

Reproductive strategy changes across latitude in a clonal sea anemone

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ABSTRACT: Understanding the forces that govern allocation to growth, asexual, and sexual reproduction is a major goal for understanding life history diversity in the sea. Environmental conditions influence the costs and benefits of various life history strategies, so different strategies may be favored across geographic gradients. We describe latitudinal patterns in population density, body size, and gamete production in the clonal sea anemone *Diadumene lineata*. Fission rate in this species is highly correlated with temperature, so the rate of asexual reproduction declines with latitude. We measured the relationships among body size, gamete production, and environmental variables using individuals from 20 sites in intertidal estuaries and harbors from Florida to Maine across the US Atlantic and Gulf coasts. Average body size increased with latitude and decreased with temperature. Body size strongly predicts both the probability of having gametes and the number of gametes produced, such that there is a latitudinal transition from low per capita gamete production in the south to higher production in central and northern populations, with a peak in the Mid-Atlantic region. This gradient in per capita gamete production in this species runs counter to a known gradient in fission rate along the US Atlantic coast, supporting a latitudinal cline in the reproductive strategy in this species.

KEY WORDS: Gametogenesis · Life history plasticity · Asexual reproduction · Latitudinal variations · Reproductive patterns · Temperature · *Diadumene lineata*

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1. INTRODUCTION

Species ranges are composed of a complex landscape, over which abundance, growth, and reproduction may vary dramatically. Abiotic gradients in this landscape are expected to lead to geographic patterns that, in turn, influence demographic and evolutionary patterns across the range. While some species conform to simple predictions, such as the 'abundant center' hypothesis (Andrewartha & Birch 1954, Brown 1984, Alexander & Edwards 2010), the majority of species investigated show more complex geographic patterns in abundance (Sagarin & Gaines 2002) and reproductive output (e.g. Lester et al. 2007, Rivadeneira et al. 2010, Martone & Micheli 2012). Having an accurate estimate of the distribution, abundance, and reproductive behavior of individuals

across a landscape is essential for understanding the ecological and evolutionary forces shaping a species (Barrett et al. 1993, Kearney & Porter 2009, Ehrlén & Morris 2015).

For clonal organisms, predicting patterns of population growth, individual growth, and sexual reproduction across abiotic gradients requires information about the interaction of asexual and sexual reproduction. Gradients in abiotic conditions and food resources can induce plastic changes in size (Sebens 1980, Chomsky et al. 2004), growth form (Helmuth & Sebens 1993, Blackstone 2001), asexual reproduction rate (Minasian 1979, Schaible et al. 2011, Tökölyi et al. 2016), and the relative allocation of resources into somatic versus gametic tissues (Dorken & Barrett 2004). Environmentally driven changes in growth and fission rates can drive patterns of body size vari-

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ation (Ryan 2018) that may influence the degree of sexual reproduction, given that sexual maturity is often size dependent (Hall & Hughes 1996). Understanding the interaction of sexual and asexual reproduction requires detailed information about the drivers of variation in each of these processes.

The presence of threshold responses and phenotypic plasticity complicates predicting individual trait values across environmental gradients. Common metrics such as a gonadal index (i.e. gamete mass divided by total mass) assume that the weight of the gonads scales continuously with body size or tissue weight, which implicitly assumes that there is no size below which eggs are not produced (Ebert et al. 2011). However, the existence of a maturation size threshold is widely expected across taxa and is expected to play a key role in the evolution of life history (Atkinson & Sibly 1997, Harney et al. 2013). The location of a size-at-maturation threshold is likely key in mediating energetic tradeoffs among growth, reproduction, and asexual proliferation. Understanding the degree to which the maturity threshold can change, either through plasticity or local adaptation, is important for making predictions about the fitness benefits of changes in life history traits. Appreciating the relationship between size and gametogenesis is also important on a practical level, as gamete production is often estimated from population size structure (e.g. Easterling et al. 2000, Elahi et al. 2016). Likewise, environmental thresholds (e.g. temperature or food levels) that govern gametogenesis or gamete release are widely expected in ectothermic taxa (Olive 1995), but are difficult to account for in phenomenological models without system-specific data.

As with threshold responses, phenotypic plasticity is expected to be pervasive, leading to complex morphological and physiological patterns across environmental gradients (West-Eberhard 2003). Co- and counter-gradient changes in growth rate, body size, and energetic allocation in response to one or more environmental variables can contribute to idiosyncratic patterns of reproduction among sites where simple gradients are expected (Conover et al. 2009). Developmental threshold functions and reaction norms are likely to be under strong selection to become adapted to the local range of conditions (Schlichting & Pigliucci 1998, Angilletta et al. 2003). Measuring the true shape of threshold functions or reaction norms across environmental gradients often requires experimental or observational data across many levels of a variable (Murren et al. 2014). However, a detailed understanding of these mechanisms is essential for making predictions about individual

trait values across large geographic scales (Helmuth et al. 2005, Merilä & Hendry 2014).

The clonal sea anemone *Diadumene lineata* (Verrill 1869) (previously known as *Haliplanella lineata* among other pseudonyms) provides an opportunity to examine the relationship between responses to environmental stimuli and broad biogeographic patterns in density and reproduction. Originally a native to East Asia (Uchida 1932), *D. lineata* has become established worldwide, including along the east coast of the USA. Temperature is known to play a critical role in setting *D. lineata* growth and fission rates, and consequently body size (Minasian 1982, Ryan 2018). Seasonal patterns of temperature dictate the timing and magnitude of binary fission (Ryan 2018), such that body size and asexual investment fluctuate throughout the year in patterns determined by the temperature environment of the region. In the eastern USA, southern populations tend to stay smaller bodied and undergo binary fission prolifically, while northern populations tend to stay larger bodied and divide very infrequently (Ryan 2018). Central populations show a biphasic pattern, growing very large through the spring before engaging in rapid clonal proliferation that reduces the body size through the summer. Because body size often limits the number of eggs that can be produced in sea anemones (Sebens 1982a), these complex growth patterns are expected to have ramifications on patterns of gamete production across the species' range. As an estuarine species, factors associated with freshwater input, including reduced salinity and increased nutrient availability, are also expected to influence body size and gamete production. While this species can survive for months in salinities as low as 10 ppt (Miyawaki 1952), growth and fission are reduced below 24 ppt (Podbielski et al. 2016). However, the effects of temperature and freshwater exposure on geographic patterns of body size and sexual reproduction were unknown in this species prior to the current study.

To understand the pattern of gamete production across latitude, we measured population density, body size, and gamete production at 20 sites grouped in 10 latitudinal regions encompassing the eastern North American range of *D. lineata*. These data were compared with patterns in temperature, dissolved oxygen, salinity, and chlorophyll *a* (chl *a*) concentration measured by publicly available buoy- and dock-mounted sensors across the range to help identify environmental drivers of growth and reproduction. With these data, we (1) tested the hypotheses that population density and gamete production are high-

est at the geographic center of the species' latitudinal range, (2) quantified the relationships among population density, body size, and gamete production with temperature and salinity gradients, and (3) interpreted the observed biogeographic patterns of gametogenesis in light of temperature-dependent processes governing the size and asexual behavior of this species.

2. MATERIALS AND METHODS

2.1. Species and site descriptions

Diadumene lineata is a small bodied, acontate sea anemone that occurs prolifically in the high intertidal zone across a variety of environments. It is assumed to be native to East Asia (Uchida 1932), but has become established on temperate shorelines worldwide over the last 150 yr (Daly & Fautin 2019). Its success has been attributed to its broad abiotic tolerance and prolific schedule of asexual reproduction (Uchida 1932, Shick & Lamb 1977); these traits have also led many authors to speculate that sexual reproduction is not important for this species outside of the native range (e.g. Shick & Lamb 1977, Minasian 1982, Ting & Geller 2000). This species does not harbor photosymbionts and is obligatorily heterotrophic.

The 20 sampling locations were stratified across the latitudinal range of the species, including 1 to 3 beaches within each of 10 regions (see Fig. 4, and Table S1 in the Supplement at www.int-res.com/articles/suppl/m611p129_supp.pdf). Regional groupings were determined by the availability of nearby water quality data collection stations. This species occurs at varying densities in protected estuarine intertidal zones with hard substratum across the US Atlantic coast. It is particularly abundant on man-made structures such as rock jetties, breakwaters, and piers, but also inhabits a variety of natural surfaces including bivalve shells, rock pools, and driftwood. Different substrata may influence the micro-environment around individuals, but little is known about these properties at this time.

Temperature data for each of the 10 regions were retrieved from the nearest publicly available data collecting station (see Table S2 in the Supplement). Data sets were checked for error codes, or obvious instrument errors (e.g. large spikes in temperature lasting only seconds), which were removed. Mean monthly temperature over a period from 2013–2017 was calculated, and then used to estimate a mean annual water temperature (MAWT) and the coeffi-

cient of temperature variation (SD/mean) for each site. Additional properties of temperature patterns were explored, including maximum and minimum temperatures as well as weekly, monthly, and quarterly means, and the number of weeks spent below the 15–17°C fission threshold (see Ryan 2018). All tested patterns were highly correlated with mean annual temperature and did not improve any model fits, so MAWT was chosen as a simple metric to reflect the directional gradient in temperature conditions across sites. Small-scale and short-term temperature dynamics likely play an important role in the actual body temperatures experienced by individuals (Helmuth 2002), but are not examined in detail here.

To understand the distribution and influence of freshwater inputs on *D. lineata* traits, mean annual salinity (ppt) and chl *a* (mg m^{-3}) were also estimated over 2013–2017 from data collected from publicly available buoys (NERRS and others, see Table S2). Mean annual dissolved oxygen (mg l^{-1}) was also estimated, as oxygen availability is likely a critical driver of fission behavior (Ryan 2018). In all cases, data were quality controlled as described above. As with temperature, many aspects of each variable were explored. In all cases, annual mean values and the coefficient of variation best described variation among sites. Also, because the production of gametes occurs over several months in the winter and spring (Fukui 1995), it is likely influenced by environmental conditions integrated across the year.

2.2. Measuring population density and gamete production across latitudes

To characterize patterns of body size, sex ratio, and gametic investment across the latitudinal range of the species in eastern North America, all 20 sites were sampled between 1 June and 6 July 2016 (Table S1). Prior sampling indicated that *D. lineata* engages in 1 bout of gamete production beginning in early spring and culminating with mature gametes in mid- to late summer (W. Ryan unpubl. data). Gametogenesis takes several months to complete (Fukui 1995), and vestiges of gamete production (i.e. partially resorbed gametic tissue) are visible for months after peak maturation (W. Ryan pers. obs.), so there is little chance of mistaking would-be reproductive individuals as sterile by sampling a few weeks too early or late.

Starting at the southernmost location, each site was visited once during low tide between 1 June and 3 July 2016. Appropriate habitats were searched until

D. lineata were located, at which point a transect was established running parallel to the water line. A 25 cm² quadrat was used to estimate density at 5 to 10 points spaced at intervals of at least 5 m apart. Because hard substrate is often discontinuous in these habitats, the realized distance between sampling points was sometimes greater than 5 m. At each point, all visible *D. lineata* individuals were counted. Where sampling points fell on a clump of oysters or rotten wood, an effort was made to break apart substrate and include individuals found within. Samples were collected from Appledore Island, Maine (Apl), by J. McAlister; density was not estimated at that site.

At each site, up to 20 individuals were collected from each of 3 to 5 quadrats (25 cm² each) spaced at least 5 m apart along the transect line to incorporate the genetic diversity present at each site (Geller & Walton 2001). Collected individuals were sequestered by quadrat in 50 ml conical tubes of seawater on ice until processing.

All individuals collected at each time point were returned to the laboratory where they were photographed in water within 1 to 3 d of collection. Image J software (Schneider et al. 2012) was used to trace the pedal disk of each anemone. Pedal disk area (mm²) is highly correlated with dry mass (Ryan 2018), so is a reliable measure of body size.

Within 3 d of collection, subsets of 6 to 12 individuals from each quadrat were selected for dissection ($N = 6\text{--}30$ ind. site⁻¹), representing the range of body sizes present. Individuals were weighed wet, then placed in individual wells of a 12-well plate with a 1:1 solution of seawater and 7.5% magnesium chloride for approximately 20 min (Strathmann 1987) to induce a relaxed state. Individuals were then carefully dissected with cuticle scissors under a dissecting microscope by a single cut longitudinally through the mouth, allowing all mesenteries to be viewed intact. Each individual was scored as having either totally sterile mesenteries, with no sign of thickened tissues behind to the septal filament; immature gonad tissue, where tissues behind to the septal filament were thickened and yellowish, but where no mature gametes were identifiable under higher magnification; sperm-bearing gonad, where at least 1 mesentery had swollen gonadal tissue where sperm could be identified in a squash under a compound microscope; or egg-bearing gonad, where at least 1 mesentery had swollen gonadal tissue where individual eggs were visible under the dissecting microscope (Fig. 1).

To determine the relationship between pedal area and egg number, the total number of eggs present

was estimated for as many individuals as possible. After opening the gastro-vascular cavity with cuticle scissors, the body wall was pinned open, allowing easy access to fertile mesenteries (Fig. 1A,C). Fertile mesenteries have a characteristically 'folded' appearance that aids in estimating the number of eggs present (Fig. 1B). For each individual, the number of eggs in a fold segment on 5 separate mesenteries was counted and averaged, and then the total number of folds was counted and multiplied by the average number of eggs per fold to arrive at an estimated total number of eggs. Where mesenteries were damaged by recent fission or were unusual in size or shape, eggs were counted individually rather than estimated. There is no equivalently efficient method for quantifying sperm production, so sperm-bearing individuals were measured for pedal area, but male gamete production was not quantified in this study.

Because both suitable habitat and the occurrence of individuals in suitable habitat are patchy in this species, population density (individuals per 0.25 m²) can be measured for at least 2 relevant scales: density within a patch and the density of patches in space. Because the sampling design here was inadequate to assess the density of patches, quadrats with 0 individuals present were removed from the analysis to allow a comparison of within-patch density across sites.

2.3. Analysis

Regional level variation in environmental variables was described with a principal components analysis, using normalized values in the 'prcomp' function in R (R Core Team 2017). Variables included in the analysis were MAWT, salinity, dissolved oxygen, and chl *a*, as well as the coefficients of variation of weekly averages of these variables to account for differences in seasonal fluctuations across the year. Estimates of additional water quality metrics, including ammonia, nitrate, and nitrite could not be located for sites in Virginia and so were excluded from the analyses. To understand geographic patterns, each water quality metric was also regressed on latitude using generalized linear models (GLMs). The results informed the variables used below.

To describe geographic patterns, linear mixed models ('lmer' or 'glmer' in the R package 'lme4'; Bates et al. 2015) were used with an Akaike's information criterion (AIC)-based model selection approach to separately evaluate the relationships between latitude and pedal disk area, population

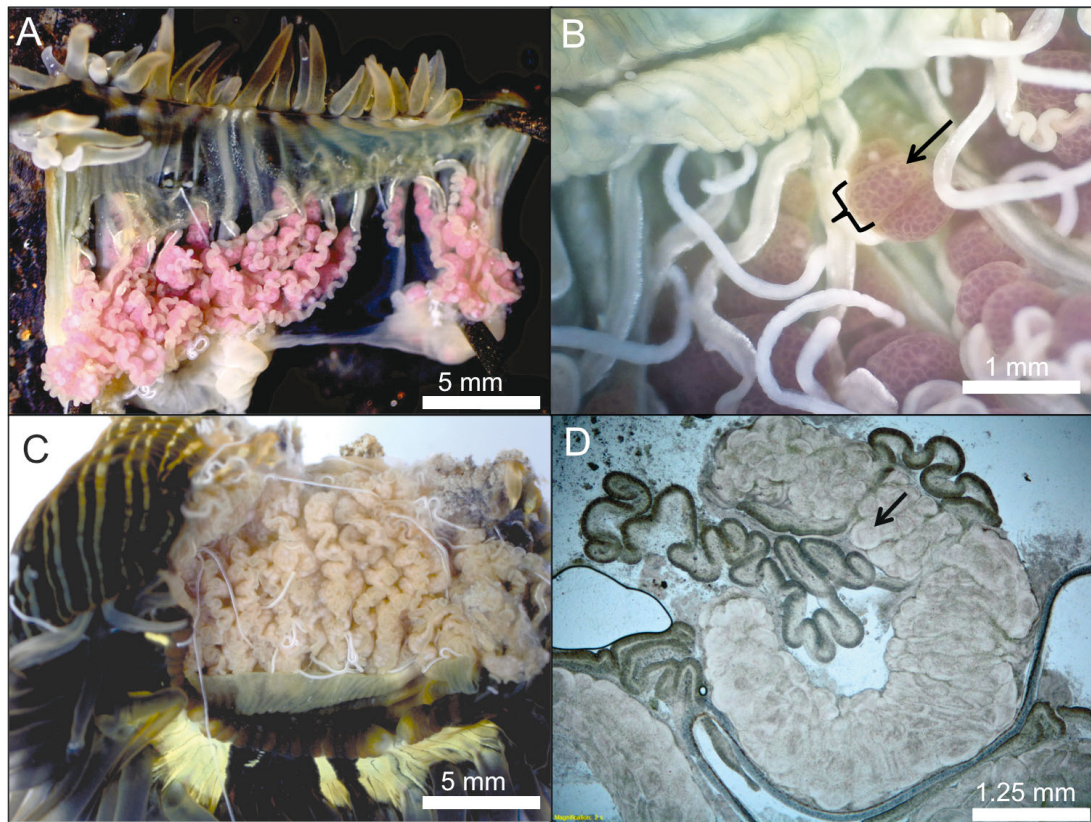


Fig. 1. Photographs of *Diadumene lineata* with mature gametes. Freshly dissected individuals showing (A,B) mature eggs and (C,D) nearly mature sperm. Bracket in (B) highlights a mesenterial 'fold' used as a subsampling unit to estimate egg number. Arrow points to an individual egg in (B) and packet of maturing sperm in (D). Scale bar distances are approximate

density, and the probability of individuals displaying any level of gametogenesis. A second set of models was used to evaluate the relationship of pedal area and population density with MAWT and salinity. Pedal area was normally distributed after natural log transformation (per Shapiro-Wilk test) and used with lmer which assumes a Gaussian distribution. Site and quadrat within site were set as random variables. Using a generalized linear mixed model (glmer) with a log-link function would be preferred, but no estimate of model fit (e.g. marginal R^2) is currently available for these models. We confirmed that the results and interpretation do not depend on the model choice. Density was modeled with a Poisson distribution. Density was calculated for each quadrat, with site as a random variable. The presence or absence of gametes was modeled with a binomial distribution using pedal area, mean temperature, and salinity, as fixed factors, with quadrat within site as a random effect. As polynomial relationships with predictors were expected, temperature was initially modeled as a third-order polynomial. Salinity was modeled as a second-order polynomial due to low replication

across the range of possible values. No interaction terms were considered, since variation in salinity was not equally represented across all temperatures (e.g. all low-salinity sites happened to occur in the middle of the latitudinal distribution). Model selection using AIC values was then used to determine the model with the best fit given the fewest parameters. Where AIC values were similar ($AIC < 2$), the less complex model was accepted. The significance of the contribution of each parameter (i.e. slope and curvature) was tested by comparing orthogonal polynomial estimates. The significance threshold was Bonferroni adjusted ($\alpha_b = 0.025$) to account for each independently estimated population metric being compared with 2 sets of models. The fraction of variance explained by each model was estimated by calculating a marginal r^2 -value using either the 'sem.modelfits' function in the R package 'piecewiseSEM' (Lefcheck 2016) or the 'r.squaredGLMM' function in 'MuMIN' (Barton 2018).

In addition to the relationship between fertility, size, and the environment estimated above, the relationship between body size and the production of

gametes was examined in 2 additional ways. To test for body size differences among sexes, differences in the average body size of individuals presenting in each of the 4 gametic states were examined with an ANOVA followed by Tukey HSD post hoc analysis. Log normality of body size was confirmed with a Shapiro-Wilk test and homogeneity of variances with a Bartlett's test. p-values below 0.05 were considered significant. To measure the scaling relationship between body size and gamete production, a GLM was used to quantify the relationship between pedal area and the number of eggs produced for fertile female individuals.

The sex ratio was also calculated for each site. Because distinguishing individual sex is only possible when mature gametes are present, the sex ratio estimated reflects only fertile individuals in the current sample. To provide a more general overview of the presence of each sex at each site, data from previous sampling periods (Ryan 2018) are also reported in order to more accurately represent the frequency of sites where both sexes are present.

Finally, geographic patterns in per capita egg production were assessed by regressing the number of eggs ind.^{-1} separately on latitude and environmental variables using a GLM with a negative binomial distribution. Model selection using AIC was used to determine best-fit models as described above. p-values below 0.05 were considered significant.

All analyses were performed in R (R Core Team 2017).

3. RESULTS

Two general axes of environmental variation were identified among sites (Fig. 2A), reflecting freshwater input and temperature differences among sites. The major axis of variation (PC1) explained 49.6% of the variation among environmental variables and generally reflected a gradient in freshwater influence among sites. Mean annual salinity ranged from 21 to 31 ppt, and was negatively correlated with mean annual chl *a* concentration, which ranged from 1.1 to 18.7 $\mu\text{g l}^{-1}$ ($R^2 = 0.59$, $p < 0.003$). Sites with a larger freshwater influence also had higher variability (estimated as coefficient of variation) in temperature, salinity, and dissolved oxygen. PC2 explained an additional 27.3% of the variation and reflected differences in temperature among sites

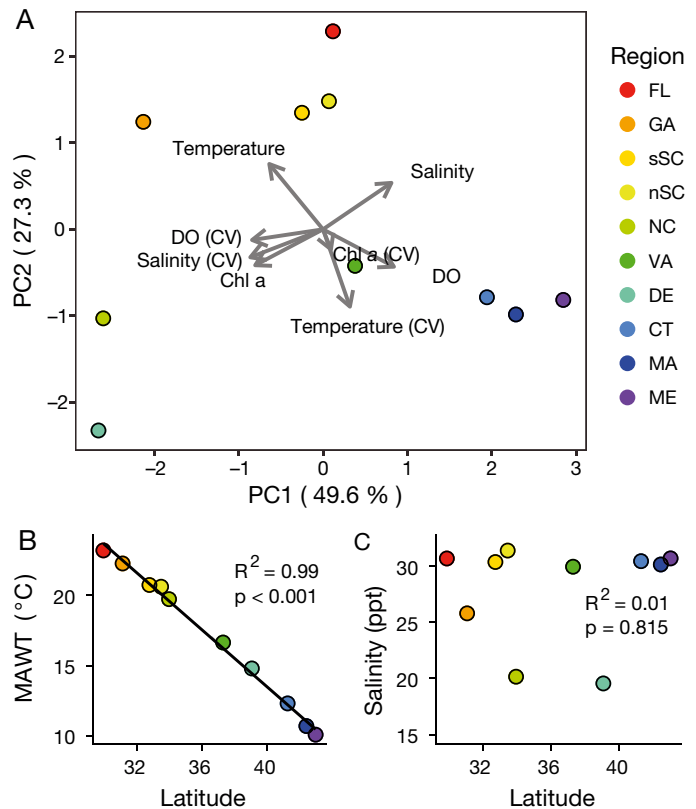


Fig. 2. Environmental variation measured for 10 regions across the North American Atlantic range of *Diadumene lineata*. (A) Principal components analysis on region-level variation in water quality metrics. Numbers in parentheses provide the proportion of variation explained by each principal component. (B) Mean annual water temperature (MAWT) is highly correlated with latitude, whereas (C) salinity is not. See Table S1 for additional site information. DO: dissolved oxygen, CV: coefficient of variation. Regions—FL: Florida, GA: Georgia, SC: South Carolina (s: southern, n: northern), NC: North Carolina, VA: Virginia, DE: Delaware, CT: Connecticut, MA: Massachusetts, ME: Maine

(Fig. 2A). MAWT, which ranged from 10.1 to 23.2°C, was highly negatively correlated with mean levels of dissolved oxygen ($R^2 = 0.69$, $p < 0.003$), which ranged from 4.7 to 9.12 mg l^{-1} . The rank order of temperature across sites was highly consistent across seasons, such that other potential temperature variables of interest were highly correlated with MAWT (e.g. the number of weeks spent above 15°C; $R^2 = 0.95$, $p < 0.001$). To simplify the interpretation of relationships among population traits and environmental variables, we focused on temperature and salinity for subsequent analyses. MAWT was strongly predicted by latitude ($R^2 = 0.99$, $p < 0.001$; Fig. 2B), while salinity was not ($R^2 = 0.01$, $p = 0.815$; Fig. 2C).

In total, the pedal area of 1125 individuals was measured in 87 quadrats distributed over 20 sites. The back-transformed mean \pm SE pedal area across the

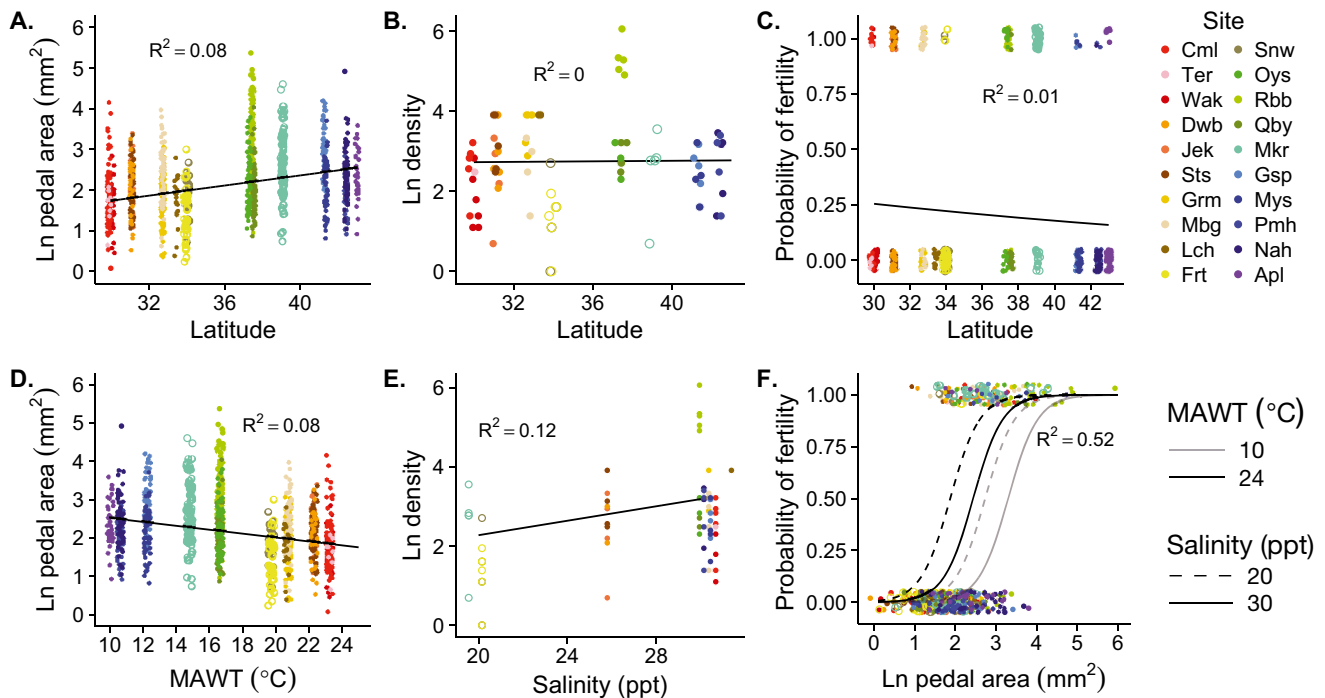


Fig. 3. Patterns of (A,D) pedal area, (B,E) within-patch density, and (C,F) the probability of fertility in *Diadumene lineata* along the Atlantic coast of North America predicted by latitude (top row) and environment variation or size (bottom row). Lines represent best-fit model estimates. For panel F, model estimates were made at 2 levels of mean annual water temperature (MAWT; black vs. grey) and 2 levels of salinity (dashed line vs. continuous) to represent the measured range of each variable. Open circles denote sites where mean annual salinity was below 24 ppt. See Table S1 for site information. See Table S3 for model fitting details

range was $9.44 \pm 0.49 \text{ mm}^2$ and ranged from 0.94 to 215.51 mm^2 (approximately 1.1 to 16.6 mm pedal diameter). Pedal area increased significantly with latitude ($t = 2.63$, $df = 18.5$, $p = 0.02$; Fig. 3A), though much of the variation was unexplained ($R^2 = 0.08$). The best-fit model of environmental predictors of pedal area retained a significant negative relationship with temperature ($t = -2.53$, $p = 0.02$) but not salinity (Fig. 3D). Quadrat within site was also retained as a random variable in the model (see Table S3 in the Supplement for full details). Body size was enormously variable within sites. The coefficient of variation of the natural log of pedal area ranged from 0.23 to 0.47, showing a marginally significant decrease across latitude (slope = -0.01 , $p = 0.06$, $R^2 = 0.14$). This may in part reflect the influence of microhabitat variation, which was not measured in this study, but may also be a consequence of the high and fluctuating fission rate in this species (discussed below).

Density was estimated for 83 quadrats over 19 sites. Density information was not collected at Appledore Island (Apl), as specimens were retrieved by a collaborator. An additional 20 quadrats, in which no anemones were found, were excluded from subse-

quent analyses. The average \pm SE within-patch density across the sampled range was 14.5 ± 1.2 individuals per 0.25 m^2 , excluding the notable outlier of the Red Bank boat ramp (Rbb) in Virginia, where the average density was 225.8 ± 52.8 individuals per 0.25 m^2 . Density showed no significant relationship with latitude ($z = 0.10$, $p = 0.92$, $R^2 = 0.00$, Fig. 3B). The best-fit model of environmental predictors showed a marginally significant positive relationship with salinity ($z = 2.076$, $p = 0.04$, $R^2 = 0.12$; Fig. 3E). Temperature was not retained in the model. The results were similar with and without including the exceptionally high values recorded at Rbb.

The presences or absence of gametes was recorded for 511 individuals distributed across all 20 sites. The highest recorded proportion fertile was 76% of individuals found at Rbb. The median proportion fertile across the range was 22.5%. The minimum value was 0 at Lichfield Bridge (Lch) in South Carolina, the only site where all individuals were sterile (Fig. 4). The probability of an individual having gametes showed no relationship with latitude alone ($z = -0.64$, $p = 0.52$, $R^2 = 0.01$, Fig. 3C). An increased probability of fertility was strongly associated with the natural

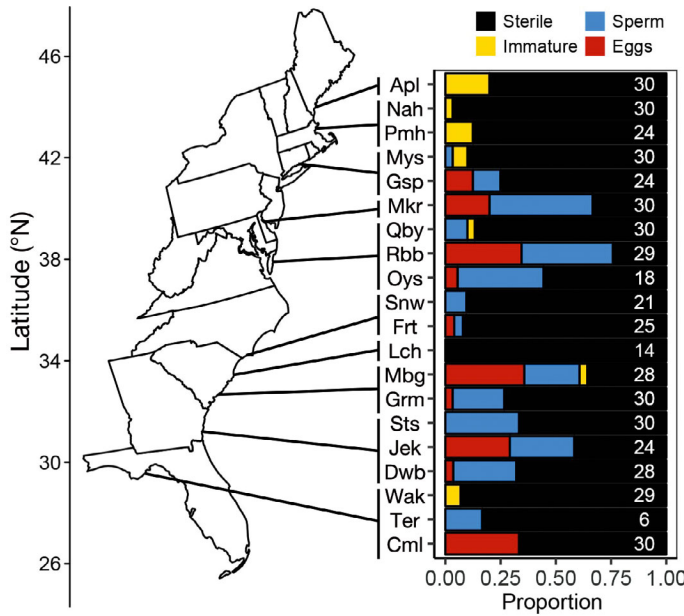


Fig. 4. Proportion of *Diadumene lineata* individuals in 4 reproductive states by site, grouped within latitudinal region, across the extent of the species' range in eastern North America. The number of individuals scored for gametes from each site is listed at the end of each bar. See Table S1 for site information

log of body size ($z = 8.61, p < 0.001$). The threshold size, above which the probability of fertility increased, decreased with temperature ($z = 2.91, p = 0.004$), and increased with salinity ($z = -2.283, p = 0.022, R^2 = 0.52$; Fig. 3F). That is, anemones at warmer or less saline sites became fertile at significantly smaller sizes. Under average conditions, the probability of being fertile is 50% at a pedal area of 16.0 mm² (4.5 mm diameter). Under the extremes of the conditions observed in this study, the predicted pedal area for individuals with a 50% probability of being fertile ranges from 6.5 mm² (2.9 mm diameter) where MAWT is 24°C and salinity is 20 ppt, to 27.1 mm² (5.9 mm diameter) where MAWT is 10°C and salinity is 30 ppt (Fig. 3F). Because sex can only be distinguished when fertile, it is not possible to assess the fertility threshold of males or females separately with these data.

At the scale of an entire beach (<0.5 km), the majority of sites had both sexes present (Table 1), but sex bias was common at the within-quadrat scale. Individuals bearing gametes were found at all but one (Lch) of the 20 sites, although the northern-most populations showed only immature gametes at the time of collection (Fig. 4). Five sites had only sperm-bearing, immature, and sterile individuals (sites Ter, Sts, Snw, Qby, Mys), and 1 had only egg-bearing and

immature individuals (Cml). The 4 remaining sites showed only immature and sterile individuals whose sex could not be determined (Wak, Pmh, Nah, Apl). Where both eggs and sperm were found ($N_{site} = 9$), the mean \pm SE ratio of male to female individuals was 0.68 ± 0.17 . Pooled across all individuals found with mature gametes ($N = 133$), the ratio of males to females was 0.60. Combined with data from prior sampling, the current survey suggests that males and females co-occur in at least 14 of the 20 sites (Table 1), a notable increase from prior reports. No trends in regional or latitudinal sex ratio were observed (Table 1), and no individuals were found bearing both eggs and sperm, confirming the status of this species as gonochoristic.

Mean pedal area differed significantly among individuals grouped by gametic state ($F_{3,477} = 62.35, p < 0.001$). Sterile individuals (mean \pm SE pedal area = 6.13 ± 0.30 mm²) were significantly smaller than those with immature gametes, eggs, or sperm (Tukey's HSD, $p < 0.05$). The size of

Table 1. Summary of *Diadumene lineata* sex distribution among sites. The sex ratio was estimated from the current survey. Gamete presence/absence data compiled from current and previous sampling. Y (Y*): confirmed presence in current (previous) sampling; N: no record of presence despite repeated sampling. A dash indicates sites where type was absent, but which were only visited once. Additional site information available in Table S1 in the Supplement. FL: Florida, GA: Georgia, SC: South Carolina (s: southern, n: northern), NC: North Carolina, VA: Virginia, DE: Delaware, CT: Connecticut, MA: Massachusetts, ME: Maine

Region	Site	Sex ratio (F:M)	Eggs	Sperm
FL	Cml	10:0	Y	Y*
FL	Ter	0:1	Y*	Y
FL	Wak	-	Y*	Y*
GA	Dwb	1:8	Y	Y
GA	Jek	7:7	Y	Y
GA	Sts	0:10	Y*	Y
sSC	Mgb	10:7	Y	Y
sSC	Grm	1:7	Y	Y
nSC	Lch	-	-	-
NC	Frt	1:1	Y	Y
NC	Snw	0:2	-	Y
VA	Oys	1:7	Y	Y
VA	Rbb	10:12	Y	Y
VA	Qby	0:3	-	Y
DE	Mkr	6:14	Y	Y
CT	Gsp	3:3	Y	Y
CT	Mys	0:1	-	Y
MA	Pmh	-	N	Y*
MA	Nah	-	Y*	Y*
ME	Apl	-	-	-

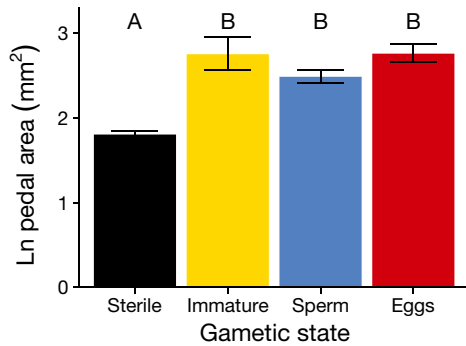


Fig. 5. Relationship between body size and reproductive status of *Diadumene lineata* individuals, pooled across sites. Mean \pm SE Ln pedal area (mm^2) is shown. Different letters indicate significantly different means tested with a Tukey's HSD test

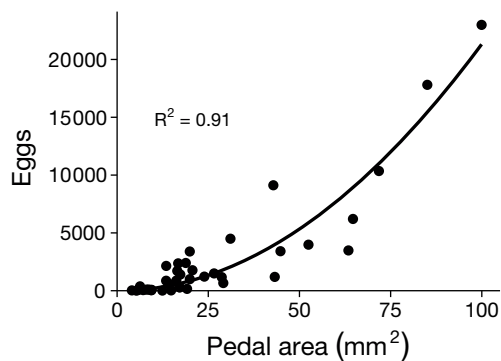


Fig. 6. Relationship between egg number and body size (pedal area in mm^2) in *Diadumene lineata*, for all individuals with eggs recorded across sites. Black line indicates the best-fit exponential function

those with immature gametes ($15.81 \pm 6.40 \text{ mm}^2$), eggs ($19.92 \pm 2.43 \text{ mm}^2$), or sperm ($12.11 \pm 1.22 \text{ mm}^2$) was not statistically distinguishable (e.g. sperm vs. eggs, $p = 0.15$; Fig. 5). For individuals with identifiable eggs, there was a strong correlation between pedal area and the number of eggs present. The best-fit model describing this relationship was an exponential function with a slope of 2.13 ($R^2 = 0.90$; $df = 1, 39$; $p < 0.001$; Fig. 6).

The relationship between per capita egg production and latitude was best fit by a third-order polynomial showing a peak in egg production across mid-Atlantic latitudes ($N = 39$; $R^2 = 0.37$; $p_1 < 0.001$, $p_2 = 0.39$, $p_3 < 0.001$; Fig. 7A). Similarly, the relationship between per capita egg production and MAWT was best fit by a third-order polynomial showing a peak in egg production where MAWT was about 15°C ($R^2 = 0.36$; $p_1 < 0.001$, $p_2 = 0.98$, $p_3 = 0.003$; Fig. 7B). The highest median per capita egg number was in Virginia (7663 eggs), followed by Delaware (2554

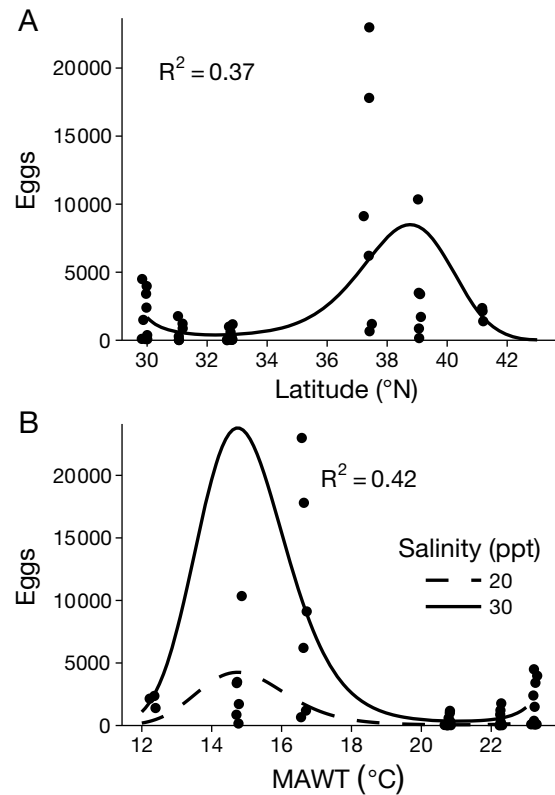


Fig. 7. Relationship between per capita egg production and (A) latitude and (B) mean annual water temperature (MAWT) estimated for 39 *Diadumene lineata* individuals found with eggs across the sampled populations in the North American Atlantic range. Lines indicate best-fit generalized linear model predictions. For (A), the best-fit model did not include the effect of salinity

eggs) and Connecticut (2145 eggs). By contrast, median egg production in southern regions ranged from 61 (South Carolina) to 1496 (Florida). No individuals bearing mature gametes were found north of Cape Cod during this study.

4. DISCUSSION

Simplistic models suggest that the biogeographic distribution of species can be explained by a unimodal peak in abundance with the greatest fitness near the center of the range (Hengeveld & Haek 1982, Brown 1984, Sexton et al. 2009). However, complex interactions between environmental variables and reproductive biology have the potential to produce more complicated patterns, particularly in organisms with complex life cycles. For example, population density in *Diadumene lineata* shows no latitudinal trend, suggesting no direct effect of temperature or related factors. However, density does

appear to be lower at sites heavily influenced by freshwater input (i.e. low salinity); sampling sites in North Carolina and Delaware, where density was the lowest, sit along the Cape Fear and Murderkill Rivers, respectively. We found that average body size increased with latitude and that per capita gamete production peaked near the geographic center of the species' range. Importantly, we demonstrated that size is a major predictor of gametogenesis, but also that the threshold size, below which no gametes are produced, varies among sites. Whether this pattern reflects environmentally mediated plasticity or genetic differentiation remains to be seen.

Body size is determined by a complex interaction of growth and fission rate, both of which are influenced by the environment. Small-scale differences in temperature, salinity, and tidal flux, for example, likely contribute the high levels of within-site size variation. Such environmental variation was far below the scale of resolution possible in this project and will need to be explored more in the future. However, fission behavior may cause a broadening of the distribution of body sizes, as suggested by the noisy but notable decline in the coefficient of variation in body size across latitude. Since fission behavior is expected to increase through the spring and summer, populations were likely in the midst of a rapid transition between a period of individual growth and fission at the time of sampling (see Ryan 2018).

The proportion of individuals in a population producing gametes is likely influenced by both temperature-mediated limitations on the maximum body size (Ryan 2018) and the availability of food resources to fuel gamete production (Dodge & Edwards 2012). The tendency for individuals to grow larger and produce more gametes at cooler temperatures is particularly interesting in light of the well-documented pattern of increased fission with temperature in this clonal species (Uchida 1932, Minasian 1979, Ryan 2018). Such inverse gradients in sexual and asexual behavior create the potential for identifying energetic tradeoffs or synergisms among these reproductive modes under differing environmental conditions that can provide insight into the evolution of clonal behavior.

The expectation that population density and gamete production will be highest in the geographic center of the range depends on the assumptions that environments change regularly across the geographic range, and that individuals respond similarly to these changes. Across the geographic distribution of *D. lineata*, temperature and its influence on the concentration of dissolved oxygen change regularly,

while freshwater input and the associated effects on nutrient availability are more stochastic. Gamete production is strongly tied to body size and water temperature in this species and so also shows a strong latitudinal pattern where north-central populations are expected to produce many more eggs per capita than those in the south. This pattern is particularly interesting given the expected inverse latitudinal gradient in fission rate (Ryan 2018). Together, these results support a negative relationship between per capita gamete production and fission rate, which may result from temperature-mediated fission rate plasticity across the geographic range of this study.

Previous work with this species has shown that individuals across this same range share similar, but not identical, reaction norms of body size and fission rate with temperature, where variation in local temperature conditions can lead to very different growth patterns at different sites through plasticity (Ryan 2018). Warm temperatures at the southernmost sites (Florida) stimulate rapid and constant fission, keeping body sizes small all year. Individuals in intermediate populations (Georgia to Connecticut) are expected to have a biphasic growth cycle where body size increases throughout the spring to achieve maximal gametogenesis, then is reduced by rapid fission as water temperatures exceed the fission threshold of 15–17°C (Ryan 2018). However, in the coldest conditions (Gulf of Maine), individuals grow slowly and delay the development of gametes (W. Ryan unpubl. data), resulting in individuals at an intermediate size during the reproductive season (Ryan 2018). Increased metabolic costs or physical limitations on capturing prey near the cold thermal tolerance limits near the northern range edge may contribute to a drop-off in body size and observed gamete production. While it is possible that northern individuals may grow more before their gametes are mature in the fall, previous observations of body size changes in the field do not indicate a major increase in body size between July and October, when sexual reproduction is known to taper off. Therefore, the reported geographic patterns in fertility are not likely to be an artifact of differences in reproductive timing across latitudes.

The balance and timing of asexual and sexual reproduction in this species is shaped by many forces that vary across the latitudinal range. *D. lineata* is an upper littoral zone species, for which desiccation risk, oxygen stress, and restricted emersion time for feeding likely all contribute to a smaller optimum body size (Johnson & Shick 1977, Sebens 1982a, Forster et

al. 2012). A need to increase the surface area to volume ratio under warmer conditions may in part explain why fission rate increases with water temperature, a feature that leads to seasonal fluctuations in size in response to local temperature patterns (Ryan 2018). Such seasonal changes in energetic dynamics have been suggested to influence the patterns of reproduction in other marine invertebrate taxa where the peaks of sexual and asexual activity are staggered in time (Sebens 1982b, McGovern 2002). The observed pattern of body size distribution across latitudes is also consistent with the hypothesis that body size is limited by high temperature, which is correlated with oxygen limitation (Atkinson 1994, Forster et al. 2012), and comports with the idea that fission is a mechanism by which positive growth rates can be achieved through the production of optimally sized units when the environment limits maximum body size (Sebens 1979, 1982a, Hughes 2005).

The consequences of such limits on body size and sexual reproduction are made clear by the exponential relationship of egg number with body size (Fig. 6) and the threshold body size below which gametes are not produced (Fig. 3F). As an individual anemone grows, its capacity to produce eggs increases. If an individual undergoes binary fission when it reaches the upper body size limit that its environment can support, it reduces its biomass by half, but reduces its egg production capacity by more than half, based on the best-fit relationship estimated between body size and egg number. The presence of a threshold below which gametes are not produced imposes an additional cost on individuals that undergo binary fission—the biomass ‘cost’ of growing to the threshold size must functionally be paid twice by the genet (the collective term for all clonal decedents) before gametogenesis can begin. Additionally, individuals risk producing daughter clones smaller than the maturity threshold, or which lack the energetic resources to support gametogenesis (Smith & Hughes 1999). For fission to increase sexual fitness (i.e. gamete production) in a species with these particular relationships between body size and gamete production, the net gain in biomass achieved by dividing clonal units into optimally sized units has to be greater than the combined loss of exponential gamete volume and double the amount of biomass that must be produced before the first egg can be made. The costs and benefits of individual growth, fission, and gamete production likely vary across environments. Fission rate plasticity may allow for genotypes to balance these costs and benefits across seasons (Ryan 2018) or along gradients. Furthermore, genetic or plastic dif-

ferences in the size at maturity threshold may ameliorate or exacerbate differences in reproductive allocation due to changes in fission rate and body size among sites. However, we are only beginning to understand the selective forces shaping such plasticity.

Interpreting the fitness consequences of variation in sexual or asexual reproduction is difficult for clonal organisms, as fitness must be integrated at the genet level, whereas data are often collected on individual ramets (e.g. Hughes 1984, Pearse et al. 1989, Baums et al. 2006), as in this study. The ramet-level patterns measured in *D. lineata* suggest a trade-off between fission and gamete production due to their opposing relationships with body size. However, if fission allows for higher growth rates because smaller individual ramets are closer to the optimal size for the environment, then the total number of gametes produced at the genet level may be equal or greater for genotypes engaging in more fission.

These considerations are further complicated by variation in the threshold size of maturity. Animals in conditions that limit their individual body size or growth (e.g. high temperature or low salinity, respectively) may trigger gamete production at a smaller size, perhaps to meet an environmentally dependent optimum size for sexual reproduction. Alternatively, animals may shift resources into sexual reproduction in response to stressful conditions (e.g. fitness-associated sex; Hadany & Otto 2009). More work is required to understand the fitness consequences of the observed variation in size at maturity and whether site-specific differences reflect plasticity, genetic differentiation, or both.

Whether the particular phenotypes observed reflect adaptive plasticity, local adaptation, both, or neither remains to be seen. However, this system holds great potential for understanding the tradeoffs involved in the evolution of a complex life cycle across a complex environment. Understanding the ecological and evolutionary forces that govern changes in fission rate has broad implications for all clonal species (Blackstone & Jasker 2003), but in particular for cnidarians. The asexual amplification of sexually produced genotypes is a core feature of the cnidarian bauplan (Fautin 2002), and is found scattered across the cnidarian tree of life (Jackson 1985). Variation in allocating energy to individual growth, asexual growth, and gamete production is expected to have far-reaching consequences for population dynamics (Caswell 1985), competitive ability (Williams 1975) and evolutionary trajectories (Orive 2001, Vallejo-Marín et al. 2010). Thus, understanding the tradeoffs that govern

allocation 'decisions' is central to understanding the diversification of cnidarian life cycles. The findings of this study move us toward the goal of understanding the multifaceted influence of the environment on growth, asexual, and sexual reproduction. In general, the expectation that key traits, such as body size, gamete production, and population density, can be predicted from broad environmental gradients is upheld by these data. However, predicting eco-evolutionary patterns from such relationships is complicated and requires knowledge of all growth and reproductive options possible for a species in the context of its environments.

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