Evolutionary Ecology Research, 2018, **19**: 669–678

The benefit of algae endosymbionts in *Paramecium bursaria* is temperature dependent

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ABSTRACT

Background: Through the effects of kinetics, biochemical reaction rates, and phenotypes, changes in temperature could alter the costs and benefits that organisms experience when in a mutualistic relationship with other species. Yet the role of temperature in determining the net benefits of mutualisms is largely unknown.

Question: How does the presence of a mutualistic relationship with endosymbiotic algae influence the temperature dependence of fitness in *Paramecium bursaria*?

Organism: *Paramecium bursaria*.

Methods: We developed paired strains of *P. bursaria* collected from the wild, one with the naturally occurring endosymbiont intact and the other with the endosymbiont removed by growing cells in the dark. We measured the per capita rate of population growth (average fitness) and per capita rate of biomass production for these two strains at seven temperatures and compared the resulting thermal performance curves.

Results: The net benefit of the endosymbiotic algae on the thermal performance curves of their host *P. bursaria* depended considerably on temperature. *Paramecium bursaria* with the algae showed higher growth rates at cooler temperatures, while *P. bursaria* without the algae showed higher growth rates at warmer temperatures. The optimal temperature for *P. bursaria* without the algae was close to the typical optimal growth rate temperature of many bacteria species, suggesting that cells without algae can make more effective use of bacterial prey resources at high temperatures when these resources are plentiful. In contrast, *P. bursaria* that have endosymbiotic algae benefit more from them at cooler temperatures.

Keywords: endosymbionts, mutualism, *Paramecium bursaria*, temperature, thermal performance curve, zoochlorellae.

INTRODUCTION

Mutualisms – mutually beneficial relationships between two organisms – are a fundamental part of ecological communities. Mutualisms can stabilize populations (Mougi and Kondoh, 2012), promote diversity (Pachepsky *et al.*, 2002), and enhance co-evolution (Guimarães *et al.*, 2011). Yet environmental factors can shift the costs and benefits of species interactions so that a

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mutualism can transform into a facultative host–parasite relationship if the costs begin to outweigh the benefits (Bronstein, 1994; Johnson *et al.*, 1997; Lowe *et al.*, 2016).

A critical factor that influences organism function – and thus the ability of species to provide benefits to other species – is temperature (Toby Kiers *et al.*, 2010; Rohrscheib *et al.*, 2016). Temperature affects many physiological and ecological processes, such as photosynthesis, respiration, and foraging rates (Brown *et al.*, 2004; Englund *et al.*, 2011; Dell *et al.*, 2014). Many biological rates and functions can be characterized by a thermal performance curve (TPC) in which the rate increases as temperature increases, reaches a peak at an optimal temperature ($T_{\mathbf{opt}}$), and then declines at higher temperatures (Angilletta, 2009; Kingsolver, 2009). Thermal performance curves also can describe the temperature dependence of maximum per capita growth rate ($r_{\mathbf{max}}$), providing a useful proxy for mean fitness of a population across temperatures (Ratkowsky *et al.*, 2005; Amarasekare and Savage, 2012; Luhring and DeLong, 2016). Shifts in the TPC for per capita growth rate under different environmental conditions can therefore indicate changes in fitness across environments. Similarly, the effect of temperature on the fitness benefits of a mutualism could be seen through shifts in per capita growth rate TPCs with and without a mutualist present (Sinclair *et al.*, 2016).

The protist *Paramecium bursaria* has a mutualistic relationship with endosymbiotic *Chlorella*-like green algae (zoochlorellae). *Paramecium bursaria* is very rarely found without its zoochlorellae in the wild (Tonooka and Watanabe, 2002). Zoochlorellae aid in ultraviolent light protection for *P. bursaria* (Summerer *et al.*, 2009), minimize photo-oxidative stress (Hörtnagl and Sommaruga, 2007), supply maltose (Brown and Nielsen, 1974), and make the *P. bursaria* less reliant on the external environment for food (Karakashian, 1963). In turn, zoochlorellae acquire protection inside *P. bursaria* cells from threats such as chlorovirus infection (Van Etten and Dunigan, 2012). The *P. bursaria*–zoochlorellae holobiont is found in temperate freshwater bodies all over the world, including the USA, Japan, Europe, and Australia (Hoshina *et al.*, 2005). Thus, *P. bursaria* experiences temperature variation associated with season, latitude, and water depth.

Paramecium bursaria faces a trade-off between allocating space within the cell to zoochlorellae and maintaining room for food vacuoles formed when consuming bacteria (Dolan, 1992). Because both foraging and photosynthesis are temperature dependent (Brown *et al.*, 2004), it is possible that variation in temperature could influence the effectiveness of different foraging modes, altering the costs and benefits of the mutualistic interaction between *P. bursaria* and zoochlorellae. Such effects could lead to a temperature dependence in the net benefit provided by zoochlorellae to *P. bursaria*, shifting their interaction from mutualistic to facultatively parasitic at some temperatures. Although division rates for *P. bursaria* with and without zoochlorellae at room temperature are similar (Siegel, 1960; Karakashian, 1963), mortality rates have been measured over a range of temperatures with results suggesting that the zoochlorellae increase the ability of *P. bursaria* to survive warmer temperatures (Iwatsuki *et al.*, 1998). Yet how the presence of zoochlorellae affects the fitness of *P. bursaria* across a range of temperatures is not known. Here we test for differences in the per capita growth rate TPC (as a proxy of mean fitness) of *P. bursaria* for cells with and without zoochlorellae endosymbionts. Our aim is to evaluate the acute thermal response of a naturally occurring mutualism between *P. bursaria* and its endosymbiont. Our results reveal that the benefits of zoochlorellae to *P. bursaria* hosts are strongly temperature dependent, with fitness benefits of the zoochlorellae occurring at colder temperatures and substantial fitness costs at warmer temperatures.

METHODS

We collected wild *P. bursaria* from a pond close to Spring Creek Audubon Center near Denton, Nebraska, in June 2013. Temperatures in freshwater lakes near our collection site vary between 4°C and 34°C over a year (Nebraska DEQ, 2008, p. 7). We maintained cultures at room temperature with natural light in protozoan media (Carolina Biological Supply) inoculated with a range of bacteria from the collection site until the fall of 2017. To achieve zoochlorellae-free *P. bursaria*, we placed cells in constant darkness for 3 months and maintained cultures on bacterized media. These cells consequently lost their associated zoochlorellae as well as the chloroviruses present in the culture. Chloroviruses are not only present in the water column of *P. bursaria* cultures but also attach to the surface of *P. bursaria* cells (Yashchenko *et al.*, 2012). We therefore employed a dilution method to obtain virus-free *P. bursaria* with zoochlorellae. We placed replicate single *P. bursaria* cells in 1.2 mL cultures in continuous light, and we transferred daughter cells from these dishes into their own individual dishes each day. Without infection to generate new chloroviruses, the chloroviruses attached to *P. bursaria* membranes became diluted (approximately half the number with each transfer) to the point of absence. We also added chlorovirus antibodies (NY-2A) for 2 weeks to aid in virus removal. We confirmed the absence of the chlorovirus in the cultures using plaque assays (Van Etten *et al.*, 1983). We kept both populations in the same conditions and media at ~23°C for a week before the experiment with zoochlorellae-bearing cells kept in the light and zoochlorellae-free cells kept in the dark.

We measured TPCs of per capita population growth rate for cultures with and without zoochlorellae. To start each replicate measurement, we transferred seven *P. bursaria* cells in 100 μL aliquots of diluted stock solution into a 35 mm Petri dish with 1.5 mL of inoculated protozoan media. We incubated six replicates dishes overnight at seven temperatures. We used 12°C, 19°C, 24°C, 29°C, 31°C, 34°C, and 37°C, thus spanning *P. bursaria*'s full range of positive growth (Luhring and DeLong, 2017). Fluorescent bulbs illuminated the microcosms with approximately 270–310 μmol/s/m^2 (measured using an LI-250A light meter). We counted all cells under a stereomicroscope after 16–20 hours of incubation. We calculated per capita population growth rate (r) using the equation for exponential growth:

$$r = \ln(N/7)/t,$$

where N is the cell count at the end of the trial at time t. We then fit the per capita growth rate data across temperatures to the Lactin-2 TPC function (Lactin *et al.*, 1995; Luhring and DeLong, 2016) using ordinary non-linear least-squares regression:

$$r = \exp(\rho T) - \exp\left(pT_{\max} - \frac{T_{\max} - T}{\Delta T}\right) + \lambda.$$

In this model, $T_{\max}$ indicates the temperature at which the TPC begins to decline at higher temperature, ΔT is a reference temperature, and the constants ρ and λ control the overall height of the curve and the steepness of the rising portions on the left, respectively.

We measured cell size from stock populations of both cultures using a FlowCAM (Fluid Imaging, Scarborough, ME). We used the equation for a prolate spheroid to calculate cell volume from width (W) and length (L) measures:

$$\frac{4\pi}{3}\left(\frac{W}{2}\right)^2\left(\frac{L}{2}\right).$$

We estimated per capita biomass production by multiplying per capita growth rate by the average stock population cell volume.

Paramecium bursaria is a mixotroph, consuming bacteria and gathering energy through photosynthesis. In order to determine whether the per capita rate of population growth TPC for *P. bursaria* reflects the productivity of its bacterial prey, we utilized data on estimated T_{opt} for growth rate for 28 bacterial strains/species from Ratkowsky (1983). We then compared the T_{opt} of the *P. bursaria* strains with the distribution of those for bacteria. All fitting, analysis, and calculations were done in Matlab.

RESULTS

Both zoochlorellae-bearing and zoochlorellae-free *P. bursaria* showed a unimodal relationship between per capita population growth rate and temperature (Fig. 1). However, cells with and without zoochlorellae exhibited different per capita population growth rates across temperatures, with significant differences in growth rate at 12°C (t = 3.84, df = 10, P = 0.003), 29°C (t = −7.82, df = 10, $P = 1.44 \times 10^{-5}$), 31°C (t = −8.71, df = 10, $P = 5.58 \times 10^{-6}$), and 34°C (t = −17.995, df = 10, $P = 6.01 \times 10^{-9}$). Zoochlorellae-bearing cells grew faster at cooler temperatures (below ~23°C), whereas zoochlorellae-free cells grew much faster at warmer temperatures. The two TPCs crossed at temperatures in the range of 22–25°C, where both cell types showed very similar per capita growth rates. Both populations transitioned to negative per capita growth rate at a similar high temperature (~35°C; Fig. 1A).

Zoochlorellae-bearing *P. bursaria* were significantly longer (t = −4.96, df = 424, $P < 0.001$), wider (t = −6.21, df = 424, $P < 0.001$), and larger (cell volume) (t = −7.88, df = 424, $P < 0.001$) than *P. bursaria* without zoochlorellae (Fig. 2). Using the average sizes to convert per capita population growth rate to per capita biomass production, we found that the curves crossed at a slightly higher temperature (approximately 27°C) but the overall pattern remained the same (Fig. 1B). Estimated biomass production was significantly different between cells with and without zoochlorellae at 12°C (t = 3.84, df = 10, P = 0.003), 24°C (t = 4.37, df = 10, P = 0.0014), 29°C (t = −2.94, df = 10, P = 0.015), 31°C (t = −3.36, df = 10, P = 0.007), and 34°C (t = −17.995, df = 10, $P = 6.01 \times 10^{-9}$).

Many species of bacteria show a T_{opt} similar to the zoochlorellae-free *P. bursaria*. The mode of T_{opt}, for the bacterial species presented in Ratkowsky *et al.* (1983), aligned more closely with the T_{opt} of the zoochlorellae-free *P. bursaria* than the zoochlorellae-bearing *P. bursaria* (Fig. 3).

DISCUSSION

Our results indicate that the benefit of mutualistic zoochlorellae in *Paramecium bursaria* is temperature dependent (Fig. 1). Zoochlorellae-bearing cells showed higher per capita growth rate than zoochlorellae-free cells at lower temperatures but lower per capita growth rate at higher temperatures. This pattern holds for both average fitness (per capita population growth rate) (Fig. 1A) and estimated biomass production (Fig. 1B). These results suggest that zoochlorellae can become a facultative parasite at higher temperatures, with potential implications for the abundance of *P. bursaria* in aquatic systems and the persistence and/or evolution of the species interaction in different climates.

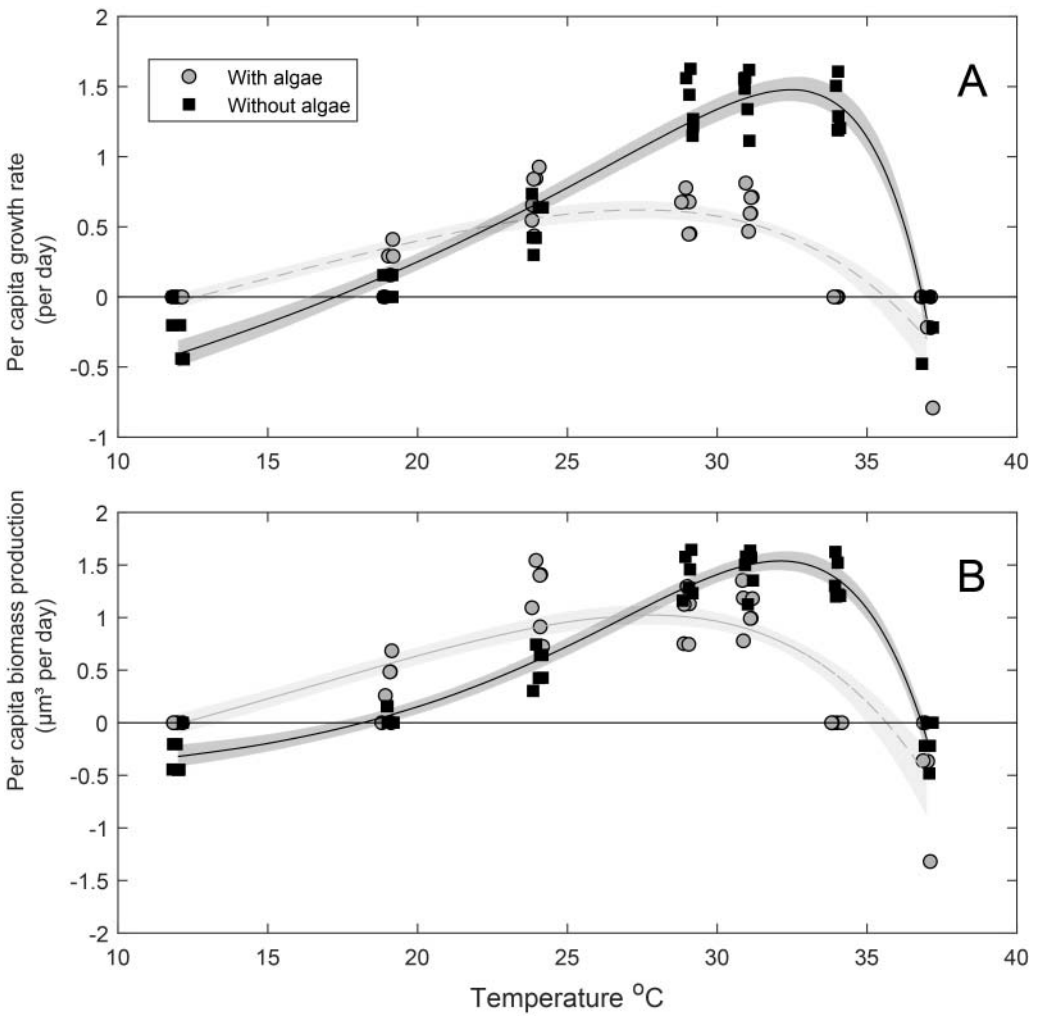

Fig. 1. (A) The thermal performance curve (TPC) for *P. bursaria* with (grey) and without (black) the algal symbiont, showing population growth in relation to temperature. (B) TPC taking into account differences in stock population sizes to show difference in estimated biomass production. All shading represents 95% confidence intervals after 1000 bootstraps.

The difference in TPCs between zoochlorellae-bearing and zoochlorellae-free cells could arise from differences in the temperature dependence of underlying drivers such as zoochlorellae photosynthesis and bacterial prey productivity. One possible explanation is that zoochlorellae photosynthesis declines at temperatures around 25°C (Converti *et al.*, 2009), suppressing per capita growth of zoochlorellae-bearing *P. bursaria* above this temperature. This explanation is unlikely, however, because free-living *Chlorella* sp. show peaks of photosynthesis around 35–40°C (Padfield *et al.*, 2016), far above the optimal growth temperature of *P. bursaria*. Yet without assessing the temperature dependence of zoochlorellae photosynthesis within *P. bursaria* cells, we cannot rule out the possibility that temperature limits

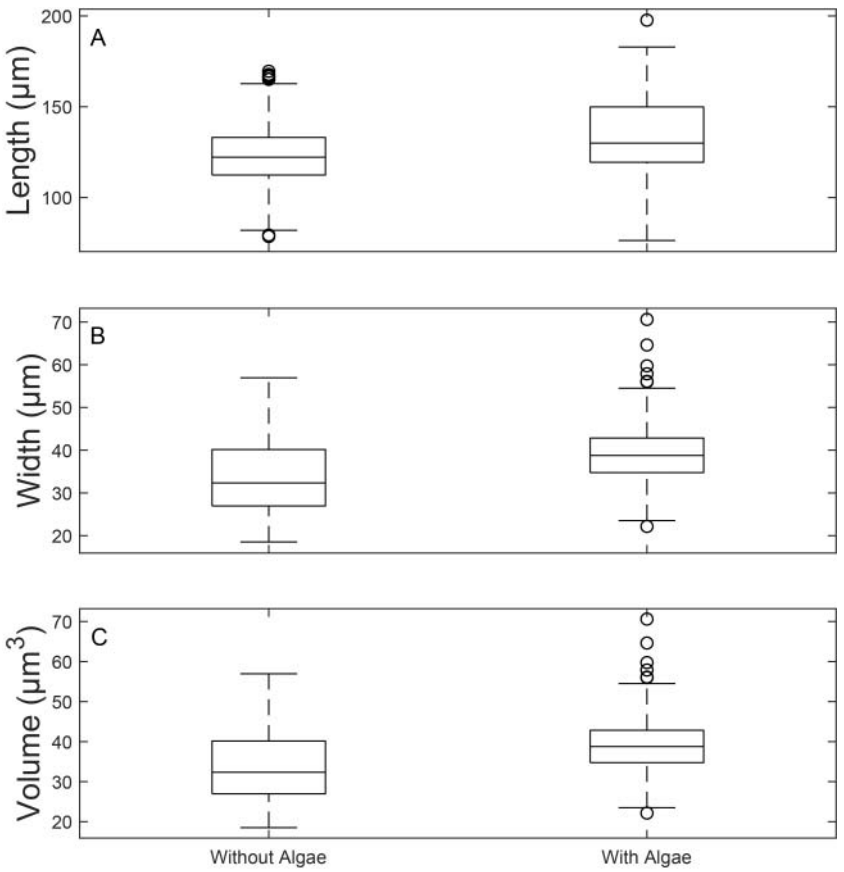

Fig. 2. Differences in (A) cell length, (B) width, and (C) volume of zoochlorellae-bearing and zoochlorellae-free *P. bursaria*. A *t*-test indicated that cells with algae had significantly greater lengths, widths, and volumes.

the photosynthetic activity of zoochlorellae above 25°C. In contrast, bacteria growth rates are temperature dependent, typically peaking at temperatures near 30–40°C (Fig. 3) (Ratkowsky *et al.*, 1983, 2005). These peaks are typically very close to the temperatures at which zoochlorellae-free *P. bursaria* perform best (Fig. 3), suggesting that the temperature dependence of per capita growth rate of zoochlorellae-free cells is linked to the temperature dependence of bacterial prey productivity.

This potential dependence on prey productivity also points to a reason why zoochlorellae-bearing cells show reduced growth at warmer temperatures. Zoochlorellae-bearing *P. bursaria* are limited in the number of food vacuoles they can make by the physical space within the cell (Dolan, 1992). This constraint is more severe when the cells also allocate space to zoochlorellae, which further limits the amount of bacteria they are able to take up from the environment. At higher temperatures, zoochlorellae-free *P. bursaria* can make better use of the more abundant bacteria, whereas the zoochlorellae-bearing *P. bursaria* do not have the space to take in as many bacteria, limiting their ability to take advantage of

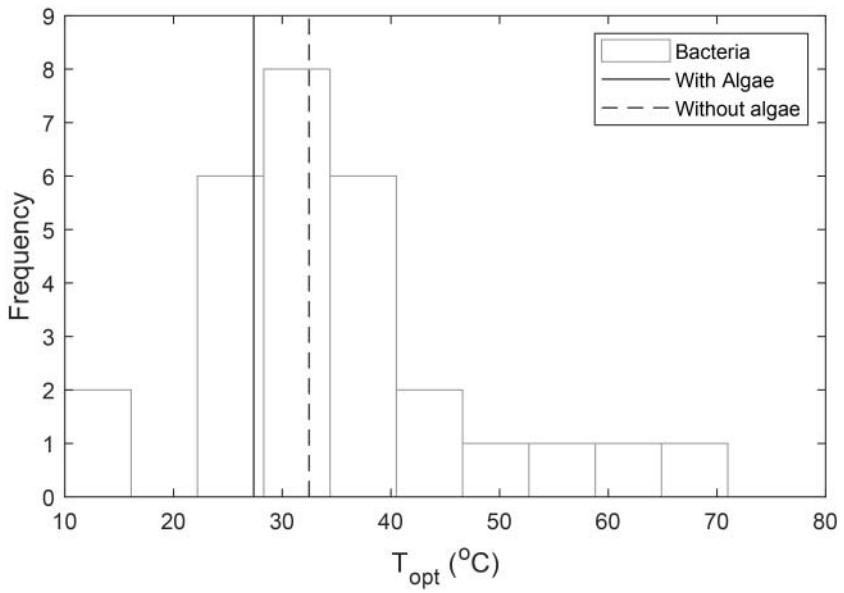

Fig. 3. Optimum growth temperatures (T_{opt}) for different species of bacteria presented by Ratkowsky *et al.* (1983) compared with the T_{opt} for *P. bursaria* with the algal endosymbiont (27.38°C) and without the endosymbiont (32.46°C).

the available energy. At colder temperatures, zoochlorellae-free *P. bursaria* have reduced growth due to low bacterial productivity and no source of photosynthetic energy, while the zoochlorellae-bearing *P. bursaria* continue to grow using the energy provided by the zoochlorellae along with what bacteria are available in the environment. Thus, the energy available in the environment at warmer temperatures surpasses that which the zoochlorellae can provide to the host, such that zoochlorellae act as a facultative parasite on the *P. bursaria* host, taking up space that could be used for food vacuoles and not providing enough energy to compensate.

Our results also highlight the importance of evaluating costs and benefits of mutualisms across a range of temperatures in natural conditions (Toby Kiers *et al.*, 2010; Rohrscheib *et al.*, 2016). Previous suggestions that there are no fitness costs or benefits to the *P. bursaria* hosts from having endosymbionts (Siegel, 1960; Karakashian, 1963) likely stemmed from conducting this assessment only at room temperature. Our results show that at room temperature (~25°C), the per capita rates of population growth of *P. bursaria* with and without zoochlorellae are very similar. The difference in reproduction rates only becomes apparent when assessing a wider range of temperatures. Furthermore, previous research addressed only a component of fitness mortality rates across temperatures (Iwatsuki *et al.*, 1998), while here we have measured a proxy of fitness itself (per capita growth rate), providing a clearer picture of the net benefits of the mutualism.

We also showed that *P. bursaria* with zoochlorellae were significantly longer, wider, and had a higher cell volume (Fig. 2) than zoochlorellae-free *P. bursaria*. This may be due to the fact that one *P. bursaria* cell can contain several hundred *Chlorella* cells (Karakashian, 1963). *Paramecium bursaria* may be buffering some of the cost of having the zoochlorellae by increasing cell volume to increase the space it can allocate to both zoochlorellae and

vacuoles. However, doing so could reduce per capita growth rates, as its time to division must generally increase as division size increases.

CONCLUSION

With the increasing temperatures predicted as a result of climate change (IPCC, 2018), the average costs and benefits linked to the relationship between *P. bursaria* and its endosymbiont could change. If warmer temperatures prevail over the long run, *P. bursaria* might shift towards disassociating from zoochlorellae. This change could have profound and unforeseen effects on the biology of these organisms as well as their ecological interactions linking *P. bursaria* to the rest of the food web. Disassociation between hosts and photosynthetic endosymbionts may be happening in some systems already. For example, coral bleaching, an event in which marine invertebrates lose their endosymbiotic photosymbiont, is already a well-known result of rising ocean temperatures (Hoegh-Guldberg, 1999). This type of change could have unpredictable cascading effects in aquatic ecosystems due to the complex associations among species, but it also may serve as an example for how climate change is unsettling previously stable relationships.

ACKNOWLEDGEMENTS

We thank S. Barrios, T. Howsden, I. Lagerstrom, T. Luhring, and S. Uiterwaal for assistance with data collection. The authors were supported by NSF EPSCOR grant #47762.

DATA ACCESSIBILITY

Per capita population growth and cell size data are available at evolutionary-ecology.com/data/3185Appendix.pdf.

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