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Body size and shape responses to warming and resource competition

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Abstract

1. Body size is a fundamental trait that impacts many aspects of species biology and ecology. It is, in turn, influenced by a suite of environmental factors, and often decreases with warming. Although environmental conditions can also impact body shape, which is another functional trait that influences locomotion, resource acquisition and potentially physiological processes, such responses are poorly understood and rarely quantified.
2. We experimentally tested the independent and combined effects of environmental temperature, resource level and interspecific competition on the body shape and size responses of two model protist species. We also tested the degree to which these individual-level phenotypic responses are associated with population densities and species coexistence.
3. Body shape was strongly influenced by resource competition, whereas body size changes were mainly driven by environmental temperature. In both species, lower resource levels resulted in body shape elongation, suggesting that relatively more elongate individuals with potentially higher swimming speed were advantaged in the resource scarce environment. However, competition had contrasting influence on the body shape of the two species. Competition decreased the population densities of *Blepharisma japonicum*, which exhibited relative body shape elongation, similar to the response at low resource levels. In contrast, competition increased the population densities of *Paramecium aurelia*, which exhibited reduced elongation similar to body shape response at high resource levels. Hence, body shape responses could be indicative of changes in resource availability, aiding our understanding of competitive hierarchies and species interactions.
4. Coexistence was observed in all treatment combinations, likely because body size of both species decreased similarly under warming, potentially maintaining constant per capita competitive intensity. These findings, along with recent research on phytoplankton, diverse pelagic invertebrates, and birds highlight the importance of body shape and morphology across different taxonomic groups. Hence, we call for body size and shape to be considered in concert when investigating ecological consequences of climate warming.

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KEYWORDS
body shape, body size, climate warming, resource competition, species coexistence

1 | INTRODUCTION

Climate warming is characterised by a rapid rise in global mean temperatures (IPCC, 2018) and an increase in the frequency and severity of extreme temperature events (Easterling et al., 2000; Meehl, 2004), which have profound impacts on global biota (Vasseur et al., 2014; Woodward et al., 2016). These changes have already shifted the distributional ranges and phenology of many species (Parmesan & Yohe, 2003), and are widely recognised as two common responses to global warming (Daufresne et al., 2009; Evans et al., 2020). More recently, reduction in body sizes has emerged as a third universal ecological response to mean increase in global temperatures (Daufresne et al., 2009; Evans et al., 2020; Sheridan & Bickford, 2011), and also potentially to extreme temperature events (Fischer et al., 2014). Such body size reductions could have profound ecological repercussions because this fundamental trait (Peters, 1983) underlies metabolic rates (Brown et al., 2004), fecundity (Arendt, 2007), species interactions and community dynamics (Brose et al., 2006; Rall et al., 2012). Hence, understanding and ultimately predicting the body size response of organisms to both long- and short-term warming is a crucial and globally important ecological challenge.

Most ectotherms mature at smaller body sizes when grown under warmer conditions (Atkinson, 1994; Atkinson et al., 2003; Forster et al., 2012). This inverse relationship between body size and temperature, the 'temperature–size rule' (TSR), is one of the most widespread phenomena in biology (Angilletta et al., 2004). It is commonly observed in organisms as diverse as bacteria, protists, invertebrates and ectothermic vertebrates (Atkinson et al., 2003; Daufresne et al., 2009; Forster et al., 2012; Horne et al., 2015). However, availability of resources also influences body size by providing the material and energy for maintenance, growth and reproduction (Sterner & Elser, 2002). In general, body size-at-stage increases at higher resource levels (Balčiūnas & Lawler, 1995), but this effect depends on the environmental temperature and community context (Tabi et al., 2019).

Body shape is also an important functional trait that directly influences motility (Beveridge et al., 2010; Gibert et al., 2017), encounter rates, and, thus species interactions (Berger, 1980). Body shape responses to changing resource level and environmental temperature have been observed in phytoplankton (Naselli-Flores & Barone, 2011), cyanobacteria (Jezberová & Komárková, 2007) and protists (DeLong et al., 2017; Gibert et al., 2017; Hammill et al., 2010). For instance, high resource levels have been found to increase protist body length (relative to other body axes), resulting in individuals being more elongate in shape (Gibert et al., 2017). Such changes likely reduce drag and improve motility (Roberts, 1981), which can increase prey encounters and hence resource uptake rates (Gibert et al., 2017). The property of body shape being conserved as size increases is termed isomorphy. By contrast, non-isomorphic 'shape-shifters' (Hirst et al., 2014) include species which become relatively more elongate (along the longest body axis, relative to other dimensions) or more flattened (more elongate in the two longest dimensions relative to the third) as they grow over ontogeny. Such body shape changes also increase the scaling of surface area to body mass compared to that achieved in isomorphic species. Increased magnitude of shape change over ontogeny has been related to increased body-mass scaling of metabolic rates in pelagic invertebrates that use their body surface for exchange of oxygen (Glazier et al., 2015; Hirst et al., 2014; Tan et al., 2019). This relationship between mass-scaling of surface area and of metabolic rate suggests that the relationship between body shape and size could underlie important physiological differences among taxa. Findings that relate morphological form to ecological function in birds (Pigot et al., 2020) further indicate that shape has broad biological and ecological implications across a wide range of taxonomic groups. However, in contrast to body size, body shape responses to environmental change are rarely studied, and it remains unknown whether and how body shape changes under the independent and combined effects of warming and resource availability.

Whereas previous work has focused on responses to abiotic variables, competition is a fundamental driver of community structure, which alters resource availability (Fox, 2002), and interacts with environmental temperature (Jiang & Morin, 2004; Lewington-Pearce et al., 2019). As higher temperatures tend to increase metabolic rates up to the thermal optimum of an organism (Brown et al., 2004), warming can increase per capita competitive intensity (Jiang & Morin, 2004) and indirectly influence both body size and shape through reducing resource availability. Hence, understanding individual responses to temperature and nutrient level in the context of competitive interactions is critical to predicting community and ecosystem consequences of climate warming (Gilman et al., 2010).

Here, we experimentally tested the independent and combined effects of short-term warming, resource availability and interspecific competition on the phenotypic changes in body size and shape of two protist species. Protists are ideally suited for controlled and replicated analyses of body size and shape responses to environmental change, as these traits are phenotypically plastic (Atkinson et al., 2003; Gibert et al., 2017) and responses can be observed after a few days of experimental manipulation (Atkinson et al., 2003). Protists are commonly used as model organisms in population and community ecology (Altermatt et al., 2015), and form an important component of natural food webs (Sherr & Sherr, 2002). Hence, an improved understanding of individual responses, which are rarely considered in previous research (Tabi et al., 2019), is important for connecting individual-level responses to changes in population and community dynamics (Atkinson et al., 2003).

We tested the hypotheses that (a) organisms will be more elongate in shape at low resource level as elongation increases swimming speed and reduces locomotion cost (Roberts, 1981), and this effect will be accentuated by interspecific competition (resource-shape hypothesis), and (b) a warmer environment will result in reduced body size (TSR), and that size reduction will be exacerbated at low resource level and under interspecific competition. We also investigated (c) whether temperature affects interspecific competition, which is governed by the temperature dependence of metabolic rates, per capita consumption rates, carrying capacity and maximum growth rate of resources (Gilbert et al., 2014), and whether (d) body shape, which plays a role in resource acquisition will, in turn, influence population densities. Our results show that body size changes are closely related to whole-organism metabolic demands, whereas body-shape changes are governed mainly by resource acquisition.

2 | MATERIALS AND METHODS

2.1 | Experimental design

To partition how experimental warming, resource level and interspecific competition influence body size and shape, we used two bacterivorous ciliate protozoa *Paramecium aurelia* and *Blepharisma japonicum*. These two species compete for common bacterial resources (Clements et al., 2013) and are morphologically distinct, which facilitates accurate identification. We obtained *P. aurelia* and *B. japonicum* as monocultures from Sciento (Manchester, UK). We maintained the single-species cultures at 21°C in protozoa medium prepared by filtering 0.56 g/L of crushed protozoan pellets (Blades Biological Ltd.) in Volvic spring water through double-layered Rombouts no. 4 coffee filters (Hammill et al., 2010). We maintained stock cultures in conical Erlenmeyer flasks containing 100 ml of autoclaved protozoan medium and a single wheat grain. While *B. japonicum* is able to form enlarged predatory morphs, these morphs tend to occur at low nutrient levels (less than half the concentration used here; Clements et al., 2013), and were not observed in our experiment. Thus, we only considered competitive interactions in this study (Clements et al., 2013).

For the competition experiment, we maintained populations of *P. aurelia* and *B. japonicum* in single-species cultures and compared their population dynamics with those in polycultures containing both species. We also factorially manipulated temperature (22 and 25°C) and resource level (low and high), to determine how body shape and size were influenced. The cultures were kept in Stuart orbital incubators (model SI500). Whereas 22°C is a common maintenance temperature for protist cultures, 25°C represents a modest scenario of 3°C global climate warming projected by IPCC (2018). The two bacteria resource levels (Supporting Information 1, Figure S1) were established by adding different amounts of protozoan pellet into the protozoa medium (0.28 and 0.56 g/L spring water, Fox, 2002). Each of the 12 treatment combinations was replicated six times, resulting in 72 experimental microcosms in total. We initiated single-species treatments by seeding 100 individuals of either *P. aurelia* or *B. japonicum* in 4 ml of protozoa medium. At the same time, we initiated two-species treatments by seeding 50 individuals of each species in 4 ml of protozoa medium. Microcosms were maintained in the dark for 11 days resulting in approximately 20–30 generations, as generation times for the two species are on the scale of 2–3 generations per day (Sonneborn, 1970). In all treatment combinations, population sizes of both species reached approximate steady state within 6–9 days (Supporting Information 1, Figure S2).

To monitor population growth, we collected samples daily during the first week of the experiment, and then every two consecutive days. At each sampling event, we subsampled 0.1 ml of medium and fixed it with a final concentration of 0.5% Lugol's media (Sherr & Sherr, 1993). We pipetted this subsample along the edge of a Bogorov counting chamber, and counted all individuals present under a Nikon SMZ1500 dissecting microscope to estimate population densities. After each sampling, we replaced the volume sampled with 0.1 ml of fresh medium. We also added 0.2 ml and 0.5 ml of fresh media on day 4 and day 7, respectively, to control for any resource depletion and evaporative water loss, allowing to maintain bacterial resources (Supporting Information 1, Figure S1).

To measure the body size and shape of protists at the end of the experiment, we fixed 0.5 ml of subsample from each microcosm with a final concentration of 0.5% Lugol's solution. We then photographed an average of 10 individuals of each species from each microcosm under a Leica DMIL inverse microscope at 40× magnification. Lugol's media was reported to have minimal effect on *P. aurelia* cell width (Hammill et al., 2010), and has also been previously used for measuring *Blepharisma* spp. size (Chapman, 2016). We measured body length and width using ImageJ analysis software (Schneider et al., 2012), calculating body size as biovolume from the length and width measurements and assuming a standard geometric shape of a prolate spheroid (Chapman, 2016; DeLong & Vasseur, 2012; Forster et al., 2013). We then obtained the mean body length, width and estimated size (biovolume) of each species for each replicate derived from 9 to 11 photographed individuals. We refer to cell size and shape as body size and shape, as it is understood more broadly in the context of the TSR (Adams et al., 2013).

2.2 | Statistical analyses

To compare the phenotypic responses of both species across all treatment combinations, we applied a factorial ANOVA with the trait of interest (*P. aurelia* or *B. japonicum* length:width ratio, body size and population density at the end of the experiment) as the response variable, and temperature, resources, interspecific competition and their interactions as the explanatory variables. We used linear regression to test for a relationship between population density and body size, and between population density and length:width ratio. All statistical analyses were performed in R statistical software version 3.6.0 (R Core Team, 2019). We detected unequal variances in the body size data of both species, but using heteroscedasticity-consistent errors with the 'white.adjust' argument in the *Anova* function in the package CAR (Fox & Weisberg, 2019) did not alter our results. We therefore report the results of our factorial ANOVA. We also quantified the temperature–size response from the formula ($\exp^{(\text{slope})} - 1$) × 100 = % change in body size per °C (Forster et al., 2012), where the slope was derived from the regression of [natural log] ln (individual body size) against temperature.

3 | RESULTS

3.1 | Body shape

The length:width ratio of *P. aurelia* was significantly influenced by resources ($p < 0.001$) and competition (i.e. presence of *B. japonicum*, $p = 0.011$, Table 1; Figure 1). At low resource level, length:width ratios were higher, indicating that individuals are relatively more elongate in form when resources are scarce, as predicted by the resource-shape hypothesis. However, interspecific competition reduced the length:width ratio of *P. aurelia* (Figure 1a), producing a less elongate body shape. Hence, interspecific competition did not accentuate body shape response observed at low resource level, partially contradicting the resource-shape hypothesis. There was a marginally significant three-way interaction effect between warming, competition and resource level on length:width ratio of *B. japonicum* ($p = 0.049$, Table 1; Figure 1b). Competition and resources generally increased the length:width ratio (Figure 1b), whereas warming reduced this ratio. Hence, in agreement with the resource-shape hypothesis, low resource levels favoured a relative elongation in the shape of *B. japonicum*, and competition further increases this shape elongation.

3.2 | Body size

In agreement with the TSR, body size of both *P. aurelia* ($p < 0.001$) and *B. japonicum* ($p < 0.001$) significantly declined with experimental warming (Table 1; Figure 1c,d). Interspecific competition and resource level interactively influenced *P. aurelia* body size ($p = 0.033$, Table 1). Their body size decreased under interspecific competition, but only at low resource level (Figure 1c). Low resource level also reduced the body size of *B. japonicum* ($p < 0.001$, Table 1; Figure 1d), but interspecific competition did not influence the effect of resources on body size of this species ($p = 0.117$, Table 1; Figure 1d). Contrary to our hypothesis based on the TSR, the effect of experimental warming on body size was not exacerbated by low resource level in both species ($p = 0.684$, *P. aurelia*; $p = 0.406$, *B. japonicum*; Table 1; Figure 1c,d).

TABLE 1 Summary statistics from an analysis of variance (ANOVA) testing the independent and interactive effects of experimental warming, resource level and interspecific competition on body shape, body size and population density of both model species. Body shape was approximated as length:width ratio

	Paramecium aurelia			*Blepharisma japonicum*		
Response/treatment	***df***	***F***	***P***	***df***	***F***	***p***
Body shape						
Competition	1, 40	7.13	0.011	1, 40	13.49	0.001
Resource	1, 40	23.16	<0.001	1, 40	7.98	0.007
Temperature	1, 40	0.62	0.435	1, 40	12.12	0.001
C × R	1, 40	0.06	0.803	1, 40	6.16	0.017
C × T	1, 40	0.06	0.812	1, 40	0.04	0.84
R × T	1, 40	0.04	0.837	1, 40	0.41	0.524
C × R × T	1, 40	0.15	0.701	1, 40	4.12	0.049
Body size						
Competition	1, 40	1.52	0.224	1, 40	2.79	0.103
Resource	1, 40	0.96	0.334	1, 40	50.11	<0.001
Temperature	1, 40	17.90	<0.001	1, 40	17.38	<0.001
C × R	1, 40	4.89	0.033	1, 40	2.56	0.117
C × T	1, 40	0.91	0.347	1, 40	2.69	0.109
R × T	1, 40	0.17	0.684	1, 40	0.70	0.406
C × R × T	1, 40	3.71	0.061	1, 40	0.40	0.533
Population density						
Competition	1, 40	8.67	0.005	1, 40	19.41	<0.001
Resource	1, 40	10.56	0.002	1, 40	0.90	0.348
Temperature	1, 40	4.01	0.052	1, 40	0.72	0.402
C × R	1, 40	10.29	0.003	1, 40	2.45	0.125
C × T	1, 40	0.58	0.451	1, 40	0.84	0.364
R × T	1, 40	0.03	0.871	1, 40	0.14	0.706
C × R × T	1, 40	0.23	0.637	1, 40	0.99	0.326

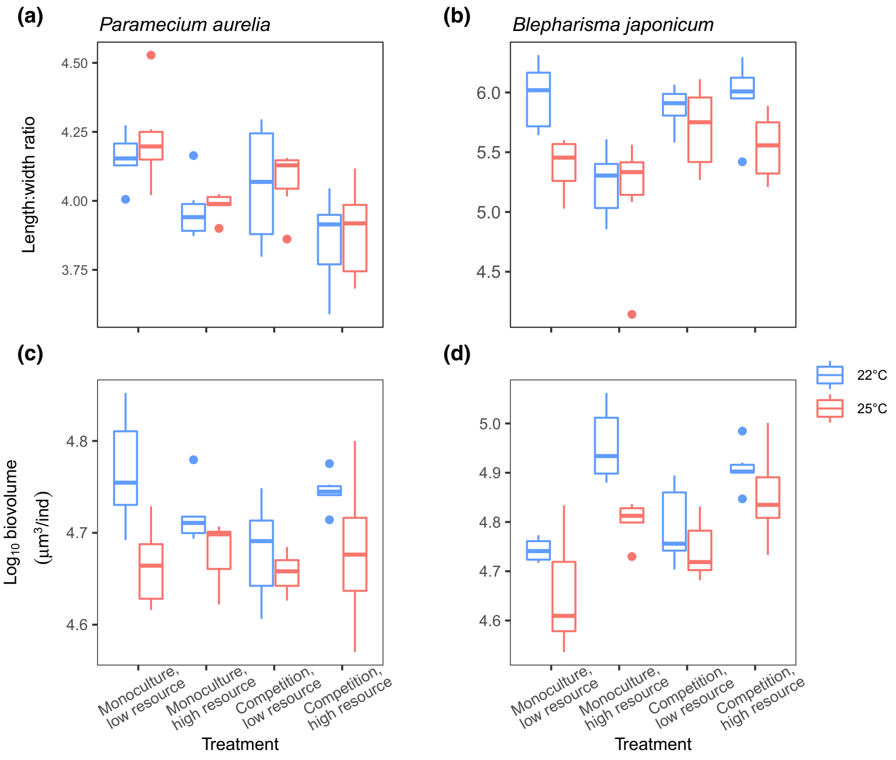

FIGURE 1 Body shape and body size responses, with greater length:width ratios indicating the elongation of *Paramecium aurelia* and *Blepharisma japonicum* across all experimental treatment combinations. (a) Resources and competition significantly influence the body shape of *P. aurelia*. At low resource level, body shape is more elongated at a given body size while competition reduces the extent of elongation. (b) In *B. japonicum*, experimental warming reduces the length:width ratio, indicating less elongated body shape. Competition and resource supply interactively influence body shape, with competition favouring elongation, but only at high resource level. (c) Experimental warming reduces *P. aurelia* biovolume. Interspecific competition also reduces the body size of *P. aurelia*, but only at low resource level. (d) Experimental warming and low resource level significantly reduce *B. japonicum* body size

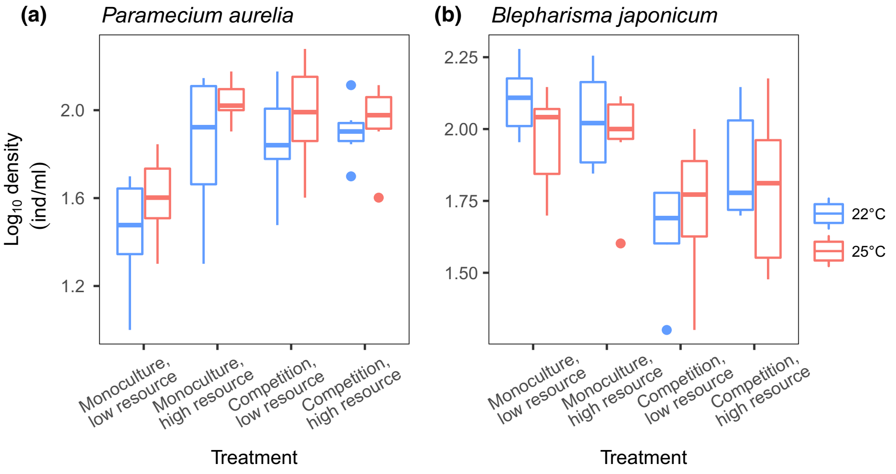

FIGURE 2 The mean body size and population density of two model species across all experimental treatment combinations. (a) *Paramecium aurelia* population density is significantly affected by the interactive effect of resource and competition. Interspecific competition increases the population density of *P. aurelia*, but only under low resource level. (b) Population density of *Blepharisma japonicum* is negatively affected by interspecific resource competition

3.3 | Population density

Resource level and competition interactively influenced the population density of *P. aurelia* ($p = 0.003$, Table 1; Figure 2a). Interspecific competition had a positive effect on the population density of *P. aurelia*, but only at low resource level. In contrast, the population density of *B. japonicum* was negatively affected by interspecific competition ($p < 0.001$, Table 1; Figure 2b), but not by resource level ($p = 0.348$, Table 1; Figure 2a). Experimental warming did not alter population densities of *P. aurelia* ($p = 0.052$, Figure 2b) or *B. japonicum* ($p = 0.402$, Table 1; Figure 2b), and coexistence was observed under all experimental conditions (Figure S2).

There is a suggestion that the population density of *P. aurelia* was negatively related to body size, although the effect was marginally non-significant ($F_{1,46} = 3.99$, $p = 0.052$, Figure 3a). However, we found no indication that the population density of *P. aurelia* is correlated with length:width ratio ($F_{1,46} = 2.2$, $p = 0.142$, Figure 3c). We also found no relationship between the population density of *B. japonicum* and either body size ($F_{1,46} = 0.10$, $p = 0.740$, Figure 3b) or length:width ratio ($F_{1,46} = 0.73$, $p = 0.400$, Figure 3d).

4 | DISCUSSION

Understanding how the environment modulates functional traits of organisms can improve our knowledge of how global changes alter biotic interactions, species coexistence and community dynamics (McGill et al., 2006). Such knowledge is increasingly important in

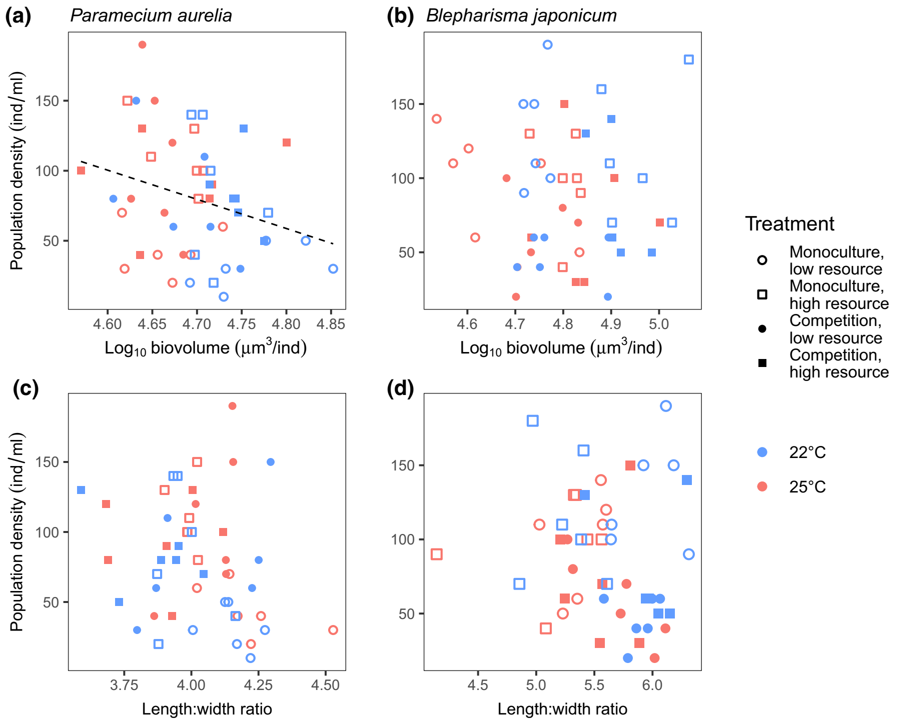

FIGURE 3 The relationship between population density and body size or between population density and length:width ratio. (a) There is an indication that population density is negatively correlated with the mean body size of *Paramecium aurelia*, although this relationship is statistically non-significant ($p = 0.052$). (b) There is no relationship between the population density of *Blepharisma japonicum* and their mean body size. (c) There is no relationship between the population density of (c) *P. aurelia* and (d) *B. japonicum* with their length:width ratio

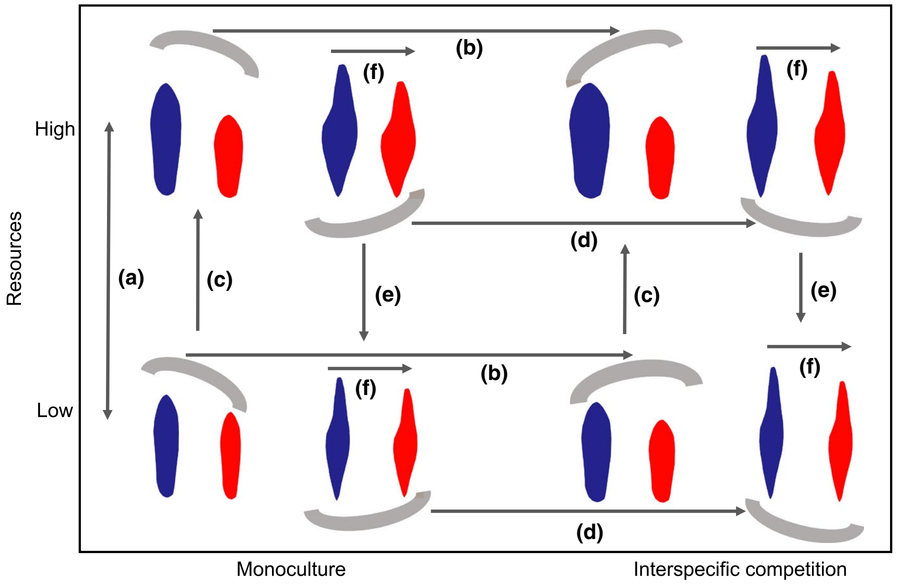

FIGURE 4 A schematic representation of how body shape and size respond to the combination of warming, resource level and interspecific competition. (a) At low resource level, both species have more elongate shape, whereas shape elongation decreases at high resource level, in agreement with the resource-shape hypothesis. (b) In *Paramecium aurelia*, interspecific competition reduced shape elongation, and body shape response is similar to that (c) observed with increased resource level, partially contrasting the resource-shape hypothesis. In contrast, (d) competition enhanced shape elongation in *Blepharisma japonicum*, resulting in a similar response as that observed (e) under reduced resource level. As a result, body shape of *P. aurelia* is similar to that found in high resource monocultures. This competitive difference between species is further corroborated by a positive competition effect on the population density of *P. aurelia* but a negative competition effect on the population density (Figure 2a,b) and biovolume density (Supporting Information 1, Figure S4) of *B. japonicum*. (f) Experimental warming (red symbols) reduces shape elongation of *B. japonicum* but not of *P. aurelia*. Experimental warming also significantly reduced body size of both model species. The grey curves group temperature levels to indicate similar body shape responses to resource competition (arrows c and e)

the face of growing pressures from rising magnitude and frequency of extreme temperature events (Meehl, 2004; Oliver et al., 2018). Our simple factorial experiment showed that the model protist species have divergent body shape responses to interspecific resource competition (Figures 1 and 4), but similar body size responses to warming (Figures 2 and 4). As these responses were observed in two modal species, it is important that future studies investigate the applicability of these findings on body shape in particular to protists and microzooplankton in general. Whereas body size responses to warming are well established (Atkinson, 1994), we highlight that body shape responds to changes in resource level. Body shape responses could therefore be important for understanding competitive abilities while the major effect of temperature on body size suggests that body size responses could be predominantly driven by metabolic demands (DeLong, 2012; Forster et al., 2012). Interactive effects of temperature and predation risk on *Paramecium* cell length, but not overall size (Uiterwaal et al., 2020), further highlight the role of multiple environmental drivers on size and shape.

Low resource levels appear to favour relatively elongate-shaped individuals in both species (Figures 1 and 4), in agreement with our resource-shape hypothesis. Changes in the length:width ratio indicate that for both species, body length and width do not grow proportionally under different environmental conditions (Gibert et al., 2017). Such body-shape elongation (i.e. greater length:width ratios) may enhance swimming speed, resource acquisition and/or reduce energetic costs of motility (Gibert et al., 2017; Pennekamp et al., 2019; Roberts, 1981), and could be favoured when resources are scarce. In contrast, reduced body shape elongation may occur under high resource levels as shorter distances between resources may reduce the advantage of reduced locomotion costs.

Interspecific competition led to shape elongation of *B. japonicum*, reflecting response observed in single-species treatments to a low level of resources. This shape response suggests that the presence of *P. aurelia* reduced resources available to *B. japonicum*. In contrast, interspecific competition reduced shape elongation in *P. aurelia*, and the resulting body shape response was similar to that observed at high resource level (Figure 1a). Although reduced elongation could indicate an anti-predator response (Hammill et al., 2010), the positive effect of *B. japonicum* on the population density of *P. aurelia* (Figure 2a) suggests that reduced elongation is not a response to predators in this case. *B. japonicum* densities are, in turn, negatively affected by the presence of *P. aurelia* (Figure 2b). This contrasting body shape response to interspecific competition suggests that *P. aurelia* could have obtained resources at the expense of *B. japonicum*. However, interspecific competition had no effect on biovolume density of the total assemblage (Supporting Information 1, Figure S5). Niche partitioning between the two species could underline the weak and asymmetric competition and coexistence of both species across the treatment conditions (DeLong & Vasseur, 2012). As the bacterial community is also affected by the protists present, *P. aurelia* may have indirectly benefited from the presence of *B. japonicum*, if the presence of *B. japonicum* increases the density of bacterial species preferred by *P. aurelia*. *P. aurelia* could, in turn, exert a weak competition effect and negatively influence the population density of *B. japonicum* if it has a broader range of preferred resources that overlaps with those of *B. japonicum*.

Contrasting influence of bacterial resources on body shape was found in another protist species, *P. bursaria*, whose individual shape is relatively more elongate at high levels of bacterial resources (Gibert et al., 2017). Shape elongation at high resource levels contrasts with our observations. However, as *P. bursaria* is a mixotrophic protist, body shape elongation may also be favoured to enhance the exposure of zoochlorellae to light (Naselli-Flores & Barone, 2011). Hence, the observed body shape response could be influenced by a space trade-off between bacterial and light acquisition (Gibert et al., 2017).

In agreement with the temperature–size rule (Atkinson et al., 2003; Forster et al., 2012), body size of both species declined under experimental warming (Figure 1c,d). However, contrary to our expectation, body size decrease was not exacerbated by low resource level or interspecific competition. Low resource level also reduces *B. japonicum* body size, but this size reduction is not altered by experimental warming or interspecific competition. Interspecific competition leads to body-shape elongation (Figure 1b), which increases swimming velocities or lowers locomotion costs, and could improve resource acquisition and compensate for lower food concentration. Such compensation may limit the extent of body size reductions under interspecific competition, thereby resulting in similar body size to that observed at high resource level. However, the maintenance of body size under interspecific competition and lower resource availability may consequently constrain population increase and result in lower *B. japonicum* population densities at steady state (Figure 2b).

Body size declined by 4.37% °C^{-1} (95% CIs: 2.18%, 6.50%) in *P. aurelia* and 6.49% °C^{-1} (95% CIs: 1.93%, 10.83%) in *B. japonicum*. Body size declines are similar in both species and are not significantly different from a size reduction of 1.7% °C^{-1} (95% CIs: 1.1%, 2.4%) calculated across a range of protist species (Forster et al., 2012). As heterotrophic organisms require essential resources such as food and oxygen, body size decline could result from greater temperature dependence of resource demand than of resource supply. Hence, temperature-enhanced food demands could increase food limitations, and thus influence body size (DeLong, 2012; DeLong et al., 2017). A convergence between oxygen demand and supply capacity could also arise at higher temperatures at large size. Metabolic rate, which influences resource demands generally increases with temperature (Brown et al., 2004). Although the passive supply of oxygen also increases with warming, its temperature dependence is weaker than that of metabolic rates (Atkinson et al., 2003; Verberk et al., 2011). Hence, smaller body size could be favoured to increase surface area for resource uptake in relation to mass, or to compensate for increased oxygen demands (Atkinson et al., 2003, 2006), or increased food demand (DeLong, 2012) at higher temperatures.

Body size is a major determinant of metabolic rate, with smaller individuals in general having lower absolute metabolic rates (Brown et al., 2004; DeLong et al., 2010). Applying an interspecific mass-scaling exponent of metabolic rate of 0.902 and an activation energy of 0.61 eV (DeLong et al., 2010), the estimated metabolic rates per individual were higher at 25°C, despite smaller body sizes in all treatment combinations but one (*B. japonicum* in high resource monocultures, Supporting Information 1, Figure S7). This suggests that size reduction could have occurred to compensate in part for the increased metabolic demands at 25°C. Higher metabolic rates and lower water viscosity at 25°C also likely enhanced swimming speed (Beveridge et al., 2010), which could improve resource capture, and the use of available energy for growth, potentially increasing carrying capacity (Gibert et al., 2017). However, warming was not associated with detectable increases in population densities (Figure 3a,b), suggesting that improved resource capture is counteracted by increased energetic costs of growth (Barneche et al., 2019), or the cost of maintenance at higher temperatures. Hence, higher per capita resource demands likely limit additional energetic allocation for growth and reproduction.

Experimental warming did not alter competitive interactions, and both species were able to coexist across all treatment combinations. *P. aurelia* benefitted from the presence of *B. japonicum* at both temperature levels, and negatively impacted *B. japonicum* population densities (Figure 2a,b) and population biovolume densities (Supporting Information 1, Figure S4) in polycultures. Similar body size reductions at higher temperature could have maintained per capita interaction strength (Sheridan & Bickford, 2011). We also found little evidence for temperature influencing densities of both species, contrasting the common suggestion that warming should decrease population carrying capacity (Bernhardt et al., 2018; Brown et al., 2004). However, as environmental changes will likely alter resource availability to protists and other groups, understanding how body shape changes alter body size response among different interacting species will be important for predicting the impact of warming on communities.

Relative body-shape elongation of both species was observed at low resource level, which highlights similar body shape response to resource levels. However, this was not the case for competition and warming (Figures 1 and 4). This study indicates that resource availability influenced body shape, whereas body size is predominantly influenced by temperature. Body shape, which influences swimming speed and cost of locomotion responds primarily to resource availability. In contrast, body size responses may be universally driven by the temperature dependence of metabolic demands. Similarity in body size reductions across different competitors could maintain the constant strength of competitive interactions under warming. However, body shape responses such as relative elongation may influence body size response to environmental changes. These results are based on two protist species, highlighting the need for further research to fully understand how body shape of other microzooplankton species respond to multiple environmental stressors. Such knowledge will be crucial for assessing the importance of the relationship between body shape and body size, and body shape response to competition as a functional trait for predicting competitive hierarchies and the extent of temperature–size responses. Given the biological implications of body shape sensu lato across diverse taxonomic groups, a deeper understanding of how body shape and size change across environmental gradients could improve the trait-based forecasting of warming impacts on natural ecosystems.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHORS' CONTRIBUTIONS

H.T. and P.K. conceived the study and designed the experiment; H.T. collected and analysed the data; H.T., P.K., A.G.H. and D.A. all contributed to the writing of the paper.

DATA AVAILABILITY STATEMENT

The data supporting this article are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.d7wm37q02 (Tan et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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