Environmental regulation of individual body size contributes to geographic variation in clonal life cycle expression

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Abstract

Clonal behavior has been hypothesized to provide an escape from allometric metabolic scaling that limits the maximum mass achieved by a single individual. Here we demonstrate the capacity of a wide-spread, non-native sea anemone to buffer its colony biomass accumulation rate across environments by modulating ramet body size through environmentally-dependent growth, fission, and catabolism. In 2015, thermal reaction norms for growth and fission behavior were constructed using clonal lines of the sea anemone *Diadumene lineata*. In 2018, variation in growth patterns under a factorial cross of temperature level and oxygen availability were examined to test the hypothesis that individual ramet size is regulated by oxygen limitation in accordance with optimal size theory. Across a wide range of temperatures, colonies accumulated a similar amount of biomass despite a radical shift from unitary to clonal growth, supporting fission as a mechanism to buffer growth rates over a range of conditions. Individual body size appears to be regulated by the environment with increased temperature and reduced oxygen modifying fission and mass-specific growth patterns, leading to the production of smaller-bodied ramets in warm conditions. However, whether anemones in common garden conditions reduce individual body size through catabolism or fission depends on the region of origin and may relate to differences in seasonal temperature patterns among coastlines, which influence the energetic benefits of fission rate plasticity.

Introduction

Despite the relative simplicity of the cnidarian body plan, an astounding diversity of life cycle and growth patterns are achieved through variation in the timing and extent of investment in clonal growth (Fautin 2002; Geller et al. 2005). While many hypotheses have been put forth to explain an adaptive advantage of clonality under specific ecological conditions (see Francis

1979, 1988; Jackson 1977; Sebens 1979, 1987; Hughes 1987,1989), traits that influence fission rate (Ferretti and Geraudie 1998; Geller et al. 2005), and how selection acts on these traits (Reitzel et al. 2011), remain poorly understood. Many studies have looked for interspecific patterns, comparing the distribution of clonal and aclonal taxa with ecological factors to illuminate putative selective pressures (e.g., Chia 1976; Sebens 1979; Jackson 1985; Jackson and Coates 1986; Francis 1988). Where intraspecific comparisons have been made, there appears to be ample variation among genotypes in many aspects of growth, physiology, and fission behavior (e.g., Shick et al. 1979; Sebens 1980; Ayre 1985; McManus et al. 1997; Edmunds 2007; Reitzel et al. 2013), suggesting that traits governing clonality are labile. Phylogenetic comparisons in anemones suggest that clonality has evolved independently several times (Geller and Walton 2001). Consequently, life cycle traits are expected to respond readily to selection in this group of cnidarians. However, our knowledge of the mechanistic pathways underlying fission behavior in cnidarians remains rudimentary despite more than a century of interest in the lives of clonal marine animals (but see Mire and Venable 1999; Geller et al. 2005; Reitzel et al. 2011). To evaluate hypotheses about which demographic and environmental factors favor the evolution of clonality, more information is needed about the nature of variation in clonal behavior among genotypes and how traits are shaped by the environment (McManus et al. 1997).

In both unitary and clonal animals, body size is a fundamental property that governs metabolic rate and reproductive potential, as well as ecological relationships (e.g., competition and predation risk). Thus, it is expected to be a key target of selection. Theories of metabolic scaling suggest that smaller-bodied individuals with large respiratory surface area to biomass ratios are more energetically efficient under diffusion limiting conditions than large-bodied individuals (e.g. Atkinson 1994; Koojiman 2010; Glazier 2014). Similar logic has been used to

63 explain the observation that for a diverse array of taxa, warmer conditions lead to smaller adult
64 body sizes, even for species where reproductive fitness is positively correlated with body size
65 (Kingsolver and Huey 2008; but see Audzijonyte et al. 2018). The risk of oxygen limitation is
66 particularly important in aquatic organisms where the strong negative correlation between
67 temperature and dissolved oxygen availability acts as a fundamental constraint on body shape,
68 size, and performance (Pörtner 2001; Forster et al. 2012; Horne et al. 2015).
69 Under conditions that limit individual body size (e.g. hypoxia), clonal animals can have an
70 energentic advantage. Genets (the collective term for a group of descendants produced through
71 an asexual process) that are capable of dividing biomass into smaller units (known as ramets) can
72 grow indefinitely (i.e., iso- versus allo-metric growth), where unitary animals typically reach a
73 maximum size and cease somatic growth (Cancino and Hughes 1985; Sebens 1987).
74 Accumulating evidence suggests that the link between ramet body size and metabolic
75 performance may be critical for explaining the evolution of clonal and colonial life histories
76 (Burgess et al. 2018). However, the degree to which clonal animals can regulate growth and
77 fission rates to achieve energetically optimal ramet sizes is not well understood.
78 The role of fission in body size regulation is difficult to characterize, as metabolic, growth, and
79 fission rates often vary with the environment (e.g., Johnson and Shick 1977; Buss and
80 Blackstone 1991; Geller et al. 2005; Reitzel et al. 2013). Such effects may be an unavoidable
81 consequence of physical or chemical properties, leading to non-adaptive variation in the
82 expressed phenotype across environments (Gotthard and Nylin 1995). Alternatively, growth and
83 fission rate plasticity may be adaptive, allowing genetically identical clones to express a locally
84 optimal body size, and investment in asexual reproduction, across a range of environmental
85 conditions (Edmunds 2007). Indeed, for species that live in fluctuating environments, body size

86 plasticity through shrinkage (e.g., Levitan 1988; Chomsky et al. 2004) or a variable fission rate
87 (Ryan 2018) may be essential for tracking changing size optima through time.
88 To better understand the role of temperature and oxygen availability in regulating individual
89 body size and shaping clonal life histories, we examined growth, fission, and body size patterns
90 produced by the clonal sea anemone *Diadumene lineata* (Verrill) under a variety of experimental
91 conditions. We also explored variation in these patterns among individuals collected from
92 different parts of the extant range that differ in seasonal temperature patterns to look for evidence
93 of variation in asexual behavior that can inform predictions about the role of thermal regieme on
94 the evolution of life cycle plasticity. Phenotypic plasticity can be described as a "reaction norm,"
95 or a linear relationship between an environmental gradient and a phenotype produced by a
96 particular genotype (Bradshaw 1965). Small differences in the curvature or slope of reaction
97 norms can lead to large differences in expressed phenotypic patterns, particularly in fluctuating
98 environments. We used this conceptual framework to understand how individuals may modulate
99 growth patterns to match energetically optimal patterns dictated by the local environment and
100 discuss the evolutionary implications of variation in reaction norms that govern clonal behavior.
101 *Diadumene lineata* is a small-bodied, clonal sea anemone that occurs in the high intertidal
102 zone. The species likely originated in East Asia, but has been spread around the world through
103 anthropogenic activity for more than 100 years (Uchida 1932; Cohen and Carlton 1995). Like
104 many non-native species, *D. lineata* persists under a broad range of abiotic conditions and occurs
105 across a range of habitat types. The success of this species has been attributed, in part, to a
106 prolific schedule of binary fission (Uchida 1932). Fission rate is also known to increase with
107 temperature (Miyawaki 1952; Minasian 1979) which may allow individuals to modulate body
108 size and reproductive effort in response to novel or fluctuating conditions and likely structures

109 seasonal and geographic patterns of sexual and clonal reproduction across its distribution (Ryan
110 2018; Ryan and Miller 2019).
111 Seasonal and geographic temperature patterns vary across the species' North American range
112 (Table 1), which may impose differential selection on life cycle expression. Highly seasonal
113 temperature patterns across the Atlantic coast mirror conditions along the species' native
114 distribution in Japan. Nearshore waters of the Gulf of Mexico are similarly seasonal, but on
115 average much warmer than the Atlantic coast. Across the Pacific coast, there are smaller seasonal
116 fluctuations and cooler average sea surface temperatures. As residents of the high intertidal zone,
117 these anemones can also experience large daily temperature fluctionations through tidal cycles,
118 though the species is often found in tidal pools, under rocks, or embedded among co-occuring
119 organisms that can help buffer short-term abiotic fluctuations. We expected highly seasonal
120 environments to favor genotypes that rapidly increased fission rate and reduce ramet body size
121 under high temperatures as such a mechanism would allow genets to track predicatable changes
122 in the optimal body size on the scale of weeks to months. Conversly, for anemones in
123 environments where daily temperature fluctuations were larger than seasonal fluctuations (i.e.,
124 Pacific US), we expected fission to be less responsive to temperature, as engaging in fission in
125 response to short-term peaks in temperature could cause ramets to be perpetually below the
126 optimal body size for the environment, thus be maladaptive.
127 In this study, a series of experiments examine both the short-term (4 week) and longer-term
128 (12 week) responses of *D. lineata* to environmental manipulation. The short-term manipulation
129 of temperature and oxygen helps illuminate mechanisms of environmental body size regulation,
130 whereas the longer-term manipulaton demonstrates the variation in growth and fission patterns
131 that emerge from such regulation. We also explore geographic variation in the shape of reaction

norms of *D. lineata* using individuals collected from the Atlantic, Gulf, and Pacific coasts of the US, which vary substantially in their seasonal temperature regiemes. We discuss our findings in the context of potential local adaptation, although remain conservative in these conclusions as patterns of genetic differentiation within and among sites are currently unknown. Specifically, we ask:

(1) What are the general shapes of the thermal reaction norms of fission rate, individual body size, and clonal biomass accumulation in this species?

(2) How do temperature, oxygen, and coastline of origin contribute to ramet body size regulation via changes in fission and growth rate?

(3) Does basal metabolic rate differ among coastlines of origin?

(4) Are reaction norm differences among anemones from different coastlines consistent with the expected effects of seasonality?

Methods

Experiment 1: Characterizing reaction norms across five levels of temperature

Producing clonal replicates

Twenty individual anemones were collected from each three field sites across the US Atlantic and Gulf coasts (Nahant, MA [Atlantic, 42^{o} N]; St. Simon, GA [Atlantic, 31^{o} N]; St. Teresa, FL [Gulf, 30^{o} N]) in January 2013. Within sites, individual anemones were collected from points spaced one to two meters apart in an effort to represent the range of genetic diversity available at each site. No clonal replicates were knowingly included in the initial collection, however the individuals used to initate lab cultures reflected the genetic structures of the field populations, which were unknown. Single-strand conformation polymorphism data from a single

155 locus performed on individuals haphazardly collected from sites along the Atlantic and Pacific
156 coasts suggests that most sites host multiple genotypes, and that repeated genotypes are common
157 within, but not among sites (Ting and Geller, 2000). Given the geographic distance among sites,
158 we are confident that the sample of individuals included in the experiment contained multiple
159 genotypes, however, none of our conclusions require this assumption.
160 Collected individuals were used to establish isolated clonal lines under common garden
161 conditions that exposed them to a seasonally adjusted range of temperatures between 15-29° C,
162 mimicking field measured conditions from St. Teresa, FL (see Ryan 2018). Between March and
163 September 2015, clonal lines were kept at 20^{o} C and fed *Artemia* nauplii (Brine Shrimp Direct,
164 Ogden, UT, USA) two times per week.
165
166 *Temperature treatments*
167 In September of 2015, individual ramets were randomly selected from each of twelve
168 genets (FL: 5, GA: 4, MA: 3) that had sufficiently large clonal populations. Individual anemones
169 from each putatively distinct genotype were measured for pedal disk area, then isolated in a 50ml
170 Falcon tube of artificial seawater (Instant Ocean; salinity 32 ppt) and randomly assigned to one
171 of five temperature chambers (including three refridgerators (see Ryan 2018), a bench top
172 incubator (PR205075G Thermo Scientific, Waltham, MA, USA), and a climate control chamber
173 (I-36VL Percival Scientific, Perry, IA, USA). Chambers differed in make and size (ranging from
174 an interior volume of 0.05 to 0.85 cubic meters), so they were left dark to standardize light
175 conditions and prevent algal fouling. This species is not known to harbor photosymbionts and
176 often occurs under rocks in total darkness, therefore does not require light for growth or
177 nutrition. All of the chambers maintained static cultures within 1°C of the target temperature.

178 The water in each tube was exchanged and anemones fed to repletion on 3 day-old *Artemia*
179 nauplii (Brine Shrimp Direct, Ogden, UT, USA) twice per week. Five temperature treatments
180 were used (6, 9, 14, 21.5, and 29°C) spanning the range of average monthly water temperatures
181 experienced by this species on the east coast of North America. Because the experiment required
182 five temperature levels, we were unable to use more than one environmental chamber per
183 temperature treatment. Thus, chamber and temperature treatment level are unfortunately
184 confounded. However, as anemones were isolated in sealed vials within chambers, we have no *a*
185 *priori* reason to suspect systematic bias due to chamber identity.
186 Each genet was initially represented by 1 to 4 ramets in each temperature condition,
187 depending on replicate availability. Eight of 12 genets had at least two replicate anemones
188 assigned to each treatment. Replicate ramets were limited for the other four genets such that
189 some temperature treatments only had one anemone assigned (see Table S1). Of these genets,
190 only two were retained in analysis (see results). The probability of survival of each genet in each
191 temperature treatment was calculated as the number of initial replicates still represented by at
192 least one ramet at the end of the experiment (week 12) divided by the initial number of genotypic
193 replicates in each treatment. Fission rate was quantified as the number of daughter clones
194 produced by each individual over the experimental period. Body size was measured by tracing
195 the outline of the attached pedal disk onto a sheet of acetate, scanning the drawings into a
196 computer and using Image J software (Rasband 1997) to calculate the area of the pedal disk
197 (mm^2). Initial pedal disk area was used to estimate dry mass using the regression measured in
198 Ryan (2018). All individuals were then rinsed in freshwater to remove extraneous salt, separated
199 into pre-tared foil boats and dried at 70° C for 72 hours. Dried tissue (µg) was weighed using a
200 microbalance. Final tissue density was calculated as dry mass per pedal disk area ($\mu g/mm^2$).

201 Change in colony weight was calculated as the difference between final and estimated initial
202 colony dry weight. Mean individual mass was calculated as the mean dry mass (μg) of all ramets
203 within a replicate.
204
205 *Analysis*
206 The individuals used were biased toward those genets that had enough ramets available.
207 Mortality during the experiment left both GA and MA with complete final data for only two and
208 one genets, respectively (see details in Table S1). Thus, no attempt was made to characterize
209 variation among sites of origin in this experiment. Genet survival, fission rate, and change in
210 colony dry weight were genet-level traits, so genet ID was used as a random factor in all models
211 (Table 3). Ramet body size and tissue density were calculated as ramet-level traits, so both genet
212 ID and replicate ID within genet were used as random factors to account for variation among
213 ramets derived from independent cultures of clonal replicates derived from the same genet. No
214 genotype by treatment interactions were considered.
215 The effect of temperature on the probability of genet survival was estimated with
216 generalized linear mixed model (GLMER) with a logit-linked binomial distribution. The effect
217 of temperature on the number of clonal descendants (fission rate) was analyzed with GLMER
218 using a negative binomial distribution. The effects of temperature on change in colony dry
219 weight, ramet dry weight, and tissue density were analyzed with GLMERs using log-linked
220 Gaussian error distributions. In all cases, temperature was initially fitted with the highest order
221 polynomial supported by the levels of temperature (4^{th} degree) to elucidate the shape of the
222 relationship. Model selection using AICc was then used to find the best-fit model using the
223 dredge function in the R (R Core Team, 2018) package MuMIn (Barton 2018). The model with

224 the lowest AICc value was chosen, except where a model with fewer parameters had a similar
225 AICc value (dAICc < 2) (see model details in Supplement 1). The signifance of the contribution
226 of each retained parameter in the best fit model was evaluated using the type II Anova function
227 in the R package car (Fox and Weisberg 2011).
228 All analyses were done in R ver. 3.5.1 (R Core Team, 2018)
229
230 Experiment 2A: Characterizing growth and fission variation among individuals collected from
231 the Pacific, Gulf, and Atlantic coasts of the United States
232
233 *Anemone collection*
234 Between June and November 2017, *Diadumene lineata* individuals were collected from
235 ten sites in the species' US range (Table 2). At each site, from one to twenty individuals were
236 collected from each of five 0.25m^2 quadrats along a 25 meter transect through the high intertidal
237 zone, parallel to the water line, except at ESL where quadrats were placed at 5 meter intervals
238 along a floating dock. This resulted in the collection of between 19 and 100 individuals per site.
239 The number collected from each quadrat at each site varied, as this species has a patchy
240 distribution. For the experiments, samples were drawn randomly from anemones available from
241 each site without respect to quadrat of origin. To reduce the effects of prior environmental
242 variation, collected individuals were maintained separately in the laboratory in 50 ml tubes of
243 artificial seawater at 30ppt, 15°C temperature, on a 12:12 hour light:dark cycle from the time of
244 colletion until use in the experiment, a period ranging from one to six months. Anemones were
245 fed weekly with Microvert liquid invertebrate food (Kent Marine, Franklin, WI, USA) until the
246 experiment began. Size variation naturally occurred among populations and persisted in collected

247 samples until the start of the experiment (Table 2). However, within site of origin, the mean
248 initial size of individuals assigned to each treatment group did not differ significantly when
249 compared with ANOVA (see results).
250
251 *Experimental design*
252 In December 2017, 20 - 25 individuals were randomly selected from among the common
253 garden-maintained cultures from each site and were assigned to one of four treatment conditions
254 representing a factorial cross of temperature (15° and 25° C) by dissolved oxygen level (50% and
255 100% of normoxia). As above, no clonal replicates were knowingly included in the experiment,
256 however the underlying genetic structure of these populations was unknown. Individuals used to
257 initate lab cultures reflected a random sample of the genetic structures of the field populations.
258 For the purposes of this experiment, each individual was treated as an independent replicate,
259 however we were careful to avoid drawning conclusions with regard to the role of genetic
260 diversity underlying the observed variation in phenotypes.
261 Each individual was wet weighed, photographed for pedal area measurements (see
262 method in Experiment 1), then placed individually into a tube with 15 ml of artificial seawater
263 (30 ppt). Live anemones in tubes of seawater were shipped with ice packs from the University of
264 Alabama at Birmingham (UAB; Birmingham, AL, USA) to the Marine Biological Association of
265 the United Kingdom (MBA; Plymouth, UK) and arrived within 36 hours of shipment. Upon
266 arrival, 20 anemones from each site (except CFP, where only 19 individuals were available) were
267 transferred individually into the wells of twenty 12-well plates (Corning Inc., NY USA) (N = 5
268 per treatment) filled with filtered natural seawater (salinity 30 ppt), which were then divided

among four, 4 L sealable plastic tanks fitted with air stones. Remaining anemones were set aside for experiment 2B (see below).

To facilitate water exchange with a surrounding tank, holes were pre-drilled into the lids of the 12-well plates and then were lined with a fine mesh to prevent anemones from escaping or moving among wells. The ability for water to exchange freely between the tank and each well was confirmed by observing the ability of food dye to diffuse easily across the mesh when a prototype plate was submerged in water. The plastic tanks, each containing five plates, were then set in water baths to control their temperature. All anemones were maintained at approximately 10^{o} C and aerated for two weeks to allow them to acclimate to the growth chambers with minimal mass change and no fission. On January 2, 2018 a factorial cross of temperature and oxygen level manipulations were initiated. Five individuals from each site, randomly positioned across plates, were subjected to each of the four treatments. The temperature of the room was raised to 15^{o}C and then submersible aquarium heaters were added to water baths surrounding half of the plates, raising their temperature to 25^{o} C over the course of two days. Temperature was monitored at five minute invertals with Hobo loggers (Onset, Bourne, MA, USA) in each plastic tank and by daily checks with an infrared thermometer. To manipulate the availability of dissolved oxygen, half of the tanks were aerated with ambient air fed from outside the building (100% ambient oxygen), the remaining tanks were aerated with a pre-mixed gas of 10.5% oxygen and 89.5% nitrogen (BOC, Plymouth, UK), equivalent to 50% of the ambient oxygen treatments. Because pre-mixed gas was used, oxygen level was not monitored during the experiment. While maintaining an independent environmental manipulation for each replicate plate would have been ideal for statistical independence, it would have required a prohibitively

large volume of mixed gas to run the experiment. Thus, each treatment consisted of one treatment tank that housed replicate plates.

Through the duration of the experiment, anemones were fed to repletion on a diet of two-day old *Artemia* nauplii (Brine Shrimp Direct, Ogden, UT, USA) every other day. Plates were removed from treatment tanks, the water in wells discarded (with care not to dislodge individuals), and a 3 ml aliquot of a well-stirred culture of nauplii was pipetted into each well. Plates were then returned to treatment tanks. This feeding protocol resulted in an 18% water replacement in treatment tanks per week.

After four weeks, anemones were returned to 15 ml tubes of filtered seawater and shipped back to UAB on ice where all anemones were wet weighed, photographed for pedal area, and then dried at 72^{o} C for 72 hours and dry weighed. Fission rate, change in colony mass, change in mass-specific growth rate, and mean individual mass were calculated for each genet as in Experiment 1.

Analysis

Variation in initial size among treatments and site of origin was assessed using a two-way ANOVA. Since initial size varied among sites of origin, initial body size was considered in all full models. Initial models also used site as a random variable to account for variation within coastline. However, in no case did including site as a random variable improve the fit, thus it was dropped for all analyses. In all cases, an initial model containing all predictor variables (temperature, oxygen, and coastline of origin) and interactions as well as a polynomial series of the natural log of initial wet weight, was constructed using GLMER in the lme4 package (Bates et al. 2015) for R (R Core Team, 2018). To determine the shape of the relationship with initial

size, the initial model used the highest order polynomial supported by available degrees of freedom (typically 5 degrees) (see supplement 2 for details). Stepwise model selection using AICc was then used to determine the best fit models (Table 3) as described for experiment 1. The probability of genet survival and probability of fission were modeled with binomial distributions. The mass-specific change in mass, calculated as the natural log of the final wet weight minus the natural log of initial wet weight, was normally distributed. The total change in colony mass, calculated as the final wet weight minus the initial wet weight, was also modeled to provide a visualization of biomass change patterns. But, given the highly leptokurtic distribution of this metric, statistical inferences are best drawn from mass-specific analyses above. The final body size of individual anemones (grams wet weight) was modeled with a log-linked gaussian distribution. Genet ID was included as a random factor to account for the non-independence of ramets produced by each genet. Analysis of Variance tables were constructed for each model to aid in interpreting the contribution of each predictor. Goodness of fit values for all models were calculated with the r.squaredGLMM function in the R package MuMIn or rsquared function in the R package piecewiseSEM (Lefcheck 2018) for GLM(M)s.

Experiment 2B: Estimating relative differences in basal metabolic rate through starvation.

Experimental design

Thirty-three individuals representing seven sites of origin on three coastlines (Pacific: 4 sites (n = 5), Gulf: 2 sites (n = 4), Atlantic: 1 site (n = 5); Table 1) were used to measure loss of body mass through starvation. Once at the MBA, these individuals were kept sequestered at 15^{o} C in individual tubes with 14 mL of artificial seawater, leaving a small headspace of air in each tube. This temperature was chosen to minimize the likelihood of fission which would complicate

337 the interpretation of the results. Approximately once per week, tubes were agitated gently, but no
338 food was provided. After four weeks, anemones were returned to UAB on ice and were weighed
339 and measured as in Experiment 2A.
340
341 *Analysis*
342 Weight loss through starvation was calculated as the final wet weight (g) minus initial
343 wet weight. To test whether the rate of biomass catabolism depended on initial body size, weight
344 loss was regressed on the natural log of initial wet mass with log-linked gaussian linear
345 regression (GLM) (Table 3). Coastline of origin was treated as a fixed factor to evaluate
346 differences in starvation-induced shrinkage as a proxy for basal metabolic rate (Sebens 1981).
347
348 **Results**
349 *Experiment 1: Characterizing reaction norms across five levels of temperature*
350 Of the 125 individuals originally included in the experiment, 23 died soon after being
351 moved into the experiment. This transplant mortality was heavily concentrated among two
352 genets, which were both removed from all subsequent analyses (see Table S1). Among the
353 remaining ten genets (FL:5, GA:3, MA: 3) mortality was low during the experiment; the average
354 probability of replicate survival was 0.93. Genet identity was the major factor contributing to
355 variance in survival (conditional $r^2 = 0.23$). Temperature was not retained as a significant
356 predictor of mortality in the best-fit binomial GLMM (Figure 1A, see analysis details in S1). For
357 two genets, all replicates in 29° C died, precluding the construction of growth and fission
358 reaction norms. Thus, only the eight genets with complete data (FL: 5, GA: 2, MA: 2) were used
359 in subsequent analyses.

The number of clonal descendants produced ranged from one (no fission) to 13 ramets and showed a significant, monotonic increase with temperature (GLMM, χ^2 (1,73) = 67.75, p <0.001; Figure 1B). All surviving colonies accumulated biomass over the 12-week experiment. Change in colony dry weight (mass accumulation) across temperature was best described by a third order polynomial (GLMM, χ^2 (3,71) = 66934, p <0.001; Figure 1C). Mass accumulation was lowest at low temperatures and highest at intermediate temperatures, peaking in the 14° C treatment. Mass accumulation remained intermediate to high across the warmer temperatures despite a rapid increase in fission; though, many genets showed a dip in mass accumulation at 21.5° C relative to 14 and 29° C. At the end of the experiment, individual ramet dry mass also differed significantly across temperatures, which was best described by a third order polynomial (GLMM, χ^2 (3,168) = 51.89, p <0.001; Figure 1D). Ramet body size was unimodal, peaking at 14° C. Individuals in the coldest treatment grew, but stayed small without dividing, whereas individuals in warmer treatments (≥ 21.5° C) accumulated colony mass through the production of daughter clones which were smaller-bodied than the founding individual. Tissue density showed a significant, monotonic decline with increasing temperature (GLMM, χ^2 (1,169) = 17.11, p <0.001; Figure S1). See Supplement 1 for model selection details and parameter estimates for all analyses above.

Experiment 2A: Characterizing growth and fission variation among genotypes collected from the Pacific, Gulf, and Atlantic coasts of the United States

Of the 199 individuals included in the initial experiment, data from 5 individuals were exclude from analysis. Two individuals from CFP were too small for initial wet weights to be measured confidently (< 0.0001 grams). Two individuals from WAS were an order of

magnitude smaller any others from the site (> 1.9 standard deviation units below the mean). One individual from WAS was an order of magnitude larger than any others from the site (> 2.5 standard deviation units above the mean). These replicates were removed to prevent statistical estimations from being extrapolation over a size range for which not all treatment levels were represented. Statistical inferences were not altered by the inclusion or exclusion of these data.

There was variation in initial body size (the natural log of wet weight) among sites of origin (Two-way ANOVA, $F(9,154) = 29.79$, $p < 0.001$), but no systematic variation among assigned treatment levels (Two-way ANOVA, $F(3,154) = 0.862$, $p = 0.46$). There was also no significant difference in initial size between treatment by site of origin (Two-way ANOVA, $F(27, 154) = 0.52$, $p = 0.52$). The median initial wet weight was highest for Pacific coast individuals, followed by Atlantic and Gulf Coast individuals (0.020, 0.018, 0.012 grams, respectively) See Table 2 for the median initial wet weights by site for individuals used in experiments 2A.

Over 4 weeks, survival was high (94%) among the 194 individuals included in the experimental analysis. Exposure to low oxygen conditions significantly reduced individual survival to 90% compared to 98% of individuals in high oxygen conditions (GLM, $r^2 = 0.18$, χ^2 $(1,193) = 6.00$, $p = 0.014$; Table S2). The lowest genet survival rate (83%) occurred for anemones of Pacific origin experiencing both high temperature and low oxygen conditions, though neither temperature nor coastline of origin were retained as significant predictors in the best-fit GLM model. Likewise, initial body size was not retained in the final model. Site of origin as a random factor was removed through model selection.

Among the individuals that survived, the probability of undergoing fission was influenced by initial body size, coastline of origin, temperature, and oxygen treatments (GLM, r^2

406 = 0.49, Figure 2). As expected, high temperature significantly increased the probability of fission
407 (GLM, χ^2 (1, 173) = 47.77, $p < 0.001$). Coastline of origin had a significant effect (GLM, χ^2 (2,
408 173) = 17.95, $p < 0.001$); Gulf Coast individuals had the highest probability of dividing across all
409 treatments, followed by the Atlantic, then Pacific individuals. The same order was reflected in
410 the mean number of clonal descendants produced across treatments (Gulf: 1.40 +/- 0.08 se,
411 Atlantic: 1.32 +/-0.10 se, Pacific: 1.08 +/- 0.03 se). The influence of intitial body size differed
412 among coastline (GLM, intial size X coastline χ^2 (2, 173) = 6.74, $p = 0.034$). The probability of
413 fission declined with initial body size for Pacific genets and increased with body size for Gulf
414 genets. Initial body size showed no average effect for Atlantic genets, partly due to the
415 significant interactive effect between oxygen and initial size. In all cases, exposure to low
416 oxygen increased the probability of fission at large initial body sizes relative to the high oxygen
417 treatment (GLM, initial size X oxygen χ^2 (1, 173) = 4.89, $p = 0.027$) (see analysis details
418 Supplement 2).

419 Mass-specific weight change, or the growth rate per unit initial biomass, show a clear
420 monotonic decline with initial body size (GLM $r^2 = 0.60$, $F(1, 176) = 258.46$, $p < 0.001$; Figure
421 3A). High temperature reduced average growth (GLM, $F(1, 176) = 6.58$, $p = 0.011$) and caused a
422 marginally significantly steeper slope in the decline of growth with body size (GLM, initial size
423 x temperature $F(1, 176) = 3.78$, $p = 0.053$). Oxygen level did not change the average mass-
424 specific weight change (GLM, $F(1,176) = 0.115$, $p = 0.115$), but did have a significant
425 interaction with initial body size (GLM, initial size x oxygen $F(1, 176) = 5.06$, $p = 0.026$).
426 Individuals exposed to low oxygen showed a trend of dampened growth among small individuals
427 where growth rates were highest, but did not alter the threshold size above which anemones lost

428 mass. Coastline of origin was not retained in the best-fit model suggesting that all regions of
429 origin showed similar treatment responses (see analysis details in Supplement 2).
430 When the raw change in colony wet weight was plotted against initial wet weight (Figure
431 3B), both the energetic benefit of optimal size and the high cost of being too large are evident.
432 The best-fit model describing the change in colony wet weight is a forth degree polynomial
433 (GLM, $r^2 = 0.67$), which demonstrates the peak in growth at an intermediate initial size, and
434 precipitous loss of mass for larger individuals (Model details are provided in supplement 2,
435 however, the influence of temperature and oxygen is best understood from the patterns of mass-
436 specific growth described above).
437 The final individual wet weight (ramet size) was influenced by initial size and
438 temperature (GLM, $r^2 = 0.56$, Figure 3C), and varied among genets within coastline (variance =
439 0.18, sd = 0.43). Final size was significantly, positively correlated with initial wet weight (GLM,
440 χ^2 (1, 223) = 59.49, $p < 0.001$); though, the slope of the relationship was consistently less than
441 one suggesting a tendency for body size to converge on a similar size within treatment over time
442 regardless of initial size. High temperature led to significantly smaller body sizes on average
443 (GLM, χ^2 (1, 223) = 14.49, $p < 0.001$) (median wet weight: 0.010 vs. 0.022 grams in low
444 temperature treatment). The rank order in body size among coastlines persisted (median wet
445 weight: 0.021, 0.015, 0.009 grams for Pacific, Atlantic, Gulf individuals, respectively), but
446 coastline of origin was not retained as an explanatory variable in the best-fit model (see
447 supplement 2). Likewise, there was little effect of oxygen treatment and this factor was not
448 retained in the final model. Interestingly, ramets from Gulf Coast genets tended to become
449 smaller through fission, whereas Pacific Coast ramets tended to shrink through catabolism
450 without undergoing fission (Figure 4). Atlantic Coast genets showed a mix of individual

451 shrinkage and fission to reduce body size. In most cases, fission produced two similarly sized
452 daughter clones (i.e., binary fission), though pronounced asymmetry in final ramet size was
453 observed particularly among Gulf individuals with large initial sizes (Figure 4). In one case, a
454 Pacific Coast individual produced a pedal lacerate.
455
456 *Experiment 2B: Estimating relative differences in basal metabolic rate among coastlines of*
457 *origin through starvation.*
458 All individuals survived the duration of the experiment and none underwent fission. All
459 individuals lost weight over four weeks, but the rate of weight loss, which is inversely
460 proportional to resting metabolic rate, was significantly influenced by both initial body size and
461 coastline of origin (GLM, $r^2 = 0.93$, Figure 5). The natural log of mass loss increased
462 significantly with the natural log of initial body size (GLM, $F(1, 22) = 288.29$, $p < 0.001$).
463 Individuals from the Gulf and Atlantic coasts lost significantly more mass than those from the
464 Pacific coast (GLM, $F(2, 22) = 5.99$, $p = 0.008$), but showed a similar slope (see Supplement 3
465 for statistical details). Because there was not complete overlap in initial body size distribution
466 available from the coasts, inferences about the performance of very large or very small
467 individuals are limited in this data set.
468
469 **Discussion**
470 Thermal growth curves for most ectothermic animals take the shape of a left skewed
471 distribution, with a gradual increase to a peak followed by a rapid decline in performance (Shulte
472 et al. 2011). The first experiment shows that the thermal reaction norms constructed for
473 *Diadumene lineata* over 12 weeks were different. They revealed the capacity of a clonal animal

474 to maintain high biomass accumulation rates across a wide range of temperatures while
475 modulating the size of ramets through changes in fission and growth. The second set of
476 experiments showed that individual body size is regulated by the abiotic environment via
477 changes in the slope of size-dependent growth curves. Over four weeks, individual growth,
478 catabolism, and fission all contributed to the pattern of individuals converging toward
479 environment-specific body sizes. Together these results support the hypothesis that life cycle
480 plasticity in clonal animals can stabilize growth across variable environments and encourages
481 more exploration into how reaction norms for clonal behavior are shaped by local environmental
482 patterns.

483 The interaction of temperature and dissolved oxygen with metabolic rate is complex. As
484 temperature increases, metabolic rate increases, which increases the demand for oxygen. At the
485 same time, higher temperatures reduce the capacity for water to hold dissolved oxygen. Thus,
486 both a direct reduction in oxygen input and temperature increase can increase the risk of oxygen
487 limitation. Metabolic demand for oxygen also increases with body size. Thus, the optimal body
488 size for avoiding oxygen limitation depends on both water temperature and rate that oxygen
489 flows into the environment.

490 In the longer-term experiment, fission rate increased linearly while individual ramet size
491 was unimodal over a broad range of temperatures. There was a transition from large and unitary
492 to small-bodied and clonal as temperature increased, though the exact slopes and inflection
493 points appeared to vary among genets. Notably, the rate of total colony mass accumulation was
494 similar for many genets between 14° to 29^{o} C despite the major transition in growth pattern. Over
495 the 12 weeks of the experiment, fission served to stabilize tissue growth rates across a span of
496 temperatures over which oxygen consumption could reasonably be expected to triple ($Q_{10} \sim 2$;

Sassaman and Mangum 1970). The reduction observed for many genets in biomass accumulation at 21.5^{o} C may suggest that asymmetry in the energetic costs and benefits of fission may lead to uneven growth across temperatures.

In the four-week experiment (2A), fission increased with temperature but showed a complicated relationship with body size, oxygen level and coastline of origin. Fission appeared to be stimulated when anemones were too large for the abiotic conditions, resulting in lost mass. However, fission also occured when anemones were growing rapidly, so were presumably well-suited to the environment. Both patterns are consistent with oxygen limitation acting as a cue for fission. These alternate roles for fission behavior may help account for the observed interaction between oxygen treatment and body size in predicting the likelihood of fission in high temperature conditions.

Overall, Gulf Coast anemones showed the highest propensity toward fission, consistent with previous observations of high fission rates and small body sizes for Gulf versus Atlantic genets of the species under a seasonal temperature cycle (Ryan 2018). The role of fission in growth also varied among coastlines. For Gulf Coast genets, fission occurred most frequently where individuals were initially large-bodied and played a role in reducing ramet size. Despite being initially larger-bodied on average, Pacific Coast genets rarely engaged in fission during the four week experiment. Pacific ramets that ended up smaller than the initial size mostly did so through catabolism rather than fission, similarly to how unitary anemones perform under high temperatures (Chomsky et al. 2004). Along with evidence of lower basal metabolic rates in Pacific genets, these results are consistent with patterns of reduced temperature sensitivity found for other organisms evolving under weak versus strong seasonal temperature fluctuation (Baumann and Conover 2011).

Recent theory suggests that non-fluctuating environments favor thermal performance curves that match optima based on mean temperature, whereas strongly seasonal environments tend to favor higher resting metabolic rates and strategies that minimize the risk of stress during summer high temperatures due to asymmetry in the energetic cost of being warmer rather than cooler than optimal (Amarasekare and Johnson 2017). Because body size influences metabolic rate, traits that modulate body size (such as growth and fission) are expected to be more responsive to temperature in seasonal environments, where the energetic cost of environmental mismatch is likely much higher (Scranton and Amarasekare 2017).

In anemones, an increased risk of exposure to heat-related hypoxia in predictably varying environments may drive the evolution of fission rates that are more responsive to temperature. Gulf anemones are at the highest risk of predictable periods of persistant hypoxia due to high mean water temperatures coupled with strong seasonal fluctuations. In contrast, Pacific anemones, which may encounter bouts of high temperature and hypoxia during low tide (Helmuth et al. 2002), do not experience sustained and predictable exposure to warm, hypoxic water to the same degree. As a consequence, Pacific anemones may use fission primarily to maintain colony growth through the production of uniformly sized ramet whereas Gulf and Atlantic populations use fission for both growth and as a means to rapidly modulate body size to avoid hypoxic stress during seasonal flux. As a point of comparison, the larger-bodied clonal species, *Anthopluera elegenatissima* occurs across a similar latitudinal distribution on the Pacific coast of the US. Fission in this species has been described primarily as a mechanism of asexual growth driven by food availability, rather than as a mechanism for modulating body size through temperature cycles (Sebens 1980,1982). However, we currently lack both the local-scale temperature data and depth of sampling for *D. lineata* to evaluate the merits of this explaination.

543 Genets from all three coastlines showed size-dependent growth, where mass-specific
544 growth rate declined steeply with increased body size resulting in a unimodal pattern of total
545 colony mass change. As predicted, the energetic cost of being smaller than optimal (reduced
546 growth rate) is much lower than the cost of being too large (mass loss), perhaps favoring fission
547 behavior when the risk of hypoxia is high. Optimal size theory extends the predictions of
548 metabolic scaling theory to suggest that the body size that maximizes energetic efficiency
549 decreases monotonically from cold to warm conditions (Sebens 2002, Kingsolver and Huey 2008
550 Forster et al. 2011, Sheridan et al. 2011). Consistent with these predictions, a combination of
551 fission, growth, and shrinkage behavior led ramets to converge toward a environment-specific
552 body size. The size onto which ramets converged differed between temperature treatments and
553 mirrored the body size that led to the highest colony biomass accumulation, consistent with
554 regulation of growth and body size via allometric metabolic scaling (reviewed by Glazier 2014).
555 These results all support the existence of energetic advantage for genets that can modulate the
556 size of ramets produced in variable environments. Thus, the patterns we observed suggest a
557 degree of adaptation in the shape of the underlying reaction norms that govern fission and
558 growth behavior in *D. lineata*. However, there is little known about how such plasticity is
559 expressed under field conditions in spatially or temporally heterogenious environments. Here we
560 have interpreted patterns through the lens of oxygen limitation, but there are likely many
561 additional effects of temperature (e.g. changes in gene expression, tissue damage, etc.)
562 influencing growth and fission in ways that we have not yet investigated. Likewise, there is
563 much to explore about the role of invasion dynamics and habitat filtering in the geographic
564 patterns observed for this species, whose range has been expanded through human activity to
565 encompass three coastlines with very different climatic patterns. Differences in the invasion

566 history of non-native populations across the US could provide an alternate explaination for the
567 observed variation among coastlines. Future work elucidating patterns of genetic diversity and
568 connectivity within and among sites will likely provide critically needed context for interpreting
569 the eco-evolutionary significance of such variation.
570 These findings also contribute to a growing pool of observations in need of synthesis on
571 how environmental parameters influence asexual reproduction in sea anemones. For example,
572 several authors have concluded that asexual reproduction is a hallmark of growth in favorable
573 conditions, such as reporting increases in asexual behavior with increased food consumption
574 (*Diadumene lineta*: Minasian 1979; *Nematostella vectinsis*: Reitzel et al. 2007), or in
575 temperatures associated with high survival and growth rates (*Metridium senile (L.)*: Glon et al.
576 2019). While others have suggested fission to be a stress response, for example during starvation
577 (*Anthopluera elegantissima*: Sebens 1980,1982; *Exaptasia diaphana*: Bedgood et al. in revision).
578 There are a number of complications preventing a general explaination for asexual reproduction
579 in anemones, chief among them being the diversity of processes included in that description,
580 including binary fission, transverse fission, and pedal laceration (Fautin 2002). There is
581 intriguing variation even within *D. lineata*, where some populations in the native range are
582 known to asexually reproduce by pedal laceration rather than binary fission (Atoda 1973). In our
583 experiment, only one out of 194 individuals considered from across the US invaded range
584 displayed pedal laceration. The factors favoring this alternate asexual mode are unknown, and its
585 apparent rarity in invasive populations (it is previously undocumented outside of the native
586 range) remains unexplained. There is also some evidence that species differ in their capacity for
587 physiological acclimation (Zamer and Mangum 1979), though this has not been explored widely.
588 Another factor limiting a unified understanding, is the tendancy to measure environmental

589 performance across a subset of conditions (e.g., two temperature treatments), using a narrow
590 range of potential body sizes. As is well appreciated in the plasticity literature, describing the
591 trend of a non-linear process depends heavily on which treatment levels are included (Murren et
592 al. 2014). The results of our study emphasize the complexity of the relationship between growth
593 and the abiotic environment, particularly for clonal organisms. It appears that fission can
594 increase in both favorable *and* stressful conditions. Reaction norm experiments are logistically
595 challenging, but necessary for understanding the true shape of environmental performance
596 curves. Likewise, concepts of metabolic scaling provide a strong framework for integrating
597 information about food and oxygen availability, temperature, body size, and shape, but require a
598 lot of data. Moreover, to understand the evolutionary basis of clonal life cycles, such
599 physiological knowledge needs to be combined with ecological and demographic data to capture
600 the multifaceted fitness effects of life cycle variation. Studies such as this one, however, suggest
601 that there are strong unifying mechanisms waiting to be characterized underneath the
602 overwhelming reproductive diversity in anemones.
603 In summary, the adaptive value of clonality depends on the degree to which the lifetime
604 fitness of a genet is increased by dividing its mass into multiple units as opposed to retaining all
605 biomass in a unitary body. For long-lived organisms in fluctuating environments, such as *D.*
606 *lineata*, the ability to manipulate body size through fission seems to offer an obvious advantage.
607 The fitness value of fission behavior must, however, be integrated across the environments a
608 genet experiences, which may be linked in different sequences, or on different time scales, all of
609 which influence the energetic costs and benefits of a given body size. Other size-dependent
610 phenomena combine with metabolic scaling dynamics to shape the adaptive landscape on which
611 organisms must “decide” if and when they should divide. Because sexual maturity and gamete

612 production depend on body size (Ryan and Miller 2019), fitness defined as the production of
613 sexual offspring might be very different between a single large or many small anemones. We
614 currently lack data to compare genet-level gamete or offspring production of replicates raised in
615 different temperature conditions, though this comparison is necessary to fully appreciate the
616 effects of temperature-dependent fission behavior on sexual fitness. Much future work is needed
617 to fully understand how and why species maintain clonal life cycles. But, with this study we add
618 to the collection of tantalizing observations that have long made this species a promising model
619 for the eco-evolutionary forces driving life cycle evolution.
620

621 **Compliance with ethical standards**

622 Data generated during the current study are available from the corresponding author on
623 reasonable request. The authors declare that they have no conflicts of interest. All applicable
624 international, national, and/or institutional guidelines for the care and use of animals were
625 followed.

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637
638

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784

Table 1. Approximate seasonal and latitudinal patterns in nearshore sea surface temperature across the known range of *Diadumene lineata* in Japan and the United States. Mean sea surface temperature ranges approximated for 2014 – 2017 using data from NOAA National Data Buoy Center https://www.ndbc.noaa.gov/). Species occurrence based on WoRMS data base (marinespecies.org) and personal observation.

Coast	Known latitudinal range of species	Seasonal range of monthly mean water temperature	Latitudinal range of mean annual water temperature
Pacific Japan (native)	Hokkaido to Oita	10 to 20° C	9 to 25 °C
Atlantic US	Cobscook Bay, ME to Cape Canaveral, FL	13 to 20° C	9 to 25 °C
Gulf of Mexico US	Corpus Christi, TX to Tampa Bay, FL	12 to 17° C	22 to 24 °
Pacific US	Vancouver, BC to San Diego, CA	2 to 5 ° C	9 to 17 ° C

Table 2. *Diadumene lineata* collection sites for geographical comparison. Individuals collected from sites in Washington were pooled to achieve sufficient replication from this region. No individuals from sites north of Virginia on the Atlantic coast could be acquired at the time of the experiment. Bold site ID denotes use in both experiments 2A and 2B. Median initial wet weight (g) was calculated for all replicates assigned to experiment 2A. SAKH: S.A. Krueger-Hadfield; GB: G. Bonthond; WHR: W.H. Ryan.

Coast	Site ID	Site	Collection date	Collector	Median initial wet weight (g)
Atlantic	ESL	VIMS Eastern Shore Laboratory, Wachaprague, VA	Sep. 2017	SAKH, GB	0.006
Atlantic	**STS**	St. Simon Island, GA	Nov. 2017	WHR	0.033
Gulf	**WAK**	Wakulla beach, FL	Nov. 2017	WHR	0.020
Gulf	**ESP**	Eastpoint, FL	Nov. 2017	WHR	0.021
Gulf	SGI	St. George Island, FL	Nov. 2017	WHR	0.009
Gulf	CFP	Copano fishing pier, Rockville TX	Jul. 2017	WHR	0.001
Pacific	**ROK**	Morro Bay, CA	Nov. 2017	WHR	0.038
Pacific	**AZO**	Azevedo Pond, Elkhorn Slough, CA	Sep. 2017	SAKH, GB	0.017
Pacific	**BRK**	Berkeley, CA	Nov. 2017	WHR	0.050
Pacific	**WAS**	Pooled from Grey's Harbor, WA & Willapa Bay, WA	Jun. 2017	WHR	0.011

Table 3. Overview of experiments and related analyses performed. Model structure reflects best-fit model resulting from model selection procedures. The predictor initial weight is the natural log transformed wet weight (g) of individual anemones at the start of the experiment.

Exp	Response variable	Model structure*	Error distribution	Link function
1	genet survival	~ 1 + (1|genetID)	binomial	logit
	number of clonal descendants	~ temp + (1|genetID)	negative binomial	log
	Δ colony dry mass	~ temp + $temp^2$ + $temp^3$ + (1|genetID)	Gaussian	log
	ramet dry weight	~ temp + $temp^2$ + $temp^3$ + (1|genetID/rep)	Gaussian	log
	tissue density	~ temp + (1|genetID/rep)	Gaussian	log
2A	genet survival	~ oxygen	binomial	logit
	occurrence of fission	~ initial weight * (coast + oxygen) + temp	binomial	logit
	mass-specific **Δ** colony wet weight	~ initial weight * (temp + oxygen)	Gaussian	identity
	Δ colony wet weight	~ temp * initial weight + initial $weight^2$ + initial $weight^3$ + initial $weight^4$	Gaussian	identity
	Individual wet weight	~ initial weight + temperature + (1|genetID)	Gaussian	log
2B	**Δ** wet weight	~ initial weight + coast	Gaussian	log

*model structures are displayed following the syntax of the R package lme4 (Bates et al. 2015)

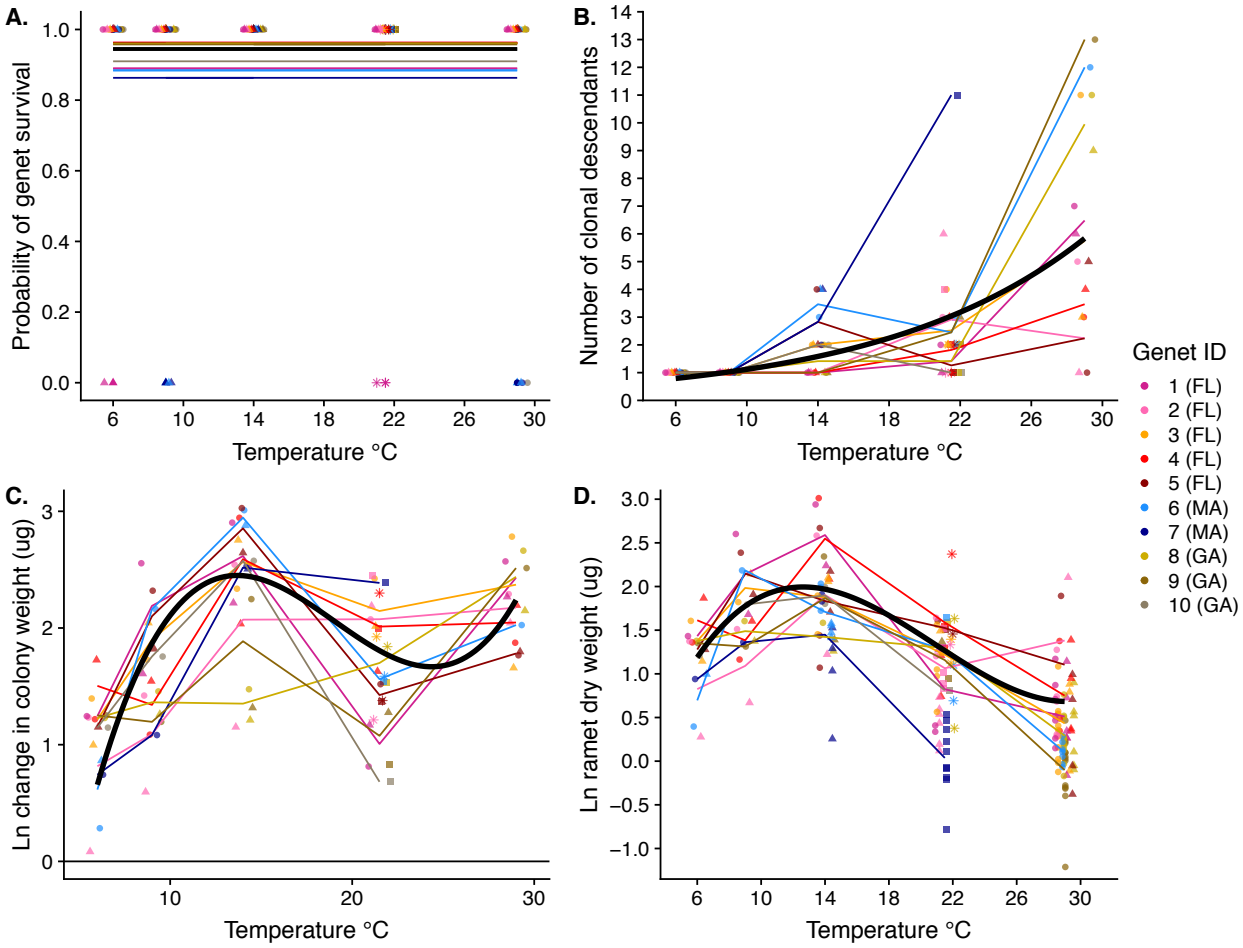

Figure 1. (Experiment 1) Reaction norms for four variables across five levels of temperature for 10 genotypes of *Diadumene lineata* grown for 12 weeks in common garden conditions (experiment 1), including (A) Probability of genet survival, (B) the number of clonal descendants produced, (C) the natural log of the change in estimated colony dry mass, and (D) ramet body size (ln dry mass (ug)). Colored lines show average pattern for each genet, black lines indicate the best-fit linear regression model. Points show either genet replicate-level (A,B,C) or ramet-level (D) values. Different point shapes of the same color designate independent replicates of a genet within temperature level. Genets 7 and 10 excluded from statistical analysis as all replicates raised at 29° C died. Points horizontally jittered to reduce overplotting.

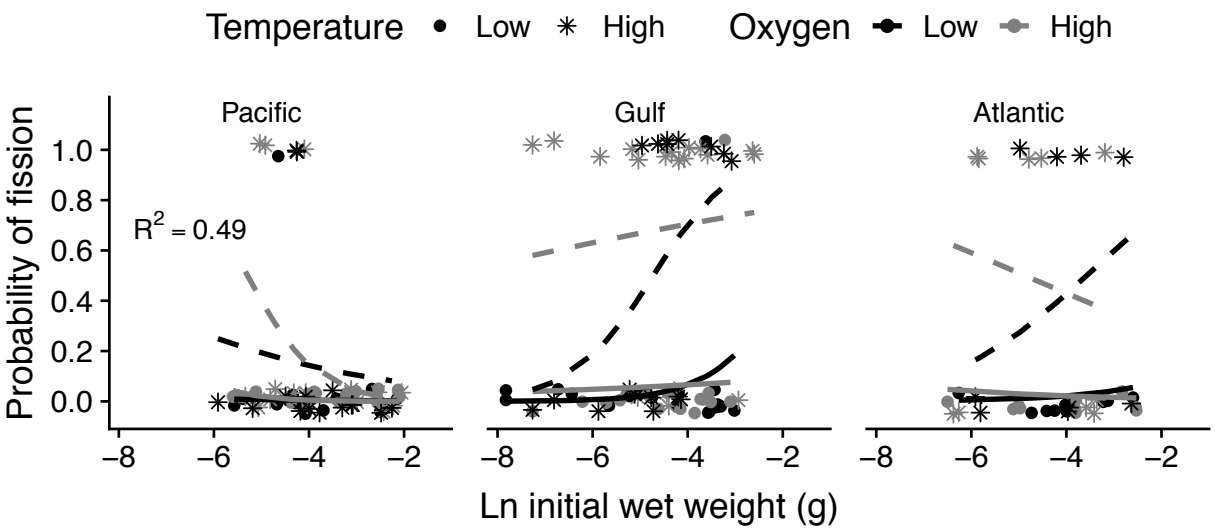

Figure 2. (Experiment 2A) The probability that *Diadumene lineata* individuals engaged in fission over 4 weeks in treatment conditions (experiment 2A) depending on initial body size and coastline of origin (panels). Lines reflect the best-fit linear model for individuals exposed to a temperature of either 15 ^{o}C (solid line, dots) or 25^{o} C (dashed line, stars), and an oxygen level of either 50% (grey) or 100% (black) of normal. Points are vertically jittered to reduce overplotting.

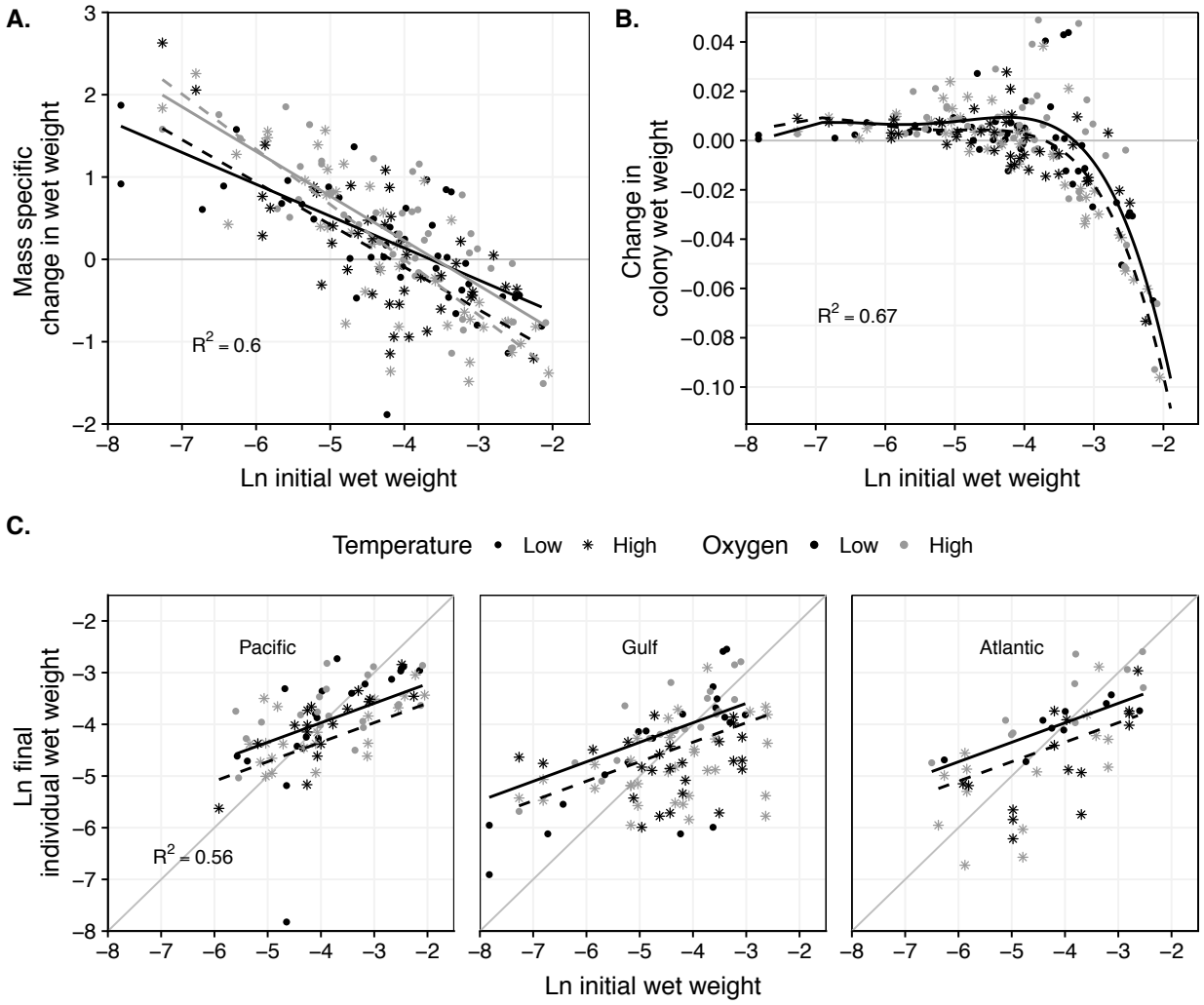

Figure 3. (Experiment 2A) The mass-specific change in wet weight (A), change in total colony wet weight (g) (B), and ramet body size (ln wet weight [g]) (C) as a function of initial body size (ln wet weight (g) of *Diadumene lineata* individuals after 4 weeks in treatment conditions (experiment 2A). Lines reflect the best-fit linear model for individuals exposed to a temperature of either 15 ^{o} C (solid line, dots) or 25 ^{o} C (dashed line, stars), and an oxygen level of either 50% (grey) or 100% (black) of normal. Model fit does not differ statistically between oxygen levels for (B) and (C). Panels in (C) show coastline of origin. The light grey line in each panel denotes a zero-change isocline.

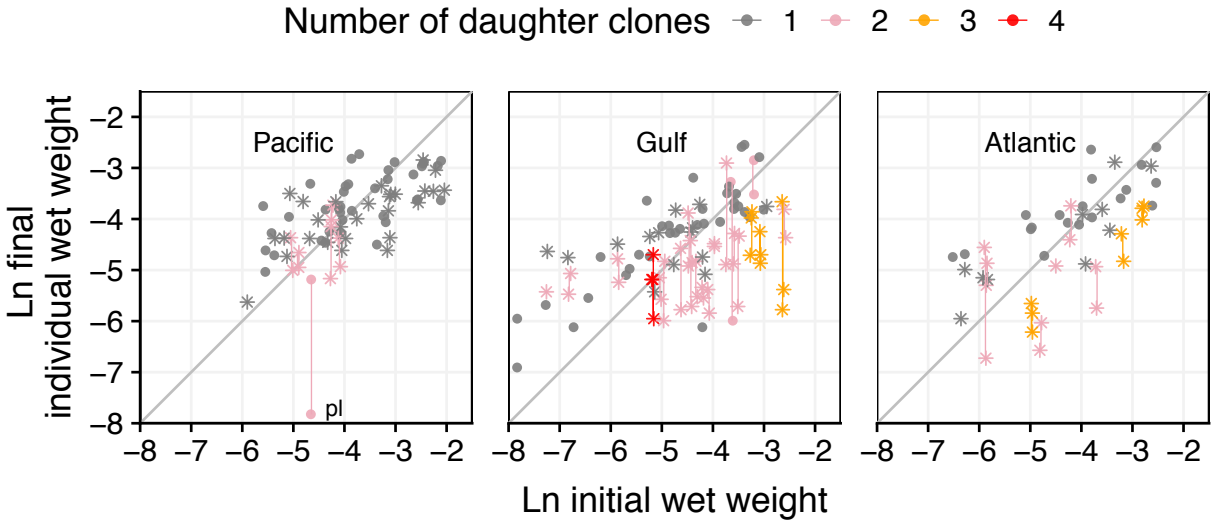

Figure 4. (Experiment 2A) Final versus initial size (ln wet weight (g)) of *Diadumene lineata* ramets after 4 weeks depending on fission activity (colors), treatment temperature (15 vs 25o C shown by dots vs. stars), and coastline of origin (panels). Lines connect ramets produced by the same genet. Points below the zero-change isocline (grey line) show ramets that are smaller than the initial individual, either due to fission or shrinkage. One case of pedal laceration was observed (pl).

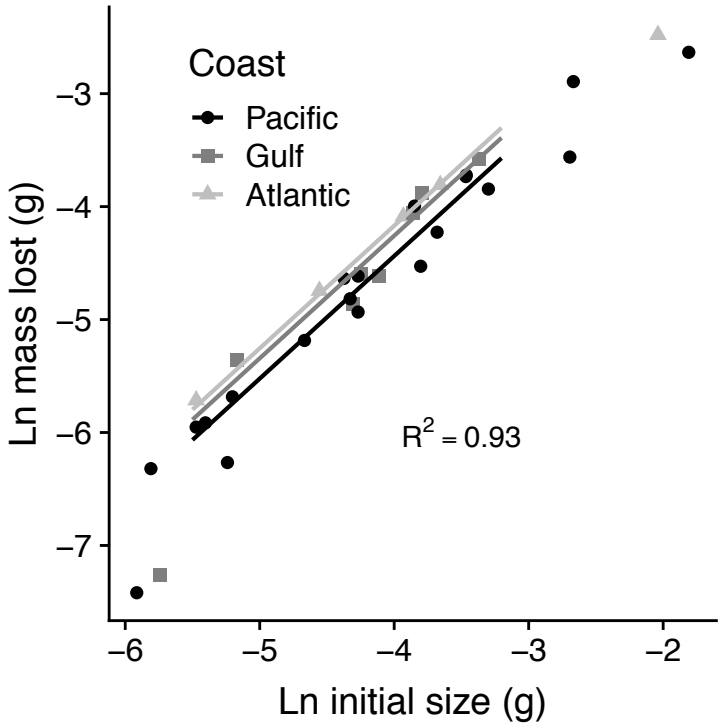

Figure 5. (Experiment 2B) The relationship between initial mass and mass lost (ln wet weight [g]) through starvation over four weeks at 15° C (experiment 2B). Weight loss through starvation is inversely proportional to resting metabolic rate. Lines reflect best fit linear model. Point shade and shape reflect coastline of origin. Regions of the initial size spectrum that did not have representatives from all three coasts available (i.e. regions beyond the best fit lines) were excluded from statistical analysis.