

Temperature-Dependent Growth and Fission Rate Plasticity Drive Seasonal and Geographic Changes in Body Size in a Clonal Sea Anemone

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ABSTRACT: The temperature-size rule is a commonly observed pattern where adult body size is negatively correlated with developmental temperature. In part, this may occur as a consequence of allometric scaling, where changes in the ratio of surface area to mass limit oxygen diffusion as body size increases. As oxygen demand increases with temperature, a smaller body should be favored as temperature increases. For clonal animals, small changes in growth and/or fission rate can rapidly alter the average body size of clonal descendants. Here I test the hypothesis that the clonal sea anemone *Diadumene lineata* is able to track an optimal body size through seasonal temperature changes using fission rate plasticity. Individuals from three regions (Florida, Georgia, and Massachusetts) across the species' latitudinal range were grown in a year-long reciprocal common garden experiment mimicking seasonal temperature changes at three sites. Average body size was found to be smaller and fission rates higher in warmer conditions, consistent with the temperature-size rule pattern. However, seasonal size and fission patterns reflect a complex interaction between region-specific thermal reaction norms and the local temperature regime. These details provide insight into both the range of conditions required for oxygen limitation to contribute to a negative correlation between body size and temperature and the role that fission rate plasticity can play in tracking a rapidly changing optimal phenotype.

Keywords: clonality, optimal size, phenotypic plasticity, sea anemone.

Introduction

Relationships among body size, metabolism, and temperature govern the acquisition and allocation of resources and so are expected to influence the diversification of life (Kingsolver and Huey 2008). The temperature-size rule, which describes a strong negative correlation between developmental temperature and adult body size, has been

observed across a wide range of taxa (Atkinson 1994; Kingsolver and Huey 2008). One mechanism that may contribute to this pattern includes temperature-mediated changes in the body size at which oxygen diffusion limitation occurs (Atkinson et al. 2006; Forster et al. 2012). When respiratory surface area scales allometrically with mass, passive diffusion through respiratory surfaces can become limiting as body size increases (Atkinson et al. 2006; Hirst et al. 2014; Gillooly et al. 2016). For aquatic species, a warmer temperature means an increased aerobic metabolic rate at the same time that water becomes less capable of holding oxygen, so the body size at which diffusion becomes limiting is smaller and results in a smaller optimum body size (Forster et al. 2012). The importance of avoiding oxygen limitation in the lives of aquatic species has led to the hypothesis that acclimatization behaviors—including growth rate, morphological, or behavioral plasticity—should be under strong selection because these traits help maintain adequate gas exchange across a range of temperatures (Atkinson et al. 2006). For animals with unidirectional growth and development (i.e., those that lack the ability to shrink or undergo fission), temperature-dependent metabolic relationships have been shown to be predictive of key life-history parameters (Atkinson 1994; Kingsolver and Huey 2008).

While the oxygen limitation hypothesis suggests a clear prediction for animals with a fixed adult size (e.g., warmer is smaller), it is less clear how temperature should influence the growth patterns of species with indeterminate or clonal growth. Such animals, however, have a wide range of potential morphological responses available when faced with environmental variation (Sebens 1987; Burgess et al. 2017). For example, individuals may alter their surface area to mass ratio through changes in shape or tissue density (Zamer and Mangum 1979; Hirst et al. 2014; Glazier et al. 2015), by shrinking or growing (Chomsky et al. 2004), or by rapidly changing their body size through fission. For an animal capable of fission, ramet (i.e., clonal unit) size depends on a balance

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between growth rate and fission rate, so temperature-dependent changes in either or both rates can change the average ramet size (Forster et al. 2011). The size of individual ramets of some clonal animals have been shown to follow the expectations of the temperature-size rule in the lab (Atkinson et al. 2006) and through time in the field (O’Dea and Okamura 1999), suggesting that the mechanisms that govern adult body size in unitary organisms might influence ramet size in clonal animals (Hughes 2005; Burgess et al. 2017). However, we have little empirical evidence to identify the role of temperature-dependent growth or fission rate in ramet size patterns or to understand how these rates evolve in clonal animals (Geller et al. 2005).

Predicting optimal behavior becomes more complicated when different forms of temperature variation are considered. Patterns consistent with the “warm is smaller” prediction have been widely documented in temperature manipulation studies (reviewed in Atkinson 1994; Kingsolver and Huey 2008) but have also been demonstrated across latitude (i.e., Bergmann’s rule; Kingsolver and Huey 2008), in response to seasonal temperature fluctuations (O’Dea and Okamura 1999; Horne et al. 2017), and through time, in association with directional climate change (Daufresne et al. 2009; Gardner et al. 2011; Sheridan and Bickford 2011). Together, these examples highlight the complex patterns of temperature influencing growth, size, and energy allocation in natural populations; thermal reaction norms are shaped simultaneously by both seasonal and spatial temperature gradients as well as by long-term environmental trends (Angilletta et al. 2003). As such, size variation across a species’ latitudinal range is expected to be a product of plastic growth and development rates (Forster et al. 2011) as well as local genetic adaptation that promotes the expression of appropriate growth and reproduction patterns for a given environment.

In strongly seasonal environments, changes in fission rate with temperature may allow individuals to vary ramet size in order to track a changing optimum through time. By dividing somatic tissue into optimally sized units, an organism can maintain positive somatic growth even when allometry constrains individual growth (Sebens 1982, 2002; Hughes 2005). Where steep environmental gradients are present, however, unavoidable trade-offs may limit any one genotype’s ability to produce an optimal phenotype across the breadth of possible environments, leading to differentiation in reaction norm shape among populations experiencing different environmental regimes (Angilletta et al. 2003; Amarasekare and Johnson 2017). Local adaptation in thermal plasticity in response to seasonal temperature patterns may also enhance (Horne et al. 2017) or mask (Conover et al. 2009) the appearance of the latitudinal clines in body size because both genotype and environment vary over the latitudinal gradient. The interplay between local

adaptation and plasticity is complex but worth investigating, because patterns of local differentiation in thermal reaction norms across the latitudinal range of a species can be a strong clue as to the selective forces that shape body size, life cycle, and life history (Conover et al. 2009). In addition, patterns of fission rate plasticity across these environments may give us clues as to the adaptive value of fission itself.

One way to understanding the role of fission in tracking a changing optimal body size is to identify the variation in fission rate expression that exists within and among populations and to see how this variation is arranged across the latitudinal range. Here I use the clonal sea anemone *Diadumene lineata* (orange-striped green anemone), which shows temperature-dependent fission (Miyawaki 1952; Minasian 1979), to explore the influence of seasonal temperature change on fission rate and body size. I test the hypothesis that fission rate plasticity is a plausible mechanism for tracking an energetically optimal body size through time in a changing environment. Specifically, I address three questions: (1) How does the pattern of seasonal temperature fluctuations affect the growth, fission rate, and body of anemones over the year? (2) Do individuals from different parts of the latitudinal species range differ in their relationship between fission rate and temperature? (3) Do seasonal changes in average ramet size follow a pattern consistent with the temperature-size rule, as predicted by the oxygen limitation hypothesis?

Material and Methods

Species Description

Diadumene lineata is a small sea anemone without photosymbionts that occurs in the upper littoral zone in harbors and sheltered estuarine waters around the world. It is often associated with oysters, mussels, barnacles, and/or green algae that trap water at low tide and may help ameliorate fluctuations in temperature and air exposure during tidal cycles. A combination of robust physiological tolerance to changes in salinity, temperature, and air exposure and a prolific rate of asexual reproduction were likely important in allowing this anemone to become globally established (Shick and Lamb 1977), following a spread from East Asia (Uchida 1932).

On the east coast of North America, *D. lineata* now occurs across a latitudinal range from the Gulf of Mexico to Maine, where it experiences average monthly water temperatures from 3° to 30°C (app. A; apps. A–D are available online), with peak temperatures spiking below freezing to above 30°C. In short-term laboratory studies, this species has shown a temperature-dependent fission rate, where

warmer water stimulates more frequent episodes of binary fission (Miyawaki 1952; Minasian 1979) and reduces average body size (Minasian 1982; Ryan 2017a). Individuals across the species' range vary by up to four orders of magnitude wet mass and typically undergo annual bouts of both asexual fission and gametogenesis (Ryan 2017a), though the timing and interaction of the two processes remains understudied.

Measuring the Effect of Seasonal Temperature Patterns on Fission Rate, Growth, and Body Size on Individuals from across the Species Range

Over 2 weeks in January 2013, *D. lineata* individuals were collected from three sites across the latitudinal range of the species. Florida (FL) individuals were collected from the underside of sedimentary cobbles on a sandy beach adjacent to the Florida State University Coastal Marine Lab boat ramp (29.915188N, -84.513690W). Georgia (GA) individuals were collected from breakwater rocks near the fishing pier at King's village, St. Simon Island, Georgia (31.134196N, -81.395825W). Massachusetts (MA) individuals were collected from a sheltered cove of bench rock near the Northeastern University's Marine Science Center in Nahant, Massachusetts (42.419810N, -70.903376W). At each site, 60 individuals were haphazardly picked from the high intertidal area using forceps, leaving 1–2 m between collection points to minimize the influence of small-scale clonal aggregations. Individuals were transported to Florida State University and separated into individual containers in a growth chamber at 10°C for 7–14 days to acclimate to laboratory conditions.

On February 1, 2013, 20 individuals from each site were isolated in 25-mL glass test tubes with artificial seawater (Reef Crystals in deionized water at 32 ppt; Instant Ocean, Blacksburg, VA) and randomly assigned to one of three temperature regimes. Treatment regimes consisted of seasonal temperature patterns mimicking each of the three collection sites, establishing a factorial design with site of origin crossed with treatment temperature. Temperatures ranging from 3° to 29°C ($\pm 1^\circ\text{C}$) were achieved with environmental chambers. Anemones were grown in the dark to minimize the growth of fouling algae. It is unknown what role light plays in the biology of this species; however, field-collected individuals are commonly found underneath rocks in total darkness.

Over the duration of the experiment, the temperature of each chamber was adjusted every 2 weeks to match the bi-weekly average temperature observed near each collection site. Temperature data were taken from the nearest publicly available data source and averaged over the 3 years before the start of the experiment (2010–2012). Three times per week, all test tubes were flushed with freshwater, refilled

with new seawater, and fed with an aliquot of 2- or 3-day-old *Artemia* nauplii. Once per week, all anemones in all tubes were counted and had their pedal disks photographed for size measurement. Body size was measured from images using a digital tablet and pen to trace the perimeter of the basal footprint with Image J software (National Institutes of Health, Bethesda, MD) to calculate the pedal area (mm^2) of each ramet (individual anemone).

Analysis

A genet (the collective term for all clonal descendants housed in each tube) was considered to have survived if at least one ramet was still alive at the end of the year-long experiment. Comparisons among treatments were made by calculating the proportion of genets surviving out of the initial number of genets assigned to that treatment. To quantify the effect of temperature regime on fission rate, the average number of ramets produced per genet in each treatment combination after 52 weeks were natural log transformed and compared with a two-way ANOVA.

The temperature threshold below which no fission occurs for each population was estimated by observing the number of clonal decedents produced over time. The inflection point where each treatment group began to show a constant or declining number of clones across multiple weeks after a period of sustained clonal increase was recorded and used to characterize the temperature at which fission ceased for each treatment. This value was compared across temperature treatments and population of origin with a two-way ANOVA.

The accumulated biomass for each genet after 1 year was quantified using the pedal disk area of each individual to estimate a dry biomass equivalent (app. B). The estimated dry biomass for all ramets of each genet were summed to estimate a total genet biomass. These values were natural log transformed and compared among treatment combinations with a two-way ANOVA.

The influence of temperature regime and site of origin on the mean body size among treatments was measured by calculating the mean natural log of the pedal area (mm^2) for all ramets in each genet each week. Site of origin, temperature treatment, and week were used as predictive variables in a set of individual growth curve models (LME using maximum likelihood) comparing the shape of the body size trajectory over time among genets with week set as a random factor. Stepwise model selection was then used to identify the combination of predictive variables that best fit the data. Details of the models and model selection are presented in appendix C.

No mature gametes were released through the duration of the experiment, so gametogenesis could not be quantified. However, individuals in colder water did have thick-

ened mesenteries (observed through the body wall), which typically occurs during early stages of gametogenesis (Fukui 1995; Ryan 2017a). Confirming or quantifying gametic investment for unreleased gametes requires sacrificial sampling; quantifying any such activity was not possible in this experiment.

Tracking Changes in Body Size in Field Populations

Seasonal changes in average body size measured in the field were compared with those under laboratory conditions over an annual cycle. On average, field sites in Florida and Georgia were visited every 2 months and in Massachusetts every 4.5 months from January 2013 through December 2015. All three sites were visited once more in June 2016. During each sampling event, one person walked a transect line parallel to the waterline through each site, and at approximately 5-m intervals, all of the anemones (up to 20 individuals) within a 25-cm² area were collected. This process was repeated until approximately 50–100 anemones were collected. Because of the extreme rugosity of some of the habitats (e.g., oyster beds) and the patchiness of *D. lineata* even where it is abundant, it is difficult to estimate population density in a meaningful way, and so population density was not measured in this study. Collected anemones were returned to the lab, photographed for pedal area, and preserved for other projects.

Constructing Body Size and Fission Rate Reaction Norms across Temperature

A second experiment was performed in order to understand the shape of the reaction norm of temperature with body size and fission rate without the confounding effects of seasonality. Clonal replicates of nine genets used above were isolated and divided among growth chambers at each of five constant temperatures for 12 weeks. Pedal area and fission rate data were used to construct a species-average reaction norm for each trait (for detailed methods, see app. D).

Regression of Dry Biomass on Pedal Area

In order to validate pedal area as a consistent proxy for comparing body sizes, 48 *D. lineata* individuals ($N_{MA} = 9$; $N_{GA} = 18$; $N_{FL} = 21$) were sacrificed to construct a regression of dry weight (mg) on pedal area (mm²). Individuals were held in artificial seawater without food for at least 2 days to ensure an empty gut, and then the pedal area of each individual was calculated from a photograph, as described above. After checking that the body column and foot were free of detritus under a dissecting microscope, individuals were flushed with freshwater to remove extrane-

ous salt before being placed in tared foil boats in a 70°C drying oven for 72 h. Dry tissue was reweighed on a microbalance. The natural log of pedal disk area was a good predictor of the natural log of dry biomass ($r^2 = 0.871$; app. B), with a relationship described by the equation $\ln(\text{dry biomass}) = 1.139 \times \ln(\text{pedal area}) - 1.931$. There was no significant difference detected in the slope of the relationship among individuals collected from the three sites ($F_{2,42} = 1.18$, $P = .317$; ANCOVA), although note that there is a difference in the range of body sizes present at each site (app. B).

Water Temperature

Surface water temperature data were retrieved from publicly available dockside monitoring stations (Florida: Florida State University Coastal Marine Lab dockside station; Georgia: National Oceanic and Atmospheric Administration [NOAA] dockside station FPKG1 at Fort Pulaski Island; Massachusetts: NOAA dockside station BHB3 in Boston). Obvious errors in the data were removed, and then data were averaged to produce biweekly temperature estimates. While the actual body temperature in the field would likely be affected by tidal cycle and intertidal microhabitat characteristics, these data provide the best available approximation of the average temperature environment. Water temperatures over the duration of the field collections varied across seasons and among sites. Massachusetts remained colder than Georgia or Florida throughout the year, although all sites showed a similar fluctuation of 15°–20°C between winter and summer (app. A).

All analyses were performed in R (v. 3.1.2; R Development Core Team 2014). Data used in all analyses are available in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.83h1h> (Ryan 2017b).

Results

Genets were lost from all treatments, with most mortality occurring in the first 4 months of the experiment. Individuals experiencing the most extreme conditions relative to their home conditions showed the fastest and overall highest genet extinction, with only 25% of genets remaining from the Florida in Massachusetts and Massachusetts in Florida treatments by the sixteenth week of the experiment (fig. 1). Despite genet level extinction occurring in all treatments, all three origins showed the highest survival (Florida, 58%; Georgia, 70%; Massachusetts, 45%) in their home conditions. Genets from the intermediate condition (Georgia) showed the smallest difference in mortality among temperature treatments (survival range, 55%–70%), whereas

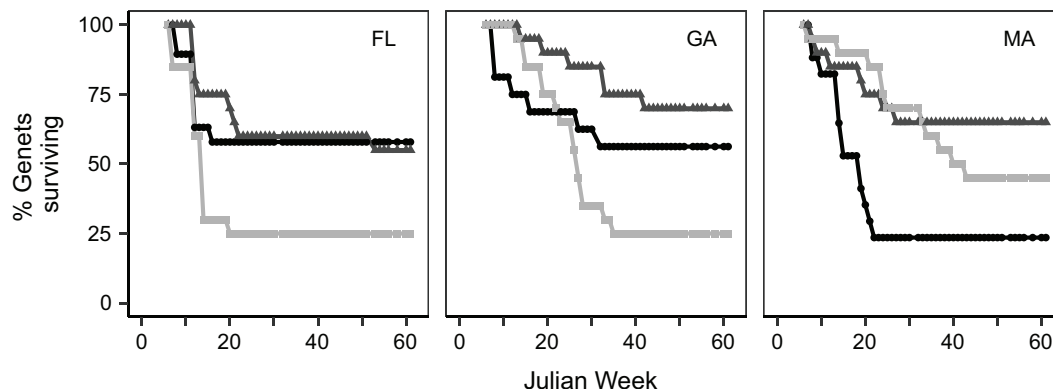


Figure 1: Proportion of genets surviving through time in common garden treatments mimicking the seasonal temperature pattern at each of three sites. Survival over 52 weeks depends on population of origin (circles, Florida [FL]; triangles, Georgia [GA]; squares, Massachusetts [MA]) and temperature treatment.

Florida genotypes did well in both Florida and Georgia conditions but suffered high mortality in Massachusetts. Massachusetts genets suffered equally high mortality in both Georgia and Florida conditions, with many more genets persisting in home conditions. Home condition survival was lowest in Massachusetts genotypes relative to Florida or Georgia genotypes. Individual ramets also suffered occasional mortality for unknown reasons. This mortality is represented in aggregate genet biomass measures but was not quantified directly.

There were also significant differences in fission rates among treatments. After 1 year, the mean number of ramets per genet for the genets that survived differed significantly among site of origin (Florida, 6.05; Georgia, 3.15; Massachusetts, 3.418; $F_{2,71} = 28.80$, $P < .001$) and temperature treatment (Florida, 6.70; Georgia, 4.33; Massachusetts, 1.58; $F_{2,71} = 43.14$, $P < .0001$; fig. 2A). Individuals from Florida produced significantly more ramets than either Georgia or Massachusetts individuals pooled across temperature treatments (distinguished with Tukey's honest significant difference [HSD]). The mean production of genets pooled across origins was highest under Florida conditions, followed by Georgia and then Massachusetts conditions, where very few clones were produced by any genet. There was a significant interaction of the main effects (origin \times temperature treatment; $F_{4,71} = 2.75$, $P = .035$) driven by a convergence onto very low clone production among origins under Massachusetts conditions.

The rate of ramet production (for genets that survived the duration of the experiment) increased predictably as temperature increased in spring and summer and declined again through the fall in all treatments. This produced an annual pattern of punctuated clonal proliferation during the warmest months interspersed with a period of stasis

when water temperatures were below approximately 14°–19°C (fig. 2A). The mean threshold temperature below which no fission occurs was marginally lower for Georgia and Florida individuals than for Massachusetts individuals ($F_{2,63} = 3.068$, $P = .054$; ANOVA; fig. 2A, inset). The threshold temperature also differed among temperature treatment ($F_{2,63} = 38.274$, $P < .001$), with individuals in Massachusetts conditions showing a significantly lower fission threshold temperature than either Georgia or Florida conditions. Most genets that survived all year underwent fission at least once during the year. Five of 13 individuals from Georgia and four of nine individuals from Massachusetts exposed to Massachusetts conditions did not undergo fission during the experiment. All surviving genotypes exposed to Georgia or Florida temperatures underwent fission at least once.

After 1 year, the mean estimated dry biomass per genet differed significantly among origins ($F_{2,71} = 8.35$, $P < .001$; fig. 2B) and showed a significant interaction between origin and temperature treatments ($F_{4,71} = 10.44$, $P < .001$) but not among temperature treatments alone ($F_{2,71} = 0.24$, $P = .791$). Anemones from Florida produced the greatest average biomass in warmer conditions (Florida and Georgia temperatures), producing significantly less biomass per genet under Massachusetts conditions (Tukey's HSD). Individuals originating in Georgia showed an opposite trend, producing equally low genet biomass in Florida and Georgia but high biomass under Massachusetts conditions. Individuals from Massachusetts produced the greatest average genet biomass under intermediate Georgia conditions, although differences among temperature treatments were not significant.

Individuals from all three sites showed similar changes in pedal disk area over time within temperature treatments,

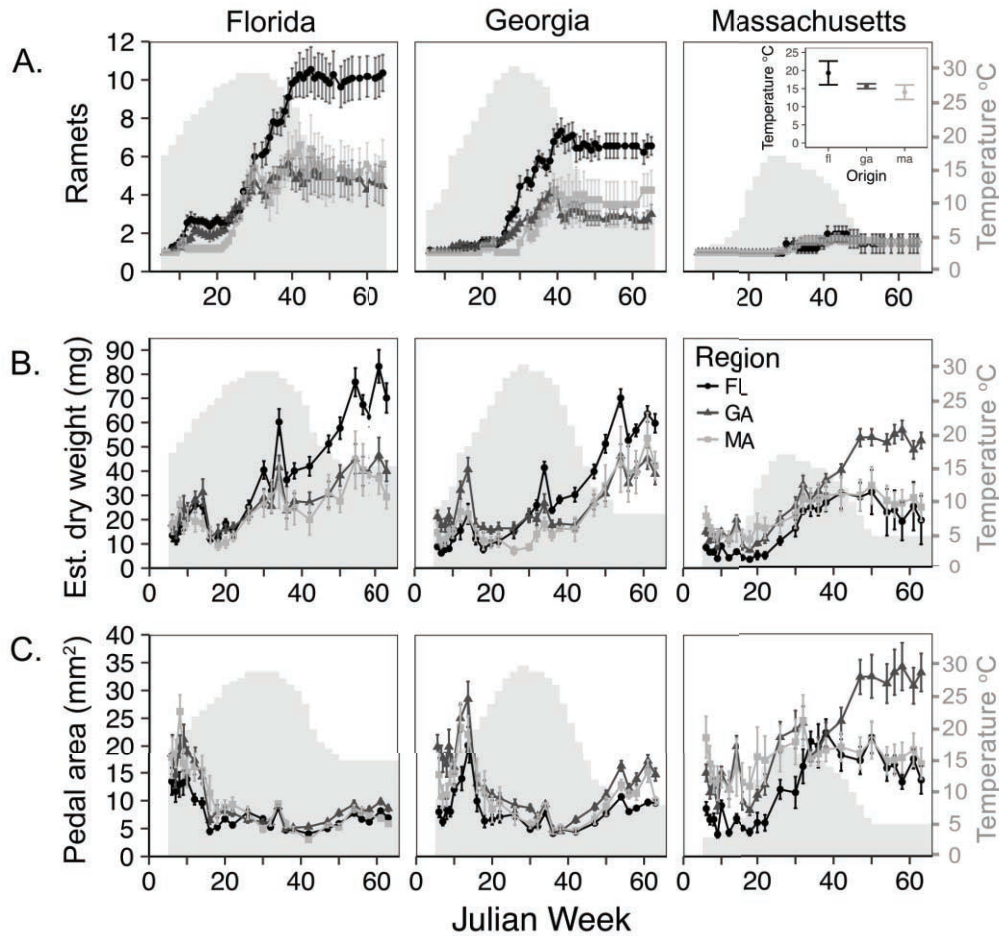


Figure 2: A, Mean \pm SE number of clonal descendants (ramets) produced by each individual collected from Florida (FL), Georgia (GA), or Massachusetts (MA) over 52 weeks in the lab at biweekly adjusted temperatures mimicking Florida, Georgia, or Massachusetts temperature conditions. Inset presents mean \pm SE temperature below which fission ceases by origin, averaged across temperature treatment. B, Mean \pm SE estimated dry biomass for each genotype (summed across ramets). Treatment combinations same as in A. C, Mean \pm SE pedal disk area (mm²) of individuals in each combination of origin and temperature treatment. Individual pedal disk areas averaged within treatment without respect to genotype to facilitate comparison with body sizes measured in the field. In all plots, gray bars indicate temperature in treatments over time.

with the notable exception of individuals from Georgia grown under Massachusetts conditions (fig. 2C). Under Florida conditions, pedal disk area initially increased, peaking at the beginning of March (by origin: Florida = 13.82 mm²; Georgia = 21.18 mm²; Massachusetts = 26.23 mm²) as the temperature increased past 18°C and fission began. Size then gradually decreased as temperature increased and fission continued through the summer. Genets from all three origins showed the smallest average pedal area (by origin: Florida = 4.08 mm²; Georgia = 4.84 mm²; Massachusetts = 3.02 mm²) just after experiencing the highest temperatures. Finally, size showed a slight increase as fission rate slowed and temperatures dropped below 20°C in the winter.

Under Georgia conditions, the average size of all three populations of origin initially dipped before rapidly increasing through March (peak size by origin: Florida = 20.13 mm²; Georgia = 28.47 mm²; Massachusetts = 21.27 mm²), with individuals from Florida and Massachusetts more than doubling in pedal area, over five weeks before declining again at the beginning of April. This sudden decrease in ramet size corresponds with the start of fission as temperatures increase above approximately 17°C around the end of March. Average pedal area reached a minimum for all origins at the end of August (by origin: Florida = 4.14 mm²; Georgia = 4.50 mm²; Massachusetts = 4.27 mm²), just after the treatment temperature peaked at 29°C. As temperatures subsided, individ-

ual body size began to increase again through the fall and winter.

When grown under Massachusetts conditions, individuals from all three sites showed an erratic decline in body size for the first few weeks of the experiment before beginning to increase again mid-May as temperatures approach 10°C. Average body size increased steadily as the temperature rose through the summer, although in Massachusetts, genets from different origins differed in their response. Individuals from Massachusetts achieved the largest average body size (26.13 mm²) in August, just before most individuals went through their sole bout of fission after the peak summer temperature of 17°C. After a drop in average pedal area following fission, sizes slightly increased until the treatment temperature dropped below 11°C, at which point the average size began to decline, suggesting either a reduced ability to take in food or an increased metabolic cost below 10°C. Individuals from Georgia also increased through the spring before exhibiting a reduction in average body size due to fission in August (minimum ~18.04 mm²). However, as temperature declined, the average size increased steadily until stabilizing (pedal area ~32 mm²) at the end of November, when temperatures fell below 10°C. Individuals from Florida grew through the spring and summer, reaching their peak body size at the end of September (22.17 mm²) before showing a steady decline in body size as temperature dropped below 13°C, even though no fission occurred.

The best-fit individual growth curve model (LME) includes a fourth-degree polynomial relationship between average pedal area and time, supporting the finding of significant seasonal fluctuations in body size over the year. The model also includes site of origin, temperature treat-

ment, and the interaction of the two factors with time as terms that significantly improve the fit of the model compared with the time-only model (log likelihood = -153.92, $P < .001$, dAIC = -121.91; for full details, see app. C). These additional factors support the observation that the magnitude and timing of fluctuations in body size are influenced by both the pattern of temperature and variation among genotypes.

Over 3 years, the average pedal area measured in the field over time (fig. 3) was smaller in Florida than in Georgia or Massachusetts (~7 vs. 15 and 16 mm², respectively). Seasonal variation in body size, however, was smaller in both Florida and Massachusetts (range of monthly means = 6.7 and 15.71 mm², respectively) than that observed in Georgia (31.24 mm²). The magnitude and timing of seasonal fluctuations in body size varied from year to year in both Florida and Massachusetts. In Georgia, a strong pattern of body size increasing through the early spring before rapidly decreasing through the summer was repeated for 3 years, although there is variation in the timing of the peak among years. The span of average pedal area measured in the field in Georgia (~6–35 mm²) is similar to that observed for Georgia individuals in Georgia conditions in the lab, with both showing an approximate threefold increase in average body size between fall and spring. Despite year to year variation, field-measured patterns at all three sites are consistent with trends in body size measured over time in experimental treatments where individuals were grown in their home conditions (fig. 2C).

The average reaction norm of mean ramet pedal area with temperature showed a unimodal pattern and peaked at 14°C (fig. 4). Fission did not occur at either 6° or 9°C but did at all higher temperatures, increasing, on average, as temper-

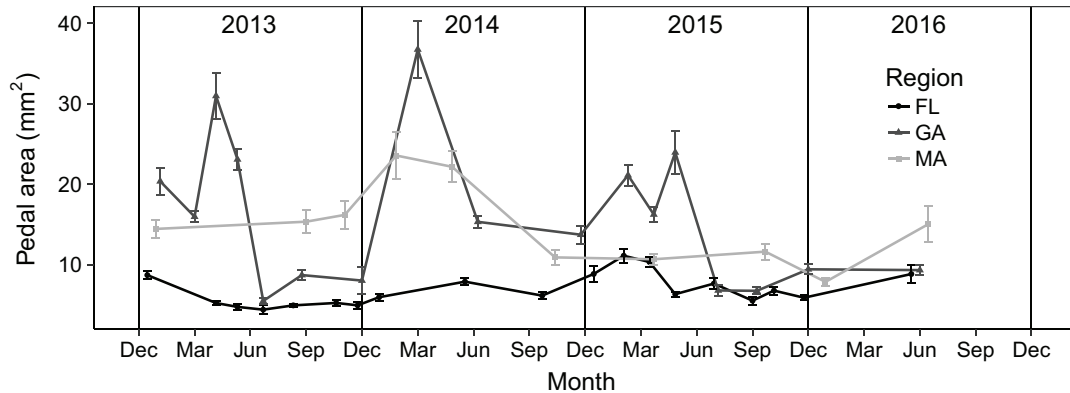


Figure 3: Mean \pm SE pedal disk area (mm²) of individuals measured in the field across 4 years in Florida, Georgia, and Massachusetts. Mean number of individuals collected per time point is 92 ± 9.8 anemones. Where four or more months have elapsed between sampling times, line slope should be interpreted with caution because fluctuations in size likely occurred but were not captured. FL, Florida; GA, Georgia; MA, Massachusetts.

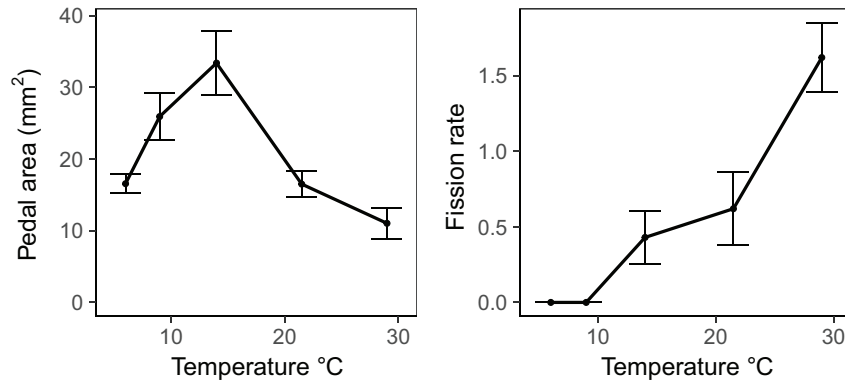


Figure 4: Mean \pm SE pedal area (mm²) and fission rate (natural log number of clonal descendants per week) calculated from clonal replicates of nine genets grown in each of five constant-temperature common garden treatments for 12 weeks.

ature increased (fig. 4). Site-level differences were not analyzed because the subset of the genets represented were nonrandom (see app. D). There was some variation in the height and slope of curves among genets; however, the general shape described above was consistent among genets.

Discussion

In the sea anemone *Diadumene lineata*, fission and body size clearly respond to temperature and contribute to region-specific patterns of growth, fission, and body size across seasons in both the laboratory and the field. Beyond the effects of temperature-mediated plasticity, differences due to site of origin were observed, potentially reflecting genetic differentiation among populations. For example, anemones from Florida grown in Florida-mimicking conditions divide more frequently and are smaller bodied than anemones collected from other regions when grown in the same Florida-mimicking conditions. Additionally, seasonal fluctuations in body size for all sites of origin show a “warmer is smaller” pattern that is consistent with the temperature-size rule (Kingsolver and Huey 2008) in Florida-mimicking conditions but show more complicated patterns under Georgia or Massachusetts temperature regimes.

Deviations from the “warmer is smaller” prediction can be explained in light of the result that pedal area follows a unimodal rather than monotonic reaction norm with temperature (fig. 4). Because the range of temperatures observed at each site is only a subset of those observed across the species range determines, only a portion of the reaction norm is expressed in conditions mimicking each site as seasons change. As such, ramet size is positively correlated with temperature in northern conditions, negatively correlated with temperature in the south, and unimodal

with temperature at intermediate sites. The expression of these patterns under seasonal conditions results in the observed site-specific patterns of body size change over the year (fig. 2C).

There appears to be a threshold temperature for fission at all sites, which interestingly falls very near the temperature at which maximum body size is attained (~14°–17°C). Above the threshold temperature, fission rate increases with temperature and ramet body size declines in a manner consistent with an increasing risk of oxygen limitation for large-bodied animals in warming conditions. In both the reaction norm and under seasonally changing conditions, a correlation between increasing fission rate and decreasing body size occurs over the warmest part of the species thermal tolerance range, where oxygen stress is most likely to occur (Pörtner 2001; Forster et al. 2011). The initiation and acceleration of fission at higher temperatures is consistent with the hypothesis that size (and surface area to mass ratio) modification through fission is an adaptive response to oxygen diffusion limitation. Because cold water is able to hold more oxygen at the same time that metabolic rate is lower, it is unlikely that the observed decline in size with low temperature is due to diffusion limitation. Cold temperatures can limit growth by inhibiting mechanical function needed to capture and digest prey as well as disrupting aerobic respiration (Pörtner 2002). Unlike the limits imposed at warm temperatures, however, these cold water limitations are not body size dependent and thus cannot be alleviated through growth or fission. The location of the threshold temperature at which fission begins may interact with body size, reflecting the point at which growth becomes inhibited by oxygen diffusion. Alternatively, individuals may respond to the temperature itself as a signal. More investigation is required to understand the mechanism underlying the relationship of temperature with fission rate.

Despite reductions in ramet size, the total biomass for genets maintained in growth chambers increased across all seasonal conditions. This is consistent with—although not a definitive test of—fission allowing for genet level biomass accumulation when unitary body size is allometrically constrained (Sebens 1982; Hughes 2005). Variation in the growth patterns among populations of origin presents an interesting clue into the types of adaptation that may occur (Angilletta et al. 2003). For example, individuals from Florida clearly show a steeper relationship between fission rate and temperature than either Georgia or Massachusetts populations. At the same time, they are able to accumulate biomass at higher rates across warm temperatures. Thus, although warm conditions limit individual ramet size, high fission rates allow for increases in genet-level biomass. In contrast, individuals from Georgia show similar rates of fission compared with Massachusetts across all temperatures but produce larger bodies and accumulate notably higher biomass even under the coldest conditions. This suggests that Massachusetts individuals either grow less efficiently or invest energy differently relative to Georgia individuals. Massachusetts individuals may invest more heavily in cold tolerance mechanisms (e.g., heat shock proteins [Sørensen et al. 2003], antifreeze proteins [Zachariassen and Kristiansen 2000], or mucus [McManus et al. 1997]). Or, tantalizingly, they could be investing proportionally more energy into gametogenesis, which was not assayed in this study. However, given their low survival rates even in home conditions, it is also possible that Massachusetts genotypes are simply maladapted.

In the field, site-specific patterns of seasonal temperature interact with population-specific growth and fission rate reaction norms to produce differing patterns of body size and clonal proliferation throughout the year. Changes in the average body size through time across multiple years show the same broad trends found in laboratory experiments, confirming that seasonal temperature is a major contributor to the size distributions of populations through time and across the species range. Because relationships among body size, metabolism, and temperature govern the acquisition and allocation of energy, the timing and magnitude of changes in fission rate and body size likely influence the timing and magnitude of gamete production. While no mature gametes were produced during the laboratory experiments, field-collected anemones from all three populations did produce eggs and sperm in the summer. While the concept of size at maturity is difficult to apply directly to an animal where size, age, and development are so decoupled, there is a threshold size below which gametes are rarely found, and the number of gametes produced above this threshold scales with body size (see Ryan 2017a). These size-dependent gametogenesis relationships suggest that the genetic and plastic fission rate

variation documented in this study are potentially important in geographic patterns of asexual and sexual investment in addition to influencing somatic growth rate.

Across the latitudinal range of this species, both geographic variation in temperature regime and genetic variation in the underlying reaction norms determine key life cycle transitions. These transitions affect patterns of body size and reproductive dynamics of local populations. The timing of fission and growth under the local temperature regime depends on the shape of the thermal reaction norm and location of thermal thresholds, so these traits are likely important targets for selection during local adaptation (Angilletta et al. 2004). Beyond providing empirical evidence for the role of temperature-mediated fission rate as a potential mechanism contributing to the temperature-size rule, the patterns presented here suggest a fruitful direction for studying the adaptive value of fission. For all clonal organisms, small variations in fission and growth rates can lead to rapid fluctuations in body size, which may allow fine-scale tuning of phenotypes to local optima, even as those optima change in rapidly fluctuating environments. Understanding the variety of mechanisms that such animals use to manipulate their shape and size in response to the environment is essential to understanding the evolution of these dynamic species.

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