



SYMPOSIUM

Variety is the Spice of Life Histories: Comparison of Intraspecific Variability in Marine Invertebrates

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Synopsis Life-history characteristics of marine invertebrates exhibit broad diversity across taxa as well as considerable variation within species. Although such variation is widely recognized, comparisons of the magnitude of variability as an outcome of evolutionary processes are relatively rare. Theory predicts, for example, that patterns of variability within and between clutches can arise as a consequence of population genetic structure, environmental variability, and the uncertainty of future ecological conditions. We review the strengths and weaknesses of several statistical methods for comparing variability across distributions, including Levene’s test, use of the coefficient of variation in *F*-tests, and analysis of covariance. We then use four case studies from our own work and from the literature to illustrate adaptive patterns of variability related to metamorphosis, habitat differentiation, physiological stress, and life-history mode. These examples demonstrate the value of comparing variability for a range of questions associated with reproductive ecology, life-history biology, and genotype-by-environment interactions. We encourage researchers studying larval ecology and life-history evolution to explicitly consider the causes and consequences of variances in traits along with their means in models, experimental designs, analyses, and interpretations.

Introduction

Marine invertebrate life histories are highly diverse, involving a broad array of larval forms and modes of development at a range of taxonomic levels (Berrill 1935; Strathmann 1985; Raff and Byrne 2006) including among close congeners (Raff 1992; Hart et al. 1998; Allen and Podolsky 2007). Within species, life histories can also be highly variable (Hadfield and Strathmann 1996). For the purposes of this review, we define such variability as inter-individual differences in life-history characteristics among and within populations, particularly among and within clutches of offspring. Other than rare cases of poecilogony, which involve distinct developmental morphs (Levin 1984; Bouchet 1989), most of this intraspecific variation is continuous in its distribution. While the evolutionary importance of interindividual variation has long been recognized

(e.g., Bennett 1987), recent work has focused not only on variation as the raw material on which selection acts, but also on patterns of variation as the outcome of evolutionary processes.

While phenotypic variation can be considered merely a byproduct of genetic, epigenetic, and environmental variation among individuals, investigators have begun to recognize ecological conditions that can select for different degrees of variation as well as patterns that signal whether differences in such variation reflect adaptation (Crean and Marshall 2009; Krug 2009). For example, population differences in the degree of variation of traits within clutches could reflect the differing levels of uncertainty in conditions faced by offspring in those populations (Donaldson-Matasci et al. 2008). Likewise, the degree of variation within clutches could be manipulated plastically by parents that have access

to information that is predictive of the variability of conditions their offspring will face. While the generation of variation in both cases represents some form of bet-hedging against environmental uncertainty, the latter example of a maternal effect involves additional constraints, requiring not only the ability to sense and predict future conditions but also the ability to mount a response on an appropriate time scale (DeWitt et al. 1998). Thus, assessing the adaptive significance of variability requires careful attention to both the biological context and the temporal scale at which variation is expressed. On a more practical level, the goal of comparing degrees of variability has raised methodological challenges that require an evaluation of the costs and benefits of different approaches.

Studies that compare variability have a broad range of potential applications. For example, comparisons of quantitative variation can help to resolve genetic or environmental histories. Phenotypic and genotypic variation have been compared to infer evolutionary patterns and processes involved in domestication (Shikano et al. 2008), species invasions (Dlugosch and Parker 2008; Ahern et al. 2009), and the occupation of ecological niches (Patterson 1983; Meiri et al. 2005). Comparisons of phenotypic variability can also help to answer questions about life histories. For example, the capacity for phenotypic plasticity expressed by individuals decreased with increasing maternal investment across species for echinoderms (Podolsky and McAlister 2005; Reitzel and Heyland 2007) and within species for cockroaches (Holbrook and Schal 2004). These results are consistent with the hypothesis that phenotypic plasticity should be most strongly expressed in cases where it provides the greatest benefits—in this case, for individuals or species that rely more heavily on exogenous nutrition (DeWitt et al. 1998; Relyea 2002). Although such comparisons of quantitative variation are still rare in the literature on marine invertebrates, we argue that in many instances, comparisons of quantitative variation can be as informative as comparisons of means.

This review has three major goals. First, we discuss some methodological approaches to the comparison of quantitative variation across distributions and review their limitations. Second, we describe several case studies, from our own work and from the literature on invertebrate life histories, to illustrate how researchers have used such comparisons to address pertinent questions about the degree of variability as an evolutionary outcome. Finally, we discuss some broader implications of comparisons of quantitative variation in marine invertebrate life histories, identify

gaps in our understanding, and address questions for future research.

Methodological options for comparing variability

Levene's test and related options

A number of procedures have been developed specifically to compare variability (reviewed by Conover et al. 1981; Zar 1999; Quinn and Keough 2002). These are often used to check the assumption of homogeneity of variance prior to other statistical procedures, although this use is not recommended by most statistics textbooks (e.g., Zar 1999; Quinn and Keough 2002). Here, we focus instead on statistical comparisons of variability that can be used to directly address ecological, evolutionary, and developmental questions.

The most useful and most frequently recommended procedure to compare variability for a broad range of experimental designs is Levene's test (Conover et al. 1981; Schultz 1985; Donnelly and Kramer 1999). This test uses an *F*-test to compare the absolute deviations of data points from each distribution's mean (or median, in the Brown–Forsythe variant of the test). Levene's test is far less sensitive to non-normality of the original distribution than commonly used alternatives such as Bartlett's or Cochran's Test (Conover et al. 1981; Quinn and Keough 2002), and use of the *F*-test allows for complex experimental designs and multiple comparisons (Donnelly and Kramer 1999). A non-parametric version of Levene's test based on weighted scores has been developed by Fligner and Killeen (1976).

Two main cautions exist regarding the use of Levene's test and its modifications. First, the deviations from the mean (or median) must satisfy the assumptions of normality and homogeneity of variance required for the *F*-test (Zar 1999; Quinn and Keough 2002). In separate studies, Conover et al. (1981) and Donnelly and Kramer (1999) used Monte Carlo simulations to compare the performance of more than 60 different tests for equality of variance. In both studies, the authors concluded that (1) Levene's test and its modifications consistently outperformed all other tests in terms of power and robustness; and (2) parametric (Brown–Forsythe) and non-parametric (Fligner–Killeen) versions of Levene's test based on medians were less sensitive to departures from normality than were versions based on means. Conover et al. (1981) also showed that discarding the median itself (or one of the two values bracketing the median) improved

performance of median-based versions of Levene's test when sample sizes were small.

Second, the outcome of Levene's test can be misinterpreted when the means of the distributions being compared are different, because the variance of measurements is expected to scale roughly as the square of the mean, a pattern that is evident in many biological systems (Lewontin 1966; Lande 1977). Log transformation as suggested by Lewontin (1966), Schultz (1985), and Donnelly and Kramer (1999) can help to correct for this problem by changing absolute deviations into proportional deviations.

Coefficient of variation

An increasingly common solution to the problem of covariance between the mean and the variance has been to carry out comparisons of the sample standard deviation divided by the sample mean, a ratio known as the coefficient of variation ($CV = \sigma/\bar{x}$). The strength of the CV is that it provides a simple, dimensionless measure of variability that is often considered to be 'scale-free,' because it is dimensionless. Quantitative comparisons of CVs using *F*-tests are common in certain fields (e.g., morphometry of vertebrates; Patterson 1983; Prevosti and Lamas 2006; McKellar and Hendry 2009), and CVs have recently been used for both quantitative (Marshall et al. 2008; Crean and Marshall 2009; Collin 2010) and qualitative (e.g., Marshall and Keough 2008; Krug 2009) comparisons of variability in life-history traits in the literature on marine organisms.

While the CV is an intuitive and easily calculated expression of variability, there are several potentially serious drawbacks associated with its use in statistical analyses. First, because the CV condenses the variability of the distribution into a single number (the standard deviation), it is far more sensitive than alternatives, such as Levene's test, to departures from normality of the original distribution. Donnelly and Kramer (1999) found that tests based on CVs were less robust to departures from assumptions and had less power than is true of many other tests, including Levene's test. Second, although CVs themselves are dimensionless, they cannot be used to compare distributions for variables with different dimensionalities. Schmalhausen (1935; cited by Lande 1977) demonstrated that the CVs for measurements of length, surface, and volume differ on a scale of 1:2:3. More broadly, CVs cannot be used to compare variation of any whole to that of its parts, because unless the variances of the parts are perfectly correlated with one another, the CV of the whole is mathematically constrained to be less than the

weighted average of the CVs of its parts (Lande 1977; Bryant 1986).

Finally, the use of a ratio (the CV) does not effectively correct for a relationship between the standard deviation and the mean. The use of ratios in statistical analyses has been heavily criticized for the way that ratios (1) generate non-normal distributions even from normally-distributed numerators and denominators, (2) can change the error distribution of the data in unpredictable ways, and (3) fail to take into account non-linear relationships between numerators and denominators (Packard and Boardman 1988; Allison et al. 1995; Jasienski and Bazzaz 1999). The error associated with calculations of ratio is particularly pronounced when there is a probability of the distribution overlapping zero (Lande 1977), or when measurement error does not decrease proportionally with decreasing mean (Polly 1998).

Ratios may be effective at eliminating the effect of a denominator only if the relationship between the numerator (in this case, the standard deviation) and the denominator (in this case, the mean) is linear and has a y -intercept of zero (Allison et al. 1995). Biological data are unlikely to meet these criteria (Hosken 2008; Houle 1992; Lajus 2001), suggesting that in many cases use of the CV is likely to obscure, rather than correct, the influence of the mean on the standard deviation (Allison et al. 1995). Figure 1 shows the standard deviation and CV for size plotted against the mean across a range of sizes for offspring of invertebrates (data from Marshall and Keough 2008). These data show the expected positive relationship between mean and standard deviation, but the relationship is logarithmic rather than linear, and the y -intercept does not pass through zero (Fig. 1).

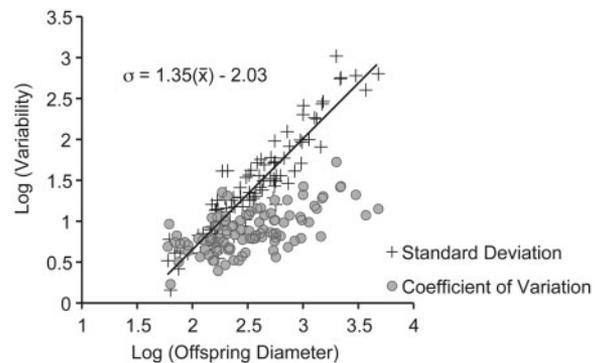


Fig. 1 Log-log plot of variability (expressed as standard deviation, +, or coefficient of variation, O) plotted against mean diameter of offspring for 102 species of marine invertebrates (data from Marshall and Keough 2008). Each point represents a single species.

Thus, it is possible that the observed positive relationship between CV and mean is an artifact of failing to meet the criteria described above, rather than a real biological effect. Several authors have attempted to eliminate this concern by separately testing for a relationship between CV and mean prior to analysis (Meiri et al. 2005; Marshall and Keough 2008), but this approach does not solve the underlying problems associated with tests on ratios, and in particular does not correct any increased error or variability that might be introduced into the dataset through use of CVs (Allison et al. 1995; Jasienski and Bazzaz 1999).

Analysis of covariance

Packard and Boardman (1988) and others have promoted analysis of covariance (ANCOVA) as an alternative to the use of ratios to standardize or scale for variables such as body size in ecology and physiology. While we have not seen ANCOVA used in comparisons of variability, in principle it could be used in an analogous way to compare standard deviations using means as a covariate. ANCOVA is the only one of the three approaches described here that can statistically control for a relationship between standard deviations and means. Whereas CVs automatically adjust the standard deviation by the mean regardless of whether such a relationship exists, and Levene's test either disregards the potential effect of the mean or relies upon log transformation to try to eliminate its influence, ANCOVA controls for variance associated with the mean as part of the statistical model rather than automatically rescaling the estimate of variability prior to analysis.

Given this potential application of ANCOVA, it is important to review its limitations. First, the use of ANCOVA typically assumes that the slope of the relationship between the independent variable (in this case, means) and the dependent variable (in this case, standard deviations) is the same across groups, although Engqvist (2005) and others have advocated use of the Johnson–Neyman procedure as a way of generalizing ANCOVA to cases with heterogeneous slopes. Second, ANCOVA assumes that values of the covariate overlap across groups (Quinn and Keough 2002), because covariance relationships should not be extrapolated beyond measured ranges. While use of ANCOVA when covariate ranges do not overlap is a potential misuse of the procedure (Miller and Chapman 2001; Quinn and Keough 2002), it should be recognized that the same caution applies to CVs, which also assume a consistent relationship between

standard deviations and means across groups. A third concern for Model I (ordinary least squares) ANCOVA procedures, which are typical of statistical packages, is that they fail to incorporate any error associated with the covariate (McCoy et al. 2006). Such a procedure could underestimate the slope of the relationship between standard deviations and means (McArdle 1988) when the standard error for the mean is substantial (that is, when means are calculated from small sample sizes). The use of Model II (reduced major axis) analogs of ANCOVA could help to avoid this problem (Warton et al. 2006; but see Smith 2009). A fourth concern in use of ANCOVA is that the variability of each distribution is represented by the standard deviation, a parameter that is highly sensitive to departures from normality in the original data set, although again it should be recognized that the same caution applies to CVs.

In summary, there exist a range of options for quantitative comparisons of variability, but some methods appear to be more reliable than others. Levene's test and its modifications are broadly recommended in the scientific literature and, because Levene's test uses an *F*-test, can be adapted to a wide range of experimental designs. ANCOVA is less well-explored for the purpose of comparisons of variance, but may present a useful alternative when a correction for the mean is required, or when raw data are not available. Although CVs have been used increasingly for variability comparisons, they are subject to several long-criticized problems associated with the use of ratios, and we caution against their use in many circumstances.

Comparison of variability in invertebrate life histories: four case studies

Case Study 1: delay of metamorphosis and size variation of offspring in ascidians

Many marine organisms have the ability to delay metamorphosis when conditions are unfavorable (Jackson and Strathmann 1981; Pechenik 1990). This kind of delay, however, can have severe consequences for lecithotrophic larvae, which must trade off energy reserves used during the delay against those available to juveniles (e.g., Pechenik et al. 1993; Takami et al. 2002; Marshall and Keough 2003). Jacobs et al. (2008) tested the hypothesis that delay of metamorphosis reduces fitness of juveniles by manipulating the length of the larval period of the ascidians *Ciona intestinalis*, *Ascidella aspersa*, and *Molgula socialis*. These authors failed to find evidence of any fitness costs associated with

prolonged larval life: delay of metamorphosis did not negatively affect juveniles' growth, survival, or vulnerability to nutritional stress for any species. The authors suggested that early activation in delayed larvae of developmental programs that build adult structures ("anticipatory development"; Degnan and Morse 1995) might allow more rapid metamorphosis and thereby compensate for some of the negative physiological consequences of delay.

Metamorphosis in ascidians involves substantial expansion of body volume over a period of several days immediately after attachment, culminating in the opening of branchial and atrial siphons and the onset of feeding (Berrill 1947). We hypothesized that the process of anticipatory development might result in more variable size at the onset of feeding, if siphons opened earlier relative to expansion of body volumes for individuals that delayed metamorphosis. Jacobs et al. (2008) measured initial size at the onset of feeding for juvenile *A. aspersa*, *C. intestinalis*, and *M. socialis* that had settled on plates at densities ranging from two to seven individuals per plate. Including only those plates that contained four or more individuals, we compared variation in initial sizes between "delay" and "no-delay" treatments using the Brown–Forsythe variant of Levene's test in JMP 5.1.2 (SAS Institute Inc.) with log transformation of absolute deviations and median values removed (Conover et al. 1981; Donnelly and Kramer 1999). In the statistical model, timing of metamorphosis (delay versus no-delay) and settlement plate nested within timing of metamorphosis were considered fixed factors (Fig. 2).

We chose to use the parametric, median-based version of Levene's test (the Brown–Forsythe test) because we had access to the raw data, and because these tests are less sensitive to departures from normality than CV-based comparisons or ANCOVA. Distributions for some plates in the 'delay' treatment for *A. aspersa* were skewed by large outlier points (third, sixth, and seventh plates) or were bimodal (fifth plate; Fig. 2A), which can reduce the reliability of the parametric versions of Levene's test (Conover et al. 1981; Donnelly and Kramer 1999). Non-parametric versions of Levene's test such as the Fligner–Killeen test would have been difficult to adapt to our complex experimental design (see Donnelly and Kramer 1999 for a discussion of this issue), so we chose instead to run a second version of the parametric test with plate five and the outlier points excluded to ensure that our findings were not biased by these non-normal distributions.

Consistent with our hypothesis, size at feeding was significantly more variable for the "delay" treatment

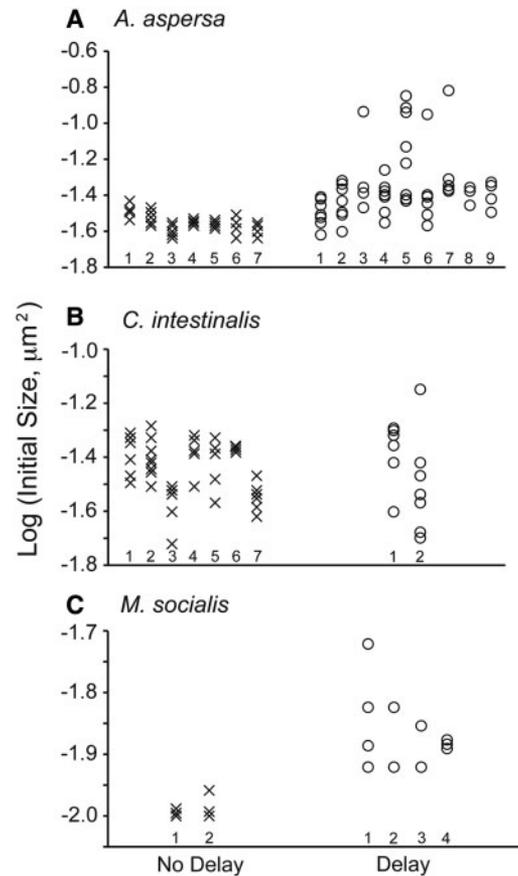


Fig. 2 Logarithm of initial size (μm^2) at the onset of feeding for no delay (X) or delay (O) treatments for juvenile ascidians *Ascidella aspersa* (A), *Ciona intestinalis* (B) and *Molgula socialis* (C). Each point represents one individual, and each column represents a separate settlement plate (numbered along x-axis).

than for the "no-delay" treatment for *A. aspersa* (Fig. 2A; $F_{1,52} = 8.5419$, $P = 0.0051$) and *C. intestinalis* (Fig. 2B; $F_{1,53} = 9.9883$, $P = 0.0026$). Exclusion of plate five and the outlier points for *A. aspersa* did not affect the outcome ($F_{1,42} = 19.6275$, $P < 0.0001$). Within-plate sample sizes for *M. socialis* were too small for a statistical analysis of variability, although the graphical pattern is consistent with the hypothesis (Fig. 2C). Anticipatory development has recently been recognized as a common feature of ascidian life histories (Jacobs et al. 2008), and our results support the hypothesis that anticipatory development in larvae that delay metamorphosis accelerates the onset of juvenile feeding relative to other metamorphic events.

Case Study 2: population-level differences in variability among larval lobsters

Harding et al. (1993) used discriminant function analysis to show significant morphological variation

in stage I (newly released) larval lobsters (*Homarus americanus*) among different locations of capture in inshore and offshore waters near Nova Scotia, Canada. Because the larvae were captured in the field, it was not possible to determine with certainty whether these differences arose from genetic or from environmental differences between populations (Collin and Salazar 2010). However, lobsters are known to display a high degree of phenotypic plasticity (Factor 1995), and lobster populations north of Cape Cod are thought to show little or no genetic substructure (Kenchington et al. 2009). Assuming that a large component of quantitative variation between sites is environmental, differences in external morphology during the first larval stage of these molting crustaceans should reflect differences in maternal environment during egg incubations. We hypothesized that the variance of morphological measurements would be higher for larvae that developed under more variable inshore conditions compared with larvae that developed offshore.

Because we did not have access to their raw data, we had to estimate variability using the standard deviations reported by Harding et al. (1993) for the five inshore and two offshore locations of capture. Offshore larvae were larger overall than inshore larvae (Harding et al. 1993), but the distributions of the standard deviations as a function of mean size overlapped between the offshore and inshore groups of larvae and were similar in slope. We chose to use ANCOVA to look at differences in standard deviations of morphological traits measured in offshore versus inshore larvae, with morphological trait and region of capture (nested within morphological trait) included as fixed factors and population mean size included as a covariate. The analysis was performed using JMP 5.1.2 (SAS Institute Inc.).

Consistent with our hypothesis, we found that standard deviations were significantly greater for inshore larvae compared with offshore larvae, for all seven morphological traits examined (Fig. 3; ANCOVA: $F_{7,34} = 2.8349$, $P = 0.0193$). Our results suggest that the maternal environment during incubation of the eggs may strongly influence morphometric variability of offspring.

Case Study 3: maternal effects on variability in size of gastropod offspring exposed to intertidal stress

Environmental variability is often associated with stress, either because variability itself is physiologically challenging (Stillman and Tagmount 2009) or because it leads to more frequent exposure to stressful conditions (Schill et al. 2002). Among marine habitats, intertidal areas experience an unusually high degree of variability in physical conditions—including temperature, salinity, desiccation, water motion, and exposure to UV radiation—as a result of tidal emersion. This variability contributes to a range of physical stresses that can be especially acute for early developmental stages of marine organisms (Pechenik 1978; Brawley and Johnson 1991; Podolsky 2003).

Podolsky (unpublished data) examined the effects of variability in temperature on the variability of growth in embryos of an intertidal gastropod, *Melanochlamys diomedea*. This species deposits gelatinous egg masses on the surface of tidal flats, where embryos experience wide fluctuations in physical conditions over their first week of development before larvae hatch and enter the plankton. In the laboratory, egg masses were subdivided and exposed to low, medium, or high daily temperature spikes with profiles that mimicked the large range of temperature fluctuations experienced by adults and embryos on tidal flats in the Pacific northwest

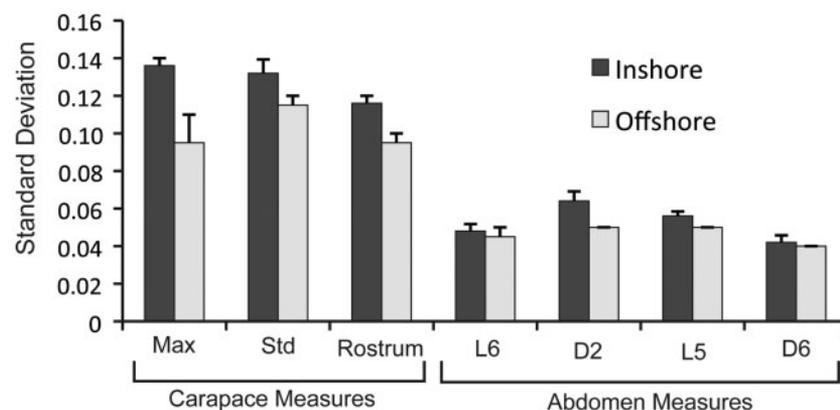


Fig. 3 Mean of the standard deviation for seven morphometric measurements of larval lobsters from five inshore (dark bars) and two offshore (light bars) locations (data from Harding et al. 1993). Error bars are standard errors.

(Helmuth et al. 2002). The peak temperatures reached in the three treatments (23, 26, or 29°C) were below, just at, or well above, respectively, the threshold temperature for embryonic expression of heat shock proteins, a signal of cellular damage (Podolsky 2003). For these experiments, the level of physiological stress experienced by embryos is therefore correlated with environmental variability. Focusing on the effects of stress rather than variability *per se* is warranted because when the two factors are separated experimentally, variance in temperature has a small impact on embryo development relative to the effect of crossing the stress threshold (Podolsky 2003; D. Fernandes and R. Podolsky, unpublished data).

One goal of this experiment was to determine whether the degree of variability in hatchling size was influenced by the degree of stress experienced by embryos. In addition, the egg masses used were obtained from adults that had themselves been exposed to one of the same three temperature profiles. The second goal of this nested design was therefore to test whether maternal exposure to temperature stress also influenced the degree of variability in size among hatchlings, as well as whether maternal and embryonic exposures had an interactive effect.

For each portion of egg mass tested under a given set of conditions, we calculated mean and standard deviation of the longest axes of the larval shells at hatching, for between 20 and 50 hatched larvae per portion. Standard deviations were then analyzed using a linear mixed model ANCOVA in PASW Statistics (v. 17.0) with the cohort mean as a covariate, adult and embryo stress levels as fixed effects, and adult as a random effect. Interactions between the covariate and fixed effects were included to test the assumption of equal slopes among treatments.

Data on size within egg masses were normally distributed, allowing us to accurately approximate variability using standard deviations. The effect of the covariate (mean) on the standard deviation was significant ($F_{1,60.7} = 4.16$, $P < 0.05$), interactions between the covariate and fixed effects were not significant (and therefore removed from the model), and ranges of means among treatments overlapped almost completely, all validating the use of ANCOVA. An analysis carried out using Levene's test with the absolute deviations log-transformed to adjust for differences in mean between treatments produced results that were statistically and graphically equivalent to the ANCOVA.

We found a significant interaction between embryonic and adult stress exposure in the degree of variability in size of hatchlings ($F_{4,58.2} = 5.05$, $P < 0.001$).

Embryos from stressed adults were more variable in size than were embryos from less stressed adults in response to a low or medium level of embryonic stress, but less variable in size than embryos from less stressed adults in response to a high level of embryonic stress. The interaction was consistent with a tradeoff in which maternal effects acted to reduce variability in size of hatchlings when embryos experienced conditions similar to those experienced by adults. The mechanism for such a maternal effect is unknown but could include, for example, changes in methylation patterns or transmission of gene products involved in thermal protection (Mamon et al. 1999; Weaver et al. 2004). Independent of the adult-by-embryo interaction, embryonic exposure to stress also contributed significantly to differences in the variability of hatchling sizes ($F_{2,64.5} = 10.81$, $P < 0.001$; Fig. 4), whereas adult exposure to stress did not ($F_{2,26.7} = 1.67$, $P = 0.21$).

A possible alternative explanation for the decline in variance under the combination of high embryonic and adult stresses is that selection reduced the range of hatchling sizes. We evaluated this hypothesis using two additional measures. First, we reasoned that the mean size of hatchlings would most likely increase if selection were responsible for the reduced variation, assuming that larger propagules would

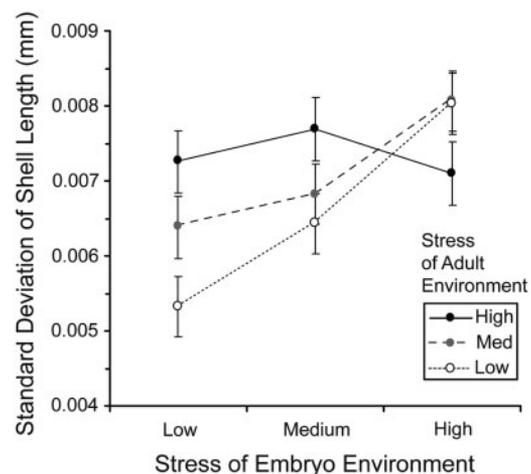


Fig. 4 Effects of exposure to stress at the embryonic and adult stages on the standard deviation of shell length. Data points in the analysis were standard deviations for cohorts of hatchlings from portions of egg masses reared in each combination of conditions, with the mean for each cohort used as a covariate. Values represent estimated marginal means ± 1 SE for the standard deviation. Sample sizes: $N = 12$ adults for the low and medium levels of stress and $N = 11$ adults for the high level of stress, with egg masses from each adult divided between the three stress levels for a total of 35 egg mass portions per stress level treatment.

better survive stresses (Moran 1999). In fact, mean hatchling size declined monotonically as a function of embryonic exposure to stress and, among embryos exposed to high stress, did not vary as a function of adult exposure to stress (ANOVA, $F_{2,32} = 1.13$, $P = 0.33$). Second, we reasoned that the number of larvae measured per treatment—which, although not a perfect proxy for survival, does reflect the ease with which the larvae were collected and photographed for measurements at hatching—would decline if selection were responsible for the reduced variation. Contrary to this prediction, the average number of hatchlings measured from adults exposed to medium and high stress was nearly identical and, if anything, greater than the number measured from adults exposed to low stress. In summary, neither measure suggests that selection is a more likely explanation than a maternal effect for the observed interactive effect of adult and embryonic stress exposure on hatchling size.

Case Study 4: interspecific differences in variability of offspring size: correlations with life history

In unpredictable environments, increased variability of offspring traits may enhance fitness of mothers by increasing the probability that at least some offspring will be well-adapted to the environment in which their early growth takes place, a form of bet-hedging (Cohen 1966). This idea is supported by some mathematical models (Geritz 1995; but see McGinley et al. 1987). However, broad interspecific comparisons of variability in size of offspring as a function of life-history mode have failed to provide evidence of this type of bet-hedging for terrestrial plants (Einum and Fleming 2004) or parasitic trematodes (Poulin and Hamilton 2000).

In the ocean, correlation in environmental conditions between mothers and offspring is likely to depend on life-history mode. Environmental conditions experienced by benthic mothers and their planktonic larvae are likely to be less strongly correlated than those experienced by benthic mothers and their crawl-away or encapsulated offspring. This observation leads to the prediction in the former case of bet-hedging through production of more variable offspring, and in the latter of production of offspring with traits that are less variable and more closely tuned to conditions experienced by the mother.

Marshall et al. (2008) tested this hypothesis by calculating CVs across a broad range of offspring sizes compiled from the literature, and by comparing within and among-brood variability for direct developers (*D*, species with encapsulated or crawl-away

juveniles), species that produce non-feeding planktonic larvae (*NF*), and species with feeding planktonic larvae (*F*). The authors predicted that for dispersing larvae (groups *NF* and *F*), variation within broods would be high relative to variation among broods, while for direct developers, variation within broods would be low relative to variation among broods. Consistent with their hypothesis, they found a significant interaction between variability and developmental mode: CV among broods was higher than CV within broods for group *D*, lower than CV within broods for group *F*, and intermediate for group *NF* (Fig. 5A).

As in Case Study 2, the authors lacked the raw data and had to estimate variability using the standard deviations reported by the various sources used in their meta-analysis. They chose to correct for any systematic effect of mean size on standard deviation by calculating CVs, for which we earlier urged caution. However, the paired nature of the analysis (the comparison of interest was of within-brood to

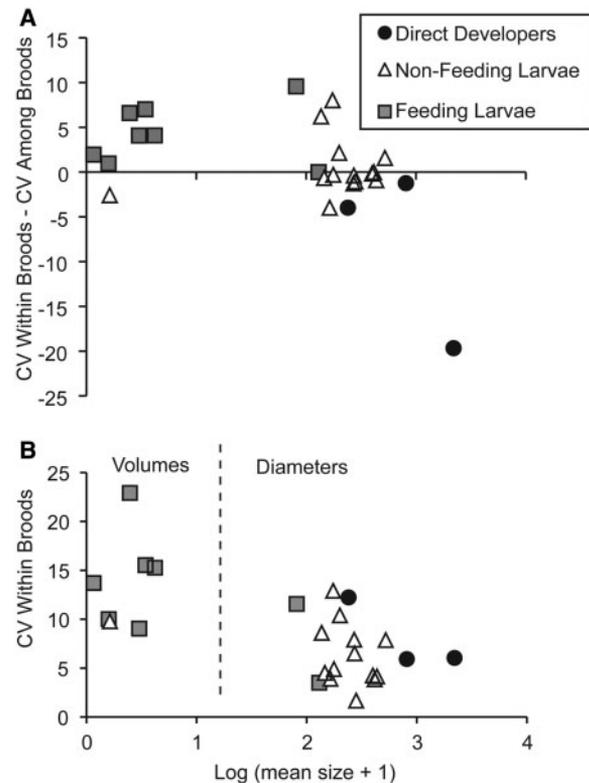


Fig. 5 (A) Difference between within- and among-brood CV as a function of offspring size for direct developers, species that produce non-feeding planktonic larvae, and species that produce feeding planktonic larvae (data from Marshall et al. 2008). (B) Within-brood CV, plotted as for (A). Points to the left of the dashed line are based on measurements of volume; points to the right are based on measurements of diameter.

between-brood variation within each species) avoided many of the pitfalls normally associated with using CVs to correct for the effects of size.

In a separate analysis, Marshall et al. (2008) compared only within-brood variation among the same three development types. They found that CVs were highest for indirect developers with feeding larvae (group *F*; Fig. 5B). This second analysis required a correction for the effect of the means on the standard deviations because offspring size covaried strongly with development type, but ANCOVA was judged not to be an option because the mean sizes of the groups under comparison did not overlap (Fig. 5). The authors chose to compare CVs, although as explained earlier, constraints on use of CVs in this respect ought to be similar to those associated with ANCOVA. In addition, the analysis was complicated by differences in dimensionality: CVs calculated from volumes (most of the *F* species) and diameters (most of the *NF* and *D* species) are not comparable (Lande 1977; Marshall and Keough 2008). When measurements of volume were excluded, within-brood CV for size of offspring was not correlated with mode of development (Fig. 5B).

This study by Marshall et al. (2008) is a significant step toward quantitatively assessing patterns of variability in life-history traits across marine invertebrates. Similar to previous findings for terrestrial plants (Einum and Fleming 2004) and trematodes (Poulin and Hamilton 2000), there was no clear evidence that within-brood variability in offspring size of marine invertebrate species is related to life-history mode. However, the comparison between among-brood and within-brood variability in offspring size, which provides an internal control for species-specific factors that could also influence variability, does offer compelling evidence of a life-history pattern and raises questions for further analysis. One unresolved issue, for example, is whether higher among-female variability for direct developers is a consequence of greater genetic variability in species with short dispersal distances, or evidence (as the authors suggest) that mothers are more likely to adjust offspring traits when the maternal environment is more predictive of the environment that offspring will experience.

Broader considerations and the biological significance of variation

The causes and consequences of variability in life history traits may often be difficult to determine. Variability can result from genetic or environmental influences, and can be adaptive, neutral, or

detrimental, depending on the strength of selection around a trait optimum and on the degree of environmental uncertainty. When environmental conditions that affect fitness are highly predictable or fitness decreases sharply away from an optimum trait value, variability becomes more disadvantageous. In this circumstance, maternal effects could increase fitness by buffering offspring against excessive variation. In contrast, when environmental conditions that will affect fitness of individual offspring are less predictable, it may be advantageous for mothers to hedge their bets by increasing the variability of their offspring (Cohen 1966; McGinley et al. 1987; Geritz 1995; Marshall et al. 2008).

Empirical support for the bet-hedging hypothesis within species has been mixed. Crean and Marshall (2009) found a correlation between the level of variability in the maternal environment and the variability of offspring size for two out of three case studies they surveyed. Mothers who experienced more variable environments produced more variably sized offspring for the soil mite *Sancassania berlesei* and the coral reef fish *Pomacentrus amboinensis*, but not for the bryozoan *Bugula neritina*. In contrast, Collin (2010) found the opposite correlation for the gastropods *Crepidula atrasolea* and *C. ustulatulina*. Mothers kept under constant conditions in a temperature-controlled incubator produced more variably sized offspring than did mothers kept under more variable conditions on a benchtop. Crean and Marshall (2009) also included two “control” studies in which variability in offspring size was not predicted to vary between treatments because maternal environment did not vary. This prediction held true for the sea slug *Chelidonura sandrana*, but not for the bryozoan *B. neritina*. Thus, it is unclear from the available evidence whether changes in variability of offspring (where observed) should be attributed to adaptive manipulations by mothers in response to environmental cues (as suggested by Crean and Marshall 2009 for *S. berlesei* and *P. amboinensis*), to a physiological response to stressful conditions (as suggested by Collin 2010 for *Crepidula atrasolea* and *C. ustulatulina*; and by Crean and Marshall 2009 for *B. neritina*), or some combination.

Our Case Study 3 is a clear example of a maternal effect that controls variability in offspring. Variability in hatchling size increased in general with increasing embryonic stress, but offspring of mothers that had themselves been exposed to highly stressful conditions were less variable under the same high stress conditions (Fig. 4). Similar to the case studies

described by Crean and Marshall (2009) and Collin (2010), increased variability in size of hatchlings after development under more stressful (and in this case, more variable) conditions is consistent with one of two hypotheses: increased variability in size is (1) an adverse physiological consequence of embryonic exposure to stress, or (2) an adaptive strategy for bet-hedging against future unpredictability in environmental conditions (Marshall et al. 2008).

For Case Study 3, the first hypothesis is supported by two lines of argument. First, the nature of the interaction between exposures to stress at the embryonic and adult stages indicates that hatchling variability was damped most when conditions experienced by adults were predictive of conditions experienced by embryos. If increased variability in hatchling size were an adaptive response to environmental unpredictability, one would have expected a synergistic interaction between embryonic and adult exposures rather than the observed counteracting maternal effect.

Second, differences in the potential for correlation in physical conditions at three stages of the life cycle—adult, embryonic, and larval—suggest that manipulation of variation in hatchling size is unlikely to be useful as a bet-hedging strategy. In this example of intertidal development, variation in the experience of embryos is greatest at about the scale of the developmental period (~7 days), owing to variation in the timing of tidal emersion (Podolsky 2003). That is, the experience of embryos will depend most strongly on whether they are deposited during a spring-tide series with high insolation, a spring-tide series with more moderate insolation, or a neap-tide series. Because of the high probability that adults' exposure to stress just before oviposition will be temporally and spatially correlated with embryos' exposure to stress, both the timing of oviposition (Podolsky 2003) and maternal effects transmitted to offspring offer a potential mechanism for buffering the exposure of embryos to stress. On the other hand, because the experience of embryos in the intertidal is unlikely to be strongly predictive of hatchlings' experience in the plankton, it is unlikely that adults or embryos could adaptively manipulate hatchling size. This example illustrates the importance of understanding the scale and context under which a plastic response would undergo selection relative to the circumstances under which environmental conditions are sensed and an appropriate response is mounted (Levins 1968).

Scale and context are also important when considering the adaptive nature of observed variability. For logistical reasons, few studies measure fitness across

entire life cycles (although see Marshall 2005; Marshall 2008). This focus on short-term measures early in life can lead to overestimation of the consequences for fitness, because selection on a phenotype integrated over a life cycle could broaden fitness peaks relative to what is observed in any single case (Podolsky and Moran 2006). An illustrative example comes from the work of Pineda et al. (2006), who followed almost 3000 barnacle recruits over an entire field season, until they either died or survived to reproductive age. They found that the eight survivors had all settled within a narrow "recruitment window" early in the season. Throughout the 3-month recruitment season, recruits had varied in size, energy content, shape, settlement behavior, and other parameters that likely influence the success of offspring in the short term. However, from the perspective of mothers, the strongest predictor of offspring success was simply the timing of reproduction relative to the recruitment window. This selection pressure could maintain greater variability in factors that control recruitment and early survival of juveniles than would be predicted based on short-term measures of individual performance or population dynamics.

Final thoughts

Size of offspring, the focus of all the case studies described above, is by far the most commonly measured life-history parameter (reviewed by Marshall and Keough 2008). However, quantitative variation is widely reported and discussed for a broad range of phenotypic measures, from shape (e.g., Vaughn 2007; Collin and Salazar 2010), to biochemical composition (reviewed by Moran and McAlister 2009), behavior (e.g., Castro and Cobb 1991; Manuel et al. 1996), and developmental timing (reviewed by Pechenik 1990; Hadfield and Strathmann 1996; Shanks 2009). Recent advances in genomic technology (reviewed by Hofmann 2005; Hofmann and Place 2007) have also revealed enormous variability in gene expression within and among groups of organisms (e.g., Marsh and Fielman 2005; Jacobs et al. 2006; Williams and Degnan 2009). Despite this widespread recognition of variability at all levels, quantitative comparisons of variation within and between populations are surprisingly rare. Although there is no single well-established methodology for comparisons of variability in the literature on marine invertebrates, there are several options and we encourage further exploration in this area, with careful attention paid to cautions applicable to the different methods.

Development can lie anywhere on a continuum from an inflexible, highly canalized, carefully orchestrated process, with sharply reduced fitness associated with deviations from a single optimal pathway, to a robust, flexible, and forgiving process, with a high degree of variability and broad fitness optima (Van Buskirk and Steiner 2009). Studies of pattern in quantitative variation and its underlying causes have the potential to greatly expand our understanding of how selection works on developmental rates, morphology, physiology, gene expression, reproductive timing, behavior, and a host of other traits. The case studies presented here, along with additional recently published work (Crean and Marshall 2009; Krug 2009; Collin 2010), highlight the value of comparisons of variability for a range of questions associated with genotype–environment interactions, reproductive ecology, and life-history biology. We encourage researchers in larval ecology and life-history biology to explicitly consider the consequences of variability in their models, experimental designs, analyses, and interpretations.

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