

## Research article

## Do marine planktonic ciliates follow Bergmann's rule?

Kailin Liu<sup>1,3</sup>, Siyu Jiang<sup>4</sup>, David J. S. Montagnes<sup>5</sup>, Hongbin Liu<sup>2,6</sup>, Liping Zheng<sup>7</sup>, Bangqin Huang<sup>1</sup>, Xin Liu<sup>1</sup> and Bingzhang Chen<sup>1,2,3</sup>

<sup>1</sup>College of the Environment & Ecology, Xiamen Univ., Xiamen, China

<sup>2</sup>Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangdong, China

<sup>3</sup>Dept of Mathematics and Statistics, Univ. of Strathclyde, Glasgow, UK

<sup>4</sup>Atmosphere and Ocean Research Inst., The Univ. of Tokyo, Kashiwa, Chiba, Japan

<sup>5</sup>Dept of Evolution, Ecology, and Behaviour, Univ. of Liverpool, Liverpool, UK

<sup>6</sup>Dept of Ocean Science, Hong Kong Univ. of Science and Technology, Hong Kong

<sup>7</sup>Inst. of Deep-sea Science and Engineering, Chinese Academy of Sciences, Sanya, China

Correspondence: Bingzhang Chen ([bingzhang.chen@strath.ac.uk](mailto:bingzhang.chen@strath.ac.uk))

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Body size is a fundamental trait determining individual fitness and ecological processes. Reduction in body size with increasing temperature has been widely observed in most ectotherms and endotherms, known as Bergmann's rule. However, we lack data to assess if ciliates, the major consumers of marine primary production, follow Bergmann's rule and what drives the distributions of their cell size. Here, we examined a data set (287 samples) collected across the global oceans to investigate biogeographic patterns in the mean cell-size of ciliate communities. By measuring the sizes of every ciliate cell (< 10 to > 300 per sample), we found that community cell-size increased with increasing latitude, conforming to Bergmann's rule. We then addressed the cause. Temperature was a main driver of the trend. Ciliate community mean cell-size decreased 34% when temperature increased from 3.5 to 31°C, implying that temperature may be a direct physiological driver. In addition, prey (phytoplankton) size also influenced the trend, with ciliate size increasing by 35% across the gradient of phytoplankton size (0.6–15.5 µm). Generally, these findings emphasized the importance of how both biotic and abiotic factors affect size distribution of marine ciliates, a key component of pelagic ecosystems. Our novel, extensive dataset and the predictive trends arising from them contribute to understanding how climate change will influence pelagic ecosystem functions.

Keywords: body size, latitudinal distribution, microzooplankton, prey size, temperature

### Introduction

Body size is a master trait governing numerous physiological and ecological processes such as metabolism, food web dynamics, and carbon flux (Turner 2002, Brown et al.



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2004, García-Comas et al. 2016). Consequently, it is used as a functional trait in ecosystem models to determine general cross-taxa patterns (Purves et al. 2013, Blanchard et al. 2017, Chen et al. 2019). In fact, it was by using body-size dependence of plankton parameters in a food-web model that Ward et al. (2012) were able to reproduce global distributions of nutrients and biomass, revealing mechanisms underlying the global size structure of marine communities. Likewise, spatiotemporal patterns of body size are central to understanding macroecology (Brown 1995), further facilitating the development of ecosystem models.

Critically, body size is influenced by temperature, and hence it will be impacted by climate change. Bergmann's rule argues that smaller species tend to reside in warmer regions at lower latitudes (Bergmann 1847, Mayr 1956) and is usually applied at the community level (Daufresne et al. 2009, Hessen et al. 2013), although intraspecific variations have also been examined (Olalla-Tárraga 2011, Horne et al. 2015). This widespread phenomenon has been observed in a diverse range of endotherms and ectotherms including mammals, birds, fish, copepods and phytoplankton (Millien et al. 2006, Walters and Hassall 2006, Saunders and Tarling 2018, Evans et al. 2020, Campbell et al. 2021). Here we examine if Bergmann's rule equally applies to a group of organisms that are known to be drivers of global biogeochemical cycling: marine planktonic ciliates (Calbet 2008).

In the global oceans, ciliates are the major consumers of primary production and important food for mesozooplankton, acting as the main trophic link between primary producers and higher trophic levels and important recyclers of carbon (Calbet and Landry 2004, Calbet and Saiz 2005). Planktonic ciliates represent a highly diverse group, ranging in size from  $< 10$  to  $> 100$   $\mu\text{m}$ , spanning more than three orders of magnitude in body volume. As planktonic food webs are highly structured by organism size, this variation in ciliates will affect food web structure and hence biogeochemical cycling, including carbon flux (Ward et al. 2012, DeLong et al. 2015). It is, therefore, surprising that the biogeographic patterns in ciliate size variations remain understudied (Weisse and Montagnes 2021).

Beyond assessing the extent to which Bergmann's rule applies to planktonic ciliates, we examine the driving mechanisms underlying any spatial patterns. Despite over a century's study, the mechanisms governing Bergmann's rule remain poorly understood. As temperature is highly correlated with latitude, it is considered the primary driver for the latitudinal-size clines (i.e. Bergmann's cline). For endotherms, temperature is the determining factor because the lower surface area to volume ratio of larger animals in colder regions helps conserve body heat (Hessen et al. 2013). This explanation is less compelling for ectotherms, as their body temperature varies with environments. An alternative explanation for ectotherms is that smaller size reduces energy loss and compensates for the lower growth efficiency at increased temperatures because of the higher temperature sensitivity of catabolism than anabolism (Atkinson 1994).

For heterotrophic protists (including ciliates), there is support for a direct physiological relation between temperature and size: meta-analysis suggests that protist size decreased with increasing temperature by about  $2.5\% \text{ } ^\circ\text{C}^{-1}$  (Atkinson et al. 2003). Such studies typically examine individual size changes under controlled laboratory conditions with constant temperatures and a surfeit of food (Diamond and Kingsolver 2010). In contrast, we know little regarding the effect of ambient temperature on body size of ciliates at community levels in natural environments. Furthermore, multiple abiotic factors (e.g. season length and oxygen concentrations) will covary with latitude and could confound temperature effects on body size; these may, in fact, also drive Bergmann's cline (Forster et al. 2012, Horne et al. 2015). For instance, the 'oxygen hypothesis' predicts that the size of aquatic ectotherms is limited by the oxygen availability, with larger body sizes in regions of high oxygen (Woods 1999, Rollinson and Rowe 2018). Likewise, variation in season length with latitude may help explain the Bergmann's cline, as this abiotic factor is argued to influence a tradeoff between growth and reproductive investments (Kozłowski et al. 2004).

Biotic factors also vary with latitudes and interact with abiotic factors (e.g. temperature) to drive the Bergmann's clines; these must be considered in the studies of Bergmann's rule (Ho et al. 2010). For instance, food availability is a plausible driver, shaping latitudinal patterns in body size as more food can lead to faster growth rate and larger body sizes (Vidal 1980), while low food availability can limit body size (Andriuzzi and Wall 2018). Conversely, larger body size can occur where food is scarce, facilitating animals to travel and forage for food (Belovsky 1997, Brown et al. 2017). In contrast to the inconsistent patterns of food availability, an increase in predator size is commonly associated with an increase in prey size (Carbone et al. 1999). Predators tend to feed on prey within a suitable range, one that provides optimal energy returns (Hansen et al. 1994, Troost et al. 2008). In planktonic food webs, the size ratio between ciliates and their optimal prey is 8 : 1 (Hansen et al. 1994). Thus, any latitudinal change in prey size (through temperature or other effects) should contribute to the latitudinal distribution of ciliate size. Here we, therefore, focus on prey size, assessing the extent to which it contributes to the observed trends; as direct measurements of prey size were not available to complement our ciliate data set, we have relied on novel estimates of the prey size structure (Brewin et al. 2010, Ward 2016), that provide insights into drivers of predator size.

In summary, this extensive study presents a much-needed assessment of geographic patterns of marine ciliate community mean cell-size, examines whether ciliates follow Bergmann's rule, and assess the potential driving mechanisms. Specifically, we then interrogate the data set and ask: 1) to what extent does temperature play a role in shaping the observed patterns? 2) to what extent does prey size affect the distribution of ciliate community mean cell-size? and 3) how do large and small ciliates in one community vary across latitudes and does this affect the mean cell-size of ciliates community? By answering these questions, we

offer mechanistic (and potentially predictive) explanations that should be valuable for predicting how shifting global temperatures may alter food web structure, and hence biogeochemical processes.

## Material and methods

### Plankton samplings and environmental factors measurements

Samples were taken during 13 research cruises (2009–2018) covering a wide range of tropical, temperate, and subpolar waters (Fig. 1, Supporting information); the 154 stations covered from 20°S to 65°N, across a temperature range of 27°C (3.5–31°C). Sampling also occurred monthly during one-year observations at three subtropical coastal stations adjacent to the South China Sea (Fig. 1, Supporting information). Temperatures at these stations varied seasonally from 13 to 31°C. Samples were collected at the surface at each station and in some cases also at the deep chlorophyll a maximum layer. From these 100–500 ml was preserved with acidic Lugol's solution (final concentration 5%) for ciliates analysis. Seawater temperature was measured (with a precision of 0.01°C) at each sampling. Chlorophyll a concentration (Chl a; a proxy for prey concentration) was determined by filtering 100–500 ml of seawater, following methods outlined by Welschmeyer (1994).

### Ciliates cell size estimates

The size of ciliates was assessed from Lugol's preserved samples following the Utermöhl method at 200× magnification. All ciliates were enumerated and photographed in each sample and identified as aloricate or loricate (i.e. tintinnids) following Montagnes and Lynn (1991) and Strüder-Kypke and Montagnes (2002). Dimensions were determined and volumes were calculated after each cell was assigned a geometrical shape (Hillebrand et al. 1999). In total  $> 2 \times 10^4$  ciliates were included in the analysis (Supporting information). The cell volume was then used to calculate the equivalent spherical diameters ( $ESD$ ) as:  $ESD = (3 \times \text{biovolume} / 4\pi)^{1/3}$ , which is commonly employed in size-structured food web models (Ward et al. 2012, Dutkiewicz et al. 2021) and was more intuitive than cell volume.

As Bergmann's rule is usually applied at the community level, we calculated the mean cell-size of ciliate community ( $\overline{ESD}$ ; of all ciliates) in each sample. As cell sizes tend to follow lognormal distribution and  $\ln ESD$  is theoretically a better metric for size than raw  $ESD$  (Supporting information; Wirtz 2013), we calculated the geometric mean  $ESD$  for all samples:

$$\overline{ESD} = \exp \left( \frac{\sum_{i=1}^n \ln ESD_i}{n} \right) \quad (1)$$

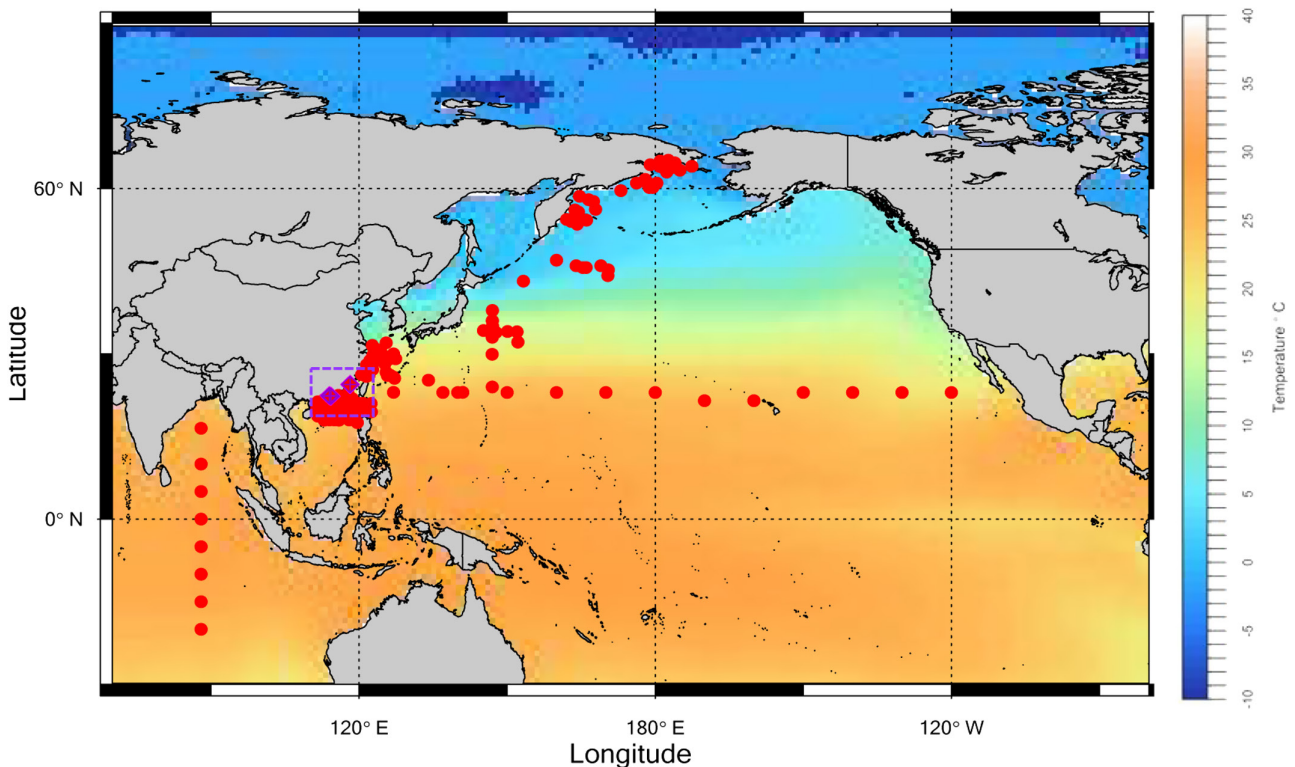


Figure 1. Ocean map with annual mean sea surface temperature (°C). Red dots are the sampling stations ( $n = 154$ ) from 13 research cruises from 2009 to 2018, and purple diamonds in the box show the sites ( $n = 3$ ) where monthly sampling was conducted (the stations in Hong Kong waters overlay in the map).



where  $ESD_i$  is the cell size of  $i$ th ciliate and  $n$  is the total number of ciliates in one sample. To minimize biases, we excluded data from samples that contained fewer than 10 ciliates. Finally, to assess the latitudinal distribution of the sizes of small and large ciliates in one community and whether they affect the community mean cell-size, we calculated the 2.5th and 97.5th percentiles of the data distribution (i.e. the log-transformed size distribution of each sample) to represent the cell size of small and large ciliates in each community, which were termed as  $\ln ESD_{2.5th}$  and  $\ln ESD_{97.5th}$ , respectively.

### Prey (phytoplankton) size estimates

The phytoplankton size was estimated in two relatively novel but established ways: i.e. the phytoplankton size fractions and the phytoplankton mean size, both of which were used when investigating the effect of prey size on the ciliate community mean cell-size.

Phytoplankton size fractions are usually assessed by size-fractionated Chl a concentration (Marañón et al. 2001) which, unfortunately, was only available at a few stations in our study. To bridge the data gap, we estimated the phytoplankton size fractions based on an empirical model that has now widely been used in studies on phytoplankton size structure (Brewin et al. 2010, Ward 2016). In this model, the Chl a concentration of three phytoplankton size classes (picoplankton:  $< 2 \mu\text{m}$ , nanoplankton:  $2\text{--}20 \mu\text{m}$ ; microplankton:  $> 20 \mu\text{m}$ ) were estimated as a function of the total Chl a concentration:

$$\log_{10}(C_s) = \log_{10}(C_s^m) + \log_{10}\left(1 - e^{-\frac{D_s C_{total}}{C_s^m}}\right) \quad (2)$$

in which  $C_s$  is the Chl a concentration of picoplankton ( $C_{pico}$ ) or combined size classes of picoplankton and nanoplankton size ( $C_{nano,pico}$ );  $C_{total}$  is the total Chl a concentration;  $C_s^m$  is the asymptotic maximum values of  $C_s$  at infinitely high  $C_{total}$ ;  $D_s$  denotes the fraction of  $C_s$  to  $C_{total}$  as  $C_{total}$  tends to zero. The values of  $C_s^m$  and  $D_s$  were referenced to Ward (2016). The proportion of picoplankton and picoplankton + nanoplankton were then calculated by  $C_{pico}/C_{total}$  and  $C_{nano,pico}/C_{total}$  respectively. The proportion of microplankton was calculated by  $1 - C_{nano,pico}/C_{total}$ .

Phytoplankton mean size was estimated based on the total phytoplankton carbon and total numerical abundances approximated by picophytoplankton abundances following Chen and Liu (2010). As the total phytoplankton abundance was mainly contributed by picophytoplankton which is one or more orders of magnitude more abundant than other larger phytoplankton (Li 2002), the average phytoplankton carbon per cell was calculated by dividing the total phytoplankton carbon by the picophytoplankton abundance. The average phytoplankton carbon per cell was then converted to biovolume by a conversion factor of  $235 \text{ fg C } \mu\text{m}^{-3}$  (Garrison et al. 2000). The mean  $ESD$  of phytoplankton was calculated via biovolume:  $PhytoESD = (3$

$\times - \text{biovolume} / 4\pi)^{1/3}$ . The total phytoplankton biomass was estimated by Chl a concentration assuming a constant C: Chl ratio of  $50 \text{ gC gChl}^{-1}$ . The picophytoplankton abundance at most stations was measured by flow cytometry. Picophytoplankton samples were collected, preserved with 0.5% buffered paraformaldehyde (v/v, final concentration), and analyzed by a Becton–Dickson FACSCalibur flow cytometer. At some stations in the South China Sea and the Pacific Ocean at  $23^\circ\text{N}$  where the samples were not collected or measured, we used the Boosted Regression Trees model described in Chen et al. (2020) to estimate the picophytoplankton abundance.

### Correlation analysis on the ciliate size and environmental factors

Relationships between size traits of the ciliate community ( $\overline{ESD}$ ,  $\ln ESD_{2.5th}$ , and  $\ln ESD_{97.5th}$ ) and potential environmental factors including Chl a concentration, temperature, latitude, and prey size ( $PhytoESD$  and the proportion of microplankton/picoplankton in total Chl a) were explored first based on Spearman's rank correlation analysis. The ciliate mean size, Chl a concentration, and the  $PhytoESD$  were logarithmically transformed to achieve quasi-normality before calculating Spearman's rank correlation coefficients.

### Generalized linear mixed-effect model: examining the effects of temperature and prey size

To explore which factors affect the ciliate community mean cell-size, we used a model-building approach by fitting a generalized linear mixed-effect model (GLMM, Zuur et al. 2009, Bates et al. 2014). As potential environmental explanatory variables, temperature and phytoplankton size were taken as fixed-effect factors. Given the variation introduced by different observers, we added the random intercept and slope for observers to the model:

$$\overline{ESD}_{i,j} = \alpha + (\beta_T + \theta_{Ti}) \times T_{i,j} + \beta_P \times \ln PESD_{i,j} + a_i + \varepsilon_{i,j} \quad (3)$$

in which  $\overline{ESD}_{i,j}$  is the  $j^{\text{th}}$   $\overline{ESD}$  value by  $i^{\text{th}}$  observer when temperature is  $T_{i,j}$  and the phytoplankton size is  $\ln PESD_{i,j}$ ;  $\alpha$  is the intercept;  $\beta_T$  and  $\beta_P$  is the slope associated with temperature and phytoplankton mean size, respectively;  $a_i$  is the random intercept for observer  $i$ , which is assumed to be normally distributed with a mean of 0 and variance  $\sigma_a^2$ ;  $\varepsilon_{i,j}$  is the  $j^{\text{th}}$  residual in the  $i^{\text{th}}$  group;  $\theta_{Ti}$  is the deviation of observer  $i$  from  $\beta_T$  and is also assumed to follow a normal distribution with a mean of 0 and a variance  $\sigma_b^2$ . We compared models with different random effects structures and found the model with random intercept and random slope of temperature was the best, based on the Akaike information criterion (AIC; Supporting information). The models would be overfitted when adding complex random effect structures with random slopes of the two predictors (Supporting information).

Ecological theory has suggested that the logarithmic cell size instead of the linear size should be used as the trait (Wirtz 2013). As such, a gamma error structure with a log-link function was used in the model.

In addition to the estimated phytoplankton mean size, we also used the proportion of microplankton or picoplankton ( $P_{micro}$  or  $P_{pico}$ ) as a proxy for prey size. We replaced  $\ln PESD_{i,j}$  with  $P_{micro_{i,j}}$  (or  $P_{pico_{i,j}}$ ) and ran the model (i.e. Eq. 3) again. The random effects were also examined for these models (Supporting information). To explore how temperature and prey size affect the cell size of small and large ciliates in communities (i.e.  $\ln ESD_{2.5th}$  and  $\ln ESD_{97.5th}$ , respectively), a linear mixed-effects model instead of GLMM was applied to the data set.

### Predicting future changes in ciliate community mean cell-size based on GLMM

Based on the final GLMM, we predicted the potential changes in the mean cell-size of ciliate community in the future warming ocean. As the ocean warms, the global Chl a concentration has been found to decrease in many Earth System models (Bopp et al. 2013, Kwiatkowski et al. 2020). The decreased Chl a concentration would change the size fractions of phytoplankton and affect the ciliate community mean cell-size. We can estimate the changes in microplankton percentage based on the changes in Chl a concentration. The corresponding model (i.e. M2 in Table 1) was then applied to predict the future changes in ciliate community mean cell-size under several scenarios where ocean surface temperature increases by 1.5, 3, 4.5°C, and the Chl a concentration decreases by 0.02, 0.05, 0.08, and 0.1  $\mu\text{g l}^{-1}$ , assuming other parameters were unchanged.

All analyses were implemented using R ver. 4.1.2 (www.r-project.org). Specifically, GLMM were fitted using the function *glmer* in the *lme4* package (Bates et al. 2014). The conditional and marginal  $R^2$  were calculated by *r.squaredGLMM* in *MuMIn* package (Nakagawa and Schielzeth 2013) to assess the overall goodness of fit and estimate the percentage of variance explained by the GLMMs (Nakagawa et al. 2017).

## Results

Marine ciliates follow Bergmann's rule with larger community mean size at higher latitudes (Fig. 2, Supporting information). The mean cell-size of the ciliate community between 20°S and 65°N was positively correlated with absolute latitude (Spearman correlation  $r=0.183$ ,  $p < 0.01$ ; Fig. 2). The cell size of large ciliates in the community ( $\ln ESD_{97.5th}$ ) was also positively correlated with latitude (Spearman correlation  $r=0.201$ ,  $p < 0.01$ ; Fig. 2), while the small ciliates' size ( $\ln ESD_{2.5th}$ ) was negatively correlated with absolute latitude (Spearman correlation  $r=-0.138$ ,  $p < 0.05$ ; Fig. 2), indicating that the increase in community mean cell-size at high latitudes mainly results from the increase in large ciliates in the community.

The ciliate community mean cell-size also significantly decreased with temperature and increased with prey size (Fig. 2). Ciliate prey size was positively correlated with absolute latitude (Fig. 2). Both the prey (i.e. phytoplankton) mean size and the proportion of microplankton increased with increasing latitude (Fig. 2), which is consistent with previous study suggesting that phytoplankton follow Bergmann's rule (Sommer et al. 2017).

Compared to latitude, temperature is a better predictor for the geographic patterns of ciliate community mean cell-size. Latitude and prey size only explained 8% variations in ciliate community mean cell-size when using them as explanatory variables in the GLMM (i.e. M4 in Table 1). The GLMM with fixed effects for temperature and prey size and random effects for observers explained 39% of the variance in the mean cell-size of ciliate community (Table 1). This model performed well, with marginal multicollinearity and the residuals being distributed approximately normally (Supporting information). Together, temperature and phytoplankton mean size explained 29%, and the random effects explained a further 10% of the variance. When using the percentage of microplankton as the index for prey size, the model explained 33% of the variance in the mean cell-size of ciliate community, of which 27% came from the fixed effects (Table 1). The slope associated with temperature was about  $-0.015 \pm 0.007$  in the two models (Fig. 3a, c, Supporting information).

Table 1. List of GLMMs and associated statistics used in this study. Temperature (or latitude) and the prey size are the fixed-effect factors. Prey size was indicated by the phytoplankton mean size ( $PhytoESD$ ) and the percentage of microplankton or picoplankton fraction in total Chl a ( $P_{micro}$  or  $P_{pico}$ ). All models include the Observer as the random intercept and random slope for temperature. AIC is the Akaike information criterion; BIC is the Bayesian information criterion; logLik is log-likelihood representing the goodness of fit; Marginal  $R^2$  represents the variance explained by the fixed effects; Conditional  $R^2$  represents the variance explained by the entire model including both fixed and random effects; the asterisk represents whether the slope of fixed-effect factor is significantly different from 0 (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ). To explore the effect of temperature and  $P_{micro}$  on  $\ln ESD_{97.5th}$ , a linear mixed-effects model instead of GLMM was used.

Model	AIC	BIC	logLik	Marginal $R^2$	Conditional $R^2$
M1: $\overline{ESD} \sim Temperature^* + PhytoESD^{***}$	1239	1263	-612.7	0.29	0.39
M2: $\overline{ESD} \sim Temperature^* + P_{micro}^*$	1397	1422	-691.5	0.27	0.33
M3: $\overline{ESD} \sim Temperature + P_{pico}^*$	1397	1422	-691.5	0.27	0.33
M4: $\overline{ESD} \sim Latitude + PhytoESD^{***}$	1243	1267	-614.4	0.08	0.29
M5: $\ln ESD_{97.5th} \sim Temperature^{***} + P_{micro}^{**}$	74	92	-32	0.10	0.18

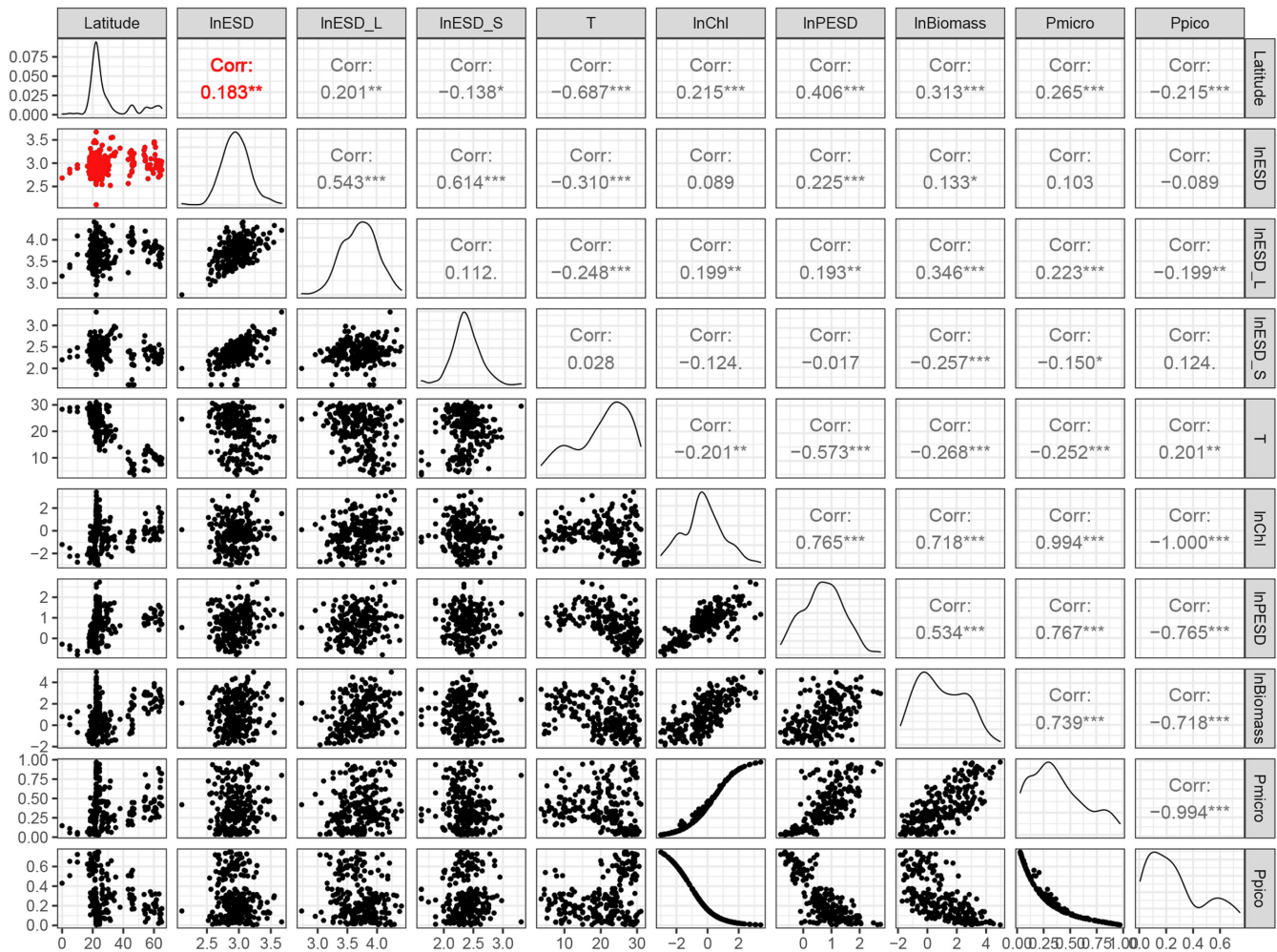


Figure 2. Spearman correlations, statistical distributions, and scatterplots among variables including latitude, ciliate mean size (lnESD), the 2.5th and 97.5th percentiles of the data distribution (lnESD\_S and lnESD\_L), temperature (T), logarithmic transformed Chl a concentration (lnChl), PhytoESD (lnPESD), and the percentage of microplankton and picoplankton (Pmicro and Ppico). The asterisk beside the Spearman correlation coefficient shows the significant levels of correlations (\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001).

The mean cell-size of the ciliate community decreased with increasing temperature (Fig. 3a, c). It decreased by 8.61  $\mu\text{m}$  from 3.5 to 31°C, which is equivalent to a 34% decrease in mean size. Assuming an approximately linear decline, the mean cell-size of ciliate community shrank by 0.31  $\mu\text{m}$  when the temperature increased by 1°C, equating to a decrease of 1.2% °C<sup>-1</sup>.

The mean cell-size of the ciliate community increased with increasing prey size (Fig. 3b, d), where it increased by a factor of 0.09 when the phytoplankton mean size (log-transformed) increased by one unit (Supporting information). The mean size increased by 6.19  $\mu\text{m}$  (~ 35%) across the phytoplankton mean size range (0.6  $\mu\text{m}$  to 15.46  $\mu\text{m}$ ). Using a linear approximation, the mean cell-size of ciliate community would be 0.44  $\mu\text{m}$  larger for each 1  $\mu\text{m}$  increase in phytoplankton mean size. Also, the mean cell-size of ciliate community increased by 3.43  $\mu\text{m}$  (~ 19%) when the microplankton percentage increased from 3 to 97%. For each 1% increase in the percentage of microplankton in total Chl a,

there would be a 0.03  $\mu\text{m}$  increase in the mean cell-size of the ciliate community.

The size of large ciliates in the community also decreased with increasing temperature and increased when microplankton were more abundant in the phytoplankton community (Fig. 4). Based on the model, the large ciliates decreased by 9.47  $\mu\text{m}$  (~21%) in cell size across the temperature range of 27°C. There was a 0.7% °C<sup>-1</sup> (i.e. 0.34  $\mu\text{m}$  °C<sup>-1</sup>) shrinkage in the cell size of large ciliates. When microplankton prey ranged from 3 to 97%, the large ciliates increased by 10.49  $\mu\text{m}$  (~29%). For each 1% increase in microplankton percentage, the large ciliate would be 0.11  $\mu\text{m}$  larger in cell size.

Based on the GLMM (M2 in Table 1), the predicted ciliate community mean cell-size significantly decreased with increasing temperature and decreasing Chl a concentration (Fig. 5). The ciliate community mean cell-size was predicted to decrease by 6.9% (from 19.4 to 18.1  $\mu\text{m}$ ) under the scenario where ocean surface temperature increases by 4.5°C and Chl a concentration decreases by 0.1  $\mu\text{g l}^{-1}$  (Fig. 5).



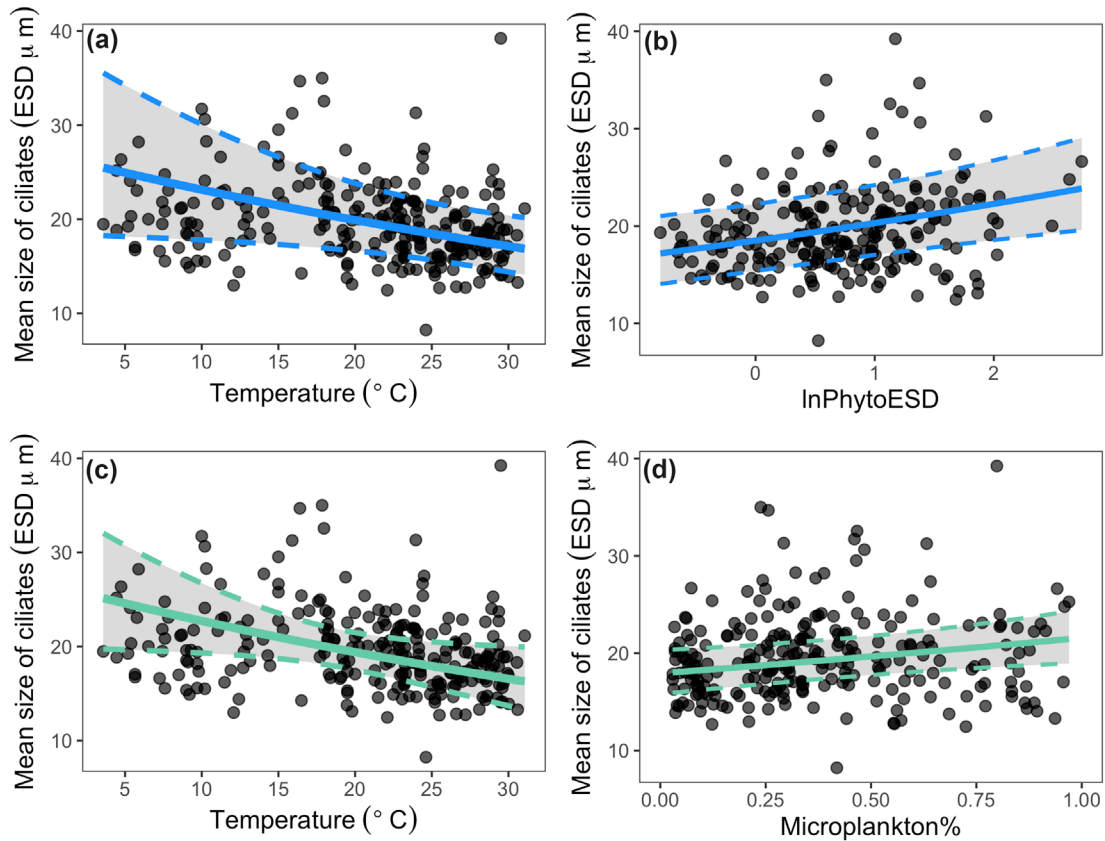


Figure 3. Relationship between mean cell-size of ciliate community and temperature (a), (c) and phytoplankton size (b), (d). Blue solid lines in (a) and (b) represent expected values of mean size based on the GLMM with temperature and estimated phytoplankton mean size as predictors (M1;  $R^2=0.39$ ,  $n = 223$ ). Green solid lines in (c) and (d) represent expected values of mean size based on the GLMM with temperature and the percentage of microplankton as predictors (M2;  $R^2=0.33$ ,  $n=249$ ). The grey band represents the 95% confidence interval for the expected values.

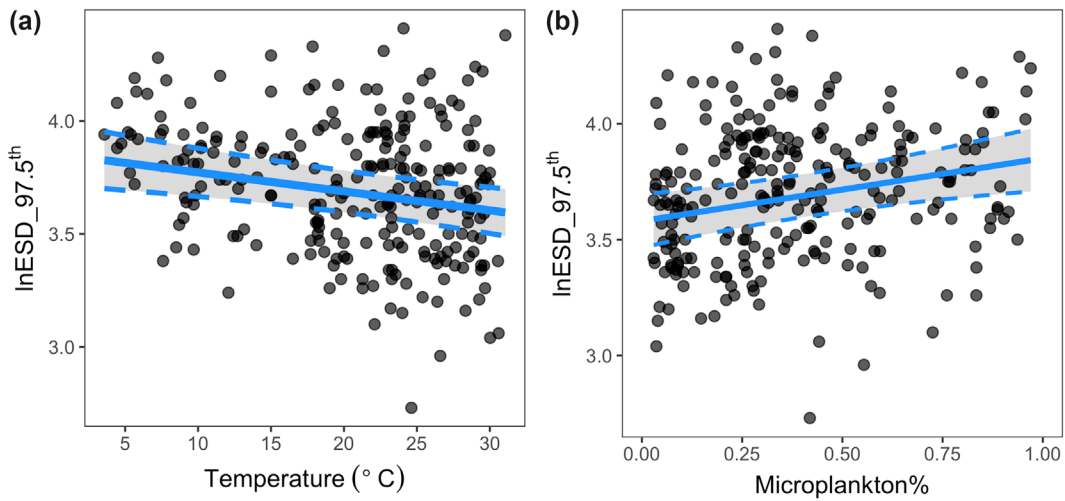


Figure 4. Relationship between the 97.5th percentiles of the size distribution in ciliate community and temperature (a) and the percentage of microplankton (b). Green solid lines represent expected values of the large ciliate size based on the linear mixed-effect model with temperature and the percentage of microplankton as predictors (M5;  $R^2=0.18$ ,  $n = 249$ ). The grey band represents the 95% confidence interval for the expected values.

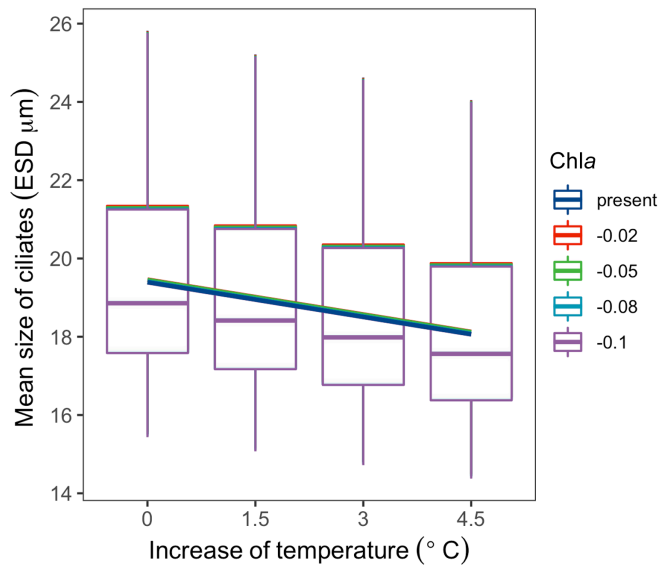


Figure 5. Predicted mean cell-size of ciliate community ( $\overline{ESD}$ ,  $\mu\text{m}$ ) under scenarios of increases in temperature and decreases in Chl a concentration. The values are displayed by a box and whisker plot. The top and bottom of the box are the 75th and 25th percentiles, respectively and the horizontal line are the median value. The whiskers represent the smallest and largest values less than or no greater than the 1.5 time of the interquartile range. The lines represent the trends between the mean of predicted values and temperature under each Chl a scenario with the corresponding colour.

## Discussion

Our results revealed a significant positive relationship between ciliate community mean cell-size and latitude (Fig. 2), extending Bergmann's rule to marine planktonic ciliates. Our evidence is robust because the dataset consisted of a wide variety of samples from tropical, temperate, and sub-polar waters (Fig. 1). In contrast to previous studies focusing on comparing the abundance of size-fraction compositions of ciliates (Wang et al. 2020) or intraspecific patterns in ciliates size (Atkinson et al. 2003), our study estimated the ciliate mean cell-size at the community level by measuring the sizes of all ciliate cells in one community (i.e. in one sample, Supporting information). This method allowed us to uncover the interspecific patterns whereby Bergmann's rule was originally proposed (Bergmann 1847), and is, therefore, reliable for examining Bergmann's rule in the underexplored marine planktonic ciliates.

### Potential drivers of Bergmann's rule in marine planktonic ciliates

Temperature is a crucial driver for the latitudinal size cline of ciliate community as we found a strong inverse relationship between their mean size and temperature (Fig. 2, 3). The decline in ciliate mean cell-size at the community level could be attributed to both intraspecific and interspecific changes in size. According to the temperature size rule, ectotherms living at warmer temperatures matured at smaller

body sizes because warming reduces their growth efficiency (Atkinson 1994, Atkinson and Sibly 1997). As temperature rises, the energy loss by catabolism might be greater than that gained by anabolism, leading to decreases in growth efficiency (Atkinson and Sibly 1997, Perrin 1995). Becoming smaller could also be an adaptive strategy for ectotherms to reduce energy loss in response to the warming environments. In addition, marine planktonic ciliates may reduce their cell size to compensate for the decreased ratio of supply to resource demand in the context of increasing temperature (Atkinson et al. 2003). Although the demand for resources is highly temperature-sensitive, the diffusion of dissolved oxygen is relatively inert to the temperature changes, causing an insufficient supply of dissolved oxygen with respect to resource demand. As a counter-strategy, a reduction in size provides an increase in resource uptake and compensate for the imbalance between supply and demand by enhancing the surface-to-volume ratio. Such phenotypic plasticity at the individual level may reduce ciliate community size in the warming ocean, and it is a promising avenue for further study (Evans et al. 2020).

The interspecific changes – a shift of community structure with more smaller species in warmer environments – could be another reason for the decrease in ciliate community mean cell-size with warming (Daufresne et al. 2009). For instance, a recent study has found that the small-sized ciliates (10–30  $\mu\text{m}$ ) are more abundant in the tropical West Pacific than sub-polar Bering Sea (Wang et al. 2020). Unfortunately, the lack of detailed taxonomic information in our dataset hinders us from testing the interspecific shift hypothesis. However, as a first attempt to address this question, we divided ciliates into two groups (i.e. aloricate and tintinnids). The aloricate ciliates are generally smaller than tintinnids (Supporting information) and are intuitively supposed to occupy a larger proportion in warmer environments (Daufresne et al. 2009). However, we did not observe any obvious increase in the abundance of the aloricate subgroup in the community with increasing temperature. In most cases, the aloricate ciliates dominated the community, while the tintinnids only accounted for a small portion (Supporting information), which is in line with the previous observations (McManus and Santoferrara 2013, Weisse and Montagnes 2021). Meanwhile, we found that the proportion of tintinnids did not vary with temperature. Thus, we deduce that the mean size of ciliate community is mainly determined by aloricate ciliates. For both aloricate ciliates and tintinnids, their mean size also decreases with increasing temperature, exactly as the communities (Supporting information). These consistent patterns support Bergmann's rule in these two main subgroups of planktonic ciliates.

In addition, we found that the size of large ciliate (i.e.  $\ln\text{ESD}_{97.5th}$ ) decreased with increasing temperature, whereas the size of small ciliate (i.e.  $\ln\text{ESD}_{2.5th}$ ) remained unchanged with temperature (Fig. 2, 4). It is most likely that the ciliate communities shift from more larger taxa/species to smaller ones as temperature increases. As such, the large ciliates in one community are smaller species in warmer waters,



which contributes to the reduction in the mean cell-size of ciliate community. This speculation is supported by studies on phytoplankton that found larger mean cell size of phytoplankton in colder water was due to the larger contribution of large phytoplankton rather than the absence of the small ones. (Chisholm 1992, Irigoien et al. 2004, Sommer et al. 2017). Nevertheless, due to the lack of taxonomic information, we cannot further examine whether the large ciliates in these communities are the same species that reduce their size in warmer waters or distinct smaller species. Although it is exceptionally labour intensive, we encourage future studies to assess the full biodiversity of the community to resolve the intraspecific and interspecific changes for further understanding their contributions to the inverse relationship between temperature and the ciliate community mean cell-size.

Another factor supporting Bergmann's rule for marine planktonic ciliates is their prey size, represented by phytoplankton size in our study. Although some ciliates feed on bacteria, bacterivorous ciliates are rare in oligotrophic and mesotrophic oceans – they are only trophically important in eutrophic waters where bacteria are abundant (Pierce and Turner 1992, Weisse and Montagnes 2021), and thus our omission of bacterial size and abundance will have little to no consequence. In addition, assuming a linear predator prey ratio of 8 : 1, there would be no ciliate that prefers to feed on bacteria (~0.5 µm). Critically, we reveal that the mean cell-size of ciliate community was positively correlated with phytoplankton mean size (Fig. 2). When controlling the effect of temperature in the GLMM, the relationship between ciliate community mean cell-size and prey size is still significantly positive (Fig. 3). As phytoplankton size increases with increasing latitude following Bergmann's rule (Sommer et al. 2017), being positively correlated with phytoplankton size indirectly drives the latitudinal size clines of ciliate community. In addition, the cell-mean size of ciliate community increased when more microplankton were available (Fig. 3). Likewise, the ciliate community mean cell-size became smaller when there were more picoplankton (Supporting information). The consistent results of our two methods on phytoplankton size estimates strongly support the importance of prey size in shaping the latitudinal size cline of ciliate community (Azam et al. 1983, Ward et al. 2014).

However, it is worth noting that the phytoplankton size is also significantly correlated with temperature (Fig. 2). We examined the collinearity between the explanatory variables in GLMM (i.e. temperature and prey size) and indicated that the collinearity was marginal (Supporting information; Zuur et al. 2009). Therefore, by using the GLMM, our study revealed the significant effects of temperature and prey size on the latitudinal size cline of ciliate community. In stark contrast with previous studies focusing exclusively on temperature (Walters and Hassall 2006, Evans et al. 2020), we emphasized the equal importance of prey size. In addition to prey size, other biotic factors such as food quality (Ho et al. 2010), food availability (Campbell et al. 2021), and predation risk (Manyak-Davis et al. 2013) also play a considerable role in shaping the latitudinal size clines, although they have

been largely overlooked (Ho et al. 2010). Such biotic factors usually interact with temperature to drive the geographic patterns of body size. For instance, the latitudinal decline of phytoplankton cell size was driven by not only temperature but also grazing and nutrient availability (Sommer et al. 2017). Consequently, using the combination of biotic and abiotic factors can better explain Bergmann's rule rather than any single factor.

Moreover, mixotrophy could be another potential factor affecting protists community mean cell-size, especially for ciliates. Many planktonic ciliates sequester the chloroplasts of their prey and use them to obtain energy through photosynthesis (Weisse and Montagnes 2021). The degree to which ciliates use mixotrophy is highly variable, with the dominant oligotrichs mostly being mixotrophic to varying degrees and their equally dominant sister group, the choreotrichs, being strict heterotrophs. Our analysis was not sufficiently detailed to even make this distinction, although as both groups feed on similarly sized prey and likely follow the 8 : 1 predator:prey ratio, they would undoubtedly be subject to similar size-restricting pressures associated with the decrease in prey size, described above. Likewise, as phytoplankton tend to follow Bergmann's rule, we might expect that ciliates acting autotrophically will similarly follow the rule. Nevertheless, more detailed data is required to verify the impact of mixotrophy and we would encourage future, more detailed analysis of ciliate biodiversity to be linked to the potential for taxa to act as mixotrophs – to varying degrees – and then these data to be used in conjunction with temperature to assess the interaction of mixotrophy and temperature on cell size.

### Implications for marine food web in warming oceans

Bergmann's rule depicts the spatial patterns in body size, which may not necessarily occur through time. Nevertheless, in ecological modelling, space-for-time substitution is widely used and has proved to be a robust approach for modelling responses to climate change (Blois et al. 2013). Our model that generalizes the effects of temperature and prey size on ciliate community mean cell-size can provide insights into the future changes in marine planktonic ciliates. Consistent with the third universal ecological response to climate warming, i.e. body size reductions (Daufresne et al. 2009, Gardner et al. 2011), we also observed a significant decrease in ciliate size (~ 34%) across a 27°C temperature range (Fig. 3). According to the Coupled model intercomparison project phase 6 (CMIP6), under high-emission scenario SSP5-8.5, sea surface temperature is projected to increase by ~ 3.47°C by 2080–2099 (mean values compared to 1870–1899); under low-emission, high-mitigation scenario SSP1-2.6, a ~ 1.42°C increase in the sea surface temperature is predicted (Kwiatkowski et al. 2020). Based on our estimates, a 3.47°C increase in sea surface temperature would lead to a 4.2% shrinkage in ciliate mean size. Under the SSP1-2.6, the mean size of ciliate may decrease by 1.7% when the temperature increases by 1.42°C.

In addition to the separate effect of temperature, changes in the prey size also help shape the ciliate size structure in the warmer ocean (Fig. 3). As small phytoplankton are predicted to proliferate in warmer oceans (Morán et al. 2010, Yvon-Durocher et al. 2011), ciliates may confront more smaller prey in the future ocean. Most Earth System models project a decline in primary production and Chl a along with warming (Bopp et al. 2013, Lefort et al. 2015, Kwiatkowski et al. 2020). We estimated the changes in the size fractions of phytoplankton (i.e. the percentage of microplankton) based on the projected Chl a change (Eq. 2; Ward 2016). Under the SSP5-8.5 and SSP1-2.6, the primary production is predicted to decrease by ~ 3 and ~ 0.6%, respectively (Kwiatkowski et al. 2020). According to the empirical equation in Marañón et al. (2014), such reductions in primary production are roughly equal to a global decrease in Chl a of 0.03 mg m<sup>-3</sup> and 0.006 mg m<sup>-3</sup>, respectively. Combining the effect of temperature and prey size, the 3.47°C warming and 0.03 mg m<sup>-3</sup> decrease in Chl a under the SSP5-8.5 can lead to a 5% shrinkage in ciliate mean size globally (Fig. 5). Under the SSP1-2.6, 1.42°C warming and 0.006 mg m<sup>-3</sup> decrease in Chl a can reduce the ciliate mean size by ~ 1.8% (Fig. 5). Such a decline in ciliate community mean cell-size will result in a decrease in global microzooplankton biomass in warming oceans, assuming abundance remains unchanged.

Reduction in ciliate community mean cell-size with rising temperature may lead to several undesirable consequences. Shrinkage in ciliate size not only affects secondary productions (Atkinson et al. 2003), it also influences the trophic interactions and biogeochemical processes that are size-dependent in marine ecosystems (Beaugrand et al. 2010). The smaller size may lead to longer food chains, depressing the energy transfer efficiency through marine food webs. As important food sources of mesozooplankton (Calbet and Saiz 2005), a decrease in ciliate size may also affect their predators, leading to declines in the body size of predators. It has been found that marine copepods that dominated mesozooplankton also reduce their body size as temperature increases, following Bergmann's rule (Evans et al. 2020, Campbell et al. 2021). Such decreases in copepod body size could be attributed to their smaller prey including ciliates in the warmer ocean, although the effects of prey size have never been examined in copepods. Smaller copepod body size would further exert negative effects on global fisheries and biological carbon pump (Beaugrand et al. 2010, Sheridan and Bickford 2011, Cavan et al. 2019). These potential consequences triggered by the decrease in ciliate size with warming will provide feedback to climate change (Pörtner et al. 2019).

## Conclusions

In this study, we test Bergmann's rule in marine planktonic ciliates by investigating the geographic patterns in the mean cell-size of ciliate community and provide comprehensive evidence for their Bergmann's clines. We found that temperature and prey size drive the latitudinal size cline of the

ciliate community and emphasized the importance of both biotic and abiotic factors in driving the large-scale patterns of ciliate size. We estimated a 1.2% °C<sup>-1</sup> reduction in ciliate community mean cell-size and projected a 5% shrinkage in ciliate community mean cell-size under high-emission scenario SSP5-8.5. Such a decrease in the ciliate size with warming may result in a series of undesirable ecological consequences such as reducing fish resources and weakening the ocean's capacity as a carbon sink.

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## Author contributions

**Kailin Liu:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Resources (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Siyu Jiang:** Data curation (equal); Resources (equal); Writing – review and editing (equal). **David J. S. Montagnes:** Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Hongbin Liu:** Conceptualization-Supporting, Formal analysis (equal); Funding acquisition-Supporting, Project administration-Supporting, Resources-Supporting, Writing – review and editing (equal). **Liping Zheng:** Data curation (equal); Resources (equal); Writing – review and editing (equal). **Bangqin Huang:** Data curation (equal); Funding acquisition-Supporting, Resources-Supporting, Writing – review and editing-Supporting. **Xin Liu:** Data curation (equal); Funding acquisition-Supporting, Resources-Supporting, Writing – review and editing-Supporting. **Bingzhang Chen:** Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (lead); Resources-Supporting, Supervision (lead); Visualization-Supporting, Writing – original draft-Supporting, Writing – review and editing (equal).

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## Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.j0zpc86jt> (Liu et al. 2023).

## Supporting information

The Supporting information associated with this article is available with the online version.

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