

LETTER

Should mothers provision their offspring equally? A manipulative field test

Hayley Cameron,* 
 Keyne Monro and
 Dustin J. Marshall

Centre for Geometric Biology
 School of Biological Sciences Monash University Melbourne, Vic.
 3800, Australia

*Correspondence: E-mail:
 hayley.cameron@monash.edu

Abstract

Within-brood variation in offspring size is universal, but its causes are unclear. Theoretical explanations for within-brood variation commonly invoke bet-hedging, although alternatives consider the role of sibling competition. Despite abundant theory, empirical manipulations of within-brood variation in offspring size are rare. Using a field experiment, we investigate the consequences of unequal maternal provisioning for both maternal and offspring fitness in a marine invertebrate. We create experimental broods of siblings with identical mean, but different variance, in offspring size, and different sibling densities. Overall, more-variable broods had higher mean performance than less-variable broods, suggesting benefits of unequal provisioning that arise independently of bet-hedging. Complementarity effects drove these benefits, apparently because offspring-size variation promotes resource partitioning. We suggest that when siblings compete for the same resources, and offspring size affects niche usage, the production of more-variable broods can provide greater fitness returns given the same maternal investment; a process unanticipated by the current theory.

Keywords

bet-hedging, complementarity, fitness, life-history theory, maternal provisioning, niche partitioning, offspring size, sibling interactions, within-brood variation.

Ecology Letters (2017) 20: 1025–1033

INTRODUCTION

According to classic life-history theory, mothers within a given environment are expected to maximise their fitness by producing uniformly sized offspring that optimise the trade-off between offspring size and number (Vance 1973; Smith & Fretwell 1974). Thus, classic theory predicts that offspring size is under stabilising selection. In nature, however, offspring sizes are variable, not only among and within populations, but also among and within broods of the same female. Variation in offspring size within broods (or clutches, litters, etc.) is ubiquitous across all taxa (Kamel & Williams 2016). Coefficients of variation (CV) for offspring volume within broods of the same female typically exceed 4%, and in some groups, exceed the variation observed among females (Lips 2001; Marshall *et al.* 2008; Kosman & Pernet 2011). For example, estimates of within-brood CVs for egg diameter average ~ 8% in a neo-tropical tree frog (Lips 2001). Assuming a normal distribution, this CV can translate to an approximate fourfold difference in volume between the smallest and the largest 5% of offspring in a single reproductive bout – a non-trivial inequality in investment. Yet, why mothers differentially provision their offspring remains unclear.

In constant environments, mothers that unequally provision their offspring inevitably produce some offspring that deviate from the optimal size, and will therefore suffer reduced mean fitness (Marshall *et al.* 2008; Fig. 1). As such, when mothers can anticipate local conditions they should produce offspring of a single, optimal size (Marshall & Uller 2007; Fischer *et al.* 2011; Burgess & Marshall 2014). In unpredictable environments, however, increasing variance in offspring size within a brood may ensure that at least some offspring phenotypes are

matched to the prevailing conditions. As such, within-brood variation in offspring size is commonly invoked as a bet-hedging strategy in unpredictable environments, whereby mothers trade-off reduced arithmetic mean fitness *within* a generation for reduced variation in fitness *among* generations, thus increasing long-term (geometric mean) fitness (reviewed in Crean & Marshall 2009).

Bet-hedging is the most cited explanation for within-brood variation in offspring size, but evidence for this is mixed. Some studies support this idea (Marshall *et al.* 2008; Crean & Marshall 2009; Olofsson *et al.* 2009), while others do not except under rare circumstances (McGinley *et al.* 1987; Einum & Fleming 2004). Empirical evidence in favour of bet-hedging is mainly correlative; for a range of taxa, within-brood variation increases with environmental variability and (or) unpredictability (Crump 1981; Lips 2001; Einum & Fleming 2002; Koops *et al.* 2003; Marshall *et al.* 2008; Morrongiello *et al.* 2012). Such correlative approaches are understandable, given it is extremely difficult to observe the multi-generational effects of bet-hedging in field populations (but see Metz *et al.* 2010). We propose, however, that direct manipulations of within-brood variance offer the opportunity to test whether within-brood variation in offspring size may provide immediate fitness benefits within a generation, in contrast to the multi-generational benefits associated with.

Another fundamental assumption of bet-hedging theory is that offspring size-fitness effects are additive – namely, that the fitness of an offspring of a particular size is unaffected by offspring (siblings) of other sizes (Fig. 1). In many systems, siblings co-occur across very small spatial scales, such that interactions are likely to alter the fitness of these neighbouring

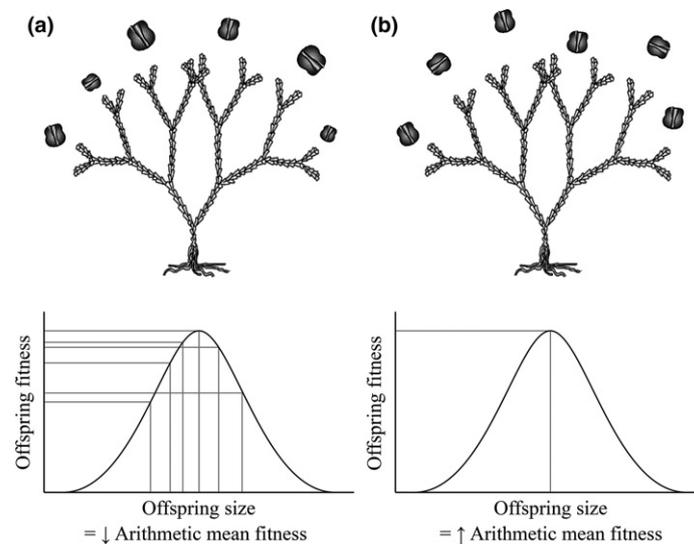


Figure 1 Schematic showing the predictions of bet-hedging theory, whereby in constant and/or predictable environments (a) mothers that unequally provision their offspring will produce more offspring that deviate from the optimal size, and thus have lower arithmetic mean fitness, compared to (b) mothers that equally provision their offspring.

siblings (Cheplick 1993; Geritz 1995; Geritz *et al.* 1999; Veliz *et al.* 2006; Plaistow *et al.* 2007; Kamel *et al.* 2010; Aguirre *et al.* 2013; Cameron *et al.* 2016). Such sibling interactions can determine selection on maternal provisioning strategies among females (Parker & Begon 1986; Plaistow *et al.* 2007; Cameron *et al.* 2016; Kamel & Williams 2016). For example, Plaistow *et al.* (2007) found that competition among cohorts of younger, smaller soil mites, and their older, larger siblings selected for larger offspring with increasing maternal age (i.e. among-brood variation). Within a single cohort, increasing densities of siblings altered offspring-size related performance relative to when offspring were isolated from siblings (Cameron *et al.* 2016). Thus, it is plausible that sibling interactions may also mediate selection on within-brood variation in offspring size, especially when limited dispersal causes siblings to interact.

Alternative theory has considered how interactions among siblings may maintain within-brood variation in offspring size, but these have received far less attention than bet-hedging (e.g. Geritz 1995; Geritz *et al.* 1999; Kamel & Williams 2016). For example, in plants, seed-size variation within broods is predicted when there is small-scale spatial variation in the density of seeds, and competition among siblings is sufficiently asymmetric in favour of larger seeds (Geritz 1995; Geritz *et al.* 1999). Thus theory predicts that within-brood variation may be adaptive if offspring-size variation intensifies competition among different-sized siblings. However, competition for shared resources can also promote phenotypic diversification (both within and among species) if this variation reduces niche overlap among individuals (Bolnick *et al.* 2003; Bolnick 2004; Day & Young 2004; Pfennig *et al.* 2007). Surprisingly, this process has been ignored in theory on within-brood variation in offspring size, but novel evidence that offspring-size can mediate resource use (Martin & Pfennig 2010; Davis & Marshall 2014) supports this exciting possibility. Nevertheless, empirical manipulations of within-brood variation in offspring size are required to test this hypothesis.

Here, we investigate the consequences of within-brood variation in offspring size for both maternal and offspring fitness. In a field experiment using the marine invertebrate, *Bugula neritina*, we create experimental broods of siblings with identical means, but different variances, for offspring size and expose them to different sibling densities. We then monitor the performance (survival and growth) of these offspring across their life-time, a good proxy for life-time fitness. As far as we are aware, this is the first study to simply manipulate variance in the size of siblings to determine the consequences for fitness. Specifically, we test the hypotheses that: (1) Within-brood variation in offspring size *reduces* maternal (i.e. arithmetic mean) fitness within a generation, in congruence with bet-hedging theory; (2) Within-brood variation in offspring-size *increases* maternal fitness because phenotypic diversity reduces competition among siblings; and (3) Density-dependence alters the fitness consequences of within-brood variation in offspring size.

METHODS

Study species

Bugula neritina Linnaeus, 1758, is a bryozoan common to sessile marine communities worldwide and is used extensively as a model for studies on life-history strategies (Wendt 1998; Allen & Marshall 2013; Pettersen *et al.* 2015; Cameron *et al.* 2016). *B. neritina* grows by asexual budding of zooids to form branched, arborescent colonies. Colonies are simultaneous hermaphrodites and fertilisation is internal; colonies cast sperm into the water column, but retain the eggs and developing larvae. Larvae are brooded in chambers called ovicells, and each ovicell contains one larva at a time. Colonies simultaneously provision several broods of larvae at various stages of development via a placenta-like system (Woollacott & Zimmer 1975). Larvae are brooded for up to one week, after which broods of fully developed larvae are released into the

water column. In *B. neritina*, larvae can vary considerably in size, both within and across populations (Marshall *et al.* 2003; Kosman & Pernet 2011). Estimates of within-brood CVs in larval volume range between 6.7 and 14.5%, and accounts for 54% of the variance in offspring size observed in some populations (Kosman & Pernet 2011).

In *B. neritina*, the non-feeding larvae are immediately competent to settle following release, and most settle within hours under field conditions, limiting the potential for dispersal (Burgess & Marshall 2011). Siblings can preferentially aggregate at settlement relative to unrelated larvae in the laboratory (Keough 1984; Aguirre *et al.* 2013), such that siblings are likely to co-occur across small-spatial scales in the field. Multiple lines of evidence suggest that sibling recruits interact in the field, and that offspring size can determine the outcome of such interactions (Burgess & Marshall 2011; Aguirre & Marshall 2012; Aguirre *et al.* 2013; Cameron *et al.* 2016). In this species, interactions among conspecifics (and presumably siblings) can involve both exploitative and interference competition. For example, neighbours may directly compete for food (Svensson & Marshall 2015), or can physically disrupt local flow regimes and the delivery of resources (e.g. food and oxygen) for neighbouring settlers (Cameron *et al.* 2016). Colonies can also chemically detect neighbouring colonies, which can induce changes in growth form (Thompson *et al.* 2015). *B. neritina* is thus ideal for testing the fitness consequences of variation in offspring size within broods, given its natural degree of within-brood variation, and the scope for sibling interactions after settlement.

Experimental methods

Spawning and measuring larvae

Reproductive *B. neritina* colonies were collected from Altona Pier, Victoria, Australia (37°52'26.6" S, 144°49'00.5" E) during December 2015. To collect larvae, colonies were returned to the laboratory, held in constant darkness at 17 °C for 2 days, then spawned using standard techniques (Marshall & Keough 2003). Briefly, colonies were removed from the dark and placed in individual beakers of seawater, then exposed to bright light to stimulate larval release. As colonies had been fertilised in the field, only maternal identity was known. Multiple paternity has not been demonstrated in bryozoans, but is common in other sperm-casting marine invertebrates (Johnson & Yund 2007). Multiple paternity is therefore likely in *B. neritina*, such that all offspring spawned from a single colony were at least half siblings, although some were likely full siblings.

To measure offspring size, each larva was photographed with a Moticam 10 MP digital camera (Motic, Hong Kong, China) mounted on a dissecting microscope at 100× magnification. As per standard techniques, larvae were photographed with the ciliary groove facing directly upwards (Marshall & Keough 2003). The length (μm) of this groove was measured using image analysis software (IMAGEJ, v.1.47; Bethesda, MD, USA), then converted to larval mass (μg), using an equation that describes this relationship (Pettersen *et al.* 2015). We settled larvae (following measurement) onto individual pre-roughened, biofilmed acetate squares. We gave these larvae 2 h to settle, after which we rinsed any unsettled

larvae from the acetate squares. We measured and settled all larvae within 3 h from the time they were spawned to minimise the effects of delayed settlement on larval quality (Wendt 1998). For each parental colony ($n = 6$), we measured and settled between 250 and 300 larvae within 24 h. In *B. neritina*, settlers develop feeding structures over the first 2–5 days after settlement, and development time does not depend on larval size (Pettersen *et al.* 2015). Thus, the fact that larvae were settled over a 24 h period was unlikely to confound the effects of larval size with differences in developmental stage.

Design and field deployment

We systematically assigned our settlers of known larval size to experimental 'broods' of siblings that had the same mean ($\mu = 12 \mu\text{g} \pm 0.053 \text{ SD}$), but different variance, in offspring size. We manipulated within-brood variation in offspring size continuously within two broad 'clumps' of offspring size variation: less-variable broods (CV for larval mass (μg): 0.61–5.12%; $\mu = 2.71\% \pm 1.10 \text{ SD}$), and more-variable broods (CV: 18.84–39.17%; $\mu = 24.80\% \pm 4.07 \text{ SD}$). In our study population, the natural range of within-brood CVs for larval mass ranges between 10.45 and 17.66% (H. Cameron, unpublished data). While the CVs of our experimental broods lie outside these estimates, our broods were generated by drawing from the natural offspring-size distribution of each parental colony (and thus uses a realistic sub-sample of offspring sizes produced by that parent). Our manipulation of CV therefore reflects the likely small-scale variation in offspring sizes generated by the settlement of sibling larvae in the field. In fact, theory predicts that small-scale spatial variation in the distribution and density of different-sized siblings can maintain within-brood variation in offspring size when these siblings compete with one another (Geritz 1995; Geritz *et al.* 1999).

To create our experimental broods, we glued our settlers onto PVC plates ($5.5 \times 5.5 \text{ cm}^2$) at one of four densities; 2, 4, 6 and 9 settlers per plate (25 cm^2) that reflect the range of settlement densities observed in natural populations (Allen *et al.* 2008; Burgess & Marshall 2011; Cameron *et al.* 2016). We used larvae from six parental colonies for our manipulations, and for each colony we replicated our variance and density combinations twice (two colonies were not replicated because of insufficient settlement; $n = 10$ replicates per treatment combination). We circled all experimental settlers in pencil and noted their position in each brood (i.e. plate), using a unique grid reference so that their performance could be monitored across their life-time, and to distinguish experimental settlers from field recruits. We removed any non-experimental settlers (both *B. neritina* and other species) from the plates weekly to eliminate competition from other organisms.

We deployed our experiment at Blairgowrie Yacht Squadron, Victoria, Australia (38°21'20.2" S, 144°46'22.8" E). We haphazardly attached the plates bearing experimental broods to PVC backing panels ($55 \times 55 \text{ cm}^2$) that were hung at 1 m depth with plates facing downwards. The entire experiment was deployed across two backing panels, and each panel received experimental broods from three parental colonies, such that all broods from a single parent were deployed on the same panel (total of 40 plates per panel). Due to logistic

constraints, we processed the larvae of a single colony per day in the laboratory. Deployment of experimental broods was therefore staggered across a 2-week period, such that panels combine family level, spatial, and temporal variation. Intraspecific competition among *B. neritina* typically occurs at the scale used in our study (plates), and density effects beyond this are small and undetectable (Hart & Marshall 2009; Hart *et al.* 2012). Thus, individuals on different plates within a panel were unlikely to interact. In total, the experiment consisted of 80 experimental broods (i.e. plates; our unit of replication) and a total of 420 larvae of known offspring size.

We measured several fitness components by monitoring the survival and size of all colonies in the field over 9 weeks. We scored survival as the presence of the colony, while absent colonies were assumed to be dead. We measured colony size as the number of bifurcations along the longest branch, which is a good indication of colony biomass in this species (Keough & Chernoff 1987). Colonies can live for several months in our study region (Pettersen *et al.* 2016), however, a heat-wave killed most of our colonies after 9 weeks. Thus, our performance estimates represent a good proxy for the life-time fitness of our individuals.

Statistical analyses

Does within-brood variation in offspring size decrease mean performance when siblings interact?

Here, we test the consequences of within-brood variation and sibling density on the mean performance of experimental broods of *B. neritina* siblings. To estimate the performance of our broods, we multiplied the mean survival (as proportion) and mean growth (bifurcations) of each brood (i.e. plate) after 8 weeks in the field. We note that individual analyses for growth and survival showed the same trends (i.e. within-brood variation tended to positively affect both growth and survival; see Table S1 in Supporting Information). We therefore used our composite performance measure as the response variable in a linear-mixed model, where within-brood variation in offspring size (CV) and sibling density were continuous fixed effects. Experimental run (that combines parental identity, date of deployment, and panel) was a continuous random effect, and was appropriate given we detected a systematic ordinal signal of run on the fitness of our broods (Bell & Jones 2014). Model reduction was performed by removing any non-significant interactions (random effects: $P > 0.25$; fixed effects: $P > 0.05$; Quinn & Keough 2002). All analyses were done in SYSTAT v.13 (San Jose, CA, USA), and assumptions were checked using diagnostic plots, and were met in all cases.

How does offspring performance influence the mean performance of experimental broods?

We found that within-brood variation in offspring size increased the mean performance of experimental broods (see Results). Three processes could drive these effects: (1) the purging of smaller, poorer-performing offspring (which would reduce brood densities, and thus competition among surviving siblings); (2) the over-representation of colonies from larger, better-performing offspring; or (3) offspring-size variation

reduces competition among siblings. To determine whether offspring-size related performance drove the benefits of more-variable broods, we first estimated the relationship between offspring size and offspring performance. Here, we performed two separate analyses that used different predictor and response variables for offspring size and performance, respectively. First, we analysed the effects of absolute offspring size on the absolute performance of that offspring (the product of survival and growth) after 8 weeks in the field. Our second model accounted for the nested structure of individual offspring within experimental broods, and used the size of an offspring relative to other offspring in that experimental brood (relative offspring size) as the predictor, and the relative performance of that offspring within the brood as the response variable. We generated offspring size-performance functions for broods that exceeded 15% CVs for offspring size, because otherwise, variances were too low to examine performance across a sufficient range of offspring sizes (our results were unchanged by using all the broods). For all models, (absolute or relative) offspring size and sibling density were continuous fixed effects, experimental run was a random continuous effect, and model reduction was performed as previously described.

To test whether offspring-size variation influences phenotypic variation and potentially decreases competition among siblings, we compared the minimum and maximum sizes of colonies in our experimental broods. We used linear-mixed models with the same model structure and model reduction procedure as our analyses of mean performance, but here, we excluded all plates with a single survivor (that would act as both the minimum and maximum performer). We also tested whether more-variable broods produced colonies of more variable sizes. Here, standard deviation and CV for colony size were response variables in separate linear-mixed models that had the same model structure and reduction procedure described previously. Again, we excluded plates with single survivors because there was no variance to estimate.

RESULTS

Within-brood variation increases mean performance and determines interactions among siblings

After 8 weeks in the field, we found that more-variable broods had higher mean performance (calculated as survival \times growth) than less-variable broods ($F_{1,74} = 14.201$, $P < 0.0001$; Fig. 2), and these benefits were consistent across our range of sibling densities (CV \times density: $F_{1,73} = 0.004$, $P = 0.953$; excluded from final model). However, the magnitude of these benefits were context dependent (CV \times experimental run interaction: $F_{1,74} = 17.64$, $P < 0.0001$); the positive effect of within-brood variation on performance was significantly stronger in earlier runs, but weaker or non-existent in later runs where performance was relatively lower. Given that run combined parental identity, panel and date of deployment in the field, any of these factors could have contributed to this interaction and cannot be disentangled here. Sibling density did not affect the mean performance of our broods, indicating sibling interactions were not density dependent ($F_{1,74} = 0.763$,

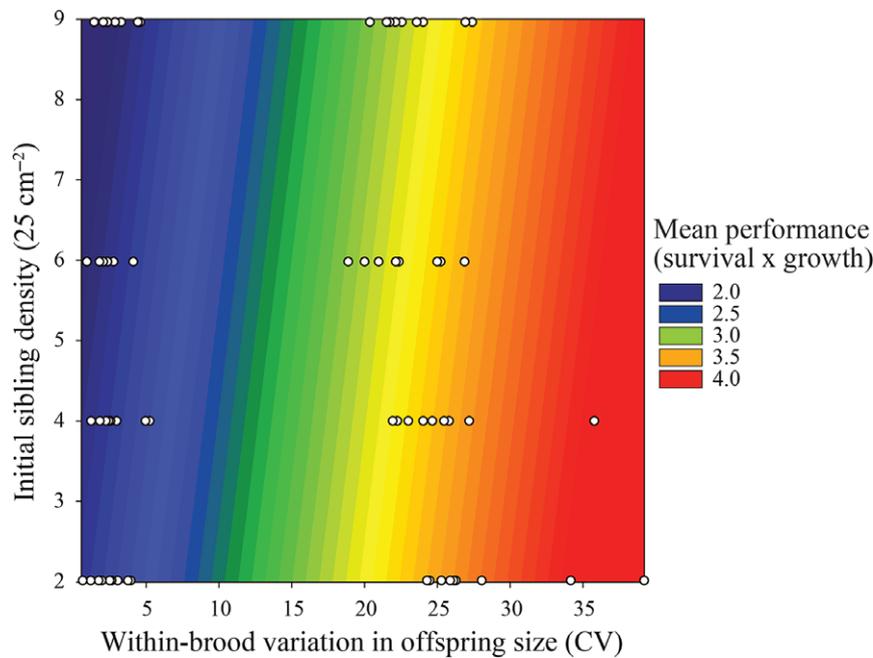


Figure 2 Estimates of the relationship between within-brood variation in offspring size and sibling density on the performance (calculated as survival \times growth; side bar) of experimental broods of *Bugula neritina* siblings after eight weeks in the field. Data points (white circles) show the raw data for within-brood variation and sibling density combinations.

$P = 0.385$). Rather, we found sibling interactions were strongly affected by the level of phenotypic (i.e. offspring size) diversity within our broods.

Within-brood variation enhances the performance of offspring irrespective of their size

After 8 weeks in the field, we found no correlations between absolute offspring size, or sibling density, and the absolute performance of individual offspring in broods where CVs for offspring size exceeded 15% (absolute offspring size: $F_{1,202} = 0.690$, $P = 0.407$; sibling density; $F_{1,202} = 0.807$, $P = 0.370$). We also found no correlations between relative offspring size or sibling density on the relative performance of offspring within these broods ($F_{1,203} = 2.525$, $P = 0.114$; $F_{1,203} = 0.238$, $P = 0.627$, respectively). Thus, higher mean performance in more-variable broods was not driven by the over-representation of colonies from larger, better-performing offspring, nor by the purging of smaller offspring. Instead, more-variable broods produced colonies with larger minimum ($F_{1,41} = 4.439$, $P = 0.041$; Fig. 3a) and maximum ($F_{1,40} = 9.594$, $P < 0.004$; Fig. 3b) colony sizes, suggesting that both extremes of offspring performance were enhanced in more-variable broods relative to less-variable broods. Interestingly, maximum colony size was also positively affected by sibling density ($F_{1,40} = 6.736$, $P = 0.013$; Fig. 3b), suggesting that increasing sibling densities facilitated the highest performing individuals. More-variable broods also had larger standard deviations for colony size after 8 weeks in the field ($F_{1,43} = 4.076$, $P = 0.05$; Fig. 3c), but within-brood variation in offspring size did not affect the coefficient of variation for colony size ($F_{1,60} = 2.342$, $P = 0.131$). Together, these

findings suggest that all offspring (regardless of initial size) performed better in more-variable broods, driving the higher mean performance of those broods (summarised in Fig. 4).

DISCUSSION

Adaptive explanations for within-brood variation in offspring size typically invoke bet-hedging in unpredictable environments (but see Geritz 1995; Geritz *et al.* 1999; Kamel & Williams 2016). According to bet-hedging arguments, mothers that produce more-variable offspring sacrifice reduced arithmetic mean fitness within a generation in return for increased geometric mean fitness across generations. In contrast, we found that within-brood variation in offspring size increased arithmetic mean fitness (and associated variation in fitness) in broods of *B. neritina* siblings. We therefore show that within-brood variation can increase maternal fitness within a generation, in contrast to the multi-generational benefits associated with bet-hedging theory. Furthermore, we found that the performance of all offspring (irrespective of their size) was enhanced in more-variable broods. Thus, within-brood variance in offspring size appears to reduce competition among siblings to enhance the mean performance of these broods; a novel finding.

Within-brood variation in offspring size may simultaneously enhance maternal and offspring fitness via complementarity effects. Complementarity effects often drive the benefits of increasing species and genetic diversity, whereby the performance of individual species or genotypes is enhanced in polyculture relative to their performance in monoculture (Loreau & Hector 2001). Here, we suggest that complementarity effects may extend to broods of siblings with greater variation

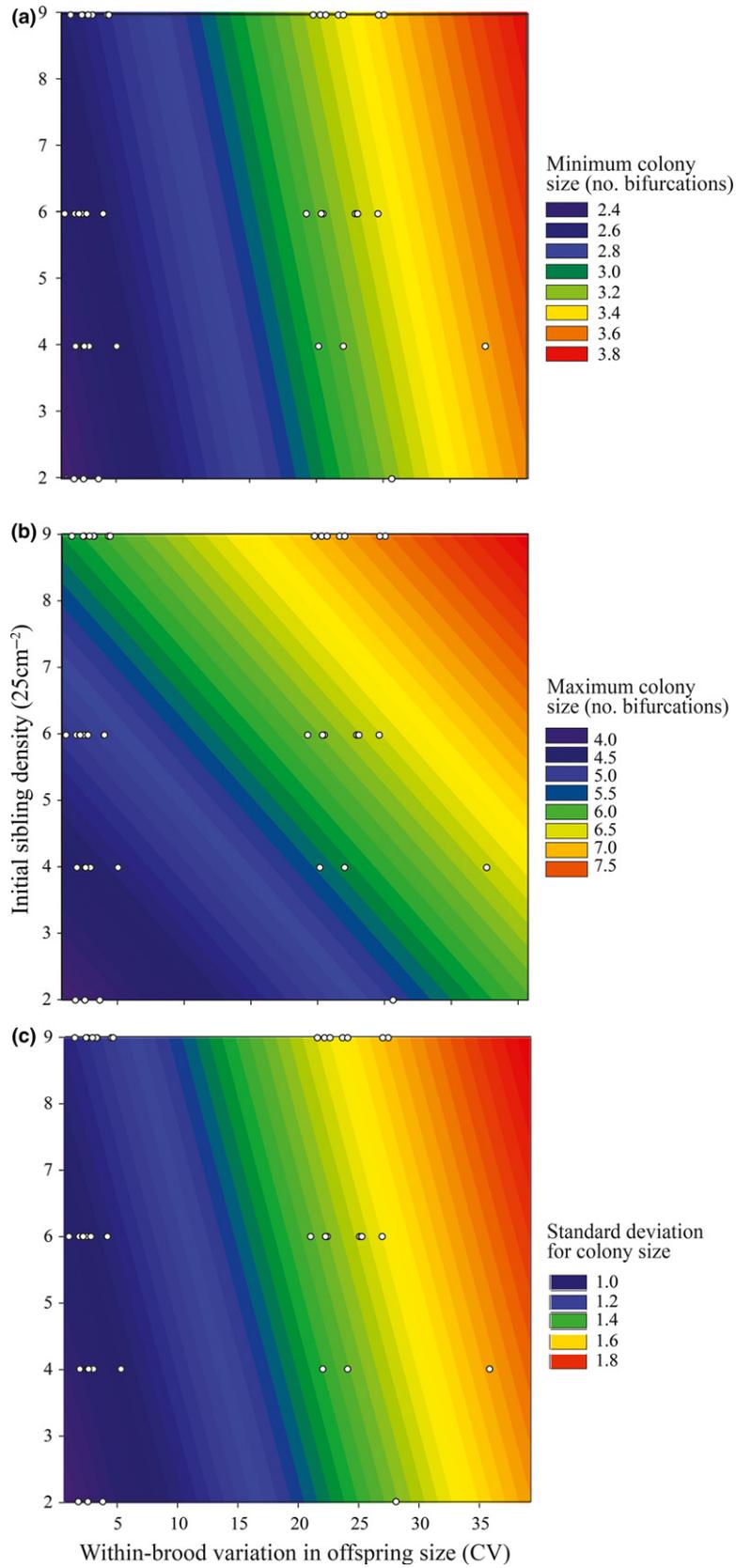


Figure 3 Estimates for the effect of within-brood variation in offspring size and sibling density on the (a) minimum, (b) maximum and (c) standard deviation for the size of colonies in experimental broods of *Bugula neritina* siblings after eight weeks in the field. Data points (white circles) show the raw data for within-brood variation and sibling density combinations.

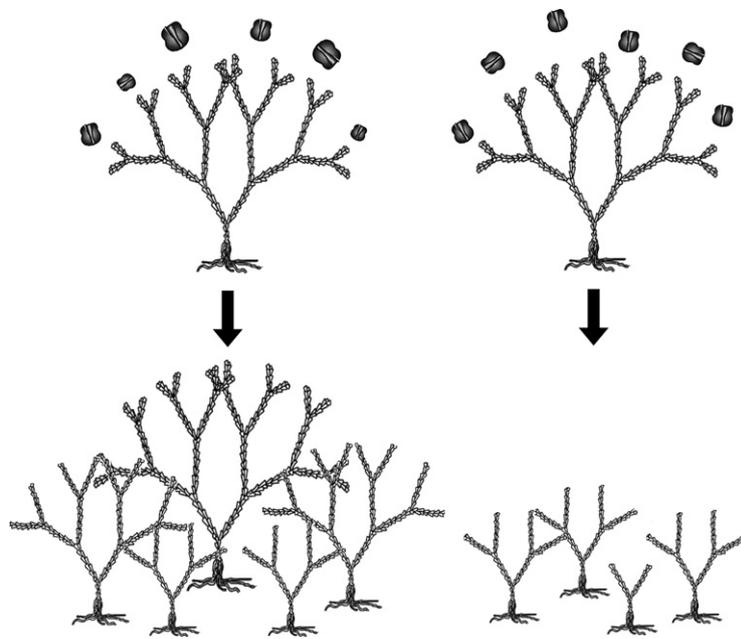


Figure 4 Schematic summarising the effects of within-brood variation in offspring size on the performance of experimental broods of *Bugula neritina*. Broods with greater offspring size variation had higher mean performance (for both survival and growth), and larger variation (standard deviation) in the size of colonies, than less variable broods after eight weeks in the field. Offspring in more-variable broods also reached larger minimum and maximum colony sizes than offspring in less-variable broods.

in offspring size. We acknowledge, however, that our design precludes formal tests of complementarity (*sensu* Loreau & Hector 2001). Nevertheless, our results support complementarity effects in several ways. First, the offspring size–performance relationship is well resolved in *B. neritina*. Typically, when offspring are grown in isolation, larger offspring have higher survival, growth and reproductive output relative to smaller offspring, but the presence of siblings can alter this relationship (Marshall *et al.* 2003; Allen *et al.* 2008; Cameron *et al.* 2016). Taken together, these lines of evidence suggest that offspring-size performance effects are non-additive in aggregations of siblings, and our finding that all offspring, irrespective of their size, are enhanced in more-variable broods is congruent with complementarity effects.

Complementarity effects can arise from either facilitation or niche partitioning among interacting individuals, although these two processes are difficult to distinguish (Loreau & Hector 2001). In our system, offspring-size mediated niche partitioning seems most likely, and may occur via two (non-mutually exclusive) processes. First, larval size positively affects the size of feeding structures (lophophores) in *B. neritina*, such that more-variable broods likely have greater lophophore-size variation among siblings (Kosman & Pertnet 2011). If lophophore-size determines the size of particles ingested, then this may facilitate niche partitioning in more-variable broods. Indeed, larger colonies (that are typically formed by larger offspring) consume larger particles than smaller colonies (Okamura 1990). Second, we find that more-variable broods produce colonies of more-variable sizes, such that the tips of these colonies would be positioned at different heights in the water column (Fig. 4). Because most feeding occurs at the tips of arborescent bryozoan colonies (Okamura 1984), more-variable colony sizes may generate spatial variation

in foraging, thereby reducing niche overlap in more-variable broods. Certainly, gape-size and body-size variation can generate niche partitioning in other systems (reviewed in Bolnick *et al.* 2003; Bolnick 2004), and we suggest offspring-size variation may contribute to such forms of niche partitioning. We acknowledge, however, that the benefits of within-brood variation observed here may arise via other, less likely, forms of offspring size-mediated facilitation among siblings (e.g. buffering predation risk; Kudo 2006).

Theory that considers the role of sibling competition in maintaining within-brood variation in offspring size does exist, but this has received less attention than bet-hedging. For example, theory developed for plants predicts that within-brood variation is maintained by small-scale spatial variation in seedling density, and asymmetric competition in favour of larger seeds (Geritz 1995; Geritz *et al.* 1999). A key assumption of these models is that larger seeds always out-compete smaller seeds, but smaller seeds persist because they are the more numerous phenotype (due to the size-number trade-off), and are better able to disperse to unoccupied habitats. In contrast to the assumptions of these models, however, we find that within-brood variation facilitates offspring of all sizes, even at high densities. We therefore suggest complementarity effects as an alternative mechanism via which sibling interactions could maintain within-brood variation in offspring size.

Our suggestion that within-brood variation in offspring size ameliorates sibling competition may apply to other organisms, but two conditions are required for this to be broadly applicable. First, siblings must co-occur across small-spatial scales, such that competition and local depletion of resources is possible. Genetic studies show that kin aggregation occurs over relatively small scales in a number of taxa, including those with prolonged dispersive propagule stages (Cheplick 1993;

Selkoe *et al.* 2006; Veliz *et al.* 2006; Kamel *et al.* 2012; Aguirre *et al.* 2013). Interactions among siblings are therefore likely across a range of life histories. Second, offspring size must affect niche use, and there is some evidence for this across disparate taxa (Martin & Pfennig 2010; Davis & Marshall 2014). For example, differential maternal investment in egg size among females induces resource-use polymorphisms and reduces competition in (unrelated) tadpoles – tadpoles from larger eggs became carnivores while tadpoles from smaller eggs became herbivores (Martin & Pfennig 2010). Thus the benefits of offspring size variation that we observe within-broods may not be restricted to our system. Given our results, we predict that within-brood variation in offspring size may be particularly beneficial in resource-limited environments to ameliorate sibling competition. Importantly, this prediction has some empirical support. In both fish and plants, mothers in lower resource environments produce more variable offspring (Halpern 2005; Crean & Marshall 2009). In neither study was offspring size-mediated niche partitioning invoked, but our findings suggest this intriguing possibility.

It is worth noting, however, that the fitness consequences of within-brood variation observed here could arise from variation in genetic relatedness, rather than variation in offspring size *per se*. Multiple paternity can affect maternal investment in offspring, including in marine invertebrates (Temme 1986; Hammerschmidt *et al.* 2011; Kamel & Williams 2016). Therefore, it is possible that our less-variable broods contained a higher proportion of full siblings. Importantly, full siblings tend to compete more intensely than half sibling and this may explain our results (Aguirre & Marshall 2012). Disentangling these two explanations would require careful breeding designs. Regardless, our main result that within-brood variation in offspring size increases brood performance remains unchanged.

Traditional theory on within-brood variation in offspring size focuses on bet-hedging, although alternative theory considers the role of sibling competition (Geritz 1995; Geritz *et al.* 1999; Kamel & Williams 2016). Here, we find benefits of within-brood size variation that arise independently of these theories. We note, however, that this does not preclude bet-hedging or sibling competition as drivers of within-brood size variation in this, or other, systems. Rather, we suggest offspring size-mediated niche partitioning and/or facilitation as a novel explanation for why mothers may unequally provision their offspring, which may also apply to other systems (Martin & Pfennig 2010). Theory has not yet accounted for such effects, but given their potential to be widespread, we suggest this is an important next step.

ACKNOWLEDGEMENTS

We are grateful to the Blairgowrie Yacht Squadron for access to our field sites. We thank M. Amor, M. Cameron, W. Cameron, A. Guillaume, H. Wootton, T. Landells and D. Barnechè for assistance in the laboratory and field. We also thank B. Taborsky and two anonymous reviewers for providing insightful advice that greatly improved our manuscript.

AUTHORSHIP

HC and DJM designed and conducted the experiments, and performed the analyses, HC wrote the first draft of the manuscript and all authors contributed substantially to revisions.

DATA ACCESSIBILITY STATEMENT

Data is available from the Dryad Digital Repository: doi:10.5061/dryad.n3k93

REFERENCES

- Aguirre, J.D. & Marshall, D.J. (2012). Genetic diversity increases population productivity in a sessile marine invertebrate. *Ecology*, 93, 1134–1142.
- Aguirre, J.D., Miller, S.H., Morgan, S.G. & Marshall, D.J. (2013). Relatedness affects the density, distribution and phenotype of colonisers in four sessile marine invertebrates. *Oikos*, 122, 881–888.
- Allen, R.M. & Marshall, D.J. (2013). Phenotypic links among life-history stages are complex and context-dependent in a marine invertebrate: interactions among offspring size, larval nutrition and postmetamorphic density. *Funct. Ecol.*, 27, 1358–1366.
- Allen, R.M., Buckley, Y.M. & Marshall, D.J. (2008). Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *Am. Nat.*, 171, 225–237.
- Bell, A. & Jones, K. (2014). Explaining fixed effects: random effects modeling of time-series cross-sectional and panel data. *Polit. Sci. Res. Meth.*, 3, 133–153.
- Bolnick, D.I. (2004). Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution*, 58, 608–618.
- Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulseley, C.D. *et al.* (2003). The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.*, 161, 1–28.
- Burgess, S.C. & Marshall, D.J. (2011). Field estimates of planktonic larval duration in a marine invertebrate. *Mar. Ecol. Prog. Ser.*, 440, 151–161.
- Burgess, S.C. & Marshall, D.J. (2014). Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos*, 123, 769–776.
- Cameron, H., Monro, K., Malerba, M., Munch, S. & Marshall, D. (2016). Why do larger mothers produce larger offspring? A test of classic theory. *Ecology*, 97, 3452–3459.
- Cheplick, G.P. (1993). Sibling competition is a consequence of restricted dispersal in an annual cleistogamous grass. *Ecology*, 74, 2161–2164.
- Crean, A.J. & Marshall, D.J. (2009). Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.*, 364, 1087–1096.
- Crump, M.L. (1981). Variation in propagule size as a function of environmental uncertainty for tree frogs. *Am. Nat.*, 117, 724–737.
- Davis, K. & Marshall, D.J. (2014). Offspring size in a resident species affects community assembly. *J. Anim. Ecol.*, 83, 322–331.
- Day, T. & Young, K.A. (2004). Competitive and facilitative evolutionary diversification. *Bioscience*, 54, 101–109.
- Einum, S. & Fleming, I.A. (2002). Does within-population variation in fish egg size reflect maternal influences on optimal values? *Am. Nat.*, 160, 756–765.
- Einum, S. & Fleming, I.A. (2004). Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evol. Ecol. Res.*, 6, 443–455.
- Fischer, B., Taborsky, B. & Kokko, H. (2011). How to balance the offspring quality-quantity tradeoff when environmental cues are unreliable. *Oikos*, 120, 258–270.
- Geritz, S.A.H. (1995). Evolutionarily stable seed polymorphism and small-scale spatial variation in seedling density. *Am. Nat.*, 146, 685–707.

- Geritz, S.A.H., van der Meijden, E. & Metz, J.A.J. (1999). Evolutionary dynamics of seed size and seedling competitive ability. *Theor. Pop. Biol.*, 55, 324–343.
- Halpern, S.L. (2005). Sources and consequences of seed size variation in *Lupinus perennis* (Fabaceae): adaptive and non-adaptive hypotheses. *Am. J. Bot.*, 92, 205–213.
- Hammerschmidt, K., Pemberton, A.J., Michiels, N.K. & Bishop, J.D.D. (2011). Differential maternal allocation following mixed insemination contributes to variation in oocyte size in a sea squirt. *Mar. Ecol. Prog. Ser.*, 422, 123–128.
- Hart, S.P. & Marshall, D.J. (2009). Spatial arrangement affects population dynamics and competition independent of community composition. *Ecology*, 90, 1485–1491.
- Hart, S.P., Burgin, J.R. & Marshall, D.J. (2012). Revisiting competition in a classic model system using formal links between theory and data. *Ecology*, 93, 2015–2022.
- Johnson, S.L. & Yund, P.O. (2007). Variation in multiple paternity in natural populations of a free-spawning marine invertebrate. *Mol. Ecol.*, 16, 3253–3262.
- Kamel, S.J. & Williams, P.D. (2016). Resource exploitation and relatedness: implications for offspring size variation within broods. *Oikos*. <https://doi.org/10.1111/oik.04034>.
- Kamel, S.J., Grosberg, R.K. & Marshall, D.J. (2010). Family conflicts in the sea. *Trends Ecol. Evol.*, 25, 442–449.
- Kamel, S.J., Hughes, A.R., Grosberg, R.K. & Stachowicz, J.J. (2012). Fine-scale genetic structure and relatedness in the eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.*, 447, 127–U164.
- Keough, M.J. (1984). Kin-recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* (L.). *Evolution*, 38, 142–147.
- Keough, M.J. & Chernoff, H. (1987). Dispersal and population variation in the bryozoan *Bugula neritina*. *Ecology*, 68, 199–210.
- Koops, M.A., Hutchings, J.A. & Adams, B.K. (2003). Environmental predictability and the cost of imperfect information: influences on offspring size variability. *Evol. Ecol. Res.*, 5, 29–42.
- Kosman, E. & Pernet, B. (2011). Intraspecific variation in larval size and its effects on juvenile lophophore size in four bryozoans. *Mar. Ecol. Prog. Ser.*, 429, 67–73.
- Kudo, S.I. (2006). Within-clutch egg-size variation in a subsocial bug: the positional effect hypothesis. *Can. J. Zool.*, 84, 1540–1544.
- Lips, K.R. (2001). Reproductive trade-offs and bet-hedging in *Hyla calypsa*, a Neotropical treefrog. *Oecologia*, 128, 509–518.
- Loreau, M. & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72–76.
- Marshall, D.J. & Keough, M.J. (2003). Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Mar. Ecol. Prog. Ser.*, 255, 145–153.
- Marshall, D.J. & Uller, T. (2007). When is a maternal effect adaptive? *Oikos*, 116, 1957–1963.
- Marshall, D.J., Bolton, T.F. & Keough, M.J. (2003). Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. *Ecology*, 84, 3131–3137.
- Marshall, D.J., Bonduriansky, R. & Bussiere, L.F. (2008). Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology*, 89, 2506–2517.
- Martin, R.A. & Pfennig, D.W. (2010). Maternal investment influences expression of resource polymorphism in amphibians: implications for the evolution of novel resource-use phenotypes. *PLoS ONE*, 5, <https://doi.org/10.1371/journal.pone.0009117>
- McGinley, M.A., Temme, D.H. & Geber, M.A. (1987). Parental investment in offspring in variable environments: theoretical and empirical considerations. *Am. Nat.*, 130, 370–398.
- Metz, J., Liancourt, P., Kigel, J., Harel, D., Sternberg, M. & Tielbörger, K. (2010). Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. *J. Ecol.*, 98, 697–704.
- Morrongiello, J.R., Bond, N.R., Crook, D.A. & Wong, B.B.M. (2012). Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. *J. An. Ecol.*, 81, 806–817.
- Okamura, B. (1984). The effects of ambient flow velocity, colony size, and upstream colonies on the feeding success of Bryozoa. 1. *Bugula stolonifera* Ryland, an arborescent species. *J. Exp. Mar. Biol. Ecol.*, 83, 179–193.
- Okamura, B. (1990). Particle size, flow velocity, and suspension feeding by the erect bryozoans *Bugula neritina* and *B. stolonifera*. *Mar. Biol.*, 105, 33–38.
- Olofsson, H., Ripa, J. & Jonzén, N. (2009). Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proc. R. Soc. Lond. B*, 276, 2963–2969.
- Parker, G.A. & Begon, M. (1986). Optimal egg size and clutch size: effects of environment and maternal phenotype. *Am. Nat.*, 128, 573–592.
- Pettersen, A.K., White, C.R. & Marshall, D.J. (2015). Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. *Proc. R. Soc. Lond. B*, 282, 20151946.
- Pettersen, A.K., White, C.R. & Marshall, D.J. (2016). Metabolic rate covaries with fitness and the pace of the life history in the field. *Proc. R. Soc. Lond. B*, 283, 20160323.
- Pfennig, D.W., Rice, A.M. & Martin, R.A. (2007). Field and experimental evidence for competition's role in phenotypic divergence. *Evolution*, 61, 257–271.
- Plaistow, S.J., St Clair, J.J.H., Grant, J. & Benton, T.G. (2007). How to put all your eggs in one basket: empirical patterns of offspring provisioning throughout a mother's lifetime. *Am. Nat.*, 170, 520–529.
- Quinn, G.P. & Keough, M.J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, New York.
- Selkoe, K.A., Gaines, S.D., Caselle, J.E. & Warner, R.R. (2006). Current shifts and kin aggregation explain genetic patchiness in fish recruits. *Ecology*, 87, 3082–3094.
- Smith, C.C. & Fretwell, S.D. (1974). Optimal balance between size and number of offspring. *Am. Nat.*, 108, 499–506.
- Svensson, J.R. & Marshall, D.J. (2015). Limiting resources in sessile systems: food enhances diversity and growth of suspension feeders despite available space. *Ecology*, 96, 819–827.
- Temme, D.H. (1986). Seed size variability: a consequence of variable genetic quality among offspring. *Evolution*, 40, 414–417.
- Thompson, M.L., Marshall, D.J. & Monro, K. (2015). Non-contact competition in a sessile marine invertebrate: causes and consequences. *Mar. Ecol. Prog. Ser.*, 522, 115–125.
- Vance, R.R. (1973). Reproductive strategies in marine benthic invertebrates. *Am. Nat.*, 107, 339–352.
- Veliz, D., Duchesne, P., Bourget, E. & Bernatchez, L. (2006). Genetic evidence for kin aggregation in the intertidal acorn barnacle (*Semibalanus balanoides*). *Mol. Ecol.*, 15, 4193–4202.
- Wendt, D.E. (1998). Effect of larval swimming duration on growth and reproduction of *Bugula neritina* (Bryozoa) under field conditions. *Biol. Bull.*, 195, 126–135.
- Woollacott, R.M. & Zimmer, R.L. (1975). A simplified placenta-like system for the transport of extraembryonic nutrients during embryogenesis of *Bugula neritina* (Bryozoa). *J. Morphol.*, 147, 355–377.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Editor, Sonya Auer

Manuscript received 23 January 2017

First decision made 7 March 2017

Manuscript accepted 24 May 2017