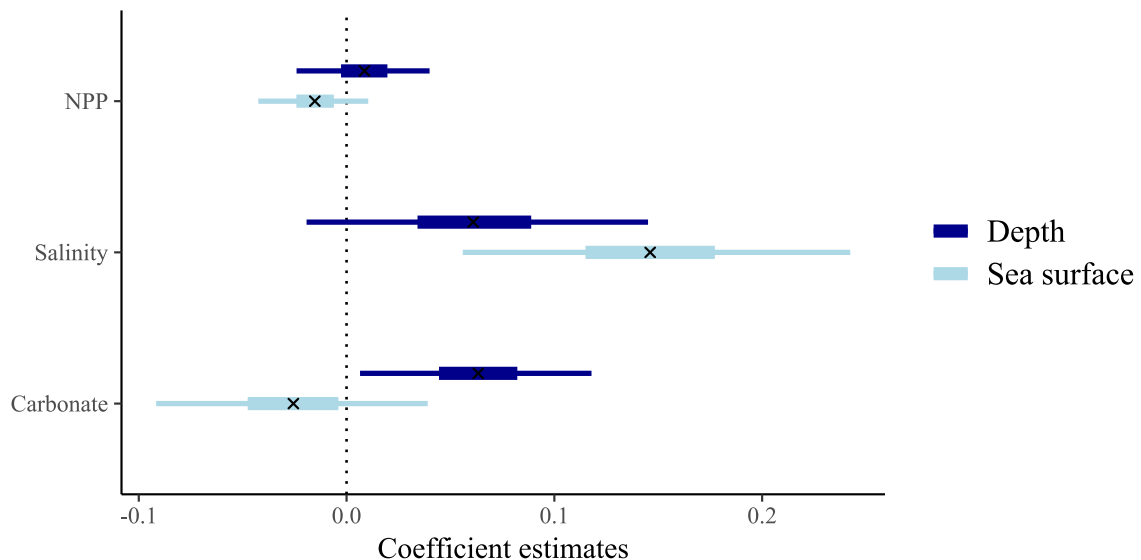
We consider the use of sea surface data ( $\leq 20$  m) for shallow dwelling species reasonable because the niche of shallow dwellers is largely dependent on sea surface temperature (Waterson et al., 2017). However, we acknowledge that our manuscript would benefit from clearly stating our choice in methodology regarding use of sea surface data. As such, **we have added text to the manuscript that provides more information on the depth of the environmental data and a paragraph which summarises the above text on problems associated with getting at exact habitat depth.** We recognise the importance of this comment and as such **have done some additional analyses to get at this problem of depth for deeper dwellers.** In the following, we detail these additional analyses.

To explore the importance of the depth of environmental data, we performed further analysis but were confronted with high collinearity ( $VIF > 10$ ) for many environmental variables. Environmental data from 200m depth was extracted from CESM2. We opted to use a depth shallower than 400m due to the latitudinal variability in depth of the pycnocline. We aimed to compare the model performance of two *G. truncatulinoides* models, one analysed with deeper (200m) environmental data and one with shallower ( $\leq 20$  m) environmental data. However, due to collinearity in the dataset it was necessary to do a principal component analysis (PCA) for the sea surface model (see note on collinearity at the end of this response to reviewers file); collinearity was also present in the model exploring deeper environmental data. Although PCA could be used to remove collinearity, this would result in differing principal components. Hence, it would be inappropriate to compare these shallow and at depth models.

To circumvent the above challenge, and as collinearity is primarily associated with sampling method in *G. truncatulinoides*, we model coretop data and sediment trap data separately. For each we run a sea surface ( $\leq 20$ m) environmental data model and a deep (200m) environmental data model. The sediment trap data models had to be abandoned due to excessive collinearity ( $VIF > 10$ ). Phosphate was removed from both coretop data models due to collinearity ( $VIF > 10$ ), leaving carbonate ion concentration, net primary productivity (NPP) and salinity.

The explained variance of coretop *G. truncatulinoides* was higher in the 200m depth model than in the sea surface model (Bayes  $R^2$  53% and 33%, respectively) showing an improvement of the link between SNW and environment for the deeper data. It is important to note that while there is a comparative improvement in model performance with the 200m depth data, still only half of SNW is explained by these three environmental variables leaving large uncertainties about other drivers for SNW in this species. We

are hesitant to overinterpret these models due to the fairly low explained variance and small sample size ( $n = 40$ ), however the overlap of the 95% credibility intervals for coefficient estimates (figure 2) suggest little difference between models. The low explained variance in SNW for these models, and for all *G. truncatulinoides* analysed using PCA (45%; table 1), might be related to the much longer life time of this species meaning that it experiences a wide range of environmental conditions given the vast distances this taxon drifts during its lifetime (van Sebille et al., 2015; Waterson et al., 2017).



**Figure 2** Effect size and credible intervals for the association between SNW and the environment for a *G. truncatulinoides* model using sea surface data (20m or less) and a model using deeper data (200m). A cross [x] represents the median value, the thicker line the 50% interval (i.e., where 50% of the posterior probability lies) and the thinner line the 95% interval. If the 95% interval does not cross zero, then there is a 95% probability there is an effect of the environmental variable. A negative value represents a negative correlation between SNW and the coefficient.

### Minor

**Line 27:** Change “are a plankton” to “are a type of plankton.” **Done.**

**Line 62:** What about seawater density? **We have added a seawater density reference, Zarkogiannis et al. (2019), to the introduction.**

**Line 108:** What are ecogroups? Consider mentioning the Aze et al. (2011) classification here, where ecogroups are first introduced. Are these ecogroups the same as those used in the group-level comparison? If so, why not refer to this comparison as ecogroup-level? Ecogroup is defined on line 67 of the original manuscript. As per your suggestion **we have added Aze et al. (2011) to the definition here.**

The group-level analysis does not refer to ecogroup-level. Instead it refers to all foraminifers being pooled together to examine whether across-species there is a universal driver of calcification. This is first defined at line 212 of the original manuscript, but we recognise the need for further clarification. **As such, throughout the manuscript we occasionally remind the reader that group-level refers to the across species analysis.**

**Lines 125-126:** I think Marshall et al. (2013) should be cited here, as they introduced area density as a normalization method against silhouette area. **We have added this reference.**

**Line 150:** Define ESMs at this point in the text. **Done.**

**Line 171: A reference for phosphate is missing.** Thank you for bringing this to our attention. We have added Demes et al., 2009; Kinsey and Davies, 1979; Lin and Singer, 2006; Paasche and Brubak, 1994.

**Line 214: I am unsure if salinity is appropriate, as its depth profile varies with latitude. In the halocline (within the first 1000 m, relevant to foraminifera), salinity increases with depth at high latitudes but decreases with depth at low latitudes. Furthermore, there is a salinity inversion in subtropical regions. If SNWs followed the salinity profile of the water column in the subtropics, there would likely be a decline in SNWs with depth, but this is not observed.**

We are confused about the point the reviewer is making here regarding the methods part of the paper. We have treated all parameters as equally possible. For the water depth we are considering, the reviewer is correct that in some locations in the high latitudes salinity is inverted due to ice melt and there can be small reductions in salinity in the surface waters of the subtropics due to freshwater injections but we do not understand how this is relevant here in the testing of drivers. As we have measurements at every location, we would consider the reduced salinity in the surface at these places.

**Lines 247-248: This explanation belongs in the methods section. It is the first time that the rationale behind using ESM data is addressed.** We have moved the sentence to the methods which introduces this rationale.

**Line 261: What happened to the merging of the 250–350 µm sieve fractions? It is unclear why the sieve fractions are separated in one instance and merged in another. Please clarify this point.**

We wanted to give the highest level of granularity possible here, and hence separated the size fractions when possible. We were able to do the separation in this part of the analysis as for this qualitative analysis, the number of data points is not so important. In contrast, in the statistical Bayesian analysis we had to combine size fractions to increase the size of the dataset being modelled. This being said, we are happy to merge the size fractions in figure 2 of the original manuscript if the editor would recommend this.

To clarify our reasoning, we have added text to section 3.2: *“To remove size fraction bias, all size fractions other than 250-300 µm and 300-350 µm have been removed and these two remaining size fractions have been merged to create a dataset sufficient for statistical analysis. Unless stated otherwise, the following statistics have been performed on this reduced dataset.”*

**Lines 264-267: *G. truncatulinoides* has variants like *excelsa*, which are hardly differentiated, while de Vargas et al. (2001) and Quillévéré et al. (2013) identify four distinct types. Therefore, it is challenging to make assumptions based on data from a single study. Zarkogiannis et al. (2022) also present *G. truncatulinoides* shell weights, volumetric data for size normalization, and CT images for variant identification, which could be useful for the present study.**

The four distinct types the reviewer mentioned are genetic types not morphologically expressed as clearly explored in de Vargas et al (2001). Therefore, the genetic knowledge of ecological adaptation cannot be used on the morphological specimen alone. The two types which can be separated are subtropical and high latitude. The Bejard study is based on specimens from the western Mediterranean which contains only one of the morphotypes (II) as per Quillévéré et al. (2013). Therefore the reference to different genetic types is not relevant here. It is further unclear to us why the genetic to morphological differentiation is mentioned here exclusively for *G. truncatulinoides* given that this is the case for many taxa (e.g. Morard et al. (2024) for a recent summary). A key theme in our manuscript is reminding future SNW researchers to consider cryptic species, so we feel that a comment is already acknowledged.

Regarding the use of the data presented in the 2022 study, as stated above, the author has not provided sufficient meta data to include their study into our analysis and has not provided the data on request. Though we appreciate the usefulness of variant identification in this dataset, its addition would not change the statement made at lines 264-267 in the original manuscript regarding the variability in the size

fraction 400-500 um, as the foraminifers in (Zarkogiannis et al., 2022) were sieved at the 300-350 um size fraction.

**Line 281: The sentence appears incomplete; something seems to be missing at the end.**

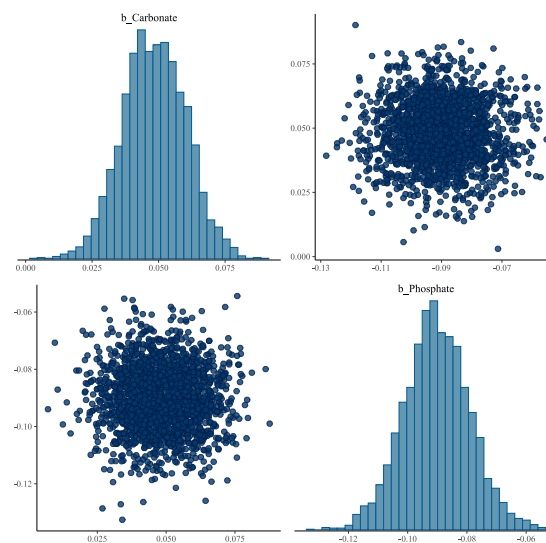
We have reworded the sentence to clarify: “As such, although the smaller size fractions are meaningful in polar and subpolar areas (as foraminifers are smaller at the poles), they must be interpreted with caution in warm, high calcite regions where this bias against small size fractions exists.”

**Line 345: Do you mean "ecogroup level"? If so, please revise for consistency.**

No – see above comment on model definitions for line 108. To improve clarity, we have added: “*Though in our group-level model (i.e., all foraminifers)*”.

**Line 400: Is there any collinearity in the current dataset between CO<sub>3</sub><sup>2-</sup> and phosphate, as suggested by Marshall et al. (2013)?**

Assessment of collinearity is covered in section 2.3.2 Model Specification. There is no collinearity in the current dataset between CO<sub>3</sub><sup>2-</sup> and phosphate. Furthermore, the Bayesian models have been run with QR decomposition analysis which reduces impacts of correlation between variables within the models. To check for any remaining collinearity pairs plots were visually assessed, and variance inflation factors (VIF) were verified using the package ‘performance’ which passes the brms model to its frequentist counterpart. A structureless i.e., “blob” like output for the visual pairwise plots indicate no collinearity between carbonate and phosphate (figure 3) and the VIF scores are under 10 (1.08 for carbonate and 1.09 for phosphate) which indicates that collinearity is not problematic (Marcoulides and Raykov, 2019).



**Figure 3** Pairwise plot showing no collinearity between carbonate and phosphate

**Line 452: Also strongly agreed. However, I did not see any raw shell weights presented in this study. The Pangea links are missing.**

Apologies, the Pangea links are currently missing as the data is awaiting a DOI. Until then, reviewers can access raw data and model code using a temporary link to the University of Bristol’s research data storage facility which we share with the editor.

**One aspect not discussed in this section or elsewhere in the paper is the cleaning protocols used for foraminifera shell weight measurements in different studies. This aspect should eventually be standardized. As shown in the HyPerCal cleaning protocol, different treatments incorporate varying amounts of sedimentary contamination, which can affect SNW, particularly for specimens with large and multiple apertures, such as *G. ruber* plexus.**

Yes, we agree this is important when measuring SNW. Based on CT data, the contamination is not important in specimens with larger apertures such as *G. ruber* as sediment can easily be removed. A larger amount of sediment is found in specimen of the globorotaliids, as it is harder for the sediment to be removed when washing.

Unfortunately, we were unable to add this level of complexity (i.e., cleaning protocol) to the modelling, as we are currently limited in how complex the models can be due to the limited sample size. Our hope is that once community agreed protocols for SNW data collection are widely used and data management improves, the size of the useable dataset will increase to support such analysis.

For now, **we have added a comment on the cleaning protocol to the discussion where we discuss other methodological limitations:** *“It would also be useful for authors to report their cleaning protocol, or the absence of cleaning. Ideally, the community would agree on a standardised cleaning method for SNW to reduce potential impacts on the weight of specimens (Béjard et al., 2023; Zarkogiannis et al., 2020).”*

**Furthermore, in a future submission please change planktic to planktonic. The correct adjective form of plankton is planktonic. The adjectives of Greek nouns ending in -on get the suffix -ic in the end like plankton – planktonic, bion – bionic, lacon – laconic. This is different to nouns ending in -os, which lose the ending -os to the previous consonant by replacing it with -ic, like bentos – benthic, cosmos – cosmic or chronos – chronic.**

While we appreciate the clear knowledge of Greek by the reviewer, it is also clear that the root of the word has been lost and both terms are used by the community.



### Reviewer #3: Pincelli Hull

I agree with the first two readers that this is an excellent, well-written manuscript that describes the results of a careful study that works to synthesize new and existing results. The current state of the literature on shell normalized weight can be generously described as a confusing and contradictory, so this is a refreshing paper to read indeed.

I have four suggestions that I do think are important to incorporate to make this current contribution clear, in terms of its findings and implications and in allowing this study to be useful for future research.

We thank the reviewer for their feedback. In the following we response to the reviewers four suggestions.

1. **Data and Code Availability:** Like Reviewer 2, I went looking for the data and couldn't find the raw data, something they explicitly implored other studies to make available. I suspect this is embargoed at present, but as a reviewer, this was unclear. Making the code available as well, would make this study truly replicable and should be done (and maybe the authors already have!).

Apologies, the Pangaea links are currently missing as the data is awaiting a DOI. Until then, reviewers can access raw data and model code using a temporary link to the University of Bristol's research data storage facility which we share with the editor.

2. **Table of Statistical models and diagnostics:** My apologies if I am missing this, but I was surprised not to see a table of the different models with diagnostics on relative model fit. The authors describe their models in words, and some of the results, but do not provide a table of these models and results. It is fine if model results are included in the supplement only, but they should be included.

Thank you for this suggestion. We include extensive diagnostics as part of our R Markdown supplement, but the link to this was missing in the preprint. This is now available via a link to the University of Bristol's research data storage facility which we share with the editor. However, for clarity we have also added a table in the supplement (Table 1). [Please see a description of this table below.](#)

Model structure: [We have added detail on the structure of the models in table 1.](#) The reader can also see the supplementary R script which contains the code needed to reproduce our analyses.

Model diagnostics:

- 1) Model convergence: We do not add the  $R_{hat}$  diagnostics to the table as this would be space inefficient. Instead, for the sake of brevity [we have added text to the methodology](#) "A  $R_{hat}$  value close to 1 (i.e., less than 1.1) indicates the chains have converged (Bürkner, 2017). All models had a  $R_{hat}$  of 1.01 or 1."
- 2) Collinearity: [In table 1 we have included the variance inflation factors \(VIF\) scores](#) for each model and their associated tolerance intervals (TI). A VIF of less than 10 and TI of  $>0.1$  indicate no collinearity.

Model fit:

- 1) Kernel density estimate plots. Figure S5 in the original supplementary information include the kernel-density estimates, which shows the goodness of fit for the individual models. The closer that "yrep" is to "y" means the better the model was able to reproduce the original data distribution. This indicates that all models have a reasonable fit.
- 2) Table S3 in the original supplementary information reports the effect size and 95% credible interval, which is equivalent to the data shown in figure 3 and 4 of the original manuscript.
- 3) [We have added Bayes R2 values](#) for all models in table 1.

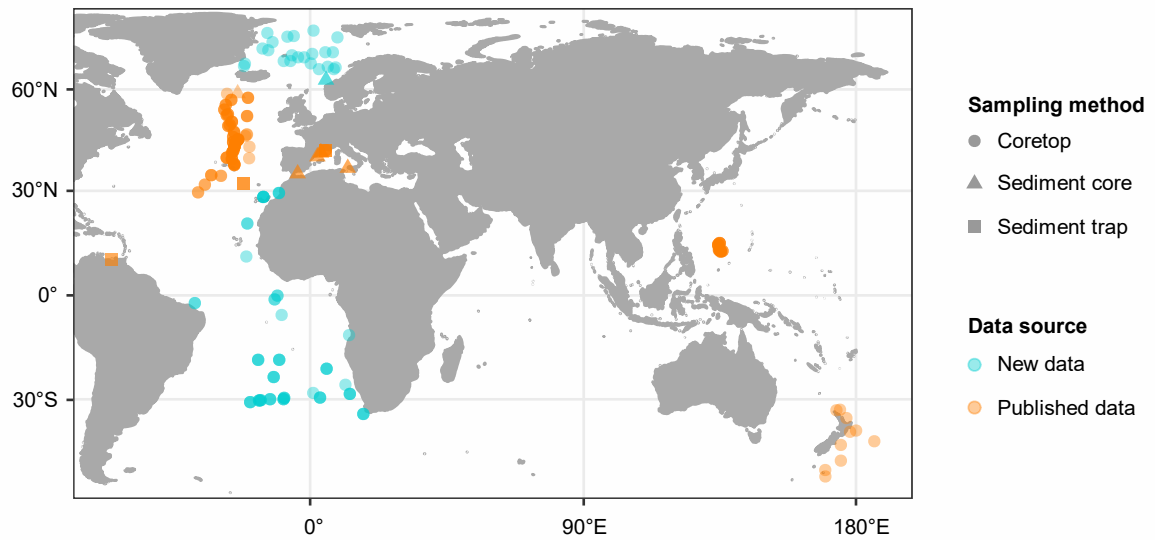
**Table 1** Bayesian model structure, collinearity diagnostics (variance inflation factor; VIF and tolerance intervals, TI, and model fit. Environmental variables include sea surface carbonate ion concentration, phosphate concentration, salinity and net primary productivity (NPP). In the ‘Group-level’ Bayesian models, Environment and Sampling method were added as fixed effects, and Species was added as a random effect (intercept only). Sampling method can include data from coretop, sediment core and sediment trap. A VIF of ten or less and tolerance interval (TI) of  $> 0.1$  indicates that collinearity is not problematic (Marcoulides & Raykov, 2019). <sup>1</sup>Models which use principal components (PCs) in place of individual environmental variables due to collinearity in the original data making dimensionality reduction necessary. Value for leave one out cross validation (LOO) are reported in  $\widehat{elpd}_{loo}$  [ $\pm$  standard error]; a lower value indicates comparatively worse performance between the two models (e.g., null model performs worse than the full model).

Model name	n	Model structure			Collinearity Diagnostics (VIF [TI])						Model fit		
		Environment	Sampling method	Species	Carbonate	Phosphate	NPP	Salinity	PC1	PC2	Bayes R2	Leave One Out Cross Validation (LOO)	
Group-level models (i.e., foraminifera pooled together)													
“full model”	491	✓	✓	✓	1.09 [0.91]	1.07 [0.93]	1.55 [0.64]	1.04 [0.96]	-	-	90%	full model	0
												null model	-247.5 [19.4]
“null model”	491	✓	✓	-	1.07 [0.93]	1.07 [0.93]	2.92 [0.34]	1.05 [0.95]	-	-	60%	null model	0
												env. only model	-114.8 [23.6]
“environment only”	491	✓	-	-	1.03 [0.97]	1.07 [0.94]	1.12 [0.90]	1.03 [0.97]	-	-	20%	-	-
Species-level models													
<i>G. truncatulinoides</i>	105	✓ <sup>1</sup>	✓	-	-	-	-	-	4.25 [0.24]	-	33%	-	-
<i>N. pachyderma</i>	53	✓	-	-	3.99 [0.25]	4.08 [0.24]	4.83 [0.21]	3.98 [0.25]	-	-	56%	-	-
<i>G. elongatus</i>	134	✓ <sup>1</sup>	✓	-	-	-	-	-	1.10 [0.91]	2.04 [0.49]	88%	-	-
<i>G. ruber</i>	53	✓	✓	-	4.67 [0.21]	1.80 [0.56]	3.35 [0.30]	3.40 [0.29]	-	-	78%	-	-
<i>G. bulloides</i>	255	✓	✓	-	3.79 [0.26]	4.63 [0.22]	2.79 [0.36]	4.03 [0.25]	-	-	65%	-	-
<i>N. incompta</i>	85	✓ <sup>1</sup>	✓	-	-	-	-	-	3.30 [0.30]	1.54 [0.65]	78%	-	-

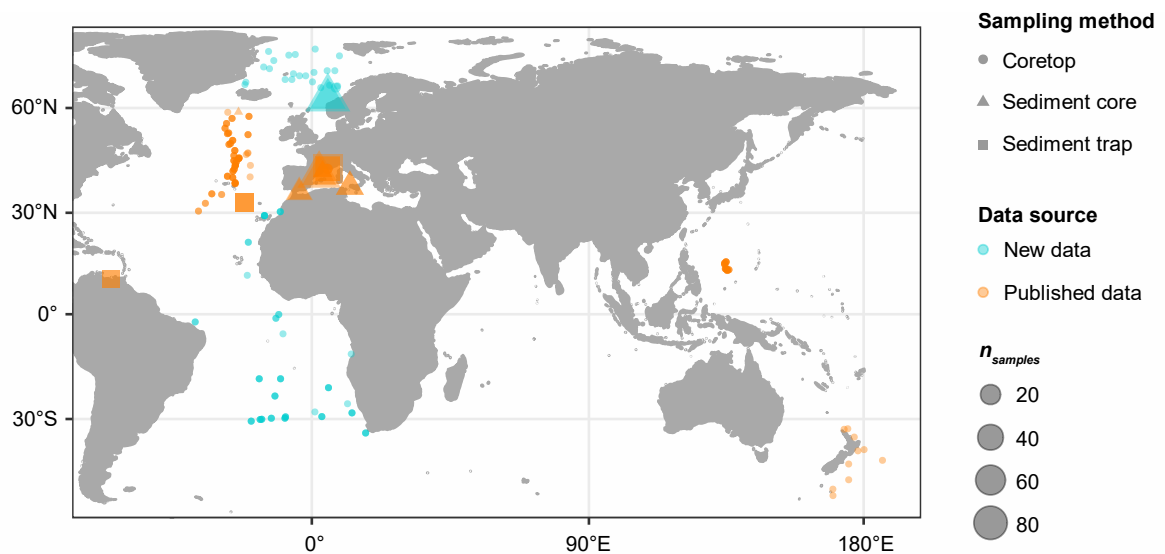
3. Handling and reporting of the fixed effect ‘sampling type’: Because I was involved in one study that found that the most important predictor of shell normalized weight was whether the test was from a sediment trap or core top, I was heartened to see this included as a fixed effect (and indeed, you discuss the literature regarding the potential importance of this factor). I was thus confused as to why the importance of this condition was not reported, nor shown, in any of the figures. I appreciate that you would want to consider the effect of this factor relative to other fixed effects, but to not report it at all nor show its effects seems like a missed opportunity. Without knowing its relative importance, it is hard to know what to do with the results that are framed in terms of the conditions present in the surface ocean around the time that the individual was alive. Here is where more information is needed on this fixed effect:

a) Figure 1: symbol type could show whether the same was a trap or from the ocean bottom

Thanks for this suggestion. We have made this change to figure 1 in the original manuscript (figure 4 in this file). We also add a supplementary figure which details the number of samples from each data type (figure 4b).



**Figure 4** Location of SNW data and sampling method.



**Figure 4b.** Location of SNW data, sampling method and number of samples per site.

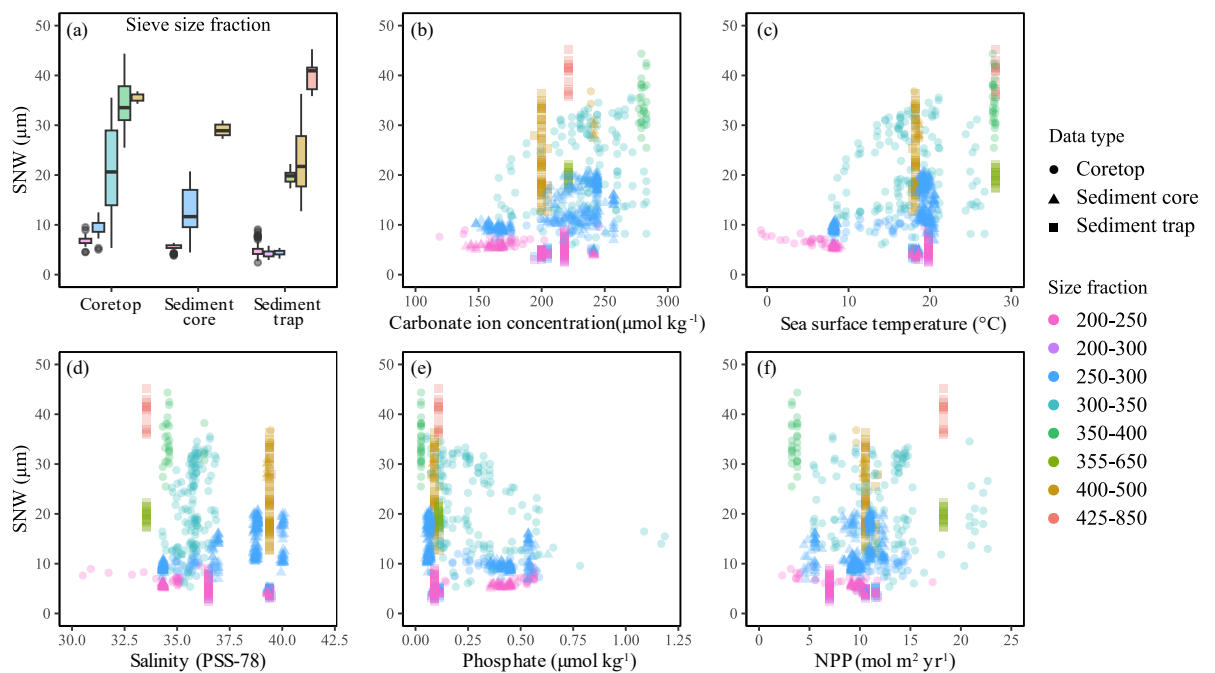
- b) **Results: please report the variance explained by the null model (i.e., fixed effects including sampling type).** I assumed from the methods and from the use of the phrase ‘environment-only’ model that the reported model with 23% of variance explained excluded the sampling method fixed effect. Did the model with random effects include sampling method? It is confusing without a table of model results to refer to (see pt #2).

The reviewer is correct that the environment-only model in the original manuscript (i.e., the 23%) excluded the sampling method fixed effect. In the original manuscript, the model with random effects (i.e., the 86%) did not include sampling method. We wanted to disentangle the impact of adding species, but we acknowledge that assessing the importance of sampling method is of interest and hence have expanded the analyses **by including the following text to the results:**

*“A model that is environment only explains 20% of the variability in SNW (Bayes R<sub>2</sub>; Gelman et al., 2019). The addition of sampling method (i.e., the “null model”) improves model performance ( $\widehat{elpd}_{100}$  improved by -114.8 [ $\pm 23.6$ ]) and explained variance increases to 60% The “full” model (i.e., environment, sampling method and species) performs better than the “null” model ( $\widehat{elpd}_{100}$  improved by -247.5 [ $\pm 19.4$ ]) and explained variance increases to 90%. Together, this shows that the choice of sampling method can influence the SNW recorded and that species-specific responses are important in determining SNW.”*

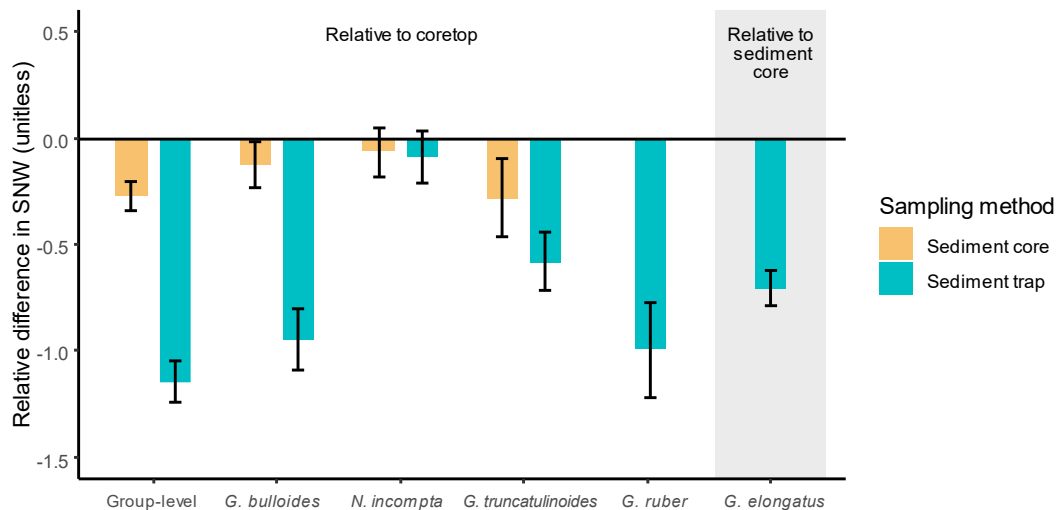
- c) **Figures: Please add this fixed-effect to Figure 2,3,4, so the effect and directionality of the effect can be understood.**

1. **Update figure 2:** In the interest of figure readability, we have added this to the supplementary information and referenced it in the main text:



**Figure 5** (a) Boxplot showing SNW distribution across sieve size fractions, split by sampling method. (b-f) Planktic foraminiferal size-normalised weight (MBW) against environmental variables extracted from the CMIP6 modelling suite (see methods). Colour indicates the size-fraction foraminifers were initially sieved at before being normalised to their length or area. See Fig. S7 for planktic foraminiferal SNW separated by species, with sieve size fraction information.

2. **Update figure 3 and 4:** Because the fixed effect output for sampling method is categorical, the Bayesian model output presents the impact of sampling method as relative to another sampling method. Therefore, we do not think it is appropriate to add to figure 3 and 4. Instead we create a separate figure (figure 6) and add this to the supplementary information. This shows that in the Group-level model, SNWs are lighter from sediment core and sediment trap data than from coretop data, with the lightest weights observed for sediment traps (figure 6).



**Figure 6** Bayesian model outputs showing the median difference in SNW from different sampling methods, relative to coretop data (unless stated otherwise). The more negative the value, the lighter the SNW. Black capped lines represent the 95% probability interval. *N. pachyderma* is not present because this dataset contained only one sampling method (Coretop). <sup>1</sup> *G. ruber* does not contain any sediment core data. <sup>2</sup> *G. elongatus* does not contain any coretop data, and the sediment trap data shown is relative to sediment core data. Models correspond to those detailed in table 1.

**We add the following text to the results:** “In all models (excluding *N. pachyderma*, which only had one sampling method), the lightest SNWs were recorded from sediment traps (figure 6). Sediment core data are lighter than coretop data, hinting towards questions of preservation not visible externally, but are more similar to each other than to sediment trap data (figure 6).”

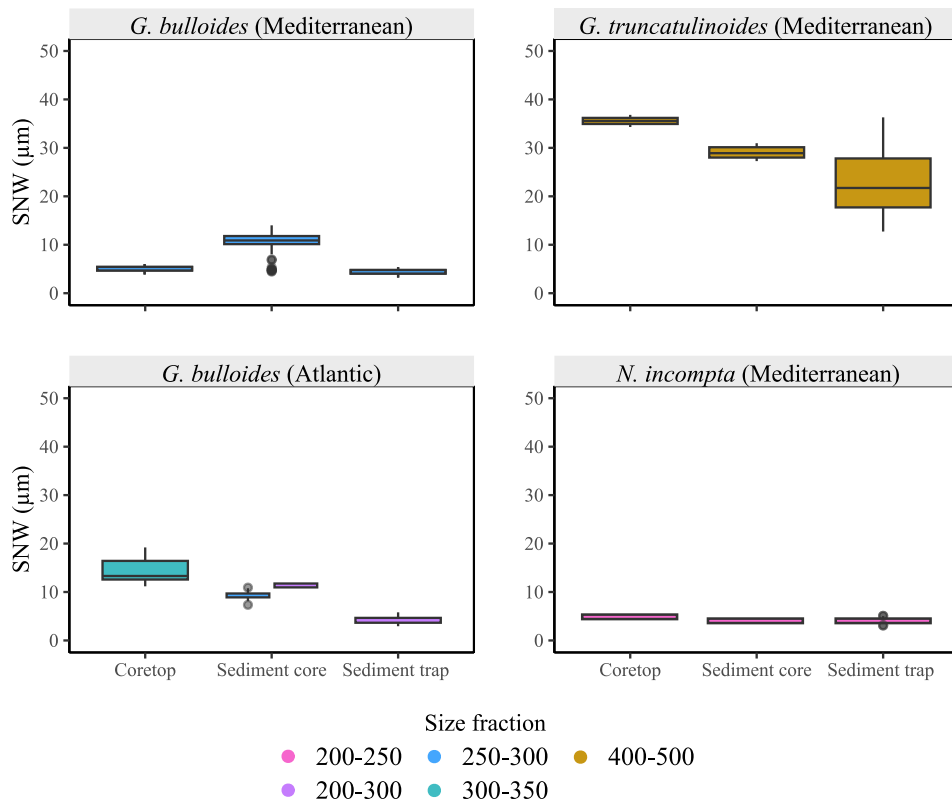
- d) **Discussion: given that the model includes this factor, you are in the position to discuss the relative importance (and overprinting) of diagenesis in the sediment versus conditions during life. I know that I, and a few others, would love to hear your thoughts on this, given the results—once presented!**

Thanks to the reviewers comments, **we have been able to discuss the impact of sampling method on SNW in more depth.** See below for a summary of our new analyses.

The choice of sampling method is important for the resulting weight of foraminifers. Despite attempting to minimise the impact of sampling method by removing plankton tow data, removing data for which dissolution is reported and removing samples approaching the CCD, the impact of sampling method on SNW is still present (figure 6). Though it is important to note that we do not

have many samples from regions which have a very shallow CCD such as the Pacific in this comparison, thereby limiting insight.

Despite having filtered the data for preservational biases in the sediment, we split the data by location to check whether sediment trap data is still lighter when the ocean basin is explicitly accounted for (figure 7). This separation shows 1) no clear trends for *N. incompta*, a relatively thick specimen, and no clear trends in the two datasets for *G. bulloides* comparing the Mediterranean with the Atlantic, though overall for this reduced dataset SNW in sediment trap data is lighter than, or equal to seafloor (coretop and sediment core) data.



**Figure 7** Boxplot showing the weight of foraminifers under different sampling methods for *G. bulloides*, *G. truncatulinoides* and *N. incompta* in the Mediterranean and the Atlantic. The data has been split in this way to remove the impact of size fraction, species, and location, which enables direct comparisons between sampling methods from the raw SNW data.

It is unlikely that dissolution in the water column (i.e., impact of intermediate and deep water) is the cause of lighter weights in sediment trap data (figure 6; figure 7), because it would have impacted coretop and/or sediment core samples even more due to longer exposure. It is unlikely that diagenetic alteration (e.g. recrystallization in the pore water) has made the SNW of coretop and sediment core data comparatively heavier as relative to the weight of the entire test it would have little impact on overall weight. We speculate here - and emphasise that this is speculation - that sediment trap data are lighter as they reflect current environmental conditions, whereas sediment core and most coretop data are preindustrial and that the lower weight may be due to impacts on lower carbonate ion on the calcification process due to ocean acidification (Moy et al., 2009; Pallacks et al., 2023).

4. **Depth of Environmental factors: it is unclear from the methods (but could be clear in a data table) at what depth(s) environmental factors were extracted and considered from the models. Were these all from the ‘sea surface’?**

In the methodology, we have provided clarity about what depth is extracted. “*we use surface ocean environmental data ( $\leq 20$  m depth)*”. Please see the earlier discussion in response to comment #2 from Reviewer 2 on limitations associated with exploring conditions in subthermocline habitats.

**Are bottom water conditions considered for the sediment samples?**

We do not use bottom water conditions for sediment samples. Please see our response to the comment above on sampling method (comment ‘d) Discussion’) for a detailed response.

**Are deeper depth conditions considered for those species that live at deeper depths? Would this change the coherence and direction of results for those taxa?**

Please see our response to Reviewer 2 regarding the depth of environmental data (comment #2).

**If these general concerns could be addressed, as I suspect they might readily be, this will make an excellent contribution in my opinion! I list a minor note below and I look forward to seeing the final version!**

**Minor: Line 170: change ‘inhibits’ to ‘inhibit’**

We have changed this.



### **A note on collinearity**

During this review because we now consider the impact of sampling method more in depth, we identified collinearity in two species-level models: *G. truncatulinoides* and *G. elongatus*. Additionally, as stated in the original manuscript we previously removed *N. incompta* from analyses due to collinearity. However, in the interest of sharing these data we have included these species in our analyses by adapting our methodology to use principle component analysis (PCA) for these three species. By using PCA to reduce the dimensionality of the environmental data, we are able to eliminate the problem of collinearity and investigate the impact of sampling method on these species, and can in-part understand the response of SNW to environmental drivers.

We plan to add the following text that details the PCA to the supplementary text, and summarise PCA results in the methodology of the main text. We have adapted figure 4 in the main text to remove collinear species *G. truncatulinoides* and *G. elongatus*, and instead presented the PCA results separately.

### **Text S3 – Principal component analysis (PCA)**

For species *G. truncatulinoides*, *G. elongatus* and *N. incompta* collinearity was problematic (variance inflation factor (VIF) score was over ten (Marcoulides and Raykov, 2019)). To remove collinearity we reduce the dimensionality of the data with PCA and use the principal component outputs instead of individual environmental drivers in the Bayesian models. For these three species, the PCA is based on the same four environmental drivers as all other modelling; carbonate ion concentration, phosphate concentration, net primary productivity (NPP), and salinity. These data were centred and scaled prior to PCA to normalise environmental data.

#### ***G. truncatulinoides***

Over half of the environmental data is represented by principal component 1 (PC1; 59%; table 2). Although including PC2 would increase the theoretical explanatory power, it leads to collinearity in the resulting Bayesian model. As such, we only use PC1 for *G. truncatulinoides*. For this species, PC1 primarily represents salinity and phosphate (34% and 29% representation respectively; table 1), while carbonate contributes 20% and NPP 17%. PC1 increases with salinity (i.e., positive eigenvector) and decreases with an increase in phosphate, carbonate and NPP (i.e., negative eigenvector; table 1; figure 8).

#### ***G. elongatus***

Together, PC1 and PC2 explain 88% of the environmental data for *G. elongatus* (table 2). PC1 primarily represents phosphate, NPP and salinity (34%, 34% and 27%, respectively), while PC2 primarily represents carbonate ion concentration (80%). PC1 increases with phosphate and carbonate ion concentration, but decreases as salinity and NPP increase (table 1; figure 8). PC2 decreases most strongly with an increase in carbonate ion concentration but also decreases with salinity, phosphate and NPP.

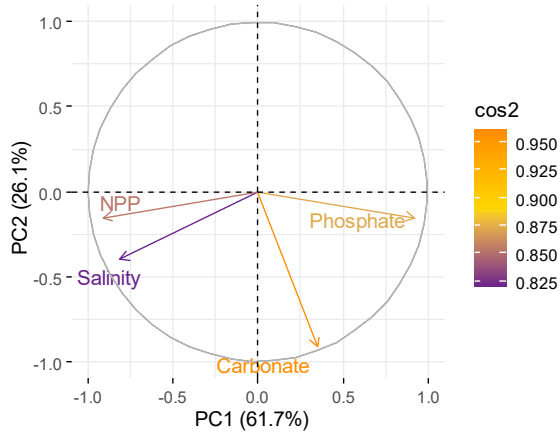
#### ***N. incompta***

PC1 and PC2 explain 98% of the environmental data for *N. incompta* (table 2). PC1 fairly evenly represents the four environmental variables, with slightly better representation for salinity (29%) and phosphate (28%). PC2 represents NPP and carbonate (60% and 39%). PC1 increases with carbonate, and decreases with an increase in salinity, phosphate and NPP. PC2 increases with NPP and decreases with an increase in carbonate.

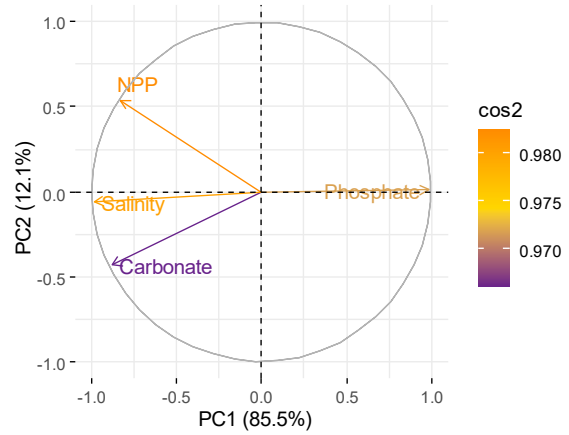
**Table 2** Results from Principal Component Analysis (PCA) for *G. truncatulinoides*, *G. elongatus* and *N. incompta*. Eigenvalue and variance explained (%) indicate how well the principal component explains the environmental data. Squared cosines ( $\cos^2$ ) and percentage contribution show how well a particular environmental variable is represented in the principal component. Loadings (Eigenvectors) are indicative of the correlation between variables.

	Eigenvalue	Variance explained (%)	Quality of representation of variable; $\cos^2$ and [percent contribution]				Variable loadings (Eigenvectors)			
			Salinity	PO <sub>4</sub>	Carbonate	NPP	Salinity	PO <sub>4</sub>	Carbonate	NPP
<b><i>G. truncatulinoides</i></b>										
PC1	2.37	59.30	0.81 [34.11%]	0.68 [28.55%]	0.48 [20.34%]	0.40 [16.99%]	0.58	-0.53	-0.45	-0.41
<b><i>G. elongatus</i></b>										
PC1	2.46	61.67	0.66 [26.84%]	0.84 [34.44%]	0.12 [5.03%]	0.83 [33.68%]	-0.52	0.59	0.22	-0.58
PC2	1.04	26.11	0.16 [15.22%]	0.02 [2.31%]	0.84 [80.17%]	0.02 [2.29%]	-0.39	-0.15	-0.90	-0.15
<b><i>N. incompta</i></b>										
PC1	3.42	85.47	0.98 [28.58%]	0.97 [28.43]	0.78 [22.75%]	0.69 [20.24%]	-0.53	-0.53	0.48	-0.45
PC2	0.48	12.05	0.00 [0.68%]	0.00 [0.01%]	0.18 [39.01%]	0.29 [60.28]	-0.08	0.01	-0.62	0.78

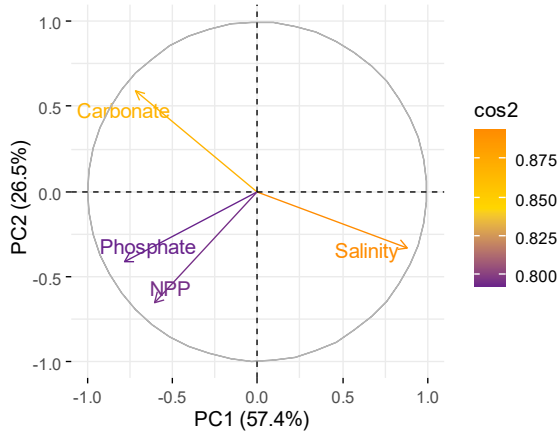
*G. elongatus*



*N. incompta*



*G. truncatulinoides*



**Figure 8** Biplot outputs from principal component analysis for *G. truncatulinoides*, *G. elongatus* and *N. incompta* for principal component (PC) 1 and PC2 for each species. The percentage presented in each axis label is the Eigenvalue expressed as percentage (i.e., how representative that PC is of the data). Squared cosine values ( $\cos^2$ ) are indicative of the quality of representation of a variable; the higher the value the better represented the variable is in that PC. The direction of the arrow along the axis describes the correlation between variables; positively correlated variables point to the same side of the plot and negatively correlated variables point to opposite sides of the plot.

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