



MONASH University

The Eco-evolutionary dynamics of maternal provisioning

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BSc (Honours)

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Abstract

The size of offspring that mothers produce varies at all scales – from among species, to among and within clutches of the same female. Offspring size is a key trait that affects performance across all phases of life – from fertilisation and birth, through to reproduction and lifespan. Life-history theorists have long sought to understand the evolutionary forces underlying offspring-size variation, but despite significant progress, the drivers within populations of the same species remain poorly understood. Theory predicts that competition among offspring plays a key role in maintaining this variation, although rigorous empirical tests are rare. In this thesis, I test several long-held assumptions regarding offspring size and competitive ability. I focus on models that predict offspring size variation at two scales of organisation – among females from the same population, and within clutches from the same female. I combine field and laboratory experiments with modelling approaches to test how offspring size affects competitive outcomes at two life-history stages: competition among settlers for environmentally supplied resources, and competition among eggs for fertilisations. I show that in contrast to classic assumptions, larger offspring do not always outcompete smaller offspring. Additionally, I find that offspring interactions are not exclusively competitive – rather positive interactions are possible and are mediated by offspring size. Life-history theory has traditionally viewed offspring interactions through the lens of competition alone. In light of findings from this thesis, I argue that theory should now accommodate positive interactions in future explorations of offspring-size variation.

Declaration

This thesis is an original work of my research and contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and, to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference is made in the text.

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Publications during enrolment

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Bellgrove A, McKenzie PF, **Cameron H**, Pocklington JB (2017) Restoring rocky intertidal communities: Lessons from a benthic macroalgal ecosystem engineer. *Marine Pollution Bulletin*, 117 (2): 7-27.

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Thesis including published works declaration

I hereby declare that this thesis contains no material accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes three original papers published in peer-reviewed journals, one un-submitted manuscript, and two appendices published (or accepted) in peer-reviewed journals. The core theme of the thesis is the eco-evolutionary dynamics of maternal provisioning. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the primary supervision of Prof Dustin Marshall. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

My contribution to the works contained in this thesis involved the following:

| Thesis Chapter | Publication Title | Status | Nature and % of student contribution | Co-author name(s) Nature and % of co-author contribution | Co-author Monash student Y/N |
|----------------|---|-----------|--|--|------------------------------|
| 2 | Why do larger mothers make larger offspring? A test of classic theory | Published | Design, data collection, analysis, writing of manuscript 65 % | 1. Dustin Marshall, 25 % Concept, design, analysis, manuscript contribution. 2. Keyne Monro, 8 % Analysis, manuscript contribution. 3. Martino Malerba, 1 % Analytical advice, manuscript contribution. 4. Stephan Munch, 1 % Analytical advice, manuscript contribution. | N |
| 3 | Can asymmetric competition maintain offspring size variation? A manipulative field test | Published | Concept, design, data collection, analysis, writing of manuscript 90 % | 1. Dustin Marshall, 10 % Concept, design, analysis, manuscript contribution. | N |
| 4 | Should mothers provision their offspring equally? A manipulative field test | Published | Concept, design, data collection, analysis, writing of manuscript 75 % | 1. Dustin Marshall, 20 % Concept, design, analysis, manuscript contribution. 2. Keyne Monro, 5 % Manuscript contribution. | N |

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|--------|--|---------------|--|---|---|
| 5 | Multilevel selection on offspring size and the maintenance of variation | Not submitted | Concept, design, data collection, analysis, writing of manuscript 75 % | 1. Dustin Marshall, 10 % Concept, design, manuscript contribution. 2. Darren Johnson, 10 % Analysis, manuscript contribution. 3. Keyne Monro, 5 % Design. | N |
| App. A | A global synthesis of offspring size variation, its eco-evolutionary causes and consequences | Published | Data collection, analysis, manuscript contribution 10 % | 1. Dustin Marshall, 85 % Concept, manuscript contribution. 2. Amanda Pettersen, 5 % Analysis, manuscript contribution. | Y |
| App. B | Size and density mediate transitions between competition and facilitation | Published | Concept, design, data collection, analysis, writing of manuscript 70 % | 1. Dustin Marshall, 20 % Concept, design, manuscript contribution 2. Tim Coulson, 10 % Concept, analysis, manuscript contribution | N |

I have renumbered sections and slightly altered the text of some submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature:

Date: 23/08/2019

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main supervisor signature:

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Table of Contents

| | |
|---|-----------|
| Copyright notice..... | 1 |
| Abstract..... | 2 |
| Declaration..... | 3 |
| Publications during enrolment | 4 |
| Thesis including published works declaration | 5 |
| Acknowledgements..... | 7 |
| 1. General Introduction | 12 |
| Patterns and processes of offspring size variation | 13 |
| Variation among females..... | 13 |
| Variation within females | 15 |
| Competition and the eco-evolutionary dynamics of offspring size | 16 |
| Does offspring size affect competitive ability? | 17 |
| Thesis aims and scope..... | 18 |
| Literature cited..... | 19 |
| 2. Why do larger mothers produce larger offspring? A test of classic theory | 23 |
| Abstract..... | 23 |
| Introduction | 24 |
| Materials and methods..... | 26 |
| Results..... | 31 |
| Discussion..... | 31 |
| Acknowledgements..... | 36 |
| Tables | 37 |
| Figures..... | 38 |
| Literature cited..... | 41 |
| 3. Can competitive asymmetries maintain offspring size variation? A manipulative field-test..... | 44 |
| Abstract..... | 44 |
| Introduction | 45 |
| Material and methods | 49 |
| Results..... | 52 |
| Discussion..... | 54 |
| Acknowledgements..... | 59 |
| Figures..... | 60 |
| Literature cited..... | 62 |

| | |
|---|------------|
| Appendix 1: Expected frequency of offspring pairings and interaction types in the field | 66 |
| 4. Should mothers provision their offspring equally? A manipulative field test | 67 |
| Abstract..... | 67 |
| Introduction | 68 |
| Materials and methods..... | 71 |
| Results..... | 77 |
| Discussion..... | 79 |
| Acknowledgements..... | 83 |
| Figures..... | 84 |
| Literature cited..... | 87 |
| Appendix 1: Effects of within-brood variation on survival and growth..... | 90 |
| 5. Multilevel selection on offspring size and the maintenance of variation. | 91 |
| Abstract..... | 91 |
| Introduction | 92 |
| Materials and methods..... | 96 |
| Statistical analyses and modelling approach | 98 |
| Results..... | 101 |
| Discussion..... | 104 |
| Tables | 109 |
| Figures..... | 110 |
| Literature cited..... | 113 |
| 6. General Discussion..... | 116 |
| Bigger is not always better..... | 117 |
| Positive interactions: are they widespread and can they maintain offspring-size variation?..... | 118 |
| Size-mediated facilitation | 119 |
| Complementarity via niche partitioning | 121 |
| Conclusions and future directions | 123 |
| Literature cited..... | 124 |
| Appendix A. A global synthesis of offspring-size variation, its eco-evolutionary causes and consequences. | 127 |
| Abstract..... | 127 |
| Introduction – offspring size, a universal functional trait? | 128 |
| Offspring size and the duration of development..... | 130 |
| Biogeographical patterns in offspring size..... | 131 |
| Developmental windows as a driver of latitudinal offspring size variation..... | 132 |
| Offspring size-fitness relationships..... | 133 |

| | |
|---|------------|
| Intraspecific variation in offspring size | 134 |
| Environmental sources of variation | 135 |
| Intrinsic sources of variation | 137 |
| Offspring size variation within mothers..... | 139 |
| Within-brood variation as a bet-hedging strategy..... | 139 |
| Within-brood variation mediates sibling competition..... | 140 |
| Why does offspring size affect fitness? A functional trait approach | 141 |
| Energy acquisition and energy loss..... | 141 |
| Outstanding questions..... | 144 |
| Whose fitness is it? | 144 |
| Offspring size number trade-off | 145 |
| Offspring size and predation..... | 146 |
| Conclusions | 146 |
| Figures..... | 147 |
| Literature cited..... | 149 |
| Appendix 1: Latitudinal patterns in offspring size | 153 |
| Appendix 2: Latitudinal patterns in selection | 155 |
| Appendix B. Size and density mediate transitions between competition and facilitation. | 158 |
| | |
| Abstract..... | 158 |
| Introduction | 159 |
| Materials and methods..... | 163 |
| Results..... | 167 |
| Discussion..... | 170 |
| Acknowledgements..... | 176 |
| Figures..... | 177 |
| Literature cited..... | 180 |
| Appendix 1: Field set up..... | 184 |
| Appendix 2: Heterospecific (<i>Bugula</i>) size and density affect conspecific survival | 187 |
| Appendix 3: Model description..... | 191 |
| Appendix 4: Heterospecific size and density affect the size-specific vital rates of a focal species | 194 |

“To strike a balance ... between the disadvantages which follow from the production of a numerous [smaller] progeny, and the advantages (such as the escape of at least some individuals from various dangers) is quite beyond our power of judgement.”

Charles Darwin, 1871

Chapter 1

General Introduction

For most organisms, parental care is restricted to the provisioning of offspring before they must fend for themselves. The size of offspring at independence from their parents reflects this provisioning – larger offspring generally receive more parental resources than smaller offspring (Bernardo 1996). Offspring size varies remarkably across time and space, and all scales of biological organisation – from among species and populations of the same species, to among and within broods of the same female (Leishman *et al.* 2000, Marshall and Keough 2008a, Krist 2011). Offspring size strongly determines performance – larger offspring typically have higher survival, growth and reproduction than smaller offspring (Fox and Czesak 2000, Leishman *et al.* 2000, Krist 2011, Ronget *et al.* 2017). Links between offspring size and performance are particularly evident early in the life history, but persist at later-stages and can affect subsequent generations (Benton *et al.* 2005, Krist 2011, Moore *et al.* 2019). Overall then, offspring-size effects are ubiquitous, pervasive and of fundamental interest to life-history theory.

Classic optimality theory predicts that for a given environment (or population), a single offspring size maximises maternal fitness – in other words, selection on offspring size is stabilising (Smith and Fretwell 1974). A fundamental assumption of this model is that mothers trade-off the size and number of their offspring – larger offspring perform better, but smaller offspring are cheaper so mothers make them in larger numbers (Vance 1973, Smith and Fretwell 1974). Mothers therefore balance the relative benefits of better performing offspring with those of increased fecundity, and this balance depends on the relationship between offspring size and performance. Empirical parameterisations of the Smith-Fretwell model reliably predict variation in offspring size among populations, as well as mean values

within populations (Einum and Fleming 2000, Marshall and Keough 2008b). Yet this classic theory cannot account for the striking variation observed within populations of the same species (Stearns 1992, Marshall *et al.* 2018).

Subsequent theory now predicts variation in offspring size within populations (reviewed below). Nevertheless, the seminal work of Smith and Fretwell (1974) pervades subsequent theory, with most models assuming that offspring size positive affects offspring performance, and a trade-off between offspring size and number.

Patterns and processes of offspring size variation

This thesis focuses on offspring-size variation at two scales of organisation – among mothers (Chapters 2 and 3) and within mothers (Chapters 4 and 5) of the same population. I therefore focus on these two sources of variation below – but see Appendix A for a comprehensive review of offspring-size variation at all scales of organisation.

Variation among females

Females living within the same environment produce offspring of disparate size. Much of this variation is correlated with maternal phenotype, most notably maternal size and age (Marshall *et al.* 2010). For example, larger mothers produce larger offspring and this pattern is ubiquitous – from plants to mammals (Lim *et al.* 2014). Yet the underlying drivers remain unclear (Rollinson and Rowe 2015). While non-adaptive physiological constraints have been proposed (Congdon and Gibbons 1987, Moles *et al.* 2004), theory mostly discredits these ideas (Rees and Venable 2007, Marshall *et al.* 2010).

Adaptive explanations follow two schools of thought (reviewed in Rollinson and Rowe 2015). The first assumes that the intrinsic qualities of mothers vary with maternal size (e.g. probability of survival: Jørgensen *et al.* 2011, Kindsvater and Alonzo 2014, Kindsvater

and Otto 2014; energetic reproductive costs: Sakai and Harada 2001, Filin 2015), and these factors drive selection on maternal provisioning. The other assumes that maternal size affects the quality of the offspring environment, such that the offspring-size fitness function (and therefore optimal offspring size) vary for different-sized mothers (Parker and Begon 1986, McGinley 1989, Venable 1992, Hendry and Day 2003). For example, Parker and Begon (1986) assumed that larger females acquire more resources, and therefore produce more offspring, than smaller females – an assumption supported by data (Lim *et al.* 2014, Barneche *et al.* 2018). Parker and Begon (1986) also assumed that larger, more fecund mothers generate higher density environments for their offspring. This model, and those of others (Brockelman 1975, Parker and Begon 1986, Venable 1992), therefore predict that larger mothers produce larger offspring to offset sibling competition. Despite the intuitive appeal of such models, there have been few direct parameterisations – this is the aim of Chapter 2.

Alternative theory predicts competition among offspring maintains offspring-size variation within populations more generally (i.e. independently of correlations with maternal size and age). Game theoretic models of offspring size predict small-scale variation in offspring density, and asymmetric competition that favours larger offspring, protects offspring-size polymorphisms (Geritz 1995, Geritz *et al.* 1999). A fundamental assumption of these models is a competition-colonisation trade-off in favour of larger and smaller offspring, respectively. These ideas are frequently tested among plant species that differ in seed size (e.g. Freckleton and Watkinson 2001, Leishman 2001, Coomes and Grubb 2003, Turnbull *et al.* 2004, Eriksson 2005, Ben-Hur and Kadmon 2015), but intraspecific tests are rare (but see Gribbin and Thompson 1990, Rodriguez-Girones *et al.* 2003). In Chapter 3, I provide one of the only intraspecific field tests of offspring competitive asymmetries.

Variation within females

Within a single reproductive bout offspring sizes are rarely equal – although this variability is often underestimated (Turnbull *et al.* 2006). For example, size may vary more than 5-fold between two offspring produced by the same parent (Lips 2001, Turnbull *et al.* 2006, Smith *et al.* 2019). Although widespread, the reason for variation within broods remains unclear. Non-adaptive arguments invoke physiological constraints (Fox and Czesak 2000), or suggest that variance arises as a by-product of family conflict over maternal resources (Kamel and Williams 2016). Other studies suggest such variation persists due to negligible selection (Childs *et al.* 2011), although there are few formal estimates for this trait.

Variation within broods is likely inevitable, but at least some of this variation may be adaptive (Marshall and Uller 2007). Adaptive explanations mostly focus on bet hedging in unpredictable environments – whereby mothers produce variable offspring so that some survive the prevailing conditions (Laaksonen 2004, Marshall *et al.* 2008, Olofsson *et al.* 2009, Rees *et al.* 2010). Mothers that bet hedge trade-off (arithmetic) mean fitness in any one generation for reduced variation in fitness across generations – increasing long run (geometric mean) fitness (Philippi and Seger 1989, Starrfelt and Kokko 2012). Within-brood variation often increases with environmental predictability (Crump 1981, Crean and Marshall 2009, Morrongiello *et al.* 2012), but no study has demonstrated the essential mean-variance trade-off in fitness across generations – thus, evidence for bet hedging remains inconclusive.

Competition among siblings may also drive within-female variation (Geritz 1995, Mock and Forbes 1995, Schrader and Travis 2012). For example, in birds asynchronous hatching generates competitive hierarchies where older, larger siblings outcompete later-hatched, smaller siblings for parentally supplied resources (Reed *et al.* 2009). Competitive hierarchies may maximise parental fitness by reducing brood numbers to match local

resource availabilities (Lack 1947, Laaksonen 2004); replacing failed young (Forbes *et al.* 1997); or facilitating other family members (Mock and Forbes 1995). Most explorations of these ideas come from systems with post-natal care (e.g. birds and mammals), where evolved behaviours such as begging or parental favouritism can mediate sibling competition (Mock and Forbes 1995). Extreme cases of sibling rivalry are most obvious, but sibling competition is ubiquitous (Cheplick 1993, Kamel *et al.* 2010, Aguirre and Marshall 2012) – yet the consequences for within-brood variation are relatively unknown for groups without parental care and experimental manipulations are particularly rare. In Chapters 4 and 5, I explore the fitness consequences of sibling interactions within broods that differ in offspring-size variation (Chapter 4), as well as brood-mean offspring size (Chapter 5).

In most taxa, siblings co-occur at scales where competition and local resource depletion are possible – including systems with prolonged dispersive propagule stages (Cheplick 1993; Selkoe *et al.* 2006; Veliz *et al.* 2006; Kamel *et al.* 2012; Aguirre *et al.* 2013). Such kin structure generates scope for multilevel selection, whereby the performance of a given offspring depends on both its own size, as well the sizes of group (i.e. brood) members with which it interacts (Goodnight *et al.* 1992). As such, brood-level traits such as the mean size of offspring, degree of variation and offspring number represent important components of the environment experienced by offspring. Multilevel selection is a potentially important force acting on offspring size in natural populations, but few studies provide formal estimates (but see Reed *et al.* 2009, Bouwhuis *et al.* 2015). In Chapter 5, I explore multilevel selection on offspring size within clutches of eggs that compete for sperm during external fertilisation.

Competition and the eco-evolutionary dynamics of offspring size

As summarised above, competition among offspring may play a key role in the eco-evolutionary dynamics of offspring-size variation at all levels of organisation – from among

species (e.g. Rees and Westoby 1997), to within-broods produced by the same female (e.g. Mock and Forbes 1995). Virtually all theory that considers competition among offspring assume that larger offspring have a competitive advantage over smaller offspring – but few studies explicitly test these assumptions, particularly at scales relevant to theoretical predictions (for example, between siblings in the case of within-brood variation). Below, I briefly summarise the evidence that offspring size determines competitive ability.

Does offspring size affect competitive ability?

There are good reasons to expect larger offspring win contests over smaller offspring. For instance, larger offspring are provisioned with more energy reserves, and use proportionally less of these reserves during development – thus, larger offspring have more energy for growth, survival and reproduction (Leishman *et al.* 2000, Pettersen *et al.* 2015, Pettersen *et al.* 2017). Greater reserves can increase the resistance of larger offspring to starvation (Berkeley *et al.* 2004), and their capacity to withstand low resource environments – for example, when high neighbour densities deplete local resources (Freckleton and Watkinson 2001, Allen *et al.* 2008, Bashey 2008). Once feeding commences, larger offspring access more resources than smaller offspring (Stanton 1984, Martin and Pfennig 2010). Yet whether larger offspring access disproportionately more resources (i.e., whether competition is asymmetric: Weiner 1990) remains unclear – yet this assumption is fundamental to offspring-size models (Geritz 1995, Geritz *et al.* 1999). Smaller offspring occasionally perform as well (or better) than larger offspring at high densities (Moore *et al.* 2015, Larios and Venable 2018) – perhaps because smaller individuals have lower resource requirements, and better tolerate resource depletion, than larger individuals (Persson 1985). Overall then, evidence that larger offspring outcompete smaller offspring is mixed and empirical tests are rare – yet this assumption continues to pervade most models of offspring size.

Thesis aims and scope

Life-history theory has long sought to understand patterns of variation in maternal provisioning. While theory is extensive, empirical tests of key assumptions lag behind, severely limiting our understanding of the drivers of offspring-size variation (Bernardo 1996, Marshall *et al.* 2018). To this end, my thesis provides much-needed tests of long-held assumptions regarding offspring size. In particular, I investigate theory specific to two scales of organisation – among mothers (Chapters 2 and 3) and within mothers (Chapters 4 and 5) of the same population. A unifying theme of this thesis is the role of offspring competition in driving the eco-evolutionary dynamics of maternal provisioning. I combine field and laboratory experiments with modelling approaches to test how offspring size affects competitive outcomes at two life-history stages: competition among settlers for environmentally supplied resources (Chapters 2, 3 & 4), and competition among eggs for fertilisations (Chapter 5). I focus on marine invertebrates as they provide a tractable system for experimental manipulations of offspring size and density, as well as long-term observations of fitness in the field.

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Chapter 2

Why do larger mothers produce larger offspring? A test of classic theory¹

Abstract

Across a wide range of taxa larger mothers produce larger offspring. Theory assumes that larger, more fecund mothers create higher local densities of siblings, and so larger mothers produce larger offspring to offset sibling competition. This assumption has been debated for over 30 years, but direct empirical tests are surprisingly rare. Here, we test two key assumptions of classic theories that predict sibling competition drives maternal size-offspring size (MSOS) correlations: 1. independent effects of offspring size and sibling density on offspring performance, or 2. as a product of an interaction between these two factors. We simultaneously tested these alternative assumptions, by manipulating offspring size and sibling density in the marine invertebrate, *Bugula neritina*, and monitoring offspring performance in the field. We found that depending on the fitness metric, offspring size and sibling density either independently, or interactively, affected offspring performance. Yet sibling density did not affect offspring performance in the ways that classic theories assume. Given our results, it is unlikely that sibling competition drives the positive MSOS correlation observed in this species. Empirical support for these classic theories remains lacking, suggesting alternative explanations are necessary.

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Introduction

Within species, larger mothers tend to produce larger offspring than smaller mothers. Maternal size-offspring size (MSOS) correlations are common within a range of taxa, from invertebrates to vertebrates (reviewed in Lim *et al.* 2014). Despite the ubiquity of MSOS correlations, their underlying drivers remain unresolved (Rollinson and Rowe 2015). Classic life-history theory predicts that within a given environment, all mothers should produce offspring of an optimal size that maximizes maternal fitness (Smith and Fretwell 1974). That larger mothers often produce larger offspring therefore challenges classic theory, but subsequent theories have explicitly considered adaptive explanations for this source of offspring-size variation (e.g. Parker and Begon 1986, Venable 1992, Hendry and Day 2003, Kindsvater *et al.* 2010, Jørgensen *et al.* 2011).

Parker and Begon (1986) were among the first to examine why offspring size increases with maternal size by introducing two main innovations to the Smith-Fretwell model. First, females vary in resource status, such that larger females have more resources for reproduction, and therefore have higher fecundities, than smaller females. Second, for species with limited dispersal, larger, more fecund mothers, create higher local densities of siblings that compete with each other. In their model, offspring performance not only increases with offspring size, as per the Smith-Fretwell model, but offspring performance also declines with maternal fecundity due to density-dependence (Fig. 1a). Under these assumptions, Parker and Begon (1986) predict that selection should favour larger mothers that produce larger offspring to offset the negative effects associated with the higher fecundities of larger mothers.

Building on Parker and Begon's model, whereby positive MSOS correlations are driven by independent effects of offspring size and sibling density on performance, Venable

(1992) explored MSOS correlations as the product of interactions between offspring size and sibling density. Specifically, a positive MSOS correlation can arise when the performance benefits of larger offspring are stronger at higher densities of siblings, but weaker at lower densities (Fig. 1b). This assumption seems reasonable given that previous studies find conspecific density typically alters the offspring size-performance relationship in this direction, although these studies did not examine competition among siblings (Marshall *et al.* 2006, Allen *et al.* 2008). Thus, theory predicts that positive relationships between maternal size and fecundity can drive adaptive MSOS correlations in two ways: 1) by generating sibling densities that negatively affect offspring performance, independently of offspring size (Parker and Begon 1986), or 2) by generating sibling densities that alter the offspring size-performance function (Venable 1992; Fig. 1).

In a recent review, Rollinson and Rowe (2015) argued that theories that invoke sibling competition as drivers of MSOS correlations are unlikely to be generally applicable to a broad range of taxa. They conclude that the few indirect tests of these ideas provide little support for sibling competition hypotheses, and that alternative theories are more likely to explain MSOS correlations. However, Rollinson and Rowe (2015) also noted that few studies have explicitly tested the assumptions of sibling competition models, especially under field conditions (but see Rollinson and Hutchings 2010). To our knowledge, there have been no formal tests examining the assumption that interactive effects of offspring size and sibling density may drive MSOS correlations (Venable 1992). Given that sibling competition has been widely invoked to explain MSOS correlations, it is surprising that there are few direct tests of these influential theories.

Identifying the mechanism underlying MSOS correlations is not only of fundamental interest to life-history theory, but also has important ecological implications. Anthropogenic activities have reduced the average body size of individuals in many systems (Gardner *et al.*

2011, Hixon *et al.* 2014). For example, commercial fishing truncates size distributions in favour of younger, smaller spawners (reviewed in Hixon *et al.* 2014). Many studies also show that larger offspring out-perform smaller offspring within a given environment (Einum and Fleming 2000, Marshall *et al.* 2003, Berkeley *et al.* 2004). Therefore, the likelihood that larger mothers produce offspring that are intrinsically fitter than those of smaller mothers is one argument for the preservation of larger females in exploited populations (Hixon *et al.* 2014). However, if females provision their offspring to compensate for sibling competition associated with maternal fecundity, theory then predicts that offspring from different-sized mothers will have the same per capita fitness (Parker and Begon 1986, Marshall *et al.* 2010). Determining the drivers of MSOS correlations, therefore, has important implications for the management of natural populations.

We simultaneously test two key assumptions of classic theory that predict sibling competition drives MSOS correlations: 1. Sibling density reduces offspring performance independently of offspring size (Parker and Begon 1986); or 2. Sibling density alters the offspring size-performance relationship, such that larger offspring perform better at high densities, but all offspring perform equally well at low densities (Venable 1992; Fig. 1). To test these assumptions, we manipulate offspring size and sibling density using the marine invertebrate, *Bugula neritina*, and monitor the consequences for offspring performance in the field.

Materials and methods

Study species

Bugula neritina (hereafter *Bugula*) is an arborescent bryozoan common to sessile marine communities world-wide. *Bugula* is clonal and grows by asexual budding of individual modules (zooids) of relatively fixed size (Thompson *et al.* 2015). Growth is

therefore indeterminate, such that colony size (number of individual zooids) is the key index of maternal size. Indeed, MSOS correlations occur at the scale of colonies in this species; a 2-fold increase in colony size is shown to correspond to a 72 % increase in larval volume (Marshall *et al.* 2003, Marshall and Keough 2003).

Bugula displays many traits that make it ideal for testing the assumptions of sibling competition models (e.g. Parker and Begon 1986, Venable 1992). For instance, offspring size positively affects the survival, growth and reproduction of *Bugula*, particularly at high conspecific densities (Marshall *et al.* 2003, Allen *et al.* 2008). Furthermore, larval durations are typically short, which limits the potential for long-distance dispersal in the field, and there is evidence for sibling aggregation and interactions at settlement (Keough 1984, Burgess and Marshall 2011, Aguirre *et al.* 2013). Maternal fecundity also positively correlates with colony size, with a doubling in colony size corresponding to an approximate doubling in fecundity (Marshall *et al.* 2003). Thus larger mothers likely create higher local densities of siblings relative to smaller mothers (D. Marshall, unpublished data), and post-settlement interactions between siblings are mostly negative (Allen *et al.* 2008, Aguirre and Marshall 2012).

Experimental methods

To determine whether sibling interactions influence offspring performance according to the assumptions of Parker and Begon (1986) and Venable (1992), we experimentally manipulated sibling density and offspring size. We manipulated sibling density to simulate the assumption that the offspring of larger, more fecund mothers, face higher local settlement densities. We manipulated offspring size by allocating a single individual of known larval size (focal individual) into each density treatment, and monitored several performance metrics of these focal individuals in the field.

To obtain larvae for our experiment, we collected reproductive *Bugula* colonies from Altona Pier (Port Phillip Bay, Victoria, Australia) from December 2014 to February 2015.

We used standard techniques to induce these colonies to spawn (Marshall *et al.* 2003). Briefly, colonies were held in separate dark, insulated aquaria at 19°C for 2 days before being exposed to bright light to stimulate larval release. Seven colonies were spawned in separate beakers to ensure our manipulations were applied to sibling larvae. As we collected colonies that had been fertilised in the field and were already brooding their larvae, only maternal identity was known. Multiple paternity is common in sperm-casting marine invertebrates (Johnson and Yund 2007), although this has not been confirmed in Bryozoans. All offspring from a single colony were therefore at least half siblings, although some were likely full siblings.

We photographed the focal larvae with their ciliary groove facing upwards on a glass slide at 100 x magnification. We then measured larval length along the axis of the ciliary groove using image analysis software (Image J). For each parental colony, we measured between 80 and 110 focal larvae. We then settled the focal larvae (after measurement) on pre-roughened, biofilmed acetate sheets. We haphazardly settled non-focal larvae (without measurement) onto pre-roughened, biofilmed PVC plates (5 x 5 x 0.6 cm) to generate our sibling density treatments. We settled all larvae (focals and competitors) within three hours after they had been released from the parental colonies to avoid delayed settlement affecting larval quality (Wendt 1998). After this time, we rinsed the unsettled larvae from settlement surfaces with filtered seawater. We left the settlers to complete metamorphosis overnight in trays of filtered (0.22 µm) seawater at 19°C.

The next day, we cut successful focal settlers from acetate sheets and glued them to the PVC plates bearing sibling competitors. A single focal settler was systematically assigned to each sibling density treatment, ensuring that larval sizes were equally distributed among densities. Our manipulation of sibling density (per 25 cm²) had four levels: no competition (0 competitors + 1 focal settler), low competition (3 competitors + 1 focal settler), intermediate

competition (6 competitors + 1 focal settler) and high competition (12 competitors + 1 focal settler). These densities reflect those from pilot studies, where a four-fold difference in maternal size produced a four-fold change in sibling densities (D. Marshall, unpublished data). We circled all experimental settlers with a pencil to distinguish them from field recruits.

We deployed our experiment at the Royal Brighton Yacht Club (Victoria, Australia). We attached the settlement plates to PVC backing panels (55 x 55 x 0.8 cm) and hung them 1 m below the water surface with the plates facing downwards to avoid smothering by sediment. Each backing panel was allocated 64 plates, such that each of the four levels of sibling density were replicated 16 times per panel. Intraspecific competition among *Bugula* typically occurs at the scale of plates, while density effects beyond this scale are undetectable (Hart and Marshall 2009, Hart *et al.* 2012). Thus, plates within a backing panel were unlikely to interact with one another. We replicated the experiment across seven panels, with each panel representing a family unit (i.e. all individuals within a panel were maternal siblings). We processed a single family per day in the laboratory, such that we staggered the deployment of panels in the field. Panels therefore combine family-level phenotypic variation, as well as spatial and temporal variation.

We measured the performance of focal individuals (N = 448) by monitoring their survival and growth after 4 weeks in the field. Survival was determined by the presence of the focal individual (scored as 1), while absent focal individuals were assumed to be dead (scored as 0). We measured growth as the number of times the colony had bifurcated along the longest branch, a good proxy for colony biomass (Keough and Chernoff 1987). We randomized the position of plates on PVC backing panels weekly and removed any new settlers of any species to eliminate spurious competition.

Statistical analysis

We analysed the effects of larval size and sibling density on offspring performance after four weeks in the field using generalized linear mixed models (GLMM). Survival was modelled as a binomial GLM with a logit link function, while subsequent colony size (as bifurcations) was modelled as a quasi-Poisson GLM with a log link function. For all analyses, we modelled larval size and sibling density as continuous, fixed effects, and backing panel as a random, fixed effect. For survival, we modelled sibling density as the initial number of settlers on a plate. For colony size, we modelled sibling density as the average number of initial settlers within our four levels of sibling density. We modelled sibling densities differently for survival and growth for several reasons. First, mortality mostly occurred early in the experiment (though it did continue throughout) and varied among plates, such that initial density was not always maintained throughout the experiment. Given most mortality occurred early, we regarded initial density as the most relevant environmental predictor of a focal colony's survival. In contrast, colony growth was affected slightly by initial settlement densities, but effects were much stronger at later densities. Given we had no *a priori* expectation of when density affects would be strongest, our averaging approach best captures the broad differences in densities experienced during colony growth. Second, a model for colony growth that used average settlement densities was an equivalent (or slightly better) fit than a model that used initial sibling densities ($\Delta\text{AIC} = 0.6$).

We reduced both models by removing non-significant interactions if their inclusion did not improve model fit (Quinn and Keough 2002). Variance components and fixed effects were estimated using restricted maximum likelihood and maximum likelihood, respectively. Model fit and significance was assessed via AIC values and likelihood-ratio tests for both fixed and random effects (at $P = 0.05$). Neither GLMM showed signs of over-dispersion, although the quasi-Poisson regression was slightly under-dispersed (Pearson $\chi^2 = 0.41$). We

fitted all models using PROC GLIMMIX in SAS (SAS Institute; Cary, North Carolina, USA) and pseudo-likelihood estimation (Bolker *et al.* 2009).

Results

After four weeks in the field, we found that sibling density and offspring size did not interactively affect the survival of the focal *Bugula* settlers (Table 1). We also found no effect of sibling density on offspring survival. Offspring size did slightly increase the probability of post-settlement survival, with a unit increase in larval size increasing the odds of survival by 1.15% (Table 1; Fig. 2).

We found an interaction between sibling density and offspring size on the growth (size) of focal settlers after four weeks in the field (Table 2). In the absence of siblings and at low sibling densities, offspring size strongly affected post-settlement growth – larger offspring grew into larger colonies than smaller offspring (Fig. 3). In comparison, offspring size weakly affected offspring performance at higher sibling densities, but overall, smaller offspring grew slightly more than larger offspring (Fig. 3). Importantly then, the effects of sibling density depended on offspring size. Increasing sibling densities enhanced the performance of smaller offspring relative to when they occurred in isolation – indicating positive density-dependence (facilitation). In contrast, larger offspring suffered a decrease in performance with an increase in sibling density – indicating negative density-dependence (competition).

Discussion

Theory has sought to explain the widespread tendency for larger, more fecund mothers to produce larger offspring. Here, we test two assumptions underlying classic theories that invoke sibling competition as a driver of MSOS correlations: 1) independent effects of offspring size and sibling density on performance (Parker and

Begon 1986); and 2) effects on performance that arise as a product of an interaction between offspring size and density (Venable 1992; Fig. 1). Whilst many aspects of *Bugula*'s life history suggest that the assumptions of these models should apply, sibling density did not affect offspring performance in either of the ways assumed by these theories. Our results, therefore, suggest that sibling competition is unlikely to drive the positive MSOS correlation in this species.

The theory of Parker and Begon (1986) assumes that offspring performance increases with offspring size, but that performance declines with sibling density. In accordance with Parker and Begon (1986) and most offspring-size models (e.g. Smith and Fretwell 1974, McGinley *et al.* 1987), we found that larger *Bugula* offspring had higher post-settlement survival relative to smaller offspring. In contrast to Parker and Begon (1986), however, we found offspring survival was not affected by sibling density. Instead, in our study, mortality appeared to be density-independent (average survival was 69%). Other studies find mixed effects of sibling density on offspring performance (Einum and Fleming 1999, Takahashi *et al.* 2005, Rollinson and Hutchings 2010, Eberhart and Tielbörger 2012). Surprisingly, *Bugula* siblings have been shown to compete more intensely relative to non-related conspecifics (Aguirre and Marshall 2012), but in our study at least, the consequences of sibling competition are not density-dependent.

We found an interaction between sibling density and offspring size on post-settlement growth, again contradicting the assumptions of Parker and Begon (1986). Venable (1992) anticipated an interaction between offspring size and sibling density – his model predicts a positive MSOS correlation when larger offspring have superior performance at high sibling densities, but all offspring perform equally well at low densities (Fig. 1b). Instead, we found the reverse: at low sibling densities larger offspring grew better than smaller offspring, but at higher densities, smaller offspring grew as large (or even slightly

larger) than large offspring. In other words, sibling density positively affected smaller offspring, but negatively affected larger offspring. This contrasts previous studies (including studies in our system) that find smaller offspring typically experience negative density-dependence more strongly than larger offspring (Beckerman *et al.* 2006, Allen *et al.* 2008). Previous studies on *Bugula* that did not measure offspring size have found colony growth to decrease with increasing densities, particularly when individuals were closely related (Aguirre and Marshall 2012, Svensson and Marshall 2015). Phenotype-specific responses to density-dependence in our study, however, suggest that density affects may not be as straightforward as previously thought. Indeed, the fitness consequences of aggregating with genetically similar individuals is phenotype-specific in other systems (although in these studies these phenotypes were not offspring size; Sinervo and Clobert 2003).

In our study, phenotype-specific density-dependence may occur if the relative strength of facilitation and competition depends on offspring size at higher sibling densities. Smaller *Bugula* larvae become juveniles with smaller, less efficient, feeding structures relative to juveniles formed by larger larvae (Kosman and Pernet 2011). At high densities, however, neighbouring colonies may generate feeding currents and reduce flow, conditions known to increase feeding efficiencies in bryozoans (Best and Thorpe 1986). Aggregating with siblings may therefore facilitate food intake for colonies from smaller larvae, enhancing their growth at higher densities. For larger larvae, however, the benefits of facilitation may be outweighed by the negative effects of resource depletion at higher densities. Such a scenario may arise if smaller larvae have lower resource requirements than colonies from larger larvae (which seems likely given colonies from larger larvae have larger feeding structures and greater biomass; Pettersen *et al.* 2015). While our proposed mechanism is speculative, this could explain why larger larvae grew less at higher densities relative to when they were isolated from siblings. Regardless of the mechanism underlying offspring-size specific

density effects, the direction of this interaction in our study contradicts the assumptions of major offspring size theories, suggesting that sibling competition is unlikely to explain the MSOS correlation in *Bugula*.

While we found no support for the assumptions of Parker and Begon (1986) or Venable (1992), the positive correlation between maternal size and offspring size may still have an adaptive explanation in *Bugula*. For example, larger mothers may produce larger larvae to facilitate their dispersal to habitats where they perform best (i.e. in isolation from siblings), whereas smaller mothers may produce smaller, less-dispersive offspring that perform best among siblings. This idea is supported by previous studies showing that relatively larger *Bugula* larvae swim for longer before settlement, and are more likely to access habitats away from siblings (Marshall and Keough 2003, Burgess and Marshall 2011). Phenotype-specific dispersal has been considered in the context of optimal provisioning strategies, and is predicted to maximise maternal fitness under some conditions (e.g. McGinley 1987, Burgess *et al.* 2013). However such models are yet to consider whether phenotype-specific dispersal may provide an adaptive explanation for MSOS correlations.

Non-adaptive arguments have also been invoked to explain MSOS correlations. It has been argued that allometric relationships between maternal size and the size of the brood space or reproductive tract may determine offspring size (Congdon and Gibbons 1987). Such an explanation seems unlikely in a colonial organism such as *Bugula*. Allometric relationships between colony size and brood chamber (ovicell) size are yet to be investigated, but individual module size (lophophore volume) does not appear to be correlated with colony size (Thompson *et al.* 2015). We argue, therefore, that brood space is unlikely to impose a constraint on offspring size in this species. Instead, we suspect that resource state at the level of the entire colony determines MSOS correlations for *Bugula*. For example, Sakai and Harada (2001) predict that if larger mothers can provision their offspring more efficiently,

they should also produce larger offspring. Their model assumes that because larger mothers have larger resource stocks, they can deploy resources more quickly and waste less energy while provisioning offspring. While Sakai and Harada (2001) considered plants specifically, similar arguments could be applied to our system, since offspring provisioning appears to be determined by total colony size and resource state (Marshall and Keough 2004).

In a recent review of competing MSOS models, Rollinson and Rowe (2015) argued that theories that invoke sibling interactions lack empirical support. Our experimental results support this argument, since sibling density did not affect offspring performance in the ways that classic theories assume. Here, we have suggested that the positive MSOS correlation may be driven by selection for phenotype-specific dispersal in *Bugula*. Alternatively, theories that invoke differential offspring provisioning efficiencies for mothers of different sizes (Sakai and Harada 2001) or larger over-head metabolic costs associated with brooding larger offspring (Filin 2015, Pettersen *et al.* 2015) are more general, and could provide a unifying explanation for why larger mothers produce larger offspring (Rollinson and Rowe 2015). Empirical tests of these theories remain a challenging next step in increasing our understanding of why offspring size positively covaries with maternal size across a wide range of taxa.

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Tables

Table 1. Generalised mixed-model (binomial) showing the effects of offspring size and sibling density on the survival of *Bugula neritina* settlers after four weeks in the field[†].

| Source | Parameter (95% CI) | χ^2 | <i>p</i> |
|--------------------------|------------------------|----------|----------|
| <i>Fixed Effects</i> | | | |
| Offspring size | 0.012 (0.00009, 0.024) | 3.91 | 0.048* |
| Density | 0.009 (-0.042, 0.061) | 0.12 | 0.732 |
| Offspring size x density | | 0.03 | 0.873 |
| <i>Random Effects</i> | | | |
| Panel | 0.344 (0.120, 3.180) | 28.66 | <0.0001* |
| Panel x density | | 1.95 | 0.163 |
| Residual | 0.993 (0.870, 1.145) | | |

Table 2. Generalised mixed-model (quasi-Poisson) showing the effects of offspring size and sibling density on the growth (size) of *Bugula neritina* settlers after four weeks in the field[†].

| Source | Parameter (95% CI) | χ^2 | <i>p</i> |
|--------------------------|----------------------------|----------|----------|
| <i>Fixed Effects</i> | | | |
| Offspring size | 0.004 (0.0013, 0.0075) | 8.17 | 0.004* |
| Density | 0.13 (0.012, 0.25) | 5.41 | 0.02* |
| Offspring size x density | -0.0006 (-0.001, -0.00006) | 5.62 | 0.018* |
| <i>Random Effects</i> | | | |
| Panel | 0.084 (0.059, 0.130) | 8.97 | 0.003* |
| Panel x density | | 1.23 | 0.267 |
| Residual | 0.370 (0.337, 0.472) | | |

[†] Tests for non-significant interactions are included for completeness, but note we removed these from the final models because they did not improve model fit (see methods). All *df* = 1.

**p* < 0.05.

Figures

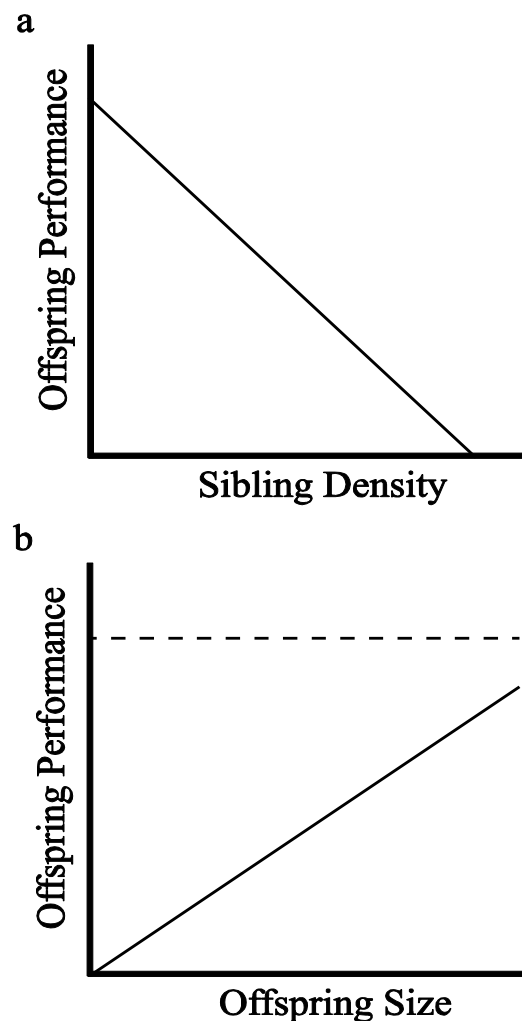


Figure 1. Schematic showing the two alternative assumptions of classic offspring-size models that predict larger, more fecund mothers produce larger offspring to offset sibling competition: a) increasing sibling densities negatively affect offspring performance, and does so independently of offspring size (Parker and Begon 1986), and b) sibling density alters the offspring size-performance relationship (Venable 1992) such that larger offspring perform better at high sibling densities (solid line), but all offspring perform equally well at low densities (broken line).

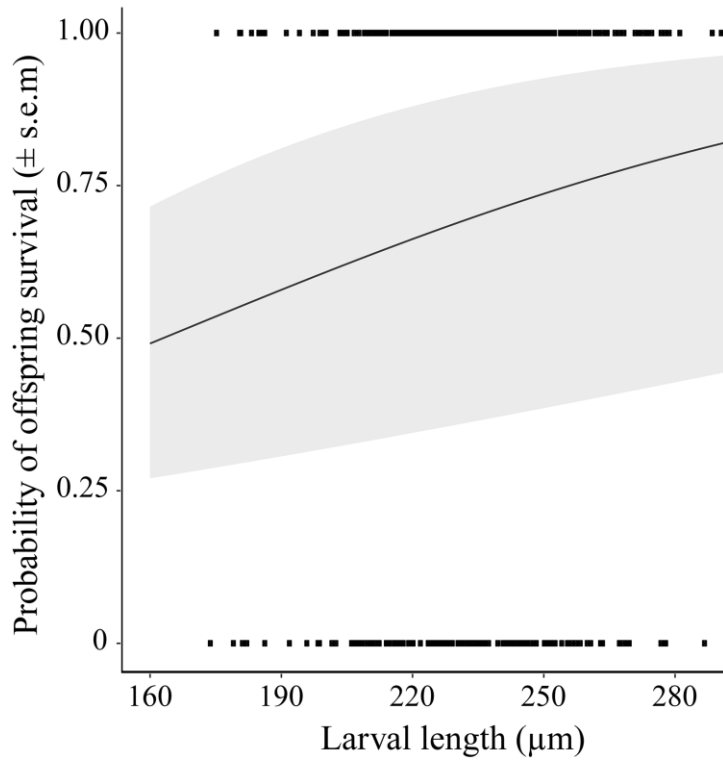


Figure 2. Estimated relationship between larval size and the probability of survival (\pm s.e.m) of *Bugula neritina* colonies after four weeks in the field. Data points show the raw data for survival at this time point (N = 448).

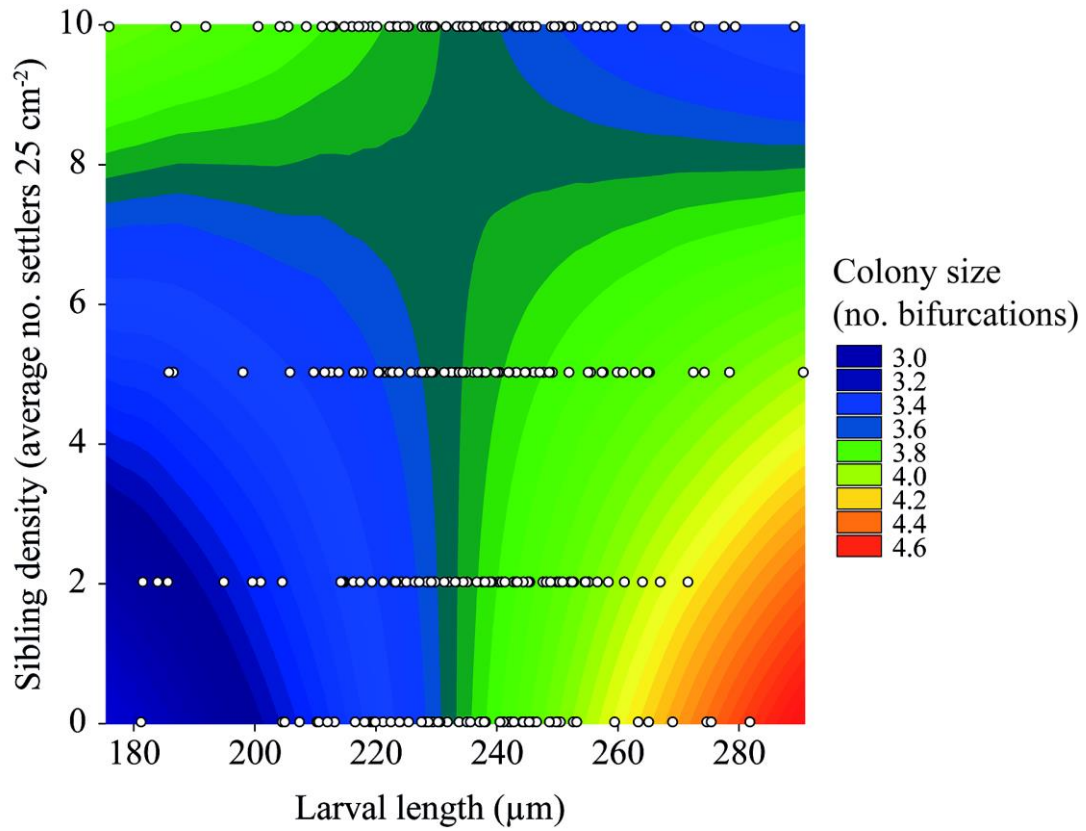


Figure 3. Estimated relationship between larval size and sibling density on colony size (number of bifurcations; side bar) for *Bugula neritina* settlers after four weeks in the field. Data points show the distribution of offspring sizes surviving at this time point (N = 292).

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Chapter 3

Can competitive asymmetries maintain offspring size variation? A manipulative field-test¹

Abstract

Offspring sizes vary within populations but the reasons are unclear. Game-theoretic models predict that selection will maintain offspring-size variation when large offspring are superior competitors (i.e. competition is asymmetric), but small offspring are superior colonisers. Empirical tests are equivocal, however, and typically rely on interspecific comparisons, while explicit intraspecific tests are rare. In a field study, we test whether offspring size affects competitive asymmetries using the sessile marine invertebrate, *Bugula neritina*. Surprisingly, we show that offspring size determines whether interactions are competitive or facilitative – large neighbours strongly facilitated small offspring, but also strongly competed with other large offspring. These findings contradict classic assumptions – that is, large offspring were not superior competitors. Instead, our results suggest that asymmetric facilitation, rather than asymmetric competition, operates in our system. We argue that facilitation of small offspring may be more widespread than currently appreciated, and may maintain variation in offspring size via negative frequency-dependent selection. Offspring size theory has classically viewed offspring interactions through the lens of competition alone, yet our results and those of others, suggest that theory should accommodate positive interactions in explorations of offspring-size variation.

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Introduction

For most organisms, parental care is restricted to the provisioning of offspring before they must fend for themselves. The size of offspring at independence from their parents reflects this provisioning – larger offspring receive more parental resources than smaller offspring (Reviewed in Mousseau and Dingle 1991; Bernardo 1996; Marshall *et al.* 2018b). Offspring vary remarkably in size across time and space, and all scales of organisation (Reviewed in Leishman *et al.* 2000; Krist 2011; Marshall *et al.* 2018b). For example, two offspring can differ five-fold in the resources they receive from the same parent (Lips 2001; Turnbull *et al.* 2006). Offspring size strongly determines performance – larger offspring typically have higher survival, growth and reproduction than smaller offspring (Krist 2011; Marshall *et al.* 2018b). Links between offspring size and performance are particularly evident early in the life history, but can persist at later-stages and may even affect subsequent generations (Benton *et al.* 2008; Krist 2011; Marshall *et al.* 2018b). Overall then, offspring-size effects are ubiquitous, pervasive and of fundamental interest to life-history theory.

Nevertheless, life-history theory has struggled to explain variation in offspring size within populations of the same species (Stearns 1992; Marshall *et al.* 2018b). Classic optimality theory predicts that for a given environment (or population), a single offspring size will maximise maternal fitness – in other words, selection on offspring size is stabilising (Smith and Fretwell 1974). A fundamental assumption of this model is that mothers trade-off the size and number of their offspring – larger offspring perform better, but smaller offspring are cheaper so mothers make them in larger numbers. Mothers therefore balance the relative benefits of better performing offspring with those of increased fecundity, and this balance depends on the relationship between offspring size and performance. Empirical parameterisations of the Smith-Fretwell model can reliably predict mean values of offspring size, and even variation among populations (Einum and Fleming 2000; Marshall and Keough

2008). Yet this classic theory cannot account for the striking variation observed within populations.

Elaborations to the Smith-Fretwell model applied game-theory to incorporate small-scale, density- and frequency-dependent interactions among offspring (Geritz 1995; Geritz *et al.* 1999). In these models, the trade-off between offspring size and number generates a competition-colonisation trade-off that favours larger and smaller offspring, respectively. Larger offspring are competitively superior and win contests over smaller offspring, but smaller offspring frequently colonize unoccupied sites because they are the most abundant propagule. These models predict that under asymmetric competition, where larger offspring disproportionately acquire resources relative to their size (Weiner 1990), no single size is evolutionarily stable and variation is maintained. Conversely, if competition is symmetric and all offspring receive the same resources (Weiner 1990), a single offspring size is optimal and will exclude all others (Geritz 1995; Geritz *et al.* 1999). Importantly, a qualitative prediction of these models is that competition must be *strongly* asymmetric to overcome the colonisation advantage of smaller offspring – that is, the degree of asymmetry is crucial for the maintenance of offspring-size variation (Geritz *et al.* 1999). Analogous models predict the coexistence of species with different seed sizes (Rees and Westoby 1997). Thus, while optimality theory cannot account for offspring-size variation within populations, game-theoretic models suggest competitive asymmetries and positive frequency-dependent selection drive this variation.

So do competitive asymmetries maintain offspring-size variation in natural populations? There are good reasons to expect that larger offspring will win contests over smaller offspring. For instance, larger offspring are provisioned with more energy reserves, and use proportionally less of these reserves during development – thus, larger offspring have more energy for growth, survival and reproduction (Leishman *et al.* 2000; Pettersen *et al.*

2015; Pettersen *et al.*). Greater reserves can also increase the resistance of larger offspring to starvation and their capacity to withstand low resource environments – although these advantages are not universal (Berven and Chadra 1988; Freckleton and Watkinson 2001; Fisher *et al.* 2007; Allen *et al.* 2008; Bashey 2008). Once feeding commences, larger offspring typically access more resources than smaller offspring (Stanton 1984; Leishman 2001; Martin and Pfennig 2010), though it is unclear whether they access disproportionately more resources (Marshall *et al.* 2018b) – a requirement of asymmetric competition (Weiner 1990). Furthermore, while larger individuals acquire more resources, smaller individuals often have lower resource requirements and may tolerate resource depletion better than their larger neighbours (Persson 1985). Overall then, while some studies support the assumption that competitive asymmetries favour larger offspring, others provide instances where these assumptions may be violated – although direct empirical tests are rare.

For the few studies that directly estimate competitive asymmetries, evidence is equivocal. So far, most tests have used comparisons of competitive abilities among plant species with different seed sizes. In general, larger-seeded species tend to outcompete smaller-seeded species (Freckleton and Watkinson 2001; Leishman 2001; Turnbull *et al.* 2004), although there are exceptions (Eriksson 2005; Ben-Hur and Kadmon 2015). Furthermore, studies that report asymmetric competition coefficients suggest these asymmetries are not sufficiently strong to compensate for the colonisation advantage of smaller-seeded species (Rees and Westoby 1997; Coomes and Grubb 2003; Turnbull *et al.* 2004). As such, the validity of competitive asymmetries as drivers of multispecies coexistence has been questioned (Coomes and Grubb 2003).

While undoubtedly informative from a community perspective, interspecific comparisons cannot predict the processes that generate offspring-size variation within species. Most studies within species focus on competitive asymmetries generated by body

size and/or ontogenetic stage (de Roos and Persson 2003; Bassar *et al.* 2016), but few have explicitly explored asymmetries among newly independent offspring to test the validity of game-theoretic models of offspring size (Geritz 1995; Geritz *et al.* 1999). Although rare, some intraspecific tests suggest that larger offspring outcompete smaller offspring (Gribbin and Thompson 1990; Rodriguez-Girones *et al.* 2003; Marshall *et al.* 2006). For example, in laboratory studies, small damselfly larvae grow less and develop more slowly due to interference competition from larger larvae, but are unaffected by other small larvae (Gribbin and Thompson 1990). Other intraspecific studies suggest that variation in offspring size mediates interactions via mechanisms other than competition. For example, in tadpoles and marine invertebrates, offspring size appears to promote resource partitioning that may reduce competitive inequalities (Martin and Pfennig 2010; Cameron *et al.* 2017). Interactions among offspring may even be beneficial – for instance, high densities of neighbours facilitate offspring of certain sizes across a range of systems (e.g. in birds: Russell *et al.* 2007; marine invertebrates: Cameron *et al.* 2016; and plants: Zepeda and Martorell 2019). Given the limited number of studies that manipulate the sizes of interacting offspring, however, generalisations about offspring size-mediated competition appear premature.

In a manipulative field experiment, we tested the assumption that offspring size generates intraspecific competitive asymmetries using the sessile marine invertebrate, *Bugula neritina*. We used a trait-specific, response-surface design to manipulate the size differences between offspring involved in pairwise interactions. We then measured the outcomes of these interactions by comparing the performance (survival, growth and reproduction) of different-sized offspring in the presence and absence of neighbours under field conditions across their lifetime.

Material and methods

Study species

Bugula neritina (hereafter *Bugula*) is a bryozoan common to sessile marine communities worldwide. *Bugula* grows by asexual budding of zooids to form branched colonies. Colonies are simultaneous hermaphrodites and fertilization is internal; colonies cast sperm into the water column, but retain the eggs and developing larvae. Colonies brood the larvae in external reproductive structures (ovicells) for up to one week before the fully developed, non-feeding larvae are released.

Within populations, offspring (larval) size varies up to four-fold both among and within females. Larval size determines post-settlement performance – larger offspring typically survive, grow and reproduce more than smaller offspring (Marshall *et al.* 2018a). Larger offspring appear to cope better with competition, although this can be context-dependent (Allen *et al.* 2008; Cameron *et al.* 2016). Importantly, these previous studies manipulated the size of focal offspring, but only the densities (and not the phenotypes) of neighbouring offspring. In contrast, our current study manipulates the size differences between interacting offspring to address competitive asymmetries explicitly.

Experimental methods

We conducted our experiments at Blairgowrie Yacht Squadron, Victoria, Australia (38°21'20.2"S, 144°46'22.8"E) from March to June 2016. To obtain larvae for our experiments, we collected mature colonies from the field and held them within dark, aerated aquaria at 17°C for 2 days. We induced the colonies to spawn their brooded larvae by exposing them to bright light and measured these larvae using standard techniques (Cameron *et al.* 2017). We settled all larvae (after measurement) onto pre-roughened, biofilmed acetate squares. For each experimental run, we measured between 250 and 350 larvae that we pooled from ~ 30 parental colonies.

In a field experiment, we tested whether offspring size mediates the outcomes of pairwise interactions using a trait-specific, response-surface design (Inouye 2001). For our manipulations, we used the continuous range of larval sizes from our source population (5.65 – 15.29 μg) to generate pairwise combinations of offspring (absolute size difference range: - 7.83 to 7.83 μg ; relative size ratio range: 0.43 – 2.35; Fig. 1). To create our treatments, we glued the acetate squares bearing the offspring onto PVC plates (5.5 x 5.5 cm) at a distance of 1 cm from each other. We treated both these offspring as the focal and neighbour simultaneously to test for reciprocal interactions (Inouye 2001). We also estimated the baseline relationship between offspring size and performance by gluing a blank acetate square 1 cm from focal settlers of various sizes (neighbour-free controls; Fig. 1a). We circled all experimental settlers and noted their position on the plates so we could distinguish them from field recruits and monitor their performance over time.

We deployed our experiment in the field by attaching the plates (bearing the settlers) to PVC backing panels (55 x 55 cm) that hung 1 m below the water surface. We replicated the experiment across three runs that we deployed into the field at one-week intervals. Runs one and two had five panels, run three had four panels. Each backing panel received 14 plates that represented the full range of focal and neighbour size pairings (i.e. covariate ranges overlapped). Overall, the experiment included 196 plates deployed across 14 backing panels, and we measured the performance of 336 individuals of known offspring size.

We measured several fitness components for all experimental colonies weekly. For survival, we scored the colonies as alive if they were present on the plates or dead if they were absent. We measured growth as the number of times the colonies had branched (bifurcated) – a good proxy for colony biomass (Keough and Chernoff 1987). We measured fecundity as the number of ovicells on the colonies each week to give a cumulative value of reproductive output for each colony (Pettersen *et al.* 2016). At each census, we removed non-

experimental settlers (both *Bugula* and other species) from the plates to eliminate competition from other organisms and randomised the plates within the backing panels. We ended the experiment after 11 weeks because by this time most plates had only a single survivor.

Statistical analyses

We tested for size-mediated interactions among settlers of known larval size using generalised linear models (GLMs). For these models, we included the size-specific performance of offspring in neighbour-free conditions (i.e. controls) by setting their corresponding value for neighbour size to zero (unless otherwise specified). For survival, we used a binomial GLM with a logit-link function, where focal and neighbour offspring size were continuous fixed effects and experimental run was a categorical fixed effect. For growth, we analysed the size (bifurcations) of the focal colonies after 11 weeks using a Gaussian GLM with the same model structure as above.

We also tested whether the size of focal and neighbour offspring affected the probability that focal colonies reproduced using a binomial GLM with a logit-link function and the same model structure above. Here, our binary response variable described whether colonies had reproduced (assigned a value of 1) or had not reproduced (assigned a value of 0) after 11 weeks in the field. Because neighbour size did not affect the probability that focal colonies reproduced (see results), we again analysed our binary response for reproduction in a binomial GLM, but this time we included neighbour presence/absence and backing panel as categorical fixed effects and focal offspring size as a continuous fixed effect. For those individuals that survived to reproduce, we also analysed cumulative reproductive output (fecundity) after 11 weeks in the field using a quasi-Poisson GLM with a log-link function and the same model structure described above. For all analyses, we first fit full models and reduced these where appropriate by removing non-significant interactions, assessed from log-

likelihood ratio tests (binomial GLMs) or F -ratio tests (Gaussian and quasi-Poisson GLMs) where $\alpha > 0.05$.

Results

After 11 weeks in the field, we found that focals from larger offspring survived better than those from smaller offspring ($\chi^2 = 7.961$, $df = 1$, $p = 0.005$; Fig. 2a), and this was not affected by the offspring size of their neighbours (neighbour size: $\chi^2 = 0.593$, $df = 1$, $p = 0.441$; focal x neighbour size: $\chi^2 = 0.450$, $df = 1$, $p = 0.503$). Larger offspring also grew into larger colonies ($F_{1,105} = 7.590$, $p = 0.007$), but again the growth of the focals was not affected by their neighbour's size (neighbour size: $F_{1,217} = 1.023$, $p = 0.314$; focal x neighbour size interaction: $F_{1,104} = 0.895$, $p = 0.346$). Larger focal offspring had a higher probability of reproducing than smaller offspring ($\chi^2 = 13.644$, $df = 1$, $p < 0.001$), irrespective of neighbour size (neighbour size: $\chi^2 = 0.12$, $df = 1$, $p = 0.663$; focal x neighbour size: $\chi^2 = 0.001$, $df = 1$, $p = 0.974$). Interestingly, however, the probability that the focal colonies reproduced tended to be lower in the presence of neighbours (ignoring neighbour size) relative to neighbour-free conditions ($\chi^2 = 3.865$, $df = 1$, $p = 0.049$), and this was consistent across focal sizes (focal size x neighbour presence: $\chi^2 = 0.37$, $df = 1$, $p = 0.541$).

For the focal colonies that reproduced, both their own offspring size, and that of their neighbour, interactively affected their reproductive output ($F_{1,133} = 4.511$, $p = 0.036$; Fig. 2b). When offspring occurred in isolation, larger offspring had 5 times the reproductive output of smaller offspring. In pairwise interactions, however, the offspring size of the focals determined their response to neighbours – neighbours increased the fecundities of smaller offspring, but diminished the fecundities of larger offspring (relative to neighbour-free conditions). In other words, neighbours facilitated smaller offspring but competed with larger offspring. Interestingly, the offspring size of neighbours determined the strength of these

interactions. Most surprisingly, facilitation increased with neighbour size – the largest neighbours were the strongest facilitators of small focals (~350 % increase in fecundity relative to neighbour-free conditions), whereas large offspring competed most strongly with each other (~50 % decrease in performance relative to neighbour-free conditions). It is also worth noting that offspring around the population mean size (11-12 μg ; Fig 1.b) were unaffected by neighbour identity (both similar and dissimilar phenotypes; Fig. 2b).

The above analyses indicate that larger offspring have both a survival advantage and a higher chance of reproducing, but that smaller offspring that survive to reproduce have higher reproductive outputs relative to larger offspring in the presence of neighbours. We therefore visualised composite fitness by multiplying our estimates of fecundity and the probability of reproducing (which includes both survival to maturity and whether or not the survivors reproduced) to fully integrate the benefits of offspring size on offspring performance (Fig. 2c). Although neighbours reduced the probability that focal colonies reproduced, their positive effects on the fecundities of smaller offspring outweighed this negative effect – thus overall, neighbours facilitated smaller offspring.

Nevertheless, larger offspring in isolation or pairwise interactions with smaller offspring had the highest performance overall – although this advantage was not disproportionate to their size (Fig. 2c). To illustrate, consider pairwise interactions between offspring of 5 and 15 micrograms. While the smaller offspring is one-third the size of the larger offspring, their performance is over half that of the larger offspring. Importantly, when we account for the fecundity costs of making larger offspring, we find that mothers receive higher fitness returns if they make more numerous, smaller offspring relative to fewer, larger offspring when these offspring then interact (Fig. 2d).

Discussion

Our study contradicts a key assumption of game-theoretic models – that strong competitive asymmetries favour larger offspring (Geritz 1995; Geritz *et al.* 1999). Rather, we found that offspring size altered the strength and direction of pairwise interactions. Focal offspring size determined whether interactions were positive or negative – neighbours facilitated smaller offspring but competed with larger offspring. Meanwhile, the offspring size of neighbours determined the strength of these interactions – neighbours from larger offspring were both the strongest facilitators and competitors of small and large focals, respectively. These findings are indicative of absolute, or antagonistic, asymmetric facilitation (Lin *et al.* 2012; Schob *et al.* 2014) – that is, small offspring receive benefits from larger offspring, but large offspring only experience competition (i.e. reciprocal interactions are +/-). Our study is the first to show that asymmetric facilitation can occur between offspring of different sizes. This raises two important questions: 1. How widespread is asymmetric facilitation among offspring; and 2. What are the eco-evolutionary consequences for variation in offspring size?

Our finding that larger offspring facilitate smaller offspring contradicts previous studies – most studies find larger offspring outcompete smaller offspring (Gribbin and Thompson 1990; Freckleton and Watkinson 2001; Leishman 2001; Rodriguez-Girones *et al.* 2003; Turnbull *et al.* 2004). Nevertheless, tests that manipulate size differences among offspring at the intraspecific level are rare (but see Gribbin and Thompson 1990; Rodriguez-Girones *et al.* 2003; Marshall *et al.* 2006). Thus far, most tests of offspring competitive ability manipulate focal size across different densities of conspecifics, but not the sizes of these neighbours (Berven and Chadra 1988; Allen *et al.* 2008; Bashey 2008; Cameron *et al.* 2016). In contrast, our study investigates pairwise interactions between offspring of different sizes, but interactions obviously occur across a range of densities in nature. Density alters the

mode of competition (symmetric vs. asymmetric) across ontogenetic stages of offspring (Cameron *et al.* 2007). An important next step, therefore, would be orthogonal manipulations of both the density *and* frequency of contemporary offspring that differ in size. Such an approach would allow direct parameterisations of ESS models, to determine whether facilitative and (or) competitive asymmetries are expected to maintain offspring-size variation within populations.

One way that asymmetric facilitation may arise in our study is through correlations between offspring size and other traits that determine resource supply and demand. In aquatic systems (including our own), the physical structure of organisms can disrupt boundary currents and increase the entrainment of resources – resulting in facilitation (Cardinale *et al.* 2002; Cameron *et al.* 2016). In our study, larger offspring grew into larger colonies that may disproportionately disrupt flow and increase resources. Meanwhile, smaller offspring likely benefit most from resource amelioration because they are less efficient at resource capture, especially in high flow environments (Okamura 1984; Cameron *et al.* 2016). Conversely, larger colonies acquire resources more efficiently (even in high flows: Okamura 1984), such that they may receive less resources when neighbours are present and so experience competition (Cameron *et al.* 2016). Competition was particularly intense between large offspring, suggesting their combined resource requirements exceed the resources they entrain. This highlights an important point – in our study, asymmetric facilitation is the net outcome of interactions, while it is unclear how competition and facilitation truly scale with offspring size (or related traits: e.g. adult size, metabolism). Identifying size-scaling relationships for competition and facilitation is challenging, but would inform the development of more explicit models of offspring interactions.

Our study is the first to demonstrate asymmetric facilitation among offspring, but we suspect this phenomenon may be more widespread. For example, smaller-seeded plant

species experience both the strongest facilitation *and* competition from high densities of heterospecifics (Zepeda and Martorell 2019). While we are unaware of other intraspecific studies similar to ours, we suspect these effects occur in other systems. For example, larger seeds produce greater root biomass and form larger seedlings (Stanton 1984; Leishman 2001) that may disproportionately ameliorate unstable sediments, soil water loss or UV radiation for more vulnerable, smaller seeds. Smaller offspring also perform as well, if not better, than larger offspring when resources are abundant, and these effects can persist at high densities (e.g. in birds: Russell *et al.* 2007; marine invertebrates: Allen and Marshall 2013; Cameron *et al.* 2016; Cameron *et al.* 2017; and plants: Larios and Venable 2018). While asymmetric facilitation appears most applicable for sessile organisms, many mobile species show remarkable variation in offspring size and have sedentary juvenile stages where siblings interact in size-specific ways (e.g. in mobile invertebrates: Kudo 2006; Kamel *et al.* 2010; frogs: Martin and Pfennig 2010; and birds: Krist 2011). That facilitation occurs via different modes (e.g. symmetric, asymmetric) is a relatively new concept in ecology (Lin *et al.* 2012; Schob *et al.* 2014; Lin *et al.* 2016). We therefore encourage further empirical tests to determine the extent to which asymmetric facilitation occurs, its links with offspring size, and its eco-evolutionary consequences.

Given the potential for asymmetric facilitation to be widespread, how might such interactions affect variation in offspring size? One possibility is that size-mediated facilitation generates negative frequency-dependent selection on offspring size (Day and Young 2004). In our study, large offspring performed best in isolation, suggesting they are advantageous when population densities are low and large phenotypes are rare. As the frequency of large offspring increases, however, their performance will decrease due to intra-phenotypic competition, while smaller offspring will initially proliferate due to facilitation, but decline again as large offspring become less abundant. Frequency-dependent selection may thus

generate cycles in the abundance of offspring sizes – but neither large nor small offspring will be competitively excluded, and ultimately both will remain rare. Certainly, frequency-dependent selection generates predictable oscillations in egg sizes in lizards (Sinervo *et al.* 2000) – although asymmetric facilitation was not the driver in this instance. In our study population, small and large offspring were the rarest phenotypes, providing support for our prediction that asymmetric facilitation and frequency-dependence shape offspring-size distributions in nature (Fig. 1b). Based on this distribution of offspring sizes, and assuming that offspring settle in pairs at random, we predict that ~ 40 % of the offspring size pairings in the field would yield facilitative outcomes (Appendix 1; Table 1). Thus, asymmetric facilitation is likely an important driver of offspring-size variation in natural populations.

Game-theoretic models of offspring size were an important step that presciently anticipated frequency-dependent consequences of offspring size (Geritz 1995; Geritz *et al.* 1999). Given our findings, however, several assumptions of these models warrant modification. First, these models exclusively explore the modes of competition, but not the modes of facilitation. Second, our data suggests that resources may vary with the local sizes and densities of offspring (via asymmetric facilitation), while these models keep resource supply constant with offspring size (Geritz 1995; Geritz *et al.* 1999). That offspring size generates small-scale heterogeneity in resources (or other stress) also applies to alternative models of offspring size (Muller-Landau 2010; D'Andrea *et al.* 2013). In these models, larger offspring are more stress tolerant (c.f. competitively superior; Geritz, 1995) and so win more stressful sites, while smaller offspring are more abundant and so win less stressful sites. We provide some support for these models – smaller offspring were advantageous when stress was reduced (albeit through facilitation, rather than fecundity), while larger offspring won when stress was not ameliorated (i.e. neighbour-free conditions). Surprisingly, stress-tolerance models do not consider that facilitation may create spatial heterogeneity in stress

regimes that benefit propagules (Muller-Landau 2010; D'Andrea *et al.* 2013) – despite the well-established links between facilitation and stress-tolerance more generally (Liancourt *et al.* 2005; Zhang and Tielbörger 2019). Given our findings, explicit exploration of positive interactions may greatly extend these theories.

While ecologists have long recognised the importance of positive and negative interactions for natural populations and communities (Bruno *et al.* 2003; Gross 2008), life-history theory has traditionally viewed offspring interactions through the lens of competition (Parker and Begon 1986; Venable 1992; Geritz 1995; Rees and Westoby 1997; Geritz *et al.* 1999). To our knowledge, a single model investigates positive interactions among offspring (McGinley 1989), while no theory explicitly considers size-dependent competition and facilitation – but some models could accommodate these assumptions (Venable 1992). In light of our findings, we propose a novel mechanism that may maintain offspring size variation in natural populations – that is, asymmetric facilitation generates negative frequency-dependent selection on offspring size (Day and Young 2004). Given offspring size mediates facilitation in other systems (Zepeda and Martorell 2019), these ideas warrant further empirical and theoretical exploration.

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Figures

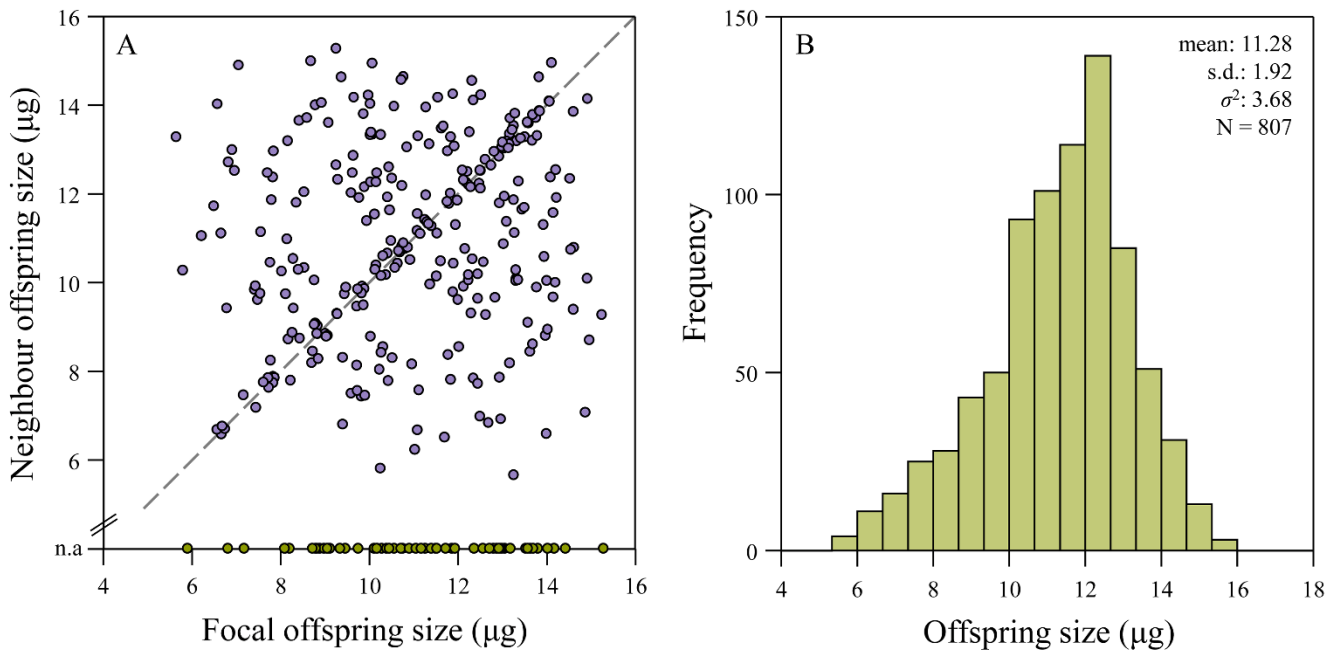


Figure 1. A. Schematic of the trait-specific, response-surface design used to test the effects of offspring size on pairwise interactions between *Bugula neritina* settlers. The purple points show the combinations of focal and neighbour sizes used in pairwise interactions (N = 330), the green points show the sizes of offspring grown without neighbours (n.f.c = neighbour-free controls; N = 56). The grey-dotted line indicates equivalence in size between focal and neighbour offspring. **B.** The natural frequency distribution of offspring-sizes in our source population (Blairgowrie Marina), obtained by measuring > 800 larvae that were spawned from ~ 90 randomly collected colonies.

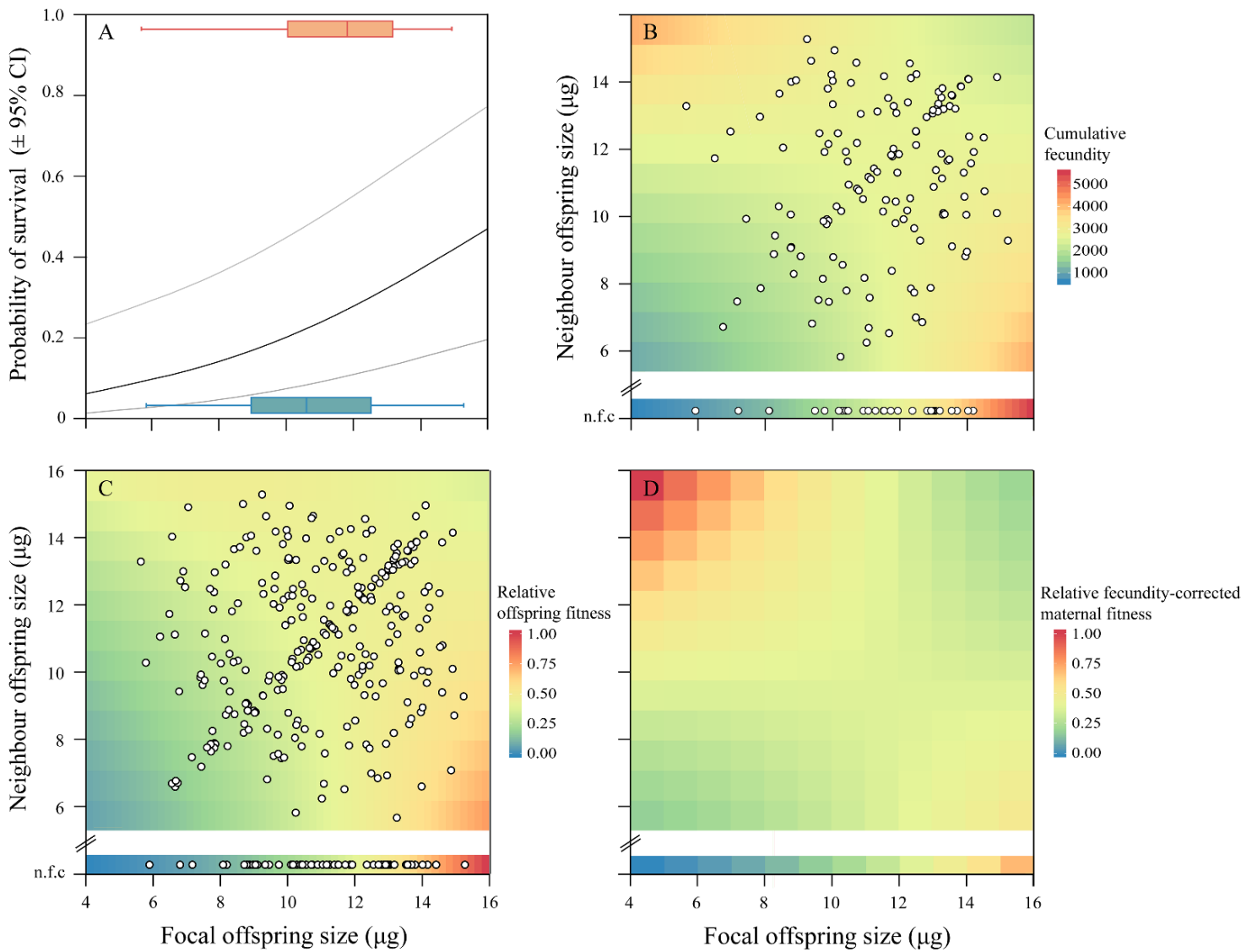


Figure 2. The effects of focal and neighbour offspring size on: **A.** the probability of survival ($\pm 95\%$ CI); **B.** the cumulative reproductive output (no. ovicells); and **C.** predicted offspring fitness (probability of reproduction \times fecundity), of *Bugula neritina* colonies after 11 weeks in the field. **D.** The relative predicted fitness of mothers that produce offspring size of a certain size, accounting for a trade-off between offspring size and number. Boxplots on **A** show the distribution of offspring that survived (red) or died (blue). White dots on **B** and **C** show the underlying data points. N.f.c = neighbour-free controls.

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Appendix 1: Expected frequency of offspring pairings and interaction types in the field

Table 1. The expected frequency (%) of pairwise interactions in the field among *Bugula neritina* offspring of different sizes (μg) given their relative proportion (shaded in grey) in the population. Shading shows the average outcome of pairwise interactions relative to neighbour-free controls in our experiment (green = facilitation: > 30 % increase in performance; yellow = neutral: < 30 % change in performance; red = competition: > 30 % decrease in performance).

| Focal size (μg) | | Neighbour size (μg) | | | | Total % |
|---------------------------------|-------|----------------------------------|--------------|---------------|---------------|---------|
| | | 5.00 - 7.99 | 8.00 – 10.99 | 11.00 – 13.99 | 14.00 – 15.99 | |
| | | 0.070 | 0.327 | 0.545 | 0.058 | |
| 5.00 – 7.99 | 0.070 | 0.48 | 2.27 | 3.78 | 0.40 | 39.66 |
| 8.00 – 10.99 | 0.327 | 2.27 | 10.70 | 17.84 | 1.91 | |
| 11.00 – 13.99 | 0.545 | 3.78 | 17.84 | 29.73 | 3.18 | 54.52 |
| 14.00 – 15.99 | 0.058 | 0.40 | 1.91 | 3.18 | 0.34 | 5.82 |

Chapter 4

Should mothers provision their offspring equally? A manipulative field test¹

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, as well as manipulating sibling density. Overall, more-variable broods had higher mean performance than less-variable broods, suggestive of benefits to 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 current theory.

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Introduction

According to classic life-history theory, mothers within a given environment are expected to maximise their fitness by producing uniformly-sized offspring that optimize the trade-off between offspring size and number (Vance 1973; Smith and Fretwell 1974). Thus, classic theory predicts that offspring size is under stabilizing 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 and 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 and 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 four-fold difference in volume between the smallest and largest 5 % of offspring in a single reproductive bout – a nontrivial 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). As such, when mothers can anticipate local conditions they should produce offspring of a single, optimal size (Marshall and Uller 2007; Fischer *et al.* 2011; Burgess and 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 and 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 and Marshall 2009; Olofsson *et al.* 2009), while others do not except under rare circumstances (McGinley *et al.* 1987; Einum and 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 and Fleming 2002; Koops *et al.* 2003; Marshall *et al.* 2008; Morrongiello *et al.* 2012). Such correlative approaches are understandable, given the extreme difficulty associated with multi-generational observations of fitness 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 reduces arithmetic mean fitness within a generation – a key, and previously untested, assumption of bet-hedging theory.

Another fundamental assumption of bet hedging 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. 1a). In many systems, siblings co-occur across small spatial scales, such that interactions are likely to alter the fitness of these neighbouring offspring (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). Sibling interactions can determine selection on maternal provisioning strategies among females (Parker and Begon 1986; Plaistow *et al.* 2007; Cameron *et al.* 2016; Kamel and 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 and Williams 2016). For example, in plants, theory predicts that small-scale spatial variation in the density of seeds and asymmetric competition that favour of larger seeds will maintain seed-size variation within broods (Geritz 1995; Geritz *et al.* 1999). Theory therefore predicts that within-brood variation may be adaptive when 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 and 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 and Pfennig 2010; Davis and 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 lifetime, a good proxy for lifetime fitness. As far as we are aware, ours is the first study to manipulate variance in the size of siblings and investigate 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 due to interactions among siblings; and 3). Density-dependence (i.e. brood size) alters the fitness consequences of within-brood variation in offspring size. To determine the processes (e.g. selection, competitive asymmetry, niche partitioning) through which within-brood variation may affect maternal fitness, we also investigated the performance of individual offspring within our experimental broods.

Materials and methods

Study species

Bugula neritina (hereafter *Bugula*), is a bryozoan common to sessile marine communities worldwide and is extensively used as a model for studies of life-histories (Wendt 1998; Allen and Marshall 2013; Pettersen *et al.* 2015; Cameron *et al.* 2016). *Bugula* grows by asexual budding of zooids to form branched, arborescent colonies. Colonies are simultaneous hermaphrodites and fertilization 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 and Zimmer 1975). Larvae are brooded for up to one week, after which broods of fully developed larvae are released into the water column. In *Bugula*, larvae can vary considerably in size, both within and across populations (Marshall *et al.* 2003; Kosman and Pernet 2011). Estimates of within-brood CVs in larval volume range between 6.7-14.5 %, and accounts for 54 % of the variance in offspring size observed in some populations (Kosman and Pernet 2011).

In *Bugula*, 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 and Marshall 2011). Siblings preferentially aggregate at settlement relative to unrelated larvae in the laboratory (Keough 1984; Aguirre *et al.* 2013), and are likely to co-occur across small-spatial scales in the field. Multiple lines of evidence suggest that siblings interact in the field after settlement, and that offspring size can determine the outcome of such interactions (Burgess and Marshall 2011; Aguirre and Marshall 2012; Aguirre *et al.* 2013; Cameron *et al.* 2016). In this species, interactions involve both exploitative and interference competition. For example, neighbours may directly compete for food (Svensson and Marshall 2015), or may 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). *Bugula* 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

We collected reproductive *Bugula* colonies from Altona Pier, Victoria, Australia (37°52'26.6"S, 144°49'00.5"E) during December 2015. To obtain larvae for our experiments, we collected mature colonies from the field and held them within dark, aerated aquaria at 17°C for 2 days. We induced the colonies to spawn their brooded larvae by placing them in individual beakers of seawater and exposing them to bright light (Marshall and Keough 2003). As the colonies had been fertilised in the field, we only knew the maternal identity of offspring. Multiple paternity is common in sperm-casting marine invertebrates (Johnson and Yund 2007), but has not been demonstrated in bryozoans. Nevertheless, multiple paternity is

likely in *Bugula*, such that all offspring spawned from a single colony were at least half siblings, although some were likely full siblings.

To measure offspring size, we photographed each larva with a digital camera (Moticam 10 MP; Motic, Hong Kong, China) mounted on a dissecting microscope at 100 x magnification. As per standard techniques, we photographed the larvae when their ciliary groove was facing directly upwards (Marshall and Keough 2003). We measured the length (μm) of this groove using image analysis software (IMAGEJ, v.1.47; Bethesda, Maryland, USA), then converted larval length to larval mass (μg) using an equation that describes this relationship (Pettersen *et al.* 2015). We settled the larvae (following measurement) onto individual pre-roughened, biofilmed acetate squares. We measured and settled all larvae within three hours after they were released from the parental colonies to avoid delayed settlement affecting larval quality (Wendt 1998). For each parental colony ($n = 6$), we measured and settled between 250 and 300 larvae within 24 hours. In *Bugula*, 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, settling larvae over a 24 hr period avoided confounding the effects of larval size and developmental stage on performance.

We systematically assigned our settlers of known larval size to experimental ‘broods’ of siblings that had the same mean ($\mu = 12 \mu\text{g} \pm 0.053 \text{ s.d.}$), 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 %; $\mu = 2.71 \% \pm 1.10 \text{ s.d.}$), and more-variable broods (CV: 18.84 - 39.17 %; $\mu = 24.80 \% \pm 4.07 \text{ s.d.}$). In our study population, the natural range of within-brood CVs for larval mass ranges between 10.45 – 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 x 5.5 cm) at one of four densities (2, 4, 6 and 9 settlers 25 cm⁻²), which reflect natural densities in the field (Allen *et al.* 2008; Burgess and 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 to distinguish our experimental settlers from field recruits. We removed any non-experimental settlers (both *Bugula* 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 x 55 cm) that we hung 1 m below the water surface. 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 two-week period, such that panels combine family-level, spatial and temporal variation. Intraspecific competition typically occurs at the scale used in our study (plates), and density effects beyond this are undetectable (Hart and

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 colonies on the plates, while absent colonies were considered dead. We measured colony size as the number of bifurcations along the longest branch, a good proxy for colony biomass in this species (Keough and Chernoff 1987). Colonies can live for several months in our study region (Pettersen *et al.* 2016), but in our study, most colonies were killed by a heatwave after 9 weeks. Thus, our performance estimates represent a good proxy for the lifetime fitness of our individuals.

Statistical Analyses

How does within-brood variation affect (mean) brood-level performance?

Our first aim was to test the effects of within-brood variation and sibling density on the overall performance of our experimental broods – a proxy for maternal fitness. To estimate brood-level performance, we calculated a composite measure of aggregate performance by multiplying the mean survival (as proportion) and mean growth (bifurcations) of offspring within each brood (i.e. plate) after 8 weeks in the field. Note that separate analyses for growth and survival showed that these performance measures did not trade-off against one another (i.e. within-brood variation tended to positively affect growth and survival; see Appendix 1; Table 1). We analysed this composite measure of performance in a linear-mixed model, where within-brood variation in offspring size (as CV) and sibling density were continuous fixed effects. We (appropriately) included experimental run (that combines parental identity, date of deployment, and panel) as a continuous random effect in

these models, because we detected a systematic ordinal signal of run on performance (Bell and Jones 2014). We first fit full models, and reduced these where appropriate by removing non-significant interactions (assessed as $p > 0.25$ for random effects; and $p > 0.05$ for fixed effects; Quinn and Keough 2002). We performed all analyses in SYSTAT v.13 (San Jose, California, USA) and assessed model assumptions using diagnostic plots (these were met in all cases).

How within-brood variation affect the performance of individual offspring?

We found that greater within-brood variation in offspring size increased brood-level performance (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) an amelioration of competition in more variable, relative to less variable, broods. We consider the first two processes as somewhat indicative of selection on offspring size and(or) asymmetric competition, whilst the third would be suggestive of size-mediated facilitation or niche partitioning.

To disentangle which of the above processes may contribute to our brood-level results, we first analysed the relationship between offspring size and the performance of individual offspring within our experimental broods using two approaches. For our first approach, we analysed the effects of absolute offspring size on the absolute performance of offspring (absolute model). For our second approach, we analysed the effects of relative offspring size, and relative offspring fitness, within a given brood to account for the nested structure of our data (relative model). For this second analysis, we relativised both offspring size and fitness according to the maximum values for each brood. We again analysed a composite measure of performance (the product of survival and growth) for each offspring

after 8 weeks in the field, but note that independent analyses on survival and growth yielded qualitatively similar results. For these analyses, we only included data for those broods where within-brood variation (CV) exceeded 15 %, because otherwise, variances were too low to examine performance across a sufficient range of offspring sizes. We note, however, that these results were unchanged when we included all the broods. For all models, (absolute or relative) offspring size and sibling density were continuous fixed effects, experimental run was a continuous random effect, and we again reduced these models where appropriate.

We also compared the minimum and maximum sizes of colonies in our experimental broods after 8 weeks in the field. For these analyses, we used linear-mixed models where within-brood variation in offspring size (as CV) and sibling density were continuous fixed effects, and experimental run was a random effect. We excluded all plates where only a single survivor remained after 8 weeks, because these survivors were both the minimum and maximum performers. We also analysed the variation in colony sizes (as standard deviation and CV) produced by offspring within our experimental broods using linear-mixed models with the same model structure above. Again, we excluded plates with single survivors because there was no variance in colony size to estimate.

Results

Within-brood variation increases (mean) brood-level performance

More-variable broods had higher mean performance (calculated as survival x growth) than less-variable broods ($F_{1,74} = 14.201$, $p < 0.0001$; Fig. 2) after 8 weeks in the field, and these benefits were consistent across sibling densities (CV x density: $F_{1,73} = 0.004$, $p = 0.953$; excluded from final model). Note, however, that the benefits of within-brood variation differed in magnitude across runs (CV x experimental run interaction: $F_{1,74} = 17.64$, $p < 0.0001$). Within-brood variation strongly and positively affected brood-level performance in

earlier runs, but weakly affected performance in later runs (where performance was relatively lower overall). Given that run combined parental identity, panel and date of deployment in the field, all these factors could contribute to the interaction between CV x experimental run, and we cannot disentangle their influence here. Sibling density did not affect the mean performance of our broods, suggesting that sibling interactions were not density dependent ($F_{1,74} = 0.763$, $p = 0.385$; Fig. 2).

Within-brood variation enhances the performance of individual offspring

Neither absolute, nor relative, offspring size affected offspring performance (absolute offspring size: $F_{1,202} = 0.690$, $p = 0.407$; relative offspring size: $F_{1,203} = 2.525$, $p = 0.114$). Sibling density also had no effect on the performance of individual offspring in either analysis (test for sibling density from the absolute model: $F_{1,202} = 0.807$, $p = 0.370$; from the relative model: $F_{1,203} = 0.238$, $p = 0.627$). In contrast, we found that more-variable broods produced colonies with larger minimum ($F_{1,41} = 4.439$, $p = 0.041$) and maximum ($F_{1,40} = 9.594$, $p < 0.004$) colony sizes, suggesting that extremes in offspring performance were enhanced in more-variable broods. Interestingly, maximum colony size was also positively affected by sibling density ($F_{1,40} = 6.736$, $p = 0.013$) – suggesting that higher sibling densities facilitated the best performing individuals. More-variable broods also had larger standard deviations for colony size ($F_{1,43} = 4.076$, $p = 0.05$), but within-brood variation did not affect the coefficient of variation for colony size ($F_{1,60} = 2.342$, $p = 0.131$).

Overall, the above analyses suggest that offspring size did not strongly affect offspring performance in more-variable broods. Thus, benefits of within-brood variation on (mean) brood-level performance were not driven by the over-representation of colonies from larger, better-performing offspring, nor by the purging of smaller, poorer-performing offspring that released more-variable broods from density dependence. Instead, the best and

worst performing offspring in more-variable broods obtained larger colony sizes than the best and worst performers in less variable broods. Taken together, these findings suggest that all offspring (regardless of initial size) performed better in more-variable broods – driving the positive effects of within-brood variation on brood-level performance (summarised in Fig. 3).

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 and Williams 2016). According to bet-hedging arguments, mothers that produce more-variable offspring suffer reduced arithmetic mean fitness 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 *Bugula neritina* siblings. We therefore show that within-brood variation can increase maternal fitness within a generation, in contrast to bet-hedging assumptions. Furthermore, we found that within-brood variation enhanced the performance of all offspring (irrespective of their size). Thus, within-brood variation 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 are enhanced in polyculture relative to their performance in monoculture (Loreau and Hector 2001). Here, we suggest that complementarity effects may extend to broods of siblings with greater variation in offspring size. We acknowledge, however, that our design precludes formal tests of complementarity (*sensu* Loreau and Hector 2001). Nevertheless, our results support complementarity effects in several ways. First, the offspring

size-performance relationship is well resolved in *Bugula*. 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. In addition, 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 and 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) and there is some indirect evidence that larger offspring (that typically form larger colonies) may consume larger particles than smaller offspring (Okamura 1990; Kosman and Pernet 2011). Second, we found that more-variable broods produced colonies of more-variable sizes, such that the tips of these colonies would occur at different heights in the water column (Fig. 3). 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 facilitation among siblings (e.g. buffering of 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 small-scale spatial variation in seedling density, and asymmetric competition in favour of larger seeds can select for within-brood variation (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 and Pfennig 2010; Davis and Marshall 2014). For example, differential maternal investment in egg size among females induces resource-use polymorphisms in (unrelated) tadpoles – tadpoles from larger eggs became carnivores while tadpoles from smaller eggs became herbivores (Martin and 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. Importantly, this prediction has some empirical support. In both fish and plants, mothers in lower resource environments

produce more-variable offspring (Halpern 2005; Crean and Marshall 2009). In neither of these previous studies were 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 and 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 siblings and this may explain our results (Aguirre and 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, while alternative theory considers the role of sibling competition (Geritz 1995; Geritz *et al.* 1999; Kamel and Williams 2016). Here, we find benefits of within-brood 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 variation in this, or other, systems. Rather, we suggest offspring size-mediated niche partitioning as a novel explanation for why mothers may unequally provision their offspring, which may also apply to other systems (Martin and 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.

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Figures

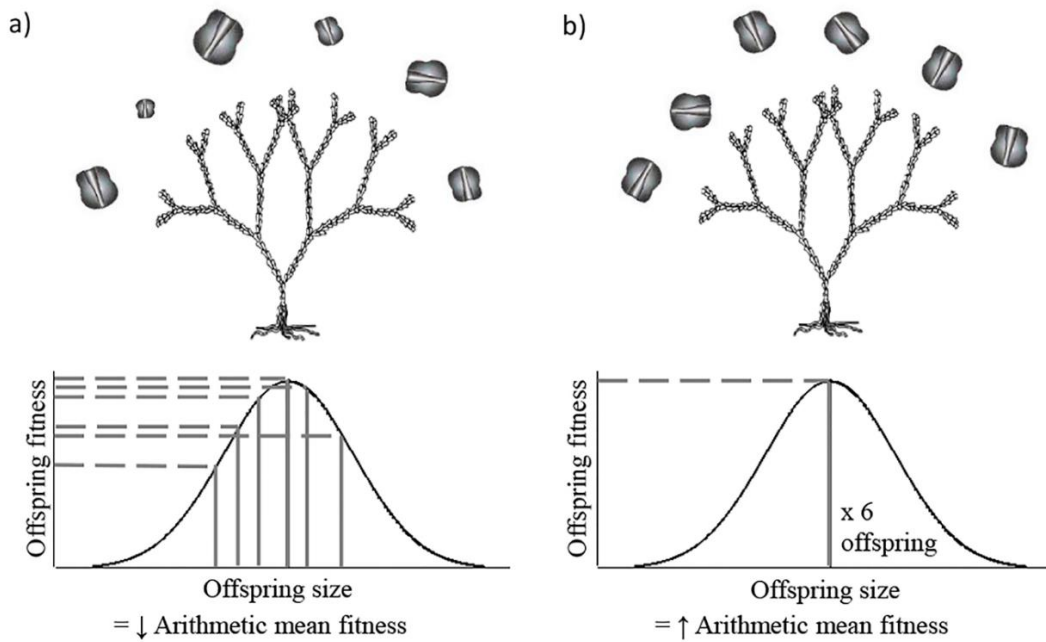


Figure 1. Schematic showing the predictions of bet-hedging theory, whereby in constant and/or predictable environments (a) mothers that unequally provision their offspring will produce more offspring that deviate from the optimal size, and thus have lower arithmetic mean fitness, compared to (b) mothers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

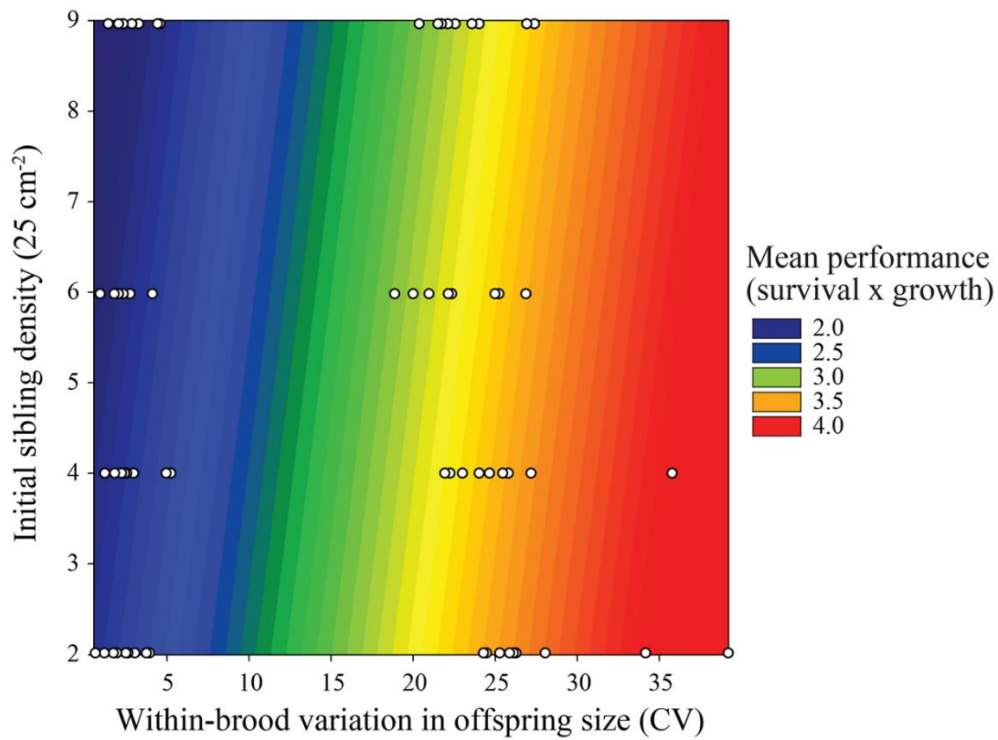


Figure 2. Estimates of the relationship between within-brood variation in offspring size and sibling density on the performance (calculated as survival x growth; side bar) of experimental broods of *Bugula neritina* siblings after eight weeks in the field. Data points (white circles) show the raw data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

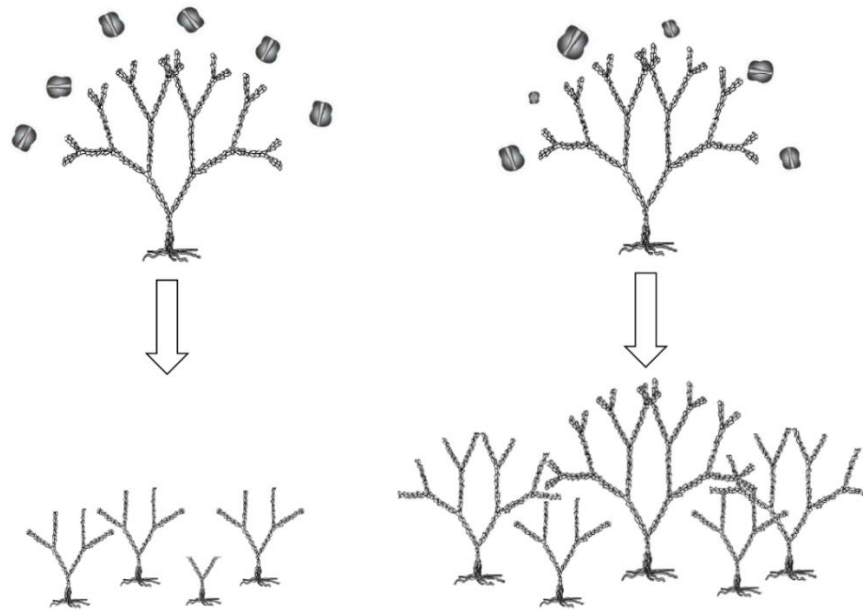


Figure 3. Schematic summarizing the effects of within-brood variation in offspring size on the performance of experimental broods of *Bugula neritina*. Broods with greater offspring-size variation had higher mean performance (for both survival and growth; see Appendix 1) and larger variation (standard deviation) in the size of colonies relative to 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 – but note that offspring performance was not correlated with initial offspring size.

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Appendix 1: Effects of within-brood variation on survival and growth

Table 1. (Reduced) linear mixed-models for the effects of within-brood variation in offspring size (coefficient of variation for larval mass; μg) and sibling density (25 cm^{-2}) on the average survival and growth of colonies in experimental broods of *Bugula neritina* siblings after eight weeks in the field.

| Source | Estimate (\pm 95% CI) | df | F | P* |
|------------------------------|--------------------------|----|--------|--------|
| Survival | | | | |
| <i>Fixed effects</i> | | | | |
| Within-brood variation | 0.006 (-0.002, 0.013) | 74 | 1.985 | 0.163 |
| Sibling density | -0.012 (-0.036, 0.011) | 74 | -1.034 | 0.305 |
| <i>Random effects</i> | | | | |
| Run | 0 (0, 0) | 74 | <0.001 | 1.00 |
| Within-brood variation x Run | -0.002 (-0.004, -0.001) | 74 | 6.688 | 0.012* |
| Residual | 0.073 | 74 | | |
| Colony size (Growth) | | | | |
| <i>Fixed effects</i> | | | | |
| Within-brood variation | 0.024 (-0.007, 0.055) | 61 | 2.438 | 0.124 |
| Sibling density | -0.009 (-0.150, 0.132) | 61 | 0.017 | 0.897 |
| <i>Random effects</i> | | | | |
| Run | -0.335 (-0.541, -0.130) | 61 | 10.621 | 0.002* |
| Residual | 0.073 | 61 | | |

* $P < 0.05$

Chapter 5

Multilevel selection on offspring size and the maintenance of variation.

Abstract

Offspring fitness can depend on both the *absolute* size of an offspring (hard selection), as well as its size *relative* to its neighbours (soft selection). When both hard and soft affect individual fitness, then multilevel selection may be operating. Multilevel selection may drive evolution of offspring size at different scales – but empirical explorations are rare. Here, we use a multilevel approach to examine selection on egg size both within and among females using a marine broadcast spawner. We conducted *in vitro* fertilisations using a split-clutch design at two levels of sperm that represented sperm limitation and saturation. We then estimated hard and soft selection on individual eggs, as well as selection on brood-level egg-size traits (means and variances), within these environments using contextual analysis. Sperm availability altered the strength and/or form of hard and soft selection – but within both environments, hard and soft selection were in opposition. Hard selection was stabilising and favoured intermediate eggs; soft selection was directional and favoured eggs relatively larger (under sperm limitation) or smaller (sperm saturation) than the brood mean. Meanwhile, in both environments, brood-level fitness was maximised at an intermediate mean-egg size, and low within-brood variance – but some (albeit weak) correlational selection occurred. We thus identify several (non-mutually exclusive) pathways for the maintenance of offspring-size variation both among and within females. In particular, the context-dependent nature of soft selection, antagonistic multilevel selection, and correlational selection on brood-means and variances should maintain variation in egg size both scales.

Introduction

Life-history theory predicts that mothers maximise their fitness by optimising the trade-off between offspring size and number. Larger offspring generally perform better with respect to characteristics such as fertilization, survival, and growth; whilst smaller offspring are cheaper so mothers can produce them in greater numbers (Smith and Fretwell 1974). Mothers therefore balance the relative benefits of better performing offspring with those of increased fecundity, and this balance depends on the relationship between offspring size and performance. Classic theory thus predicts a single offspring size is optimal in any given environment – in other words, selection on offspring size is stabilising. In nature, however, offspring sizes vary remarkably across time, space, and all scales of organisation (Leishman et al. 2000, Marshall and Keough 2008, Krist 2011, Ronget et al. 2017). For example, within the same reproductive bout (i.e. clutch, litter, brood etc.), the same parent might provision one offspring with five-fold more resources than another (Lips 2001, Turnbull et al. 2006, Marshall et al. 2008). Yet the processes that drive and maintain variability at different biological scales remain unclear (Marshall et al. 2018).

An oversight of classic theory is that offspring fitness is modelled as a function of the absolute size of offspring alone (Smith and Fretwell 1974) – but fitness can depend on both the *absolute* size of an offspring, and its size *relative* to neighbours. In other words, offspring size may be under both hard and soft selection – whereby hard selection is frequency-independent, such that fitness is determined by absolute phenotypic values; while soft selection depends on the frequency of phenotypes in the population, and is determined by relative phenotypic values (Wallace 1975). For example, offspring may compete according to their *relative* size (Geritz et al. 1999, Marshall et al. 2006, Reed et al. 2009). As such, some components of fitness may depend on absolute offspring size (e.g., trade-offs with fecundity:

Smith and Fretwell 1974; predation: Dibattista et al. 2007), whereas other components may depend on relative values of size (e.g. competition; Marshall et al. 2006).

In many taxa, offspring aggregate across spatial scales where interactions are more likely within, rather than among, family groups (Cheplick 1993; Selkoe *et al.* 2006; Veliz *et al.* 2006; Kamel *et al.* 2012; Aguirre *et al.* 2013). In systems with strong kin structure, both individual offspring size, and size relative to other brood members (hard and soft selection, respectively) may determine offspring performance. Soft selection that occurs within defined groups, such as broods or families, is considered a form of multilevel selection – where fitness is determined by both individual and group traits simultaneously (Goodnight et al. 1992). For example, the mean size, degree of variation in size, and(or) number of offspring within a given brood can form important components of the offspring environment and may alter selection on individuals (Parker and Begon 1986, Mock and Forbes 1995, Reed et al. 2009, Cameron et al. 2017). Multilevel selection on offspring size is likely widespread, and may have important implications for the evolution of offspring size at different levels of organisation. For example, selection at the level of groups (i.e. broods) may oppose or augment selection on individuals, and populations can evolve in response to selection at both levels simultaneously (Wade 1977, Goodnight et al. 1992, Agrawal et al. 2001). In particular, antagonism between group and individual selection can reduce the efficacy of selection at any given level – providing a pathway for the maintenance of phenotypic variation (Goodnight et al. 1992, Weinig et al. 2007). Despite the importance of these effects, however, most studies estimate hard selection on offspring size (e.g. see data in Rollinson and Rowe 2015), while explicit considerations of multilevel selection are much rarer (but see Reed et al. 2009, Johnson et al. 2011, Bouwhuis et al. 2015).

We investigate multilevel selection on egg size in a marine broadcast spawner (external fertilisers) – a group that has long been the subject of evolutionary theory in regards

to gamete size evolution (Parker et al. 1972, Bode and Marshall 2007, Henshaw et al. 2014, Parker and Lehtonen 2014). We focus specifically on multilevel selection on female gametes (eggs) though classic theory has traditionally emphasized hard selection on eggs and soft selection on sperm (Immler and Otto 2018). Most fish and marine invertebrates reproduce via this ancestral mode of reproduction (Monro and Marshall 2015), where gametes are spawned directly into the water column and must then meet and fuse for fertilisation to occur. External fertilisation is fraught, and over half the eggs from a single clutch often go unfertilised, generating opportunity for selection on gamete traits (Levitan and Petersen 1995).

In broadcast spawners, egg size and sperm availability determine fertilisation success. Smaller eggs are smaller targets and typically encounter sperm less frequently, while larger eggs suffer a greater risk of unviable (polyspermic) fertilisations by multiple sperm (Levitan 1993, Marshall et al. 2002). As such, selection on egg size is ultimately stabilising and mostly driven by the absolute size of eggs (hard selection). Sperm availability can, however, shift optimal values of absolute egg size (Levitan 1993). For example, when sperm are abundant, directional selection typically favours smaller eggs that best avoid polyspermy, while non-linear selection strongly penalises variance (Marshall et al. 2002, Millar and Anderson 2003). As sperm become increasingly limited, however, directional selection tends to shift in favour of larger eggs that are larger targets for sperm, while non-linear selection may weaken – suggesting some variance in egg size may be tolerated as sperm availability declines.

Egg size has traditionally been thought to be under hard selection at fertilisation (Parker et al. 1972), but it is also conceivable that the phenotypic context of neighbouring eggs may determine selection on egg size. Certainly, eggs compete for sperm during external fertilisation (Marshall et al. 2004, Marshall and Evans 2005b, Okamoto 2016), and often remain concentrated near females for extended periods of time after they are released (Yund and Meidel 2003). Given that egg size determines fertilisation kinetics, one can easily

imagine eggs of different sizes differentially affecting the local sperm environment to alter the fertilisation success of other eggs. Egg size may therefore be subject to multilevel selection in which fertilization success depends on both the absolute size of a focal egg (hard selection), as well as the size of that egg relative to the mean size of others in the brood (soft selection). Furthermore, the relative importance of individual and soft selection may vary with ecological factors, for example, the availability of resources (Donohue 2004). In particular, hard selection is expected to predominate when resources are abundant, while group (i.e. soft) selection is often relatively more important when resources are limited and individuals are forced to interact. In broadcast spawners, sperm availability may thus alter patterns of soft selection on egg size, but this remains to be tested.

Here, we estimate multilevel selection on egg size at fertilisation for a marine broadcast spawner. We used a split-clutch design to conduct *in vitro* fertilisations on natural clutches of eggs by orthogonally exposing each clutch to two levels of sperm availability representative of sperm limitation and saturation. We then measured the fertilisation success (fitness) of eggs within the clutches. We partitioned fertilisation success into components of both hard and soft selection using a modelling approach analogous to contextual analysis (Goodnight et al. 1992, Johnson et al. 2011). Contextual analysis extends the classic multiple regression approach of Lande and Arnold (1983) to include the effects of group-level traits (in our case, brood mean-egg size) on fitness, as well as accounting for the hierarchical (e.g. kin) structure of natural populations (Goodnight et al. 1992). We then used our estimates of hard and soft selection at the (individual) egg-level to explore the fitness consequences for broods as a function of brood-level egg-size traits (means and variances). Our experimental design and analytical approach thus allowed us to explore selection on egg size at two levels of organisation simultaneously: within-broods of the same female (i.e. family groups) and among the broods of different females.

Materials and methods

Study species and field collection

Galeolaria caespitosa (henceforth *Galeolaria*) is an intertidal, broadcast-spawning serpulid polychaete from southern and eastern Australia (Halt et al. 2009). *Galeolaria* is used extensively as a model in studies of selection on gamete size and egg competition (Marshall and Evans 2005b, Johnson et al. 2013, Monro and Marshall 2016). Individuals live in calcareous tubes that occur as mixed-sex clusters. Local densities can vary from sparse individuals to dense aggregations and some studies suggest spawning is locally synchronous (Kupriyanova 2006). As such, eggs encounter a wide range of sperm concentrations in the field, from saturating (or even polyspermic) when females and males spawn adjacently, to sperm-limited when they are separated by a few centimetres or more (Hollows et al. 2007). Such variability in sperm availability can function as agent of selection on egg size, and egg size often respond to this selection imposed by sperm (Levitan and Petersen 1995).

We regularly collected clusters of *Galeolaria* from St. Leonards pier, Victoria, Australia (38° 17'S, 144° 71'E) from May to June 2017. We stored these clusters at 18 °C in aerated, unfiltered seawater for up to five days before we used them for our experiments. During this holding period (< 6 days), we fed the clusters commercially prepared phytoplankton (Seachem Reef Phytoplankton), and changed their water every two days. To induce spawning, we removed the worms from their tubes and placed them into individual petri dishes with filtered seawater (0.22 µm). To minimize the effects of gamete aging on fertilization success, we used the eggs and sperm within 1.5 hr and 30 min after release from the adults, respectively (Kupriyanova 2006).

Experimental design

To estimate multilevel selection on egg size, we conducted *in vitro* fertilizations on natural clutches of eggs spawned from multiple females. We used a split-clutch design, whereby each female's clutch was divided into four subsamples, of which two were exposed to a 'low' sperm concentration (10^4 sperm mL^{-1}) and two that were exposed to a 'high' sperm concentration (10^5 sperm mL^{-1} ; Figure 1). These sperm concentrations yield fertilisation rates consistent with sperm limitation (~ 30% fertilisations) and saturation (~90 % fertilisations), respectively, and are well within the range of sperm that eggs naturally encounter in the field (Levitan and Petersen 1995, Hollows et al. 2007). We repeated the experiment across five experimental runs (6-8 females per run). For each run, we used sperm pooled from 10 males (50 males in total) to eliminate compatibility effects (Marshall and Evans 2005a). For each experimental run, we first standardized the concentration of pooled sperm, and then performed a series of 10-fold dilutions to achieve our desired concentrations (10^4 and 10^5 sperm mL^{-1}). Sperm concentrations were thus identical, but paternal identities differed, among runs. Overall then, our experiments included clutches from 34 females, which we split between two replicate fertilisations within each of two sperm environments – yielding a total of 136 replicate fertilisations and ~ 17,000 eggs.

In vitro fertilizations were conducted in 48 well plates at 20 °C. For each clutch, we first standardized the concentration of eggs to 2.5×10^3 eggs mL^{-1} such that differences in fecundity among females did not confound our measures of fertilisation success (Vogel et al. 1982, Luttikhuisen et al. 2004). For each replicate fertilisation (egg subsample), we then exposed 0.1 mL of eggs (~ 125 eggs) to 0.9 mL of the desired sperm concentration in a single well of the well plate. We randomised the position of each subsample of eggs, and the sperm treatment to which they were exposed, across the well plate. We included an additional subsample of eggs from each clutch as a negative control for errant fertilisations by adding

0.9 mL of filtered seawater (instead of sperm) to 0.1 mL of control eggs. If control eggs showed > 10% fertilizations, we excluded all replicates from that female's clutch.

Two hours after we exposed the eggs to sperm, we assessed the fertilisation success of eggs within the clutches. To do this, we took photographs of each replicate fertilisation (each well) using a digital camera attached to an inverted compound microscope (Olympus 1x73; x10 magnification). For each replicate, we scored ~100 eggs as fertilised or unfertilised and measured their diameter (μm) using Olympus CELLSSENS DIMENSION software. We scored eggs as fertilised if they had successfully cleaved without evidence of polyspermy, or not fertilised if they had not cleaved or had cleaved irregularly (a sign of polyspermy). We estimated the initial (unfertilised) size of fertilised and unfertilised eggs using equations that describe these relationships (initial egg size = $a + bx$; for fertilised eggs (x): $a = 33.24 \pm 3.77$ s.e.m., $b = 0.46 \pm 0.06$ s.e.m.; unfertilised eggs (x): $a = 5.59 \pm 5.13$ s.e.m., $b = 0.902 \pm 0.08$ s.e.m). Note that we did not include immature eggs released during strip spawning, nor eggs that had cleaved irregularly, in our measurement of egg size and fertilisation success. Importantly, our estimates of brood-means after fertilisation were comparable to those obtained from photographs of each female's clutch taken prior to fertilisation.

Statistical analyses and modelling approach

Analysing variation in fertilisation success and the opportunity for selection between sperm environments

Prior to formally modelling multilevel selection, we explored whether selection on egg size differed with sperm availability by using a sequential model building approach (outlined in Chenoweth and Blows 2005). We used binomial GLMs with a logit link function, where fitness (fertilisation success) was a binary response variable (0 = not fertilised, 1 = fertilised) and (unstandardized) initial egg size (linear and quadratic forms) and sperm

environment were included (where appropriate) as continuous and categorical predictors, respectively. In all cases, we compared model fits using log-likelihood ratio tests. We also tested for differences in the opportunity for selection (I) between sperm environments by comparing the variance in relative fertilisation success (Crow 1958, Arnold and Wade 1984) in each environment using Bartlett's Test for equal variance.

Modelling multilevel selection:

Fertilisation success and the opportunity for selection differed among sperm environments (see Results). We therefore estimated components of both hard and soft selection within each environment using the approach outlined in Johnson et al. (2011). As in other contextual models of multilevel selection (Goodnight et al. 1992), we modelled hard selection as a component whereby individual fitness depends on *absolute* egg size, and soft selection as a component in which fitness depends on *relative* egg size (as deviations from the brood-mean). By including a component of fitness that depends on deviations from the brood-mean egg size we describe frequency-dependent interactions among eggs within a clutch, where the fitness of a focal egg of size z' is a linear function of the difference in size between the focal egg and another egg it interacts with (z). We then integrate these effects across the distribution of egg sizes within the population, such that we describe the net effects of these phenotype-dependent interactions as:

$$\int \varepsilon(z' - z) p(z) dz = \varepsilon(z') \int p(z) dz - \int zp(z) dz = \varepsilon(z' - \bar{z}).$$

Note that this approach could be modified to include a component of fitness that depends on both the frequency ($p(z)$) and density (N) of eggs explicitly. For example:

$$\int \varepsilon(z' - z) Np(z) dz = \varepsilon N(z' - \bar{z}).$$

However, our experimental design held the number of eggs constant among trials, and the results we present thus describes frequency-dependent selection at a constant density of eggs. Nevertheless, egg-egg interactions for sperm are density-dependent in broadcast spawners (Okamoto 2016). Interactions among eggs are thus likely to be both frequency- and density-dependent (i.e. soft selection *sensu stricto*), and future experiments could explore these processes simultaneously.

Because both hard and soft selection can take several functional forms (i.e. directional, concave or convex), we first fit relatively flexible and complex models to the data and compared their fit to simplified models using AIC. In both environments, hard and soft selection were best represented by quadratic and linear logistic functions, respectively. As such, we modelled egg-size specific fitness (fertilisation success) as:

$$W(z) = \left(\left[\frac{1}{1 + e^{-(\alpha + \gamma(z - \beta)^2)}} \right] \times \left[\frac{1}{1 + e^{-(\delta + \varepsilon(z - \bar{z}))}} \right] \right).$$

The first term represents hard selection and describes the expected (average) probability that an egg of a given size (z) is fertilised, regardless of the frequency of other egg sizes in the brood. The second term represents soft selection and describes how the expected probability of fertilisation is modified by the relative size of a focal egg within the brood. $W(z)$ was fit to the data via maximum likelihood estimation and parameter values were estimated using the package *bbmle* in R (Ben Bolker and R Core Development Team 2017). Importantly, in both sperm environments, models that included components of both hard and soft selection provided a much better fit than models that described hard selection alone (evaluated via AIC; Table 1).

We then used our estimates of $W(z)$ to explore selection on brood-level egg-size traits (means and variances) arising from hard and soft selection on egg size within broods. To do

this, we calculated the average fitness (as a proportion of fertilised eggs within the clutch) of a given female in each sperm environment by integrating our estimates of $W(z)$ with phenotypic distributions of egg sizes. For simplicity, we assumed normal egg-size distributions for each brood, with mean and variance specified by (\bar{z}_i) and $\text{var}(z)_j$, respectively – a valid assumption, given that natural clutches from our population were largely normally distributed. We explored the fitness surface of females across a range of plausible values for means and variances. In particular, we calculated mean fitness ($\bar{W}_{i,j}$) for all combinations of means (i) and variances (j) across the observed ranges (means: 60 to 66; variances: 0.2 to 3.6; each evaluated at intervals of 0.05). We calculated the average fitness of a given female (i.e., for a given mean-variance combination) as:

$$\bar{W}_{i,j} = \int p(z|\bar{z}_i, \text{var}(z)_j)W(z)dz$$

Where $p(z|\bar{z}_i, \text{var}(z)_j)$, describes the distribution of egg sizes for a specified mean and variance. Other symbols are described above.

Results

Variation in fitness (fertilisation) and opportunity for selection between sperm environments

Overall, average fertilization rates depended on the availability of sperm ($\chi^2 = 1821.72$, $df = 1$, $p < 0.0001$) – unsurprisingly, the proportion of fertilised eggs was highest under sperm saturation (0.888 ± 0.336 s.d) relative to sperm limitation (0.315 ± 0.464 s.d). Preliminary analyses also indicated that linear ($\chi^2 = 31.605$, $df = 1$, $p < 0.0001$) and quadratic ($\chi^2 = 15.07$, $df = 1$, $p < 0.0001$) selection on absolute egg size differed between sperm environments. Variance in the relative fertilisation success of eggs also differed ($\chi^2 = 5415.607$, $df = 1$, $p < 0.0001$) – that is, the opportunity for selection (I) was greatest in the low ($I = 2.222$) relative to high ($I = 0.119$) sperm environment.

Patterns of hard and soft selection on egg size

In both sperm environments, we found that selection on egg size at fertilisation is captured by components of both hard (i.e. frequency-independent) and soft (i.e. frequency-dependent) selection (Table 1). In both environments, hard selection was best described by a three parameter logistic function (saturation: $\alpha = 3.342 \pm 0.132$; $\beta = 63.79 \pm 0.080$; $\gamma = -0.282 \pm 0.022$; limitation: $\alpha = 0.578 \pm 0.079$; $\beta = 63.76 \pm 0.043$; $\gamma = -0.379 \pm 0.021$) – suggesting that hard selection is strongly unimodal (i.e. stabilising; Fig. 2a & d). Soft selection, on the other hand, was best modelled as a two parameter logistic function – suggesting that soft selection was, on average, directional (saturation: $\delta = -1.473 \pm 0.175$, $\varepsilon = 4.824 \pm 0.397$; limitation: $\delta = 1.437 \pm 0.188$, $\varepsilon = 2.625 \pm 0.387$). Note, however, that the direction of soft selection differed among sperm environments (Fig. 2b&e). When sperm were saturating, soft selection was negative on average – that is, eggs smaller than the brood-mean had relatively highest fertilisation success (Fig. 2b). In contrast, when sperm were limiting, average soft selection was positive – that is, eggs larger than the brood-mean were favoured (Fig. 2e).

Visualisation of combined hard and soft selection revealed that in both environments, fitness-surfaces varied with mean-egg size – i.e. the relative fitness (fertilisation success) of a given egg depended on both its phenotype, and the mean phenotype of the brood to which it belonged (Fig. 2c & f). In other words, we found evidence for multilevel selection on egg size (Goodnight et al. 1992). Notably, under sperm saturation, soft selection disfavoured relatively larger eggs within a brood, and eggs whose size was near the global average (62 – 68 μm) had higher relative fitness when they were part of a brood with a larger-mean size (Fig. 2c). This occurred because eggs within this size range were closer to, or comparatively smaller, than the brood-mean. In addition, within a given brood, the egg size with the highest fertilisation success (i.e. optimal egg size) systematically shifted right with an increase in brood-mean egg size. Because soft selection had a greater influence when mean-egg size was

small, the range of fertilisable-egg sizes were narrower for broods with smaller means. Importantly then, when sperm were abundant both the optimum and width of the fitness surface increased with mean-egg size (Fig. 2c).

When sperm were limiting, fitness surfaces again differed with the mean-egg size of the broods. Overall, fertilisation success was again a unimodal function of absolute egg size (Fig. 2d) but at low sperm concentrations, soft selection disfavoured relatively smaller eggs within a brood (Fig. 2e). Consequently, eggs closer to the global average (63.5 – 64.5 μm) had higher relative fitness when they were part of a brood with a smaller-mean size (Fig. 2f). As mean-egg size increased, the overall fitness surface became laterally compressed, reflecting the greater influence of soft selection. Thus, when sperm were limiting, the fitness optima shifted right with increasing mean-egg size (as under sperm saturation), but the width of the fitness function decreased as mean-egg size increased (c.f. sperm saturation; Fig. 2c & f). Note also that the influence of soft selection was relatively stronger under sperm limitation (c.f. saturation; Fig 2).

Aggregate fitness and selection on brood-level egg-size traits

Selection surfaces of brood-level egg size traits varied between sperm environments (Fig 3). In both environments, selection on brood-mean egg size was stabilising – that is, mothers that produced broods with a mean that deviated from the population average generally suffered reduced fitness. However, the strength of stabilising selection on mean-egg size differed among environments – stabilising selection was strongest in the low sperm environment. Selection on within-brood variation also differed in magnitude among environments. In the high sperm environment, selection on within-brood variation was weak, but overall, variance was disfavoured (Fig. 3a). In the low sperm environment, selection again generally disfavoured larger variances and the fitness surface was steeper along the

variance axis (Fig 3a). Thus, in the low sperm environment, variance was penalised more strongly around the optimum for brood-mean size, relative to the high sperm environment (Fig. 3).

Finally, correlational selection on brood-mean and within-brood variation in egg size differed between sperm environments (Fig. 3). In the high sperm environment, we found no evidence for correlational selection acting on the brood-mean and within-brood variance within the phenotypic range explored (Fig. 3a). In contrast, in the low sperm environment, some negative correlational selection occurred across restricted ranges of parameter space – fertilisation rates were enhanced by high variance in broods with very small mean-egg sizes (60.5 – 61.5 μm), while broods with very large mean-egg sizes (65 – 66 μm) received highest fertilisation rates when within-brood variance was low (Fig. 3b). Nevertheless, the strength of correlational selection in the low sperm environment was weak relative to stabilising selection on mean-egg size. As such, in both sperm environments, fitness was consistently maximised for mothers that produced broods with an intermediate mean-egg size and low within-brood variation (Fig. 3).

Discussion

We found egg size was under multilevel selection – fertilisation depended on both absolute-egg size (hard selection) and egg size relative to the brood mean (soft selection). Sperm availability altered the strength and form of multilevel selection, but within a given sperm environment, hard and soft selection were in opposition. Hard (frequency-independent) selection consistently favoured intermediate eggs (i.e. was stabilising), while soft (frequency-dependent) selection was directional and favoured eggs relatively larger or smaller than the brood-mean under sperm limitation or saturation, respectively. At the brood-level, however, fitness was maximised in both sperm environments when mothers produced clutches with

intermediate mean-, and low variance in, egg size – although some (albeit weak) negative correlational selection occurred between brood-mean and within-brood variance under sperm limitation. Together, these findings have several, previously unanticipated, consequences for the maintenance of offspring-size variation both within and among females.

Our finding that hard selection on egg size was stabilising largely conformed to theoretical expectations – eggs that are too small fail to encounter sperm, while eggs that are too large suffer polyspermy (Levitan 1993, Marshall et al. 2002). In contrast, our detection of soft selection was unanticipated and provides novel evidence that sibling eggs engage in size-specific interactions for sperm. In broadcast spawners, competition for sperm is primarily exploitative – some sperm permanently bind to the egg surface after contact (even if those sperm do not successfully enter the egg), reducing the effective sperm concentration available for other eggs (Marshall and Evans 2005b, Okamoto 2016). The patterns of soft selection that we observe may thus emerge if larger eggs deplete the available sperm pool relatively more than smaller eggs. The idea that larger eggs draw-down more sperm than smaller eggs has some support – certainly, sperm-egg collisions appear to increase (at least) proportionately with egg cross-sectional area (Vogel et al. 1982), such that sperm should bind to larger eggs in higher numbers.

To illustrate, when sperm were saturating, we found that the average nature of soft selection favoured eggs that were relatively smaller than the brood-mean. This could occur if relatively larger eggs removed a higher proportion of sperm, and thus buffered their smaller siblings from polyspermy. This may explain why eggs closer to the global optima fared better when they belonged to a brood with a larger mean-egg size. Meanwhile, when sperm were limiting, soft selection favoured eggs relatively larger than the brood-mean that are able to access more sperm. As such, under sperm limitation, eggs closest to the global optimum had the lowest fertilisation rates when they belonged to a brood with larger mean-egg sizes

monopolised sperm to the detriment of their smaller siblings. Our study thus shows that soft selection can yield unanticipated costs to producing broods of larger eggs when sperm are limiting (c.f. theory: Millar and Anderson 2003) – in essence, larger eggs are more ‘wasteful’ of sperm, robbing their siblings under sperm limiting conditions. Overall then, we show that egg sizes that benefit individuals may be disadvantageous to groups (broods). Such antagonistic multilevel selection can reduce the efficacy of selection at any one level (Goodnight *et al.* 1992, Weinig *et al.* 2007) and may explain why evolutionary responses do not always match the predictions of studies that consider hard selection alone.

We also found that the combined patterns of hard and soft selection resulted in a narrowing (under sperm saturation) or widening (under sperm limitation) of the range of fertilisable egg sizes with an increase in brood-mean egg size. These patterns are suggestive of positive and negative correlational selection between brood-mean and within-brood variation in egg size under sperm saturation and limitation, respectively. Whilst there was evidence for negative correlational selection between brood-means and variances under sperm limitation, we failed to detect positive correlational selection across the range of phenotypic space investigated. This is because under sperm saturation, hard selection had a stronger influence on fitness than soft-selection, such that hard selection mostly contributed to variation in fitness at the brood-level. In contrast, the influence of soft selection was relatively stronger under resource (i.e. sperm) limitation, such that we did detect some (albeit weak) negative correlational selection. These findings are congruent with other studies of group selection, which find the relative importance of soft selection (c.f. hard selection) increases with resource limitation (Aspi *et al.* 2003, Donohue 2003, Weinig *et al.* 2007).

Estimating multilevel selection revealed that the interests of mothers and offspring are misaligned. In both sperm environments, maternal fitness was maximised for broods with an intermediate-mean egg size and low within-brood variation. Meanwhile, offspring fitness was

also maximised at an intermediate egg size – but this depended on the offspring’s context. The performance of intermediate eggs was highest when they belonged to a brood with a larger-mean (sperm saturation) or smaller-mean (sperm limitation) egg size. Importantly then, multilevel selection appears to generate conflict between offspring and mothers over optimal provisioning. Ultimately, the winner of such parent-offspring conflict will depend on the relative heritability of the maternal (mean) or offspring (individual) egg size traits (Wolf and Wade 2001). To date, few studies have estimated heritability at both these levels simultaneously, but this represents an important next step towards integrating the effects of multilevel selection on the evolution of offspring size.

Our study has a number of limitations. Our manipulations investigate egg-egg interactions for sperm within clutches, but in nature, eggs from within and among clutches are likely to interact simultaneously. An important next step would be to investigate whether incorporating higher levels of population structure (i.e. interactions among clutches) alter our estimates of multilevel selection on egg size at fertilisation. Furthermore, our experiments only explored the consequences of multilevel selection at one life-history stage (external fertilisation), because in our species, sibling interactions are less likely after fertilisation once offspring have dispersed and recruited into the population. Future studies could estimate multilevel selection at across the entire life history when offspring interact with groups of siblings or unrelated conspecifics beyond fertilisation.

Within-population variation in offspring size has long perplexed life-history theoreticians. Our study identifies several, non-mutually exclusive, pathways through which offspring-size variation may be maintained both among and within females living in the same population. In particular, our results suggest that frequency-dependent (soft) selection within clutches, antagonistic selection between adults and gametes (Immler and Otto 2018), and negative correlational selection between brood-means and variances may all play a role in

maintaining offspring-size variation within populations. Given that interactions among siblings are ubiquitous (Blaustein and O'Hara 1987, Cheplick 1993, Selkoe et al. 2006, Veliz et al. 2006, Aguirre and Marshall 2012a, Aguirre et al. 2013, Kamel and Grosberg 2013), we encourage future studies of selection on offspring size take a multilevel approach to characterise the full nature of selection on this trait.

Tables

Table 1. Comparison of models of egg-size specific fitness, $W(z)$, described by hard selection alone, or as a combination of hard and soft selection. Note that in both sperm environments, models that include both hard and soft components of selection provide much better fits to the data.

| Model | Parameters estimated | AIC |
|-------------------------|-----------------------------|------------|
| <i>High sperm</i> | | |
| Hard selection only | 3 | 1938.727 |
| Hard and soft selection | 5 | 1828.774 |
| <i>Low sperm</i> | | |
| Hard selection only | 3 | 4130.647 |
| Hard and soft selection | 5 | 3942.271 |

Figures

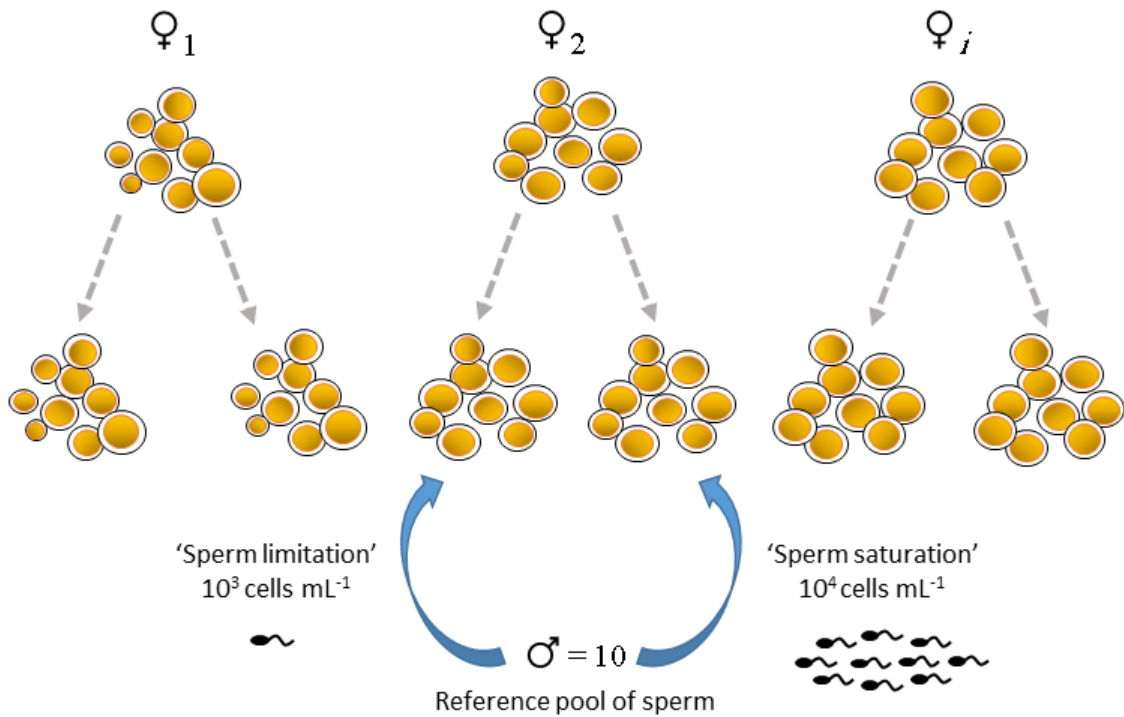


Figure 1. Schematic of the split-clutch design used to examine multilevel selection on egg size within-broods at fertilization for *Galeolaria caespitosa*. Clutches of eggs from 34 individual females were split into four subsamples and exposed to two levels of sperm availability: a high sperm concentration (10^5 cells mL^{-1} ; $n = 2$ per clutch) that reflects sperm saturation; or a low sperm concentration (10^4 cells mL^{-1} ; $n = 2$ per clutch) that reflects sperm limitation. All clutches were fertilised with sperm pooled from 10 males and diluted to the appropriate concentrations.

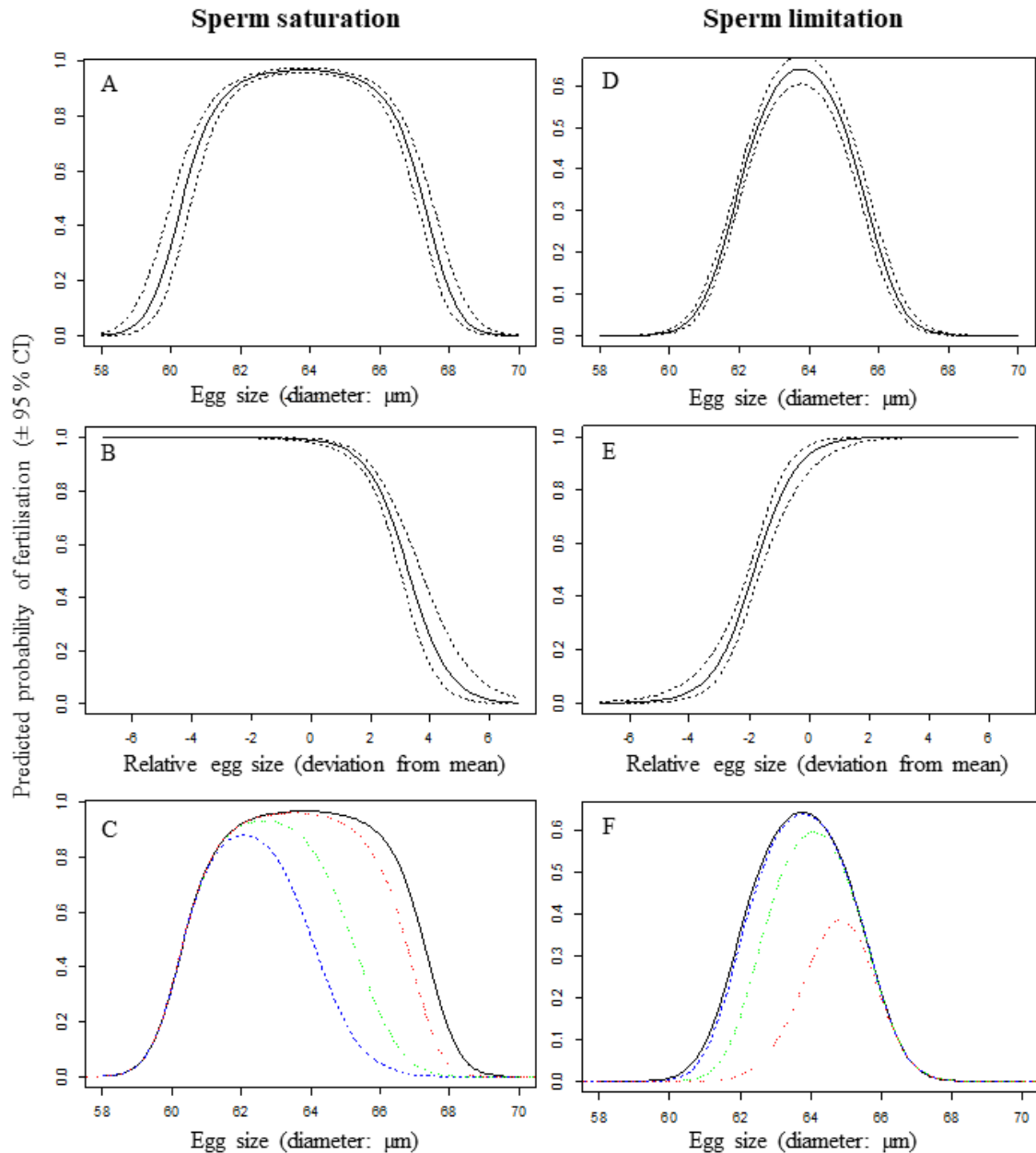


Figure 2. Components of hard and soft selection on egg size at fertilisation under sperm limitation (A-C) and sperm saturation (D-F). A and D depict hard selection, whereby fertilisation success depends on the absolute egg size regardless of neighbour frequency. B and E depict the average nature of soft selection, whereby fertilisation success depends on the size of a given egg relative to the brood-mean egg size. C and F show the combined effects of hard and soft selection for broods with small (mean diameter: 62 μm ; blue line), intermediate (mean diameter: 64 μm ; green line), and large-mean (mean diameter: 66 μm ; red line) egg sizes. Note the change in scale in D and F.

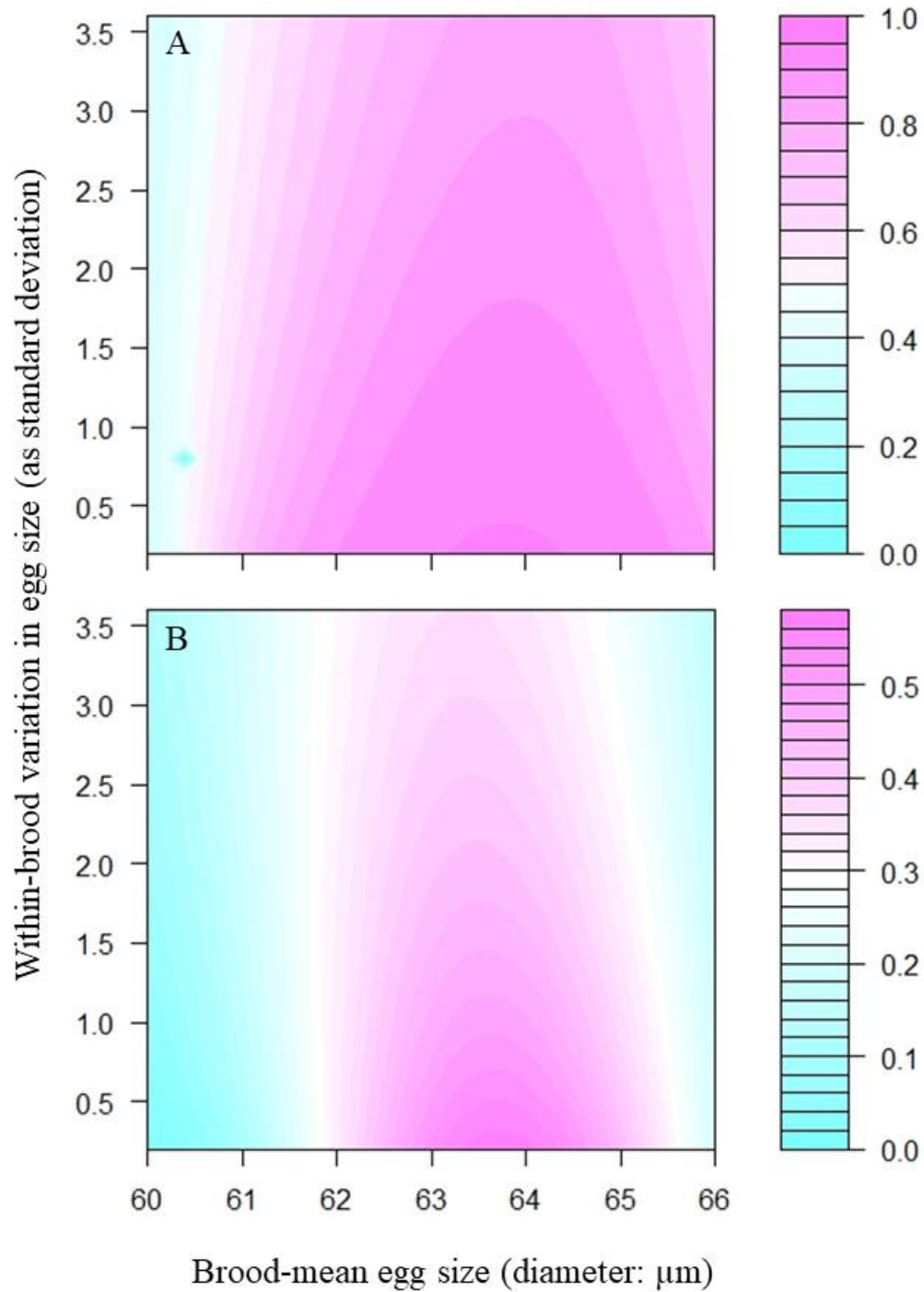


Figure 3. Brood-level fitness (as proportion of the clutch fertilised: sidebars) for females that differ in two brood-level egg-size traits (mean-egg size and variance in egg size) under **A.** sperm saturation, and **B.** sperm limitation, in the broadcast spawner, *Galeolaria caespitosa*. Note the change in scale between the two panels.

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Chapter 6

General Discussion

Biologists have long sought to understand patterns of maternal allocation towards offspring. While theory is extensive, empirical tests of key assumptions lag behind, severely limiting our understanding of the drivers of offspring size variation (Bernardo 1996, Marshall *et al.* 2018). In particular, two fundamental assumptions pervade most offspring-size theory: 1. Larger offspring perform better, such that smaller offspring only provide a fecundity benefit to mothers (Smith and Fretwell 1974); and 2. Larger offspring outcompete smaller offspring for resources (Brockelman 1975, Parker and Begon 1986, Venable 1992, Geritz 1995, Mock and Forbes 1995, Geritz *et al.* 1999). Within this context, my work makes two main contributions to our understanding of offspring size.

First, I test classic theoretical assumptions regarding offspring size and competition and show these are not universal. In particular, I show that larger offspring do not always perform better than smaller offspring – particularly, under scenarios of putative competition. Instead, I find smaller offspring may outperform larger offspring in unanticipated ways. Second, I demonstrate that offspring size-mediated interactions are not always competitive – rather, positive interactions are also possible, and arise through several, non-mutually exclusive, mechanisms including size-dependent facilitation (Chapters 2 & 3), complementarity via niche partitioning (Chapter 4) and frequency-dependent and(or) multilevel selection (Chapters 3, 4 & 5). This thesis thus identifies several alternative pathways that may maintain offspring-size variation, all of which warrant further theoretical and empirical attention. In this discussion, I focus on the two key points mentioned above – but Appendix A identifies several key priorities for future work in the field of offspring size more generally.

Bigger is not always better

Larger offspring are thought to survive, grow and reproduce more than smaller offspring (Marshall *et al.* 2018). There are important exceptions to this general trend however, for example, larger offspring may suffer reduced survival or higher predation rates than smaller offspring (Kaplan 1992, Litvak and Leggett 1992, Gomez 2004, Regnier *et al.* 2013). Nevertheless, theory anticipates that larger offspring should be particularly advantageous under harsh conditions, such as low resource or high-density environments (Brockelman 1975, Parker and Begon 1986, Goulden *et al.* 1987, Venable 1992) – and this is empirically supported in some systems (Berven and Chadra 1988, Hutchings 1991, Einum and Fleming 1999, Marshall *et al.* 2006, Bashey 2008, Dziminski *et al.* 2009). The intuitive appeal of this pattern – that larger offspring are superior when resources are scarce, makes it tempting to assume that it is universal.

This thesis largely contradicts the tenet that larger offspring win contests over smaller offspring. For example, I found that higher densities of siblings actually enhanced the performance of smaller offspring (facilitation), but negatively affected larger offspring (competition; Chapter 2). Similarly, larger offspring most strongly facilitated small offspring, and most strongly competed with other large offspring – indicating asymmetric facilitation (c.f. asymmetric competition; Chapter 3). Within-brood variation also enhanced brood-level (maternal) performance by ameliorating competition for resources among siblings – again, failing to support assumptions of competitive asymmetries (Chapter 4). Finally, larger eggs sometimes buffered their relatively smaller siblings from polyspermy during external fertilisation (Chapter 5). Importantly then, smaller offspring may obtain higher than expected fitness when they interact with siblings (Chapter 2, 4 & 5) and unrelated conspecifics (Chapter 3) – and these advantages arise via positive interactions. Thus, I show that smaller

offspring can provide fitness advantages beyond those of increased maternal fecundity – in contrast to the assumptions of classic theory (Smith and Fretwell 1974).

My findings were not entirely heterodox. For example, in Chapters 2 & 3, larger offspring grown without neighbours achieved the highest performance overall. Findings from Chapter 5 also provide support for the bigger is better hypothesis – relatively larger eggs outcompeted smaller eggs for fertilisations in sperm-limited environments. Yet egg size was under both hard (frequency-independent) and soft (frequency-dependent) selection (i.e. multilevel selection: Goodnight *et al.* 1992) – revealing unanticipated costs associated with broods of larger eggs under sperm limitation (c.f. theoretical predictions: Styan 1998, Millar and Anderson 2003) that arose from the overexploitation of sperm. As such, traits that are beneficial to individuals may be disadvantageous at the brood-level. Antagonistic multilevel selection can reduce the efficacy of selection at any one level (Goodnight *et al.* 1992, Weinig *et al.* 2007) – and may explain why evolutionary responses do not always match predictions from studies that only consider hard selection. Multilevel selection thus represents a previously unappreciated mechanism for offspring-size variation both among and within females. Kin structure and kin interactions are ubiquitous (Blaustein and O'Hara 1987, Cheplick 1993, Selkoe *et al.* 2006, Veliz *et al.* 2006, Aguirre and Marshall 2012a, Aguirre *et al.* 2013, Kamel and Grosberg 2013), thus I encourage future explorations of selection on offspring size take a multilevel approach.

Positive interactions: are they widespread and can they maintain offspring-size variation?

Ecologists increasingly recognise the importance of positive and negative interactions for populations and communities, yet life-history theory almost exclusively views offspring interactions through the lens of competition (Parker and Begon 1986; Venable 1992; Geritz

1995; Rees and Westoby 1997; Geritz *et al.* 1999). Indeed, only a handful of studies have considered positive interactions in explorations of offspring-size. For example, McGinley (1989) predicted that facilitation via predator satiation generates positive correlations between maternal size and offspring size. In cooperative breeders, mothers adaptively reduce the size of their eggs in the presence of helpers that assist with post-natal (e.g. feeding or guarding offspring), because these helpers facilitate smaller offspring to obtain equivalent performance to (initially) larger offspring that are reared without helpers (e.g. in birds: Russell *et al.* 2007; in fish: Taborsky *et al.* 2007). Positive interactions may also maintain variation in offspring size within-broods. For example, egg-guarding insects may lay smaller eggs at the peripheries of their clutches to protect larger, fitter eggs from predation (Mappes *et al.* 1997, Kudo 2001, Kudo 2006). Despite a growing list of examples, however, the eco-evolutionary consequences of positive interactions remain poorly understood – particularly with regard to life-history theory (See also Appendix B).

This thesis provides novel examples of positive interactions among offspring that arise via multiple, previously unanticipated, mechanisms. In light of these findings, I argue that positive interactions play an important, but currently underappreciated, role in shaping patterns of offspring-size variation. Below, I expand on the types of positive interactions observed in this thesis, and review their eco-evolutionary potential.

Size-mediated facilitation

Throughout this thesis, neighbouring offspring facilitated smaller offspring. For example, smaller offspring had higher performance in high-density stands (phenotype-specific density-dependence: Chapter 2) or the presence of larger offspring (asymmetric facilitation: Chapter 3) relative to when smaller offspring were isolated. These findings are unlikely to be restricted to marine invertebrates. For example, in plants, smaller-seeded

species receive the strongest facilitation *and* competition from higher densities of heterospecific neighbours (Zepeda and Martorell 2019). Furthermore, stress-tolerance typically increases with offspring size (Leishman *et al.* 2000, Muller-Landau 2010, D'Andrea *et al.* 2013), such that it is reasonable to suspect smaller offspring are more reliant on facilitation than larger offspring. Conversely, larger offspring may often be the strongest facilitators. For example, larger seeds produce greater root biomass and form larger seedlings (Stanton 1984, Leishman *et al.* 2000) that may disproportionately ameliorate unstable sediments, soil water loss or UV radiation (Lin *et al.* 2012). Therefore, while there are reasons to expect offspring-size mediated interactions are widespread, I encourage further tests to determine their generality and identify their underlying mechanisms.

Theory predicts that interspecific facilitation may have evolutionary consequences for genetic and phenotypic variation within populations (Day and Young 2004, Liancourt *et al.* 2012). In the context of my findings, I suggest that small-scale variation in neighbour density and size generates micro-environmental heterogeneity via habitat amelioration – creating favourable ‘safe sites’ that diminishes selection against smaller offspring. Safe-sites are important components of life-history models that promote offspring-size variation (Geritz 1995, Geritz *et al.* 1999, Muller-Landau 2010, D'Andrea *et al.* 2013), but these models are yet to consider that intraspecific facilitation itself may create them. In light of my findings, I propose that density- and frequency-dependent competition and facilitation among offspring generates negative-frequency dependent selection that maintains offspring-size variation (Day and Young 2004). Future studies should formally explore this possibility using tools from adaptive dynamics, and test these predictions via orthogonal manipulations of offspring sizes and densities.

Facilitation as an eco-evolutionary driver of offspring-size variation raises several avenues for future investigation. For instance, does the relative importance of facilitation vs

competition for regulating patterns of offspring-size differ with environmental stress? Can stress gradients generate local adaptation in facilitative asymmetries – as found for competitive asymmetries along resource gradients (Potter *et al.* 2019)? Do traits evolve to enhance the ability of offspring to benefit from neighbours and/or enhance (or reduce) their ability to provide benefits (Bronstein 2009)? For example, dispersal and settlement behaviours (e.g. aggregation, avoidance or kin recognition) may allow offspring to seek or avoid facilitative scenarios depending on whether they are beneficiaries (smaller offspring) or benefactors (large offspring) – generating scope for correlational selection between offspring size and other offspring traits (e.g. dispersal: Marshall and Keough 2003).

Interspecific facilitation can drive evolutionary responses when different species act as benefactors and beneficiaries (Day and Young 2004, Liancourt *et al.* 2012, Castellanos *et al.* 2014). Such dynamics are likely to play out differently within species – necessitating theory explicit to intraspecific facilitation. In light of my findings in marine invertebrates, and similar findings in plants (Zepeda and Martorell 2019), I propose that offspring size represents an ideal trait for exploring the evolutionary consequences of intraspecific facilitation (Liancourt *et al.* 2012).

Complementarity via niche partitioning

In Chapter 4, I provide novel evidence that within-brood variation enhances the aggregate performance of broods, as well as the performance of individual offspring (irrespective of their initial size). Thus, I show that within-brood variation may be adaptive in systems without parental care. Findings from this chapter were suggestive of complementarity effects – whereby phenotypic diversity non-additively enhances the performance of individuals due to facilitation or niche partitioning (Loreau and Hector 2001). In Chapter 4, I could not formally demonstrate complementarity as the mechanism

underlying these benefits, but subsequent experiments now confirm complementarity as a driver – although these effects can be context-dependent (H. Cameron, unpublished data).

Complementarity can arise via facilitation or niche partitioning, but these processes are difficult to distinguish (Loreau and Hector 2001). I argue that offspring-size mediated resource partitioning appears most likely – certainly, offspring size positively correlates with gape size and prey size in my system (Okamura 1990, Kosman and Pernet 2011). In light of findings from Chapters 2 & 3, however, asymmetric facilitation represents an alternative explanation. Theory predicts that asymmetric facilitation reduces size inequalities among interacting individuals, delaying self-thinning and increasing the survival of individuals (Lin *et al.* 2016). While survival was density-independent and tended to be higher in more-variable broods, colonies in more-variable broods showed greater variation in colony size – providing mixed support for asymmetric facilitation. Future experiments that quantify the distribution of resources consumed by broods that differ in offspring-size variation under realistic conditions (as in Ghedini *et al.* 2017) may explicitly disentangle whether the benefits of within-brood variation arise via niche partitioning or other forms of facilitation.

Theory predicts that competition for shared resources promotes phenotypic diversification when this variation reduces niche overlap among individuals (Bolnick *et al.* 2003, Bolnick 2004, Day and Young 2004, Pfennig *et al.* 2007). Theory specific to within-brood variation is yet to consider these ideas, but this thesis and other studies (Knutsen and Tilseth 1985, Martin and Pfennig 2010, Aubret *et al.* 2012) suggest this exciting possibility. Finally, size-mediated resource partitioning may not be restricted to siblings, but may also occur among unrelated offspring – providing a more general mechanism for offspring-size variation. Interactions among siblings are often more intense than among unrelated conspecifics (Aguirre and Marshall 2012a, Aguirre and Marshall 2012b, Smith *et al.* 2019) – thus resource partitioning may be particularly important for ameliorating competition among

siblings (Smith *et al.* 2019). Orthogonal manipulations of offspring size differences and genetic relatedness would test this prediction (i.e. kin vs. kind design: Ehlers and Bilde 2019). I would expect that in more related groups, offspring-size variation is more beneficial than in less related groups, but this awaits testing.

Conclusions and future directions

Offspring size is a key functional trait that strongly determines performance at all life-stages – from fertilisation to reproduction. Nevertheless, our understanding of the functional consequences of offspring size – that is, *how* offspring size affects performance – are remarkably poorly understood (Marshall *et al.* 2018). That offspring size determines the acquisition and expenditure of energy (i.e. consumption and metabolism, respectively) represents a potentially universal mechanism through which offspring size may affect performance. Yet, these relationships are currently unresolved. Appendix A extensively reviews these issues, but I reiterate them here, given this thesis repeatedly presents counter-initiative findings that may only be better understood by identifying the mechanisms through which offspring size affects performance. A clear priority moving forward would be to characterise how offspring size affects energy acquisition and loss, and how interactions with neighbours alter these relationships, under various environmental conditions (e.g. stress, resource levels, population densities, etc.).

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Appendix A

A global synthesis of offspring-size variation, its eco-evolutionary causes and consequences.¹

Abstract

Offspring size is a key functional trait that can affect all phases of the life history, from birth to reproduction, and is perhaps the most ubiquitous trait among all Metazoa. Despite its ubiquity, reviews tend to be taxon-specific. We explored the causes and consequences of offspring size variation across all taxa. We find clear latitudinal patterns in offspring size, with fish, invertebrates and birds showing positive covariation with latitude, while plants and turtles show the converse. We propose the developmental window hypothesis for why plants and turtles show negative covariance with latitude. Meanwhile we find stronger, positive selection on offspring size at higher latitudes for most animals, perhaps explaining the positive covariance between latitude and offspring size in these groups. Offspring size also varies at all scales of organization, from populations through to broods from the same female. We explore the reasons for this variation and suspect much of this variation is adaptive, but in many cases, there are too few tests to generalize. We show that larger offspring lose relatively less energy during development and gain more energy when they reach independence – thus, larger offspring may have greater net energy budgets than smaller offspring. We suggest this as one of the most common mechanisms by which larger offspring tend to outperform smaller offspring. While life history theorists have been fascinated by offspring size for over a century, we identify key knowledge gaps in this field, and find that the eco-evolutionary dynamics governing offspring size in particular, are poorly understood.

¹ This thesis appendix has been published as: Marshall DJ, Pettersen AK and Cameron HC. 2018. A global synthesis of offspring size variation, its eco-evolutionary causes and consequences. *Functional Ecology*, 32(6): 1436-1446

Introduction – offspring size, a universal functional trait?

There are few life history features or functional traits that are more ubiquitous across the metazoa than size, and offspring size is perhaps the most interesting. While sexual mode (clonal/parthenogenesis/sexual), developmental mode (direct/indirect) and life history (complex/simple) all vary enormously across species, common to all metazoan life is the necessity to allocate resources to offspring so they can complete development. But this allocation is fraught. Inevitably, allocating resources to one offspring comes at the expense of other fitness-enhancing activities, such as allocation to other offspring, current performance, or future reproduction. Intuitively we expect larger offspring, those that receive more parental resources, to have higher fitness than smaller offspring - and indeed this has been demonstrated repeatedly. Thus, at some level, parents face a universal trade-off between producing many, poorer performing offspring and fewer, better performing offspring. This offspring-size number trade-off was first modelled formally by (Vance 1973), but is better known from (Smith and Fretwell 1974), and forms the lens through which the evolutionary ecology of offspring size is viewed.

Because the offspring size-number trade-off is common to all metazoans, we are afforded the rare opportunity to gain insights from comparisons across the entire tree of multicellular life. Yet remarkably, such comparisons are lacking – much of the offspring size literature remains taxon-specific, with too little exchange among say, the seed literature (Moles and Westoby 2003), the bird-egg literature (Krist 2011) and even between the marine fish and invertebrate literature (Kamler 1992; Marshall *et al.* 2012). Broad synthetic efforts that span the major phylogenetic groups are largely lacking, despite the commonality of ideas. Indeed, most offspring size reviews are taxon-specific; the most recent general review was over 20 years ago (Bernardo 1996). While these taxon-specific reviews have been

excellent for advancing the field, their capacity for identifying patterns and contrasts among broad taxonomic groups is intrinsically limited.

Here, we seek to unify our understanding of offspring size by working across taxonomic boundaries to make what generalisations are now possible, and identify both commonalities and contrasts among groups. Such an approach is not without risk (as we stray beyond our taxonomic expertise) and downsides (we must be briefer than we would prefer). For instance, we prioritise empirical studies over theoretical ones, but we recognise theoretical approaches are essential. We review the concept of offspring size as a functional trait, and explore patterns in offspring size variation at all levels of biological organisation, from among species to within clutches from the same parent. Next, we examine how offspring size covaries with offspring fitness and why offspring size affects fitness, using energy as a common currency that applies across all taxa. Finally, we identify and prioritise the key questions that remain outstanding with regards to offspring size.

Offspring size is only one axis on which parental investment can vary but we believe it is the most useful. Offspring size at independence from parents is the perhaps the best and simplest metric by which to measure per offspring investment for most organisms that lack post-parturition care if it captures the majority of energy investment a parent makes in each offspring. Across species, larger offspring have more energy than smaller offspring. Across a wide variety of marine invertebrates (Marshall, Reitzel and McAlister 2017), plants (Leishman *et al.* 2000), birds (Williams 1994), fish (Wootton and Smith 2015) and insects (Fox and Czesak 2000), larger offspring have greater dry weights, protein and energy contents than smaller offspring, though this relationship is imperfect and composition can vary.

Offspring size and the duration of development

In 1973, Vance brilliantly intuited that offspring size must affect the duration of the developmental period (Vance 1973). Vance assumed that larger embryos take longer to reach the same developmental stage as smaller embryos. This assumption has intuitive appeal - if rates of cell cleavage are relatively constant across species, then inevitably larger eggs take longer to become embryos. While well known to marine invertebrate biologists, Vance's models are less appreciated by those working in other groups. Very early studies also supported the idea that larger eggs take longer to become larvae (Morgulis 1909), and since Vance's pioneering models, it has become increasingly apparent that embryos from larger-egged species take longer to develop than embryos from smaller-egged species. In birds, insects, fish and plants, offspring size strongly affects among-species patterns in time to hatching or germination (Rahn and Ar 1974; Pauly and Pullin 1988; Moles and Westoby 2003; Maino, Pirtle and Kearney 2017). Similar effects are observed in mammals with regards to gestation (Blueweiss *et al.* 1978). In marine invertebrates, where development is complicated by larval feeding in some groups, larger embryos take longer to hatch more generally, though the total larval period may not be longer overall (Marshall and Keough 2008; Marshall, Reitzel and McAlister 2017) – similar effects have been noted in frogs (Morgulis 1909). As discussed later, the effect of offspring size on developmental duration may be a key driver of latitudinal patterns in offspring size across species.

Within species, the effects of offspring size on development are far less clear. For some groups, intraspecific patterns mirror interspecific patterns (Kamler 1992), whereby larger eggs take longer to develop, but the converse is also true (Schenk and Sondgerath 2005; Marshall and Keough 2008). Why these effects seem so consistent among species, but so variable within species is unclear – though could simply be driven by effect size and statistical power (there is more offspring size variation among species than within).

Resolving this question is important, however, because size and developmental duration can critically affect dispersal distances of offspring, such that mothers may alter the dispersal potential of their offspring through maternal provisioning. Such effects are particularly important for plants and sedentary marine invertebrates (Leishman *et al.* 2000; Marshall and Keough 2008; Wang and Ives 2017), where the early life-history stages are also the most dispersive, but offspring size also affects dispersal in groups with highly mobile adults (such as fish; Einum *et al.* 2011; Kamler 1992)

Biogeographical patterns in offspring size

Spatial patterns in offspring size among species are often striking. For example, marine life history theoreticians noted a strong relationship between offspring size and latitude in the 1930's (Thorson 1936). Such patterns have only become more apparent as studies accumulate; across taxa, offspring size covaries with latitude but remarkably, no broad syntheses of these patterns exists. We compiled these taxon-specific syntheses of biogeographic variation in offspring size to yield the largest and broadest database of offspring size ever compiled (see supplemental materials for details).

Our synthesis found that biogeographical patterns in offspring size fell into two broad groups (Figure 1): those that show a negative relationship between offspring size and latitude; and those that show a positive relationship between offspring size and latitude. Plants show the most reliable and strongest covariation between latitude and offspring (seed) size. Moving from the tropics to the poles, seeds become much smaller. Turtles also show a negative relationship between latitude and offspring size. In contrast, offspring size for all other animal groups positively covary with latitude. We note, however, that for one group (minnows), there was no indication of a relationship. For this group, all but 1 species came from the same 10° latitudinal band, suggesting limited power to detect any differences.

Some generalisations seem possible based on our compilation. Plants generally show a consistent negative relationship between seed size and latitude. In contrast, it is clear that most animals (except turtles) show strong positive covariance between offspring size and latitude. Why turtles show the opposite pattern is unclear – this group shows the standard Bergmann size cline whereby lower latitude species tend to be smaller adults (Ashton and Feldman 2003), and so maternal body size constraints are unlikely to drive the pattern. We suspect that both turtles and plants show different patterns to most other animals because of the way offspring size and temperature affects development across all of these groups.

Developmental windows as a driver of latitudinal offspring size variation

Plant and animal life history theorists have tended to invoke different factors when considering biogeographical patterns in offspring size. Nevertheless, we suspect there is some newfound potential for unifying explanations. Discussions of latitude-seed size relationships have traditionally focused on biotic factors that covary with latitude such as herbivory and competition (Leishman *et al.* 2000; Moles and Westoby 2003; Moles *et al.* 2004). More recently however, explanations of latitude-seed size relationships have focused on scope for growth and development (Moles and Westoby 2003). Temperature strongly affects development rates in all organisms– small increases in temperature yield massive increases in developmental rate (Gillooly *et al.* 2002). Higher latitudes have far fewer warmer days and therefore provide shorter windows for rapid seed development (de Casas *et al.* 2017). Seed size, among species at least, positively covaries with development time and so it is argued that large seeds simply cannot complete development at higher latitudes (Moles and Westoby 2003). Interestingly, the same argument has been invoked independently for turtles (Iverson *et al.* 1993). Larger turtle eggs take longer to develop (Iverson *et al.* 1993), and as turtle eggs are largely at the mercy of ambient temperature regimes, large eggs may simply fail to complete development in the shorter warm periods of high latitudes.

But if developmental windows drive offspring size covariance patterns with latitude, why do birds and other animals produce larger offspring at higher latitudes, while plants and turtles show the converse? A key difference between birds and turtles of course is that birds typically incubate their eggs such that environmental temperature would more strongly affect development for turtles relative to birds. Other reptiles (e.g. squamates, crocodiles) that show limited thermoregulation of their nests would represent a key group in which to test the developmental window hypothesis, yet compilations of egg size variation in these groups are lacking.

Biogeographic patterns in offspring size for nonavian animals other than turtles however appear to contradict the developmental window hypothesis. Certainly, egg size positively covaries with developmental time among species of both invertebrates and fish (Pauly and Pullin 1988; Pepin 1991; Gillooly *et al.* 2002), so we would expect these ectotherms to follow the same patterns as turtles. One speculative explanation is that because eggs are typically much smaller in fish, amphibians and invertebrates relative to other vertebrates and the seeds of plants, developmental windows might be less severe in fish and invertebrates. We predict that once developmental window constraints are removed, all else being equal, selection for larger offspring may be stronger at higher latitudes, potentially explaining the production of larger offspring towards the poles in these groups. In the next section, we formally test whether there are any apparent geographic patterns in selection on offspring size.

Offspring size-fitness relationships

Offspring size affects fitness. While this seems straightforward there are remarkably few formal compilations of the relationship between offspring size and fitness (but see Rollinson and Rowe 2015). We expanded the dataset of Rollinson and Rowe (2015) to examine the spatial patterns in selection across latitudes and conducted a phylogenetically-

controlled analysis using weighted regression (see supplementary materials for details). As expected, selection on offspring size is generally (though not invariably) positive with an average standardised selection gradient of 0.125 and an average selection differential of 0.145 across all studies. We found that the strength of selection on offspring size increases with latitude; although this pattern is only apparent for selection differentials, but not selection gradients (Figure 2). Overall, stronger positive selection tends to favour larger offspring at higher latitudes relative to lower latitudes, providing some support for our suggestion that biogeographic patterns in selection may drive latitudinal patterns in offspring size observed in some groups (discussed above). Nevertheless, our results are very preliminary as there are few formal estimates of selection on offspring size generally, and we encourage future studies of offspring size effects to provide such estimates.

The mechanisms mediating latitudinal variation in selection are unclear. It is always tempting to invoke differences in mean conditions across latitude as drivers of latitudinal patterns. For example, earlier in this review we invoked mean temperature and its effect on development as a driver of latitudinal patterns in offspring size. However, recent studies remind us that latitudes differ in ways beyond mean conditions. For example, the seasonality and predictability of conditions also differ dramatically across latitude, and both mediate selection on, and shape spatial patterns in, offspring size (Marshall and Burgess 2015).

Intraspecific variation in offspring size

In this section we consider variation among populations, mothers and siblings. We consider two different drivers of variation in offspring size: extrinsic or environmental sources and intrinsic sources. These distinctions are largely artificial and simply for convenience – in reality, environmental factors such as food availability can quickly translate into intrinsic factors such as maternal phenotype. Nevertheless, we would argue that the field

has progressed better in understanding external rather than internal drivers of variation in offspring size, and so we make these distinctions here.

Environmental sources of variation

Life history theoreticians have long known that offspring size varies with environmental conditions such as temperature, local food regimes, competitors and stressors. Traditionally, there was a tendency to simply assume that poor quality maternal conditions would translate to the production of poor quality (i.e. smaller) offspring, otherwise known as a transmissive maternal effect (Marshall and Uller 2007). Pioneering work by Chuck Fox and others overturned these assumptions, showing that often, the opposite was the case: mothers in poor quality environments often produce larger offspring (Fox, Thakar and Mosseau 1997). Increasingly it seems that much of this variation is driven by adaptive transgenerational plasticity on the part of parents whereby mothers anticipate the environment their offspring are likely to experience and provision their offspring accordingly (Allen, Buckley and Marshall 2008). Mothers often produce larger offspring when competition is more intense or food availability/quality is lower (Plaistow, Lapsley and Benton 2006; Bashey 2008). Such anticipatory maternal effects can work both ways – mothers can also reduce the size of their offspring when conditions are benign. A beautiful meta-analysis by (Swanson *et al.* 2016) showed that, across many species of butterfly, mothers decrease the size of their eggs when laying on higher quality food sources, allowing them to lay more eggs overall. Thus offspring size is typically the functional trait by which mothers buffer their offspring from harsher environments or maximise fecundity in more benign environments. Such anticipatory maternal effects are particularly likely when the environment affects resource availability and the maternal environment is a good predictor of the offspring environment (Burgess and Marshall 2014). Importantly a recent meta-analysis of parental effects found they were often not adaptive (Uller *et al.* 2013). We suspect that this

meta-analytical result reflects the nature of the tests that have been made so far – most have manipulated maternal environment without formally considering whether such manipulations are meaningful in that they provide reliable cues to mothers about their offspring's likely environment. Maternal effects are no panacea, but we believe that more careful consideration of the reliability and predictability of parental environments as cues for offspring provisioning decisions should result in better estimates of the prevalence of adaptive parental effects.

Temperature has also long been recognised to covary with intraspecific variation in offspring size in ectotherms. Mirroring interspecific patterns discussed above with regards to latitude, in animals at least, warmer mothers tend to produce smaller offspring than cooler mothers across a range of animal taxa (Atkinson *et al.* 2001). While some have speculated that this pattern is a product of physiological processes (Van der Have and de Jong 1996), we suspect it is actually an adaptive response to differences in the costs of development at different temperatures (Kamler 1992). Interestingly, in both vertebrates and invertebrates (Fischer *et al.* 2003; Bownds, Wilson and Marshall 2010; Burgess and Marshall 2011), smaller offspring tend to perform better in higher temperatures and vice versa when both parental and offspring environmental temperatures are manipulated, indicating that temperature-mediated changes in offspring size are likely to be adaptive.

It is worth noting that maternal manipulations of offspring in response to environmental changes are unlikely to be perfect, and that particular attention should be paid to changes in the offspring-size fitness function across environments, and whether it is biologically likely for mothers to anticipate such changes (Burgess and Marshall 2014). In some instances, the environment might be so bad that no amount of provisioning will increase offspring fitness (Allen, Buckley and Marshall 2008). In these instances, mothers appear to change the size of their offspring, not so the offspring can perform better in the

local environment, but so the offspring can escape that environment. Because offspring size affects development time, dispersal probability or dormancy duration (Marshall and Keough 2008; de Casas *et al.* 2017; Wang and Ives 2017), mothers may alter the dispersal potential or dormancy of their offspring by manipulating offspring size. Thus, maternal effects on offspring size insulate offspring from particularly bad local environments by facilitating dispersal out of that environment, or delaying development such that the environment is likely to improve.

Intrinsic sources of variation

Mothers that experience the same environment may still produce offspring of very different sizes and much of this variation seems correlated with maternal phenotype – particularly maternal size and age (Marshall *et al.* 2010). Within a wide range of taxa, larger mothers produce larger offspring (Lim, Senior and Nakagawa 2014). Rollinson and Rowe (2016) recently provided an excellent summary of this topic, and we share their view that non-adaptive, constraint-based arguments regarding maternal size-offspring size (MSOS) correlations are unlikely to be broadly applicable. In their review, Rollinson and Rowe highlight two schools of thought for why MSOS correlations might be adaptive. Briefly, one school of theory assumes that because larger mothers are more fecund or select different habitats than smaller mothers, the offspring from larger mothers have a different offspring-size fitness function than the offspring of smaller mothers (Marshall *et al.* 2010; Rollinson and Hutchings 2011). Thus, larger mothers place their offspring in environments that select for larger optimal offspring sizes, and so produce larger offspring. Rowe and Rollinson are somewhat dismissive of such explanations, but we believe there is insufficient evidence to discount these ideas, and direct tests of the theory are still rare (Plaistow *et al.* 2006; Rollinson and Hutchings 2010; Cameron *et al.* 2016). We therefore encourage more empirical work along this line.

The second school of thought proposes that maternal size alters the costs of producing offspring independently of the offspring size-fitness function. This theory was pioneered by Sakai and Harada (2001), with subsequent iterations and modifications (Filin 2015). Essentially, these theories predict that if larger mothers can mobilise more nutritional resources more quickly, and offspring use resources while being provisioned, then larger mothers are able to produce larger offspring more cheaply than smaller mothers. While the key assumptions of these models seem reasonable, empiricism again lags behind, with very few direct tests.

Rollinson and Rowe suggest that maternal nutritional status, rather than maternal size *per se*, drives MSOS correlations. While nutritional status and maternal size are highly correlated in some species, we suspect that in others, mothers might be larger because of greater accumulated size from previous periods but can still have poor nutritional status in the current reproductive round. As Rollinson and Rowe suggest, a straightforward test would be to manipulate maternal size and current food regime orthogonally and examine the relationship between maternal size and offspring size.

As well as varying with maternal size, offspring size also covaries with maternal age. In some instance, this covariance appears to be driven by the fact that maternal age is a reliable predictor of offspring environment (Plaistow *et al.* 2006) but in others, it is unclear whether size or age is the driver. While theory has explored these issues specifically and sought to disentangle size and age (Kindsvater *et al.* 2010), too few empirical studies have sought to disentangle these factors (Green 2008).

Determining the drivers of MSOS correlations is particularly important given that many harvested species suffer shifts in mean body size (Hixon, Johnson and Sogard 2014). If larger mothers produce offspring of different quality than smaller offspring, then removing larger mothers will have disproportionate impacts beyond any fecundity effects alone (Hixon,

Johnson and Sogard 2014). Crucially, the two schools of thought regarding the drivers of MSOS correlations have different consequences for offspring quality - one implies that offspring quality (irrespective of offspring size) is unchanged across maternal sizes, the other does not (Marshall *et al.* 2010). Thus, resolving this issue is not only important to life-history theory, it is also crucial for the management of harvested species.

Offspring size variation within mothers

Offspring from a single reproductive bout are never perfectly uniform in size, though this variability is often underestimated (Turnbull *et al.* 2006). Indeed in some groups, offspring size variation within a clutch exceeds the variation observed among mothers (Kamler 1992; Marshall, Bonduriansky and Bussiere 2008). Although widespread, offspring size variation within broods (or clutches, litters etc.) has received less empirical attention than at other scales. As such, the causes and consequences of within-brood variation remain unclear. Some authors suggest physiological constraints (Fox and Czesak 2000) or family conflict (Kamel and Williams 2017) may limit the capacity of mothers to equally provision their offspring. But given that mothers can adaptively alter the mean size of their offspring (discussed above), we suspect that mothers may adaptively control variance, at least to some degree. We review the adaptive explanations proposed to maintain within-brood variation, which generally fit into two categories: 1. Bet-hedging hypotheses, and 2. Sibling competition hypotheses.

Within-brood variation as a bet-hedging strategy

As previously discussed, when mothers can anticipate the environment their offspring will face, they should produce offspring of a single, optimal size. But what if mothers cannot accurately predict the offspring environment? Theory predicts that in unpredictable environments, mothers ‘hedge their bets’ by producing variable clutches to ensure that at

least some offspring survive the prevailing conditions. In unpredictable environments, mothers that unequally provision their offspring therefore trade-off arithmetic mean fitness for reduced variation in fitness across generations, increasing long-run (geometric mean) fitness (Philippi and Seger 1989). Within-brood variation is most commonly attributed to bet-hedging, but evidence remains equivocal. Theory supports this idea when the offspring size-fitness function is modelled as dome-shaped (Marshall *et al.* 2008), but not when offspring fitness is a positive function of offspring size (McGinley, Temme and Geber 1987; Einum and Fleming 2004). Correlative studies suggest a positive relationship between environmental predictability and the level of offspring-size variation within broods (Morrongiello *et al.* 2012), but few studies have manipulated the offspring environment to explore maternal bet-hedging responses (but see Barbosa *et al.* 2015).

Within-brood variation mediates sibling competition

In many systems, siblings often co-occur across small spatial scales during some of the life-history, and thus interact to alter one another's fitness. For example, within-brood variation may be adaptive if competition among different-sized siblings is asymmetric. In birds, hatching asynchrony may create competitive hierarchies where older, larger siblings outcompete later-hatched, smaller siblings for parentally supplied resources (Lack 1947). Analogous theory in plants predicts within-brood variation is maintained by small-scale variation in seed density and asymmetric competition in favour of larger seeds (Geritz, van der Meijden and Metz 1999). A key assumption of these models is a colonization/competition trade-off in favour of larger and smaller seeds, respectively. Again, empirical support for these theories are rare. In contrast to competitive asymmetry hypotheses, novel evidence suggests that offspring-size mediated facilitation may maintain within-brood variation in a sessile marine invertebrate. Cameron *et al.* (2017) found that within-brood variation increases not only the collective performance of broods, but also the performance of individual

offspring, irrespective of their size. These authors speculate that their results may be driven by food-resource partitioning among different-sized siblings, and is supported by evidence in frogs (Martin and Pfennig 2010).

Why does offspring size affect fitness? A functional trait approach

The vast majority of studies measuring the consequences of offspring-size variation have focussed on the nature of the offspring-size fitness function, where ‘fitness’ may pertain to survival, growth rate, longevity or reproductive output (Bernardo 1996). These phenomenological approaches provide strong evidence that offspring size covaries with fitness (Ronget *et al.* 2017). However it is surprising that in most cases, the mechanism by which offspring size affects fitness remains unknown. While many species-specific explanations have been proposed, general mechanisms that apply across taxa are surprisingly limited. Given that the fitness benefits of having a larger size early in the life history are ubiquitous among species, we might expect to see general mechanisms driving this response. Here we discuss potential drivers of the offspring size-performance relationship.

Energy acquisition and energy loss

All organisms uptake, transform, and expend energy. The amount of energy available for key biological processes is thought to be closely linked to fitness. Because offspring often need to undergo some development before they can begin to feed, or cannot access the full range of nutritional resources available to adults, offspring are particularly reliant on parentally-derived resources. As discussed earlier, the best predictor of parental energy investment seems to be offspring size for most taxa. Increased energy reserves may be a potential advantage to producing larger offspring, but it also possible that there are higher costs associated with originating from a larger offspring size. In order for larger offspring sizes to carry an energy advantage, larger offspring must also possess or gain more energy

relative to the costs of maintaining a larger size. In other words, for energy acquisition/use to provide a general mechanism for the offspring size-performance relationship, larger offspring size should have higher net energy available for fitness-enhancing functions than smaller offspring (Leishman *et al.* 2000). These benefits can arise via two non-mutually exclusive ways: 1) bigger offspring *acquire* proportionally more energy, or 2) bigger offspring *use* proportionally less energy.

It seems that larger offspring often access more energy resources than smaller offspring. In spadefoot toads, larger tadpoles originating from larger eggs can access a more nutrient-rich carnivorous diet, and thus potentially outcompete smaller, omnivorous tadpoles (Martin and Pfennig 2010). Similarly, larger gape sizes at birth allows for more efficient energy acquisition and growth in some snakes and fish (Knutsen and Tilseth 1985; Kamler 1992; Aubret 2012). In filter feeding marine invertebrates, larger offspring initially produce more, or larger, feeding structures (Marshall, Reitzel and McAlister 2017).

In plants, seed size likely affects photosynthetic capacity (energy intake) by affecting the size of leaves that are produced by the seedling (Leishman *et al.* 2000). Numerous interspecific studies find that species with larger seeds typically produce seedlings with greater initial leaf mass (Moles *et al.* 2004), but intraspecific studies (which we are necessarily interested in when exploring mechanism) are rarer. Classic studies show that larger seeds produce larger cotyledons and have greater leaf surface area (Stanton 1984; Moegenburg 1996). Though rare, other intraspecific studies show seed size affects the size of the root mass, another way in which plants access essential resources (Bonfil 1998).

While these studies imply that larger offspring can take in more energy than smaller offspring – it is unclear whether they can take in *disproportionately* more than smaller offspring (i.e. energy intake scales hyperallometrically). At this point we think it is reasonable to assume that energy intake scales at least isometrically with offspring size,

though we encourage formal tests. Such patterns of isometry takes on new significance when we also consider size-dependent energy use in offspring.

Energy use (metabolism) is likely to scale allometrically with offspring size. In most, if not all metazoans, metabolic rates scale to mass with an exponent less than 1 – larger organisms have lower mass-specific metabolic rates (Pettersen *et al.* 2017). Thus, larger offspring should expend relatively less energy than smaller offspring and should complete any costly developmental stage with a higher proportion of their initial energy reserves. This developmental allometry is particularly crucial for offspring during non-feeding phases. There is direct evidence for allometric scaling in marine invertebrates and fish where a doubling in offspring size can yield a reduction in mass-specific metabolic rates of ~40% (Pettersen, White and Marshall 2015; Pettersen *et al.*, 2017). Similar ideas were raised by Kamler (1992) where she argued that the relative costs of development should decrease with increased offspring size when metabolism scales allometrically. There is also indirect evidence for development being more metabolically efficient in birds and fish (Duarte and Alcaraz 1989; Williams 1994). Williams (1994) showed that hatchlings from larger eggs didn't necessarily emerge with a greater size (measured as wing length) but they did emerge with a higher mass and higher proportion of yolk reserves, as one might expect if larger offspring completed development more efficiently. An analysis of the data in Duarte and Alcaraz (1989) shows that fish larvae from larger eggs are disproportionately larger than larvae from smaller eggs (our analysis of their data included both intra- and inter-specific variation). Interestingly, some studies also suggest larger offspring (particularly in fish) are more resistant to starvation than smaller offspring (Kamler 1992; Marshall and Keough 2008). Such an effect would not occur if larger offspring had the same mass-specific metabolic demands as smaller offspring. Given the ubiquity of allometric scaling, we suggest

developmental allometry is a potentially universal explanation for why larger offspring perform relatively better than smaller offspring.

Combining the effects of offspring size on energy acquisition and loss, it seems likely that a key mechanism underlying offspring size effects on offspring performance is net energy acquisition. Larger offspring are likely to access more, and expend proportionally less, energy during key early life-history stages than smaller offspring, and thus larger offspring should have comparatively more energy for fitness-enhancing processes such as growth and reproduction.

Outstanding questions

Whose fitness is it?

A number of outstanding questions regarding offspring size remain. First among these is the assignment of fitness. As Wolf and Wade (2001) brilliantly show, life history theoreticians think about offspring size differently to classic quantitative geneticists. Life history theoreticians typically assign fitness returns of different maternal investment strategies to mothers. In this sense offspring size is an odd trait because it affects the fitness of both mothers and offspring, but life history theoreticians assume that selection maximises maternal fitness (Smith and Fretwell 1974; Stearns 1992). This assumption arises because otherwise, it is hard to explain why selection would favour an offspring receiving less than perfect provisioning from the mother. Yet, the view that offspring sizes are the product of selection on mothers, it is at odds with standard quantitative genetic theory that assigns fitness strictly to the organism bearing the trait (Wolf and Wade 2001). From this perspective, given offspring size affects offspring fitness, selection should act on offspring size to maximise offspring fitness. Given the ubiquity of directional selection for increased offspring size (Rollinson and Rowe 2015), under this framework then, we should expect larger offspring to be favoured, regardless of the trade-off mothers might face.

Wolf and Wade provided a clear reconciliation of this issue – they showed that neither view (life history theory or the strict quantitative genetics view) was completely correct. Instead, it appears that the assignment of fitness will depend on the genetic covariance between offspring size in one generation and the next. We strongly recommend those interested read the paper in detail as it provides a clear path for resolving this issue. In the meantime, studies in model systems suggest that mothers generally win the parent-offspring conflict, with gene knockout studies revealing that maternal genes limit offspring size in both *Drosophila* and *Arabidopsis* (Dani and Kodandaramaiah 2017).

Offspring size number trade-off

Models of offspring size inevitably assume a size number trade-off, but such trade-offs are not likely to be observed within or among species for reasons elegantly described by Stearns on page 80 (Stearns 1992). Nevertheless, the true costs of producing offspring of different numbers and size are poorly resolved. For example, while the energy allocated to each offspring is nontrivial and reflected by size, there are other costs associated with producing offspring of different sizes or number. For example, if there are specialised reproductive structures for each offspring, then it might be more costly to produce multiple offspring than fewer offspring (Filin 2015). If offspring use resources while being provisioned, that may alter the relative costs of offspring sizes (Sakai and Harada 2001). If male gametes are limited, there are several reasons why making smaller, more numerous offspring will be costly and wasteful (Marshall, Reitzel and McAlister, 2017). Unfortunately, the energy costs of brooding offspring, or the per capita costs of making reproductive structures are largely unknown and so a true energy budget of offspring, beyond the simple energy content of those offspring, remains unknown.

Offspring size and predation

Offspring size may affect fitness simply because a physically larger offspring is fitter, regardless of energy considerations. For example, physical size may affect predation – this idea has been important to fish offspring size discussions for over 50 years (Kamler 1992). If predators are size-limited, then larger offspring may simply be less susceptible to predation (Reznick, Callahan and Llauredo 1996). Larger offspring can also have greater locomotory abilities, allowing them to better escape predators (Kamler 1992). In some cases, however, larger offspring can actually be more attractive to predators in both plants (Moegenburg 1996; Gómez and Husband 2004; Wang and Ives 2017) and animals (Gosler, Greenwood and Perrins 1995; Donelan and Trussell 2018), but more tests are needed more generally. Similarly, larger offspring may actually make more effective predators/consumers (Palmer 1990; Martin and Pfennig 2010b) but again there have been exceedingly few tests.

Conclusions

Offspring size is a fascinating functional trait that shows predictable global patterns among species. Offspring size affects every part of an organism's life, from birth to reproduction and selection tends to favour larger offspring, though the strength of selection varies in space and time. We suggest that negative relationship between offspring size and absolute latitude observed for some groups (large-egged clades that cannot insulate their offspring) is explained by the developmental window hypothesis. For other groups (birds and invertebrates) latitudinal patterns in selection appear to shape global patterns in offspring size, but more data is required for more robust tests of this prediction. We suggest that a major way in which offspring size affects subsequent offspring fitness is by altering the relative energy costs of development and subsequent energy intake at independence.

Figures

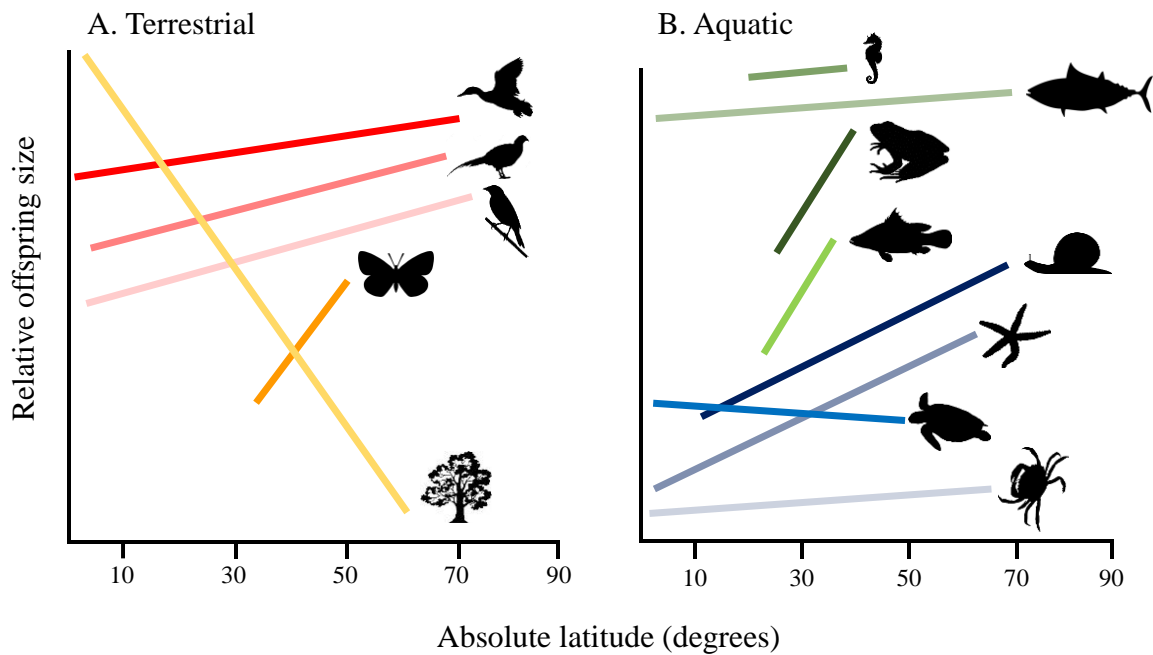


Figure 1. Spatial patterns in relative offspring size across latitude for different: **A.** terrestrial species – including different groups of birds, butterflies and plants; and **B.** Aquatic species – including different groups of marine and freshwater fish, turtles and marine invertebrates with different developmental modes. For the marine invertebrates, many phyla are included in each developmental mode and the snail, seastar and crab represent species with direct development, non-feeding and feeding larvae, respectively. Note that these lines do not represent absolute offspring sizes, nor are their relative positions on the y-axis representative. Rather the lines show the slope of the relationship between offspring size and latitude for each group, as well as the latitudinal extent of the underlying data. Importantly, the slope for plants is extremely underestimated relative to other groups because plants display much higher levels of variation (4 orders of magnitude) in offspring size across the latitudinal gradient.

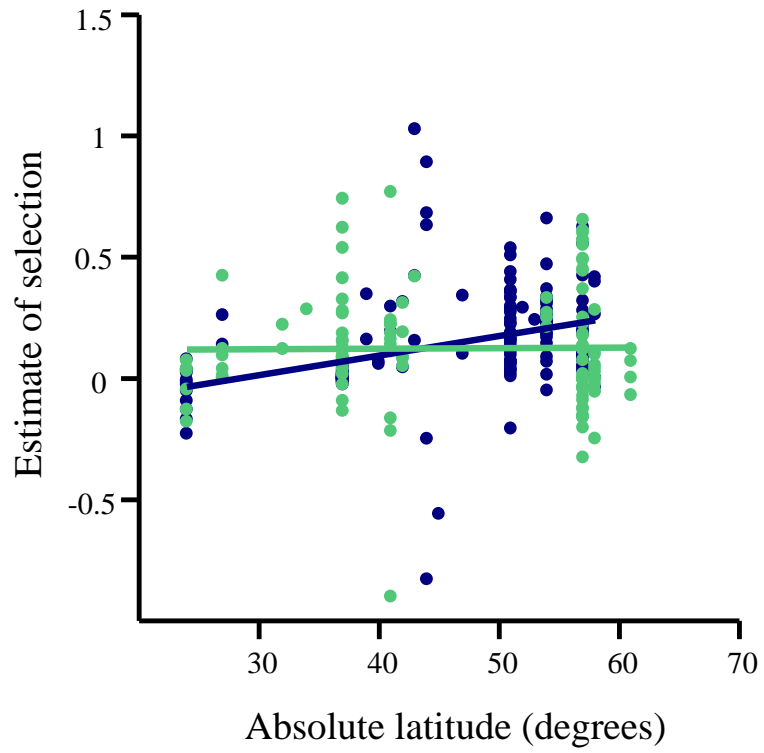


Figure 2. Spatial patterns in selection on offspring size across latitude. Each point is a single estimate of selection and selection differentials and gradients are shown in green and blue, respectively.

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Appendix 1: Latitudinal patterns in offspring size

We compiled syntheses of biogeographic variation in offspring size, restricting our search to those reviews that have already compiled geographic variation for 10 or more species (Table 1). The groups for which we could find data were eclectic but cover a wide variety of modes of life and habitat. Our compilation includes: marine invertebrates; marine and freshwater fish; butterflies; frogs; turtles; birds; and plants; yielding a total of 2523 species (mainly marine invertebrates and plants). Unfortunately, some studies could not be included because they did not include raw egg size data. We do not control for phylogeny in our entire synthesis, but several studies in our compilation did and such patterns are typically robust to including phylogenetic controls within major clades (Marshall *et al.* 2012; de Casas *et al.* 2017). Below are statistical tests for each group and regression coefficients linking relative offspring size (standardised by the largest offspring size for that group) to absolute latitude. Latitude was obtained from each study as the specific location where eggs were collected and does not take into account species ranges. This approach reflects the fact that many species show geographic variation in egg size across their range – as such egg size for a species at any one latitude may not reflect egg size at any other latitude.

Table 1. Summary of significant relationships between latitude and relative offspring size (all offspring sizes were converted to volume or mass and then standardised to the maximum observed size in that group). Blue rows represent aquatic animals, orange represent terrestrial animals and green represent plants. *Note that for de Casas *et al.* (2017), the raw seed size data are unavailable, but Figure 1 clearly depicts a strongly negative relationship between offspring size and latitude.

| Group | n | Coefficient | Phylogenetically controlled? | Study |
|--|-----|-------------|------------------------------|-------------------------------|
| Turtles | 86 | -0.0057 | no | Inverson <i>et al.</i> (1993) |
| Marine fish | 99 | 0.0046 | no | Thresher (1988) |
| Freshwater fish (percids) | 28 | 0.029 | no | Paine (1990) |
| Minnnows | 55 | 0.0042 | no | Coburn (1986) |
| Sea horses | 12 | 0.0096 | no | Foster and Vincent (2004) |
| Marine invertebrates (feeding larvae) | 458 | 0.0078 | no | Marshall <i>et al.</i> (2012) |
| Marine invertebrates (nonfeeding larvae) | 320 | 0.0108 | no | Marshall <i>et al.</i> (2012) |
| Marine invertebrates (aplanktonic) | 319 | 0.0107 | no | Marshall <i>et al.</i> (2012) |
| Frogs | 9 | 0.0277 | no | Morrison and Hero (2003) |
| Game birds | 52 | 0.0051 | no | Arnold (1988) |
| Water birds | 62 | 0.0047 | no | Rohwer (1988) |
| Plants | 309 | -15.92 | yes | Moles and Westoby (2003) |
| Legumes | 532 | -ve* | yes | de Casas <i>et al.</i> (2017) |

Appendix 2: Latitudinal patterns in selection

We tested for phylogenetically controlled latitudinal patterns in selection on offspring size by compiling variance-standardized estimates of linear selection differentials (s) and linear selection gradients (β) from the existing dataset in Rollinson and Rowe (2015). We included only those studies that had measured selection on offspring size in the field. We included studies that measured selection on raw offspring size traits only (e.g. mass at birth or fledging, hatchling or larval length, egg weight), and excluded studies that used composite or corrected measures of offspring size (i.e. PC scores or the residuals from regressions of offspring size metrics). Our dataset includes multiple entries for those studies that estimated selection using several fitness metrics, or where changes in selection were measured across different years, populations, or environments (including manipulative field studies). We only included studies where Rollinson and Rowe (2015) had obtained selection estimates directly from tables or figures in the original papers. We excluded cases where Rollinson and Rowe (2015) had calculated selection differentials from phenotypic distributions before and after selection, because it was not always clear how these were calculated from the data in the original papers.

Our final dataset included 101 estimates of selection differentials (s) and 114 estimates of selection gradients (β) on offspring size traits from 33 studies that spanned 9 classes of animals, including: birds; reptiles; mammals; fish; and marine invertebrates (215 entries in total). Our dataset incorporated studies where offspring performance was measured as: offspring survival ($n = 137$); survival to reproduction ($n = 63$); offspring growth ($n = 9$); or lifetime reproductive output ($n = 6$). We should note that the data are strongly biased toward the northern hemisphere.

A phylogenetic tree was constructed using the open tree of life (Hinchliff *et al.* 2015) with the “rotl” package (Michonneau *et al.* 2016) of R v3.3.2. Data were then analysed using

a phylogenetic mixed model implemented in “ASReml-R” (Gilmour *et al.* 2009) and R v3.0.2, which allowed us to partition the variance between changes in selection on offspring size (both s and β) with absolute latitude due to the shared evolutionary history among the study species sampled, relative to that independent of phylogeny. The mixed-effects (fixed slope, random intercepts) model included latitude as a fixed effect and the relatedness matrix from the phylogeny (produced using the R package “MCMCglmm”; Hadfield 2010) as a random effect, on the magnitude of latitude on selection on offspring size. Likelihood ratio tests were used to determine the significance of the effect of phylogeny, where phylogenetic signal was calculated as the proportion of variance – conditioned on the fixed effects – attributable to the random effect of phylogeny (Hadfield and Nakagawa 2010).

The proportion of variance attributable to phylogeny (conditioned on the fixed effects) was negligible, and not significantly different from 0 ($\chi^2 = 9.812 \cdot 10^{-6}$, $p = 0.998$). We therefore ran a general linear model, and tested whether the relationship between selection on offspring size and latitude depended on which method was used in each study (i.e. whether selection differentials (s) or selection gradients (β) were measured). We found a significant interaction between method and latitude ($\chi^2 = 4.112$, $p = 0.043$), thus each method was fit with a separate linear model. Finally, we used a weighted least squares regression to test whether standard error (SE; for those studies that did report standard error) was related to a higher precision of estimates. We calculated an estimate for the slope of latitude on selection on offspring size, weighted according to the precision of the estimate across species (i.e. smaller standard error received higher weighting). We found that the precision of the estimate (standard error) did not affect the magnitude of the relationship between latitude and s ($\chi^2 = 0$, $p = 1$), or β ($\chi^2 = 0$, $p = 1$).

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Appendix B

Size and density mediate transitions between competition and facilitation.¹

Abstract

Species simultaneously compete with and facilitate one another. Size can mediate transitions along this competition-facilitation continuum, but the consequences for demography are unclear. We orthogonally manipulated the size of a focal species, and the size and density of a heterospecific neighbour, in the field using a model marine system. We then parameterised a size-structured population model with our experimental data. We found that heterospecific size and density interactively altered the population dynamics of the focal species. Size determined whether heterospecifics facilitated (when small) or competed with (when large) the focal species, while density strengthened these interactions. Such size-mediated interactions also altered the pace of the focal's life history. We provide the first demonstration that size and density mediate competition and facilitation from a population dynamical perspective. We suspect such effects are ubiquitous, but currently underappreciated. We reiterate classic cautions against inferences about competitive hierarchies made in the absence of size-specific data.

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Introduction

Species that use the same resources were traditionally viewed as competitors, but it is increasingly apparent that facilitation is also common – some species enhance the performance of others (Bertness and Callaway 1994; Bruno *et al.* 2003). It is now clear that competition and facilitation occur simultaneously, such that the net outcomes of interactions represent a continuum – from strongly negative (competition) to strongly positive (facilitation). Both competition and facilitation influence species ranges (Bulleri *et al.* 2016), population and community dynamics (Butterfield 2009) and species coexistence (Buss and Jackson 1979; Chesson 2000; Gross 2008; Johnson and Bronstein 2019). Determining the factors that mediate competition and facilitation is therefore a priority in ecology.

A long-held view is that the size of organisms can influence the outcomes of species interactions (Buss 1980; Damuth 1981; Russ 1982; Weiner 1990). Size determines resource acquisition and consumption, so it is perhaps unsurprising that size mediates competition. For example, larger individuals both require more resources, and are better at appropriating resources, thus they often outcompete smaller organisms (Weiner 1990). On the other hand, smaller individuals have relatively lower resource requirements, such that at times, they may have a competitive advantage (Persson 1985; De Roos *et al.* 2003; Reuman *et al.* 2014).

Increasingly, it appears that size not only influences competition, but also facilitation (Lin *et al.* 2012; Lin *et al.* 2016; Malanson and Resler 2016; Zepeda and Martorell 2019). For instance, large herbivorous mammals facilitate smaller herbivores by removing grasses that would otherwise prevent these smaller species from accessing their target resources (Vesey-FitzGerald 1960; Gordon 1988). In plants, the relative influence of facilitation and competition can depend on size and age (Miriti 2006; Schiffers and Tielbörger 2006; Reisman-Berman 2007; le Roux *et al.* 2013). Theory predicts that size-mediated shifts in competition and facilitation may be important for the dynamics and size-structure of

populations (Chu *et al.* 2008; Lin *et al.* 2012; Lin *et al.* 2016), as well as species coexistence (Ranjan and Bagchi 2016). Empirical demonstrations are rare, however, such that the consequences of size-mediated interactions remain unclear.

A key impediment to understanding size-mediated interactions is that empirical studies struggle to provide information at the appropriate scale (Hart *et al.* 2018). Empirical approaches typically examine the outcomes of interactions at the level of individuals, while theory considers the consequences for populations – specifically, whether interactions ultimately lead to coexistence or competitive exclusion. Crucially then, theory defines the ‘winners’ of species interactions across multiple generations (Hart *et al.* 2018). Under this definition, a number of limitations associated with classic studies emerge.

Most empirical approaches (including earlier work of our own: Cameron and Marshall 2019) use snapshot observations or short-term performance data to make extrapolations about size and species interactions (Buss 1980; Russ 1982). These classic studies in particular were undoubtedly foundational for demonstrating that size alters species interactions, but population-level inferences based on snapshot data can be deeply misleading (Fowler *et al.* 2006; Trinder *et al.* 2013; Hart *et al.* 2016). This dissonance stems in part from the fact that competitive ability (formally defined) comprises an organism’s ability to: 1. appropriate resources and suppress other individuals (competitive effect); 2. withstand competition from neighbours (competitive response); and 3. grow in the absence of neighbours (intrinsic rate of increase – a population-level parameter; Hart *et al.* 2018). To illustrate, if Species A invariably overgrows Species B in a snapshot study, then traditionally A would be considered the superior competitor. But if B has a higher intrinsic rate of increase, then perhaps counter-intuitively, B will competitively exclude A over time (Hart *et al.* 2018).

Size-mediated interactions complicate things further, such that snapshot approaches become even less informative. For example, imagine that Species A when large, negatively affects the performance of younger, smaller, individuals of Species B, but that the reverse is true when B is larger than A – snapshot approaches might conclude that competition is intransitive (Buss 1980; Russ 1982). However, as Buss (1980) presciently suggested, the ultimate outcome will then depend on the size-frequency distribution of the population. From our example above, large individuals of Species B may be so rare in the population that ultimately, Species A will outcompete B. Ontogenetic shifts in competition and facilitation complicate things further, such that net outcomes cannot be intuited. A rare demographic example found adult plants that received facilitation as juveniles contributed little to the overall dynamics of the population relative to isolated plants (Miriti *et al.* 2001; Miriti 2006). Thus, facilitation of individuals during any one life-stage may not be important at the population-level when integrated over the life cycle. Clearly then, an approach that integrates individual-level data across different performance metrics (mortality, growth and fecundity), and ontogenetic stages, is required to reliably predict the population-level outcomes of size-mediated interactions.

Demographic models provide one such approach. These models integrate empirically estimated demographic rates (survival, growth, reproduction) through time (i.e. ontogeny) to make projections about future population growth (λ) under current ecological conditions (Caswell 2006). Demographic models can be directly parameterised with experimental data collected over the lifetime of organisms, and used to quantify the nature of species interactions at the population-level by comparing projections of λ across experimental conditions (e.g. McPeck and Peckarsky 1998; Bassar *et al.* 2015). In essence, demographic models provide the formal theoretical bridge between short-term estimates of individual-level performance and population-level outcomes.

Integral Projection Models (IPMs) are one such model that investigates the dynamics of populations structured by continuous characters – typically body size (Easterling *et al.* 2000; Coulson *et al.* 2010; Ellner *et al.* 2016). These models are amenable to parameterisation with experimental data collected using designs common to studies of species interactions, such as response surfaces (Bassar *et al.* 2016). Response surface designs estimate interaction coefficients across a range of densities to investigate the nature of species interactions under realistic conditions (Inouye 2001). As such, both IPMs and response surfaces are powerful, but underused, tools for determining the factors that mediate transitions between competition and facilitation at the population-level.

Here, we test for size-mediated transitions in competition and facilitation by parameterising a single-species IPM with data from a manipulative field experiment. We use a marine invertebrate system that has provided some of the foundations of population ecology and species interactions (Buss and Jackson 1979; Buss 1980; Russ 1982; Hart and Marshall 2013), and have attributes particularly amenable to demographic studies in the field. We elaborate on traditional response surface experiments to create a trait-specific response ‘cube’ (Fig. 1), where we orthogonally varied the size of a focal species, as well as the size and density of a heterospecific species. The inclusion of density was particularly important given that facilitation may alter the relationship between size and density in non-intuitive ways (Chu *et al.* 2008). We monitored key demographic rates (survival, growth and reproduction) of the focal species across their lifetime under field conditions. We then integrated across these lifetime measures of performance using our IPM approach. We provide novel evidence that the size and density of a heterospecific species can mediate transitions along the competition-facilitation continuum to affect the population dynamics of another species.

Materials and methods

Study system

We use two common filter-feeding bryozoans, *Bugula neritina* (hereafter *Bugula*) and *Watersipora subtorquata* (hereafter *Watersipora*), as our heterospecific and focal species, respectively. Both species are colonial, but differ in growth form: *Watersipora* is encrusting and grows by adding zooids (modular subunits) to the edges of the colony; *Bugula* is arborescent and buds new zooids from the tips of the colony branches (Appendix 1; Fig. S1). Traditional views of competition predict that these species compete via different strategies: *Watersipora* should pre-empt space and over-grow other organisms; *Bugula* should exploit resources more efficiently (Jackson 1977). Both species are common to early-successional assemblages worldwide and primarily compete for space, food and oxygen (Hart *et al.* 2012; Hart and Marshall 2013; Svensson and Marshall 2015; Lagos *et al.* 2017), but interactions may switch from competitive to facilitative with increasing environmental stress (Hart and Marshall 2013).

Experimental methods

To test for size-mediated interactions, we use a trait-specific, response cube that orthogonally varied the size of our focal species (*Watersipora*), as well as the size and density of our heterospecific species (*Bugula*; Fig. 1). For *Watersipora*, we used a continuous range of size-classes (as colony area) that spanned 0.185 to 414.2 cm². For *Bugula*, we had five size-classes based on the number of times the colony had branched (1, 2, 3, 4 and 5 bifurcations). We had six heterospecific densities (0, 1, 2, 3, 5 and 10 colonies per 121 cm²;) reflecting natural densities of *Bugula* in the field (Hart and Marshall 2013). Our manipulations of heterospecific size and density generated several biomass isoclines – whereby the total number of zooids were equivalent, but differentially ‘packaged’, as either

many small, or a few large, heterospecifics (Fig. 1c). The experiment included 30 combinations of competitor sizes and densities, and each combination received eight focal colonies (replicates) that spanned the distribution of *Watersipora* sizes (see Appendix 1; Table S1).

We performed the experiment at the Blairgowrie Yacht Squadron, Victoria, Australia (38°21'20.2"S, 144°46'22.8"E) from December 2013 to April 2014. For our manipulations, we obtained colonies of both species that varied in size and attached them to PVC plates (11 x 11 cm) using standard methods (Hart *et al.* 2012; see also Appendix 1). We deployed the plates (experimental units) into the field by attaching them to the undersides of PVC backing panels that hung 1 m below the water surface. In total, our experiment included 1080 colonies of known size of both species, distributed across 240 plates and 15 backing panels (Appendix 1; Table S1). We measured key demographic rates for 240 focal *Watersipora* colonies.

We monitored the performance of our focal colonies from photographs taken every two weeks for a total of eight weeks. We consider performance at the level of the colony (genet) rather than individual zooids (ramets), given that colony size alters demographic rates (growth, survivorship and fecundity) and drives population dynamics in modular organisms (Caswell 1985). We scored colonies as alive if they were present on the plates and contained living zooids, while we scored the colonies as dead if they were missing from the plates or all the zooids had died. We measured the growth of the colonies as their total area (cm²; Image J) at each census. We measured fecundity as the total number of embryos brooded by the colony. We note that all focal colonies were pre-reproductive at the beginning of the experiment and we first detected reproduction 6 weeks after our manipulations. Thus, focal colonies were able to adjust their reproductive schedules in response to the heterospecific environment.

We noted changes in the density of heterospecifics (*Bugula*) by monitoring their survival at each census (Appendix 2). We randomised the position of the plates within backing panels and removed any non-experimental settlers (both our study species and other organisms) every two weeks to avoid exogenous competition. Recruitment onto the plates was extremely low during the experiment – thus, other species did not affect the interactions we observe and model. We stopped the experiment after 8 weeks in the field because by 10 weeks, most of the heterospecific colonies (*Bugula*) had died. Mass die-offs are common for *Bugula* populations in our study region, and typically coincide with seasonal changes in the environment at the end of summer (Marshall 2005), as we also observed.

Modelling framework

To explore how the size and density of heterospecifics (*Bugula*) affect the population dynamics of the focal species (*Watersipora*), we integrated across our various performance measures (vital rates) using an Integral Projection Model (IPM; Easterling *et al.* (2000)). Our IPM is both a size- and age-structured model (Coulson *et al.* 2010):

$$n(z', 1, t + 1) = \sum_a \int [D(z'|z, a, y_b, N_b)M(z, a, y_b, N_b)B(z, a, y_b, N_b)n(z, a, t)]dz$$

$$n(z', a + 1, t + 1) = \int [G(z'|z, a, y_b, N_b)S(z, a, y_b, N_b)n(z, a, t)]dz$$

where the continuous trait for size, z , is the square root of colony area (cm²). In our model, the first equation deals with settlers, the second equation deals with the survival and growth of colonies. Our model is thus multigenerational – individuals reproduce and recruit into the populations but we do not model the dispersal of offspring outside their natal environment, nor their planktonic mortality.

$D(z'|z, y_b, a, N_b)$ is the conditional probability density function that describes the distribution of offspring sizes, z' , produced by parental colonies of size z at time $t + 1$. a is the

experimental age class, y_b is the size of the competitor (as bifurcations), and N_b is the density of the competitor. $M(z, a, y_b, N_b)$ is a continuous function describing the mean number of offspring produced by an individual with the colony area z , and of experimental age a , given the size and density of the competitor. $S(z, a, y_b, N_b)$ and $B(z, a, y_b, N_b)$ are continuous functions that describe the probability of an individual with colony area z , and experimental age a , at the beginning of the interval surviving and reproducing at the end of the interval, respectively, as a function of competitor density and size. $G(z'|z, a, y_b, N_b)$ is the Gaussian probability density function describing transitions from colony area z at time t to colony area z' at time $t + 1$ among survivors as a function of experimental age, competitor size and density. $n(z, t)$ is the distribution of *Watersipora* size at time t such that $N(t) = \int_x^y n(z, t) dz$ is the number of individuals between size z and y . We note that y_b and N_b are experimental factors that we manipulated and are not dynamic in the model, but heterospecific colonies obviously grew and died throughout the experiment (Appendix 2). While we do not model these dynamics explicitly, they are implicit in our phenomenological projections of focal population growth rate based on our experimental data.

For a comprehensive description of the model and the underlying vital rate functions, see Appendices 3 and 4, respectively. From this model, we then calculated a number of quantities:

1. The asymptotic population growth rate (λ) for *Watersipora* across the range of heterospecific densities and sizes we used in our experiments.
2. The elasticity of λ to each matrix element $\alpha_{i,j}$: $\frac{\partial \log(\lambda)}{\partial \log(\alpha_{i,j})}$. This quantity is a partial derivative that describes the proportional change in the population growth rate to a proportional change in the matrix element. We summed the elasticities of λ for all matrix elements across all focal sizes in the first-age class to ask under which

heterospecific environments are smaller, younger individuals most important to population growth. A large value of elasticity means that individuals in the first age class have a large impact on the dynamics of the focal species.

3. The reproductive value distribution (v) across the range of heterospecific sizes and densities used in our experiments. Reproductive values describe the expected numerical representation of the descendants of an individual of a given size and age within the population at time t at some arbitrary time point far in the future. We report the reproductive values of small (0.63), medium (14.3) and large (27.94) *Watersipora* colonies at experimental age one, but we note that we obtain equivalent results at later ages.

As per standard practice, we calculated the above-mentioned quantities at equilibrium – that is, the model had reached a stable population structure and constant population growth rate (Caswell 2006). We note this definition of equilibrium does not imply that our model (or empirical manipulations) had reached a constant population size, nor that the communities in which our species exist were at equilibrium.

Results

Heterospecific size, density and biomass affect the population growth rate of the focal species

We found that the size and density of a heterospecific species (*Bugula*) interact to regulate the size-specific vital rates (Appendix 4) and the population dynamics of a focal species (*Watersipora*). Importantly, heterospecific size mediated the transition between competition and facilitation, while heterospecific density largely determined the strength of these interactions (Fig. 2a). The change in λ across heterospecific regimes show these transitions along the competition-facilitation continuum – competition occurs when values of

λ within a given heterospecific environment are lower than under heterospecific-free conditions (when heterospecific density is 0), while the inverse indicates facilitation.

The population growth rate of *Watersipora* increased with increasing densities of smaller *Bugula* relative to heterospecific-free conditions. In particular, λ was highest when heterospecifics were small and numerous – indicating strong facilitation. These interactions transitioned from facilitative to increasingly competitive as the size of *Bugula* increased. Importantly, when *Bugula* were largest and most dense, λ was < 1 – indicative of competitive exclusion. Overall, we found a 70 % difference in estimates of λ between *Watersipora* populations that were surrounded by many, smaller heterospecifics (i.e. when λ was highest) and many, larger heterospecifics (i.e. λ was lowest). Interestingly, *Bugula* of intermediate size did not alter the population growth rate of *Watersipora* relative to heterospecific-free conditions – that is, intermediate *Bugula* had consistently neutral effects, regardless of their density.

Heterospecific size and density were stronger determinants of interspecific interactions than total heterospecific biomass across much of the parameter space (demonstrated by the superimposed biomass isoclines in Fig 2a). For example, when total heterospecific biomass was < 200 zooids, interactions changed from mildly competitive at lower densities of large heterospecifics, to facilitative at higher densities of smaller heterospecifics. Thus, the interactive effects of heterospecific size and density decouple the relationship between population growth and total heterospecific biomass.

Elasticities revealed that when *Watersipora* could persist in the presence of *Bugula* (i.e. $\lambda > 1$), smaller colonies in the first age class had the greatest contribution to our projections of λ (Fig. 2b). Indeed, these smaller, younger individuals contributed most to λ when heterospecifics were small and numerous (i.e. regions of facilitation), or large and few

(i.e. regions of mild competition). In contrast, these younger, smaller colonies were least important to population growth at high densities of larger heterospecifics (i.e. regions of intense competition).

Heterospecific size, density and biomass affect the size-specific reproductive values of the focal species

The size, density and total biomass of heterospecifics had complex, non-linear effects on the size-specific reproductive values (v) of the focal species. In heterospecific-free conditions (when heterospecific biomass is 0), reproductive values increased with colony size, as shown by the upward shift in intercept moving from the left to right panels in Fig. 3. In contrast, the presence of heterospecifics altered these size-specific reproductive values relative to heterospecific-free conditions (i.e. in regions of both facilitation and competition; compare Fig 2a to Fig 3a-c).

For the smallest and largest *Watersipora* colonies, total heterospecific biomass (rather than size and density) determined reproductive values, albeit in opposing directions. Unsurprisingly, very small *Watersipora* colonies had relatively small v , but within this size class, v was surprisingly highest at the largest heterospecific biomasses (Fig. 3a & d). Note this increase came not from greater growth of *Watersipora* under increasing biomass, but because reproduction was induced at a smaller size (see Appendix 4). In contrast, for the largest *Watersipora* colonies, v was maximised at lower heterospecific biomasses (Fig. 3c & f).

For intermediate *Watersipora*, complex interactions between heterospecific size, number and biomass altered v (Fig 3b & e). Broadly, v increased with heterospecific biomass to a point, but then declined again at very high biomasses. Additionally, while intermediate biomasses had consistent effects on v for intermediate *Watersipora*, the way that biomass was

partitioned into the size and number of individuals altered v when total biomass was very low or very high. When biomass was low, v was highest when biomass comprised fewer, large heterospecifics relative to many, smaller heterospecifics. Conversely, when heterospecific biomass was very high, v peaked at intermediate densities of large heterospecifics, but declined as these densities increased (Fig 3b & e).

To summarise then, when competition is most intense (i.e. higher densities of larger heterospecifics; see Fig. 2a) small and medium individuals contribute the most offspring to future population growth, while few large individuals reproduce (Fig. 3; see also Appendix 4). In contrast, when facilitation dominates (see Fig 2a), small and intermediate individuals appear to delay reproduction, such that only the largest individuals contribute offspring (Fig. 3; see also Appendix 4).

Discussion

We demonstrate that heterospecific size and density mediates transitions between facilitation and competition and alters the population dynamics of a focal species. Interspecific interactions shifted from facilitative when heterospecifics were small, to increasingly competitive when heterospecifics were large. Meanwhile, increasing heterospecific density strengthened these interactions – that is, size-mediated competition and facilitation were density-dependent. Importantly, these size-mediated effects were not transient; rather they persisted across the life cycle of the focal species (c.f. Miriti *et al.* 2001). We also found that heterospecific size and density had complex, non-linear effects on the size-specific reproductive values of the focal species, suggesting that size-mediated competition and facilitation can alter the pace of the life history. Other examples of complex interactions between the size and density of organisms in driving competition and facilitation are rare, but we suspect they are likely ubiquitous.

Consequences for populations

The total biomass of a given population should determine its resource use, and therefore competitive effects, on others (Damuth 1981). In our study, heterospecific size and density had interactive effects on the population-dynamics of the focal species – thus heterospecific biomass was a poor predictor of the focal species' response to interspecific interactions (see Fig. 2). Our findings therefore contradict classic theory that did not consider the role of positive interactions in regulating populations (Damuth 1981). Recent studies propose different pathways through which facilitation may decouple traditional biomass-density relationships (Chu *et al.* 2008; Ghedini *et al.* 2017). Our findings are congruent with these ideas, although explicit tests are required to identify the mechanisms via which facilitation disrupts classic assumptions regarding body size and resource use.

One explanation for our results is that heterospecific size and density alters the availability of resources for the focal species. Arborescent *Bugula* colonies (our heterospecific species) may alter water-flows and the delivery of water-borne resources to conspecific neighbours – leading to intraspecific facilitation and competition (Cameron *et al.* 2016; Cameron and Marshall 2019; see also Thompson *et al.* 2015 for studies using structural mimics). We suspect similar mechanisms determine interspecific interactions between *Bugula* and *Watersipora*. For example, smaller *Bugula* facilitated *Watersipora* and these interactions were density-dependent. Importantly then, it appears that a larger number of heterospecifics are required to ameliorate conditions for the focal species, as observed in other systems (Leslie 2005; Bishop *et al.* 2012; Svanfeldt *et al.* 2017). That *Watersipora* benefits from amelioration of flow and resource stress also has empirical support – reduced flows enhance feeding rates of encrusting byozoans in the laboratory, as well as their performance in the field (Okamura 1985; Svanfeldt *et al.* 2017). Furthermore, amelioration may be particularly important here, given the high flow rates at our site (Lagos *et al.* 2017).

As heterospecific size increased, however, interspecific interactions became increasingly competitive, and again, were density dependent. Competition likely increased with heterospecific size because of the relatively higher resource demands of larger individuals (Schoener 1983; Weiner 1990; in *Bugula*: Barneche *et al.* 2017). Overall then, our results suggest that many smaller *Bugula* disrupt water flow but do not consume all the resources they generate, therefore they facilitate *Watersipora*. In contrast, many large *Bugula* may increase resource supply, but also consume much of these resources (Cameron and Marshall 2019), thus they compete with *Watersipora*. Nevertheless, we acknowledge that other, less likely, forms of habitat amelioration (e.g. predator defence) may have contributed to our findings. An important next step, therefore, would be to estimate resource consumption by our focal species under various heterospecific regimes and realistic flow conditions (e.g. Ghedini *et al.* 2017). Such experiments would determine whether size-mediated resource amelioration and consumption drives facilitation and competition in our system, respectively.

Population-level consequences of size-based shifts in facilitation and competition are unlikely to be restricted to our study system. Rather, we suspect such interactions are widespread, but have previously gone undetected due to the rarity of studies that use population dynamical approaches. For example, facilitation occurs via resource amelioration in a range of systems, and in some cases is size-dependent (e.g. in mammals: Vesey-FitzGerald 1960; Gordon 1988; other aquatic systems: Cardinale *et al.* 2002; Whalen and Stachowicz 2017; birds: Russell *et al.* 2007; and plants: Pretzsch *et al.* 2013). Size and density may also determine interactions when facilitation occurs via non-resource related forms of amelioration. Certainly, general models of amelioration predict size-based shifts between facilitation and competition (Lin *et al.* 2012; Lin *et al.* 2016; Malanson and Resler 2016). Our study thus highlights the importance of viewing size-mediated interactions from a

population dynamical perspective, and we encourage further tests to determine their generality and identify their underlying mechanisms.

Consequences for life histories

Changes in the size-specific reproductive values that we observe suggest the focal species shifted its life history in response to the heterospecific environment. For example, smaller focal colonies contributed the most descendants to future population growth (i.e. had highest reproductive values) when competition was most intense (i.e. high heterospecific biomasses) – indicative of an acceleration in the life history. Early reproduction can occur when competition reduces the probability of future growth and(or) survival in other systems, including other colonial marine invertebrates (Harvell and Grosberg 1988; Kozłowski and Weiner 1997; Hesse *et al.* 2008). As far as we are aware, our study is the first to demonstrate these effects in response to the size of neighbours, but size-mediated competition can alter life histories within species (Bassar *et al.* 2016). Recall, however, that higher densities of larger *Bugula* competitively excluded *Watersipora*. At the population-level, therefore, shifts towards earlier reproduction did not compensate for the negative effects of competition. Indeed, elasticities reveal that small, young (reproductive) colonies are relatively unimportant for population growth when competition is most intense.

Much theory has examined the role of competition in shaping life histories (e.g. Kozłowski and Weiner 1997; Bassar *et al.* 2016), while the role of facilitation has traditionally been ignored, yet can be equally strong (Zepeda and Martorell 2019). We found that facilitation slowed the focal's life history relative to heterospecific-free conditions (see Castellanos *et al.* (2014) for a similar example in plants). In regions of facilitation, larger individuals contributed the most descendants to future population growth (i.e. had higher reproductive values) because focal colonies delayed reproduction and invested more in growth, such that they eventually reproduced more as larger colonies (Appendix 4: Table S3).

Elasticities further support this interpretation – smaller, younger (non-reproductive) colonies were important for population growth in regions of facilitation. As such, facilitation on early survival and growth persists at the population-level because a higher number of younger individuals transitioned to later stages and (eventually) reproduced more as larger colonies (Appendix 4: Table S3) – positively affecting population growth (c.f. Miriti *et al.* 2001). Crucially, we show that facilitation, not just competition, may shape life-history strategies and this warrants further exploration.

Consequences for coexistence

Classic studies presciently suggested that size promotes competitive intransitivities that maintains coexistence among species (Buss 1980; Russ 1982). While such studies were undoubtedly foundational, they could not access the population dynamical consequences of size-mediated interactions (Hart *et al.* 2018). We show that size-mediated interactions can generate profound differences in population dynamics (~ 70 % difference in population growth) that not only mediate competitive hierarchies, but also change the direction of species interactions. Given these findings, studies that aim to identify competitive hierarchies should use a size-based approach, integrated into a dynamic framework. To illustrate, our current findings contradict previous studies in our system that did not manipulate body size, but rather estimated interactions across a restricted size range (Hart *et al.* 2012; Hart and Marshall 2013). In these earlier studies, *Bugula* had, at most, weak competitive effects on *Watersipora*. In contrast, we find strong effects that depend on *Bugula* sizes and densities. Such idiosyncrasies may explain why average competitive effects *appear* weak when size-structure is ignored, and may lead to misleading conclusions about putative competitors.

Our findings highlight two important points. First, the overall outcome of species interactions will depend on the size-structure of the interacting species within a given

community (Buss 1980), which often differ in time and space (including in our species: Marshall 2005). Certainly, different population structures yield vastly different population dynamical outcomes within species (Miriti *et al.* 2001; Bassar *et al.* 2015). Second, recall that we found complex, nonlinear effects of heterospecific size, density and biomass on both the population growth, and size-specific reproductive values, of the focal species. Thus, examining performance for any one size combination of heterospecific and focal (even the most common sizes) will provide inaccurate estimates of species interactions due to non-linear averaging (i.e. Jensen's inequality: Jensen 1906). Jensen's inequality thus precludes meaningful inferences based on the average sizes of interacting species, and can have important implications for species coexistence (Bolnick *et al.* 2011; Hart *et al.* 2016). As such, we suggest an explicit partitioning of size-specific effects is necessary to fully capture the dynamics, and identify the ultimate outcomes, of species interactions.

Our findings may have further applications for theories of coexistence. Cohorts of *Bugula* will alternatively facilitate and compete with *Watersipora*. How this alternation of benefits and costs ultimately affect coexistence is unclear. Recent studies proposed that opposite-signed interactions may represent an equalising mechanism for species coexistence (Zepeda and Martorell 2019) – our finding of size-mediated competition and facilitation may have similar consequences. Importantly, current theory largely focuses on size differences among species. We show intraspecific size variation among heterospecifics simultaneously determines competition and facilitation – an alternative pathway through which species using the same resources may coexist (Gross 2008). Such theory is yet to consider body size in mediating these effects, but our results suggest this exciting possibility.

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Figures

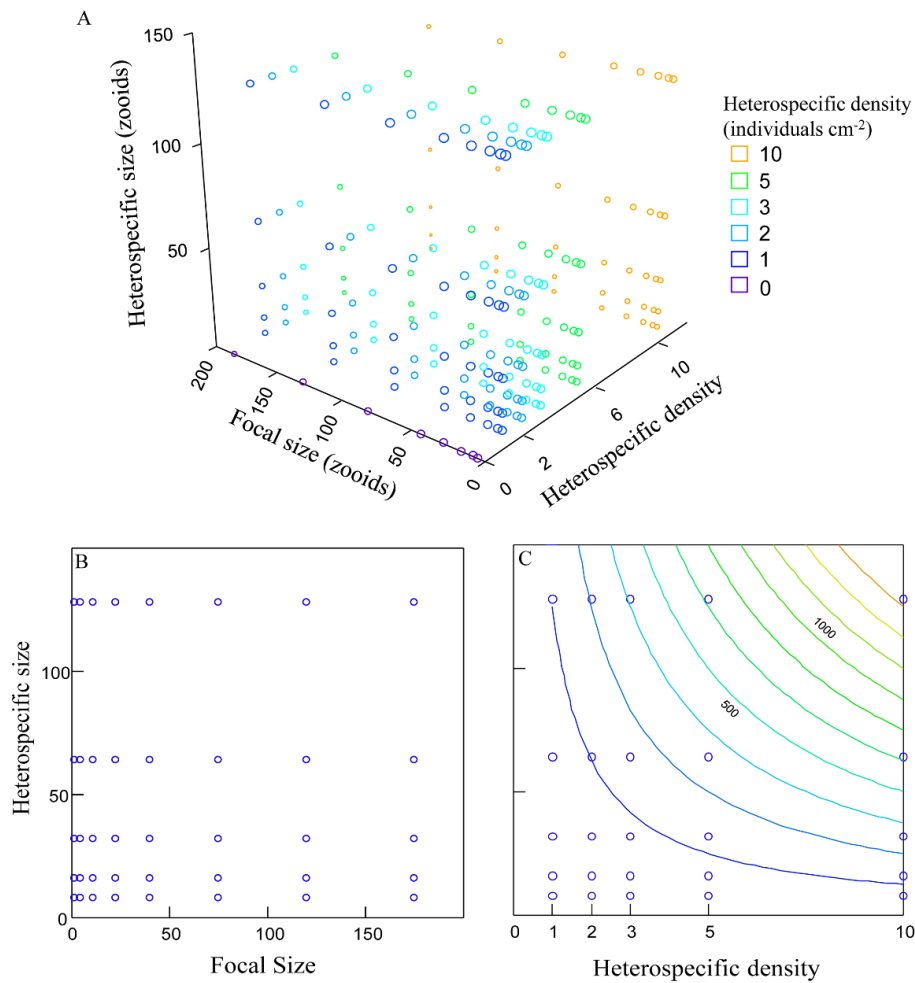


Figure 1. **A.** Schematic of the trait-specific, response cube used to test the effects of the size and densities (legend) of heterospecifics (*Bugula*) on the size-structured population dynamics of a focal species (*Watersipora*). **B.** Variation in the absolute and relative sizes (zooids) of the focal and heterospecific species (absolute size difference range of focal to heterospecific: -126 to 167 zooids; relative size ratio range of focal to heterospecific: 0.01 to 21.88). **C.** The heterospecific environments (*Bugula* sizes and densities) used to generate isoclines of heterospecific biomass (total zooids; coloured lines). Overall, the experiment included 30 heterospecific size and density combinations replicated across eight focal colonies of variable size, and we measured key demographic rates for 240 focal *Watersipora* colonies in the field across their lifetime.

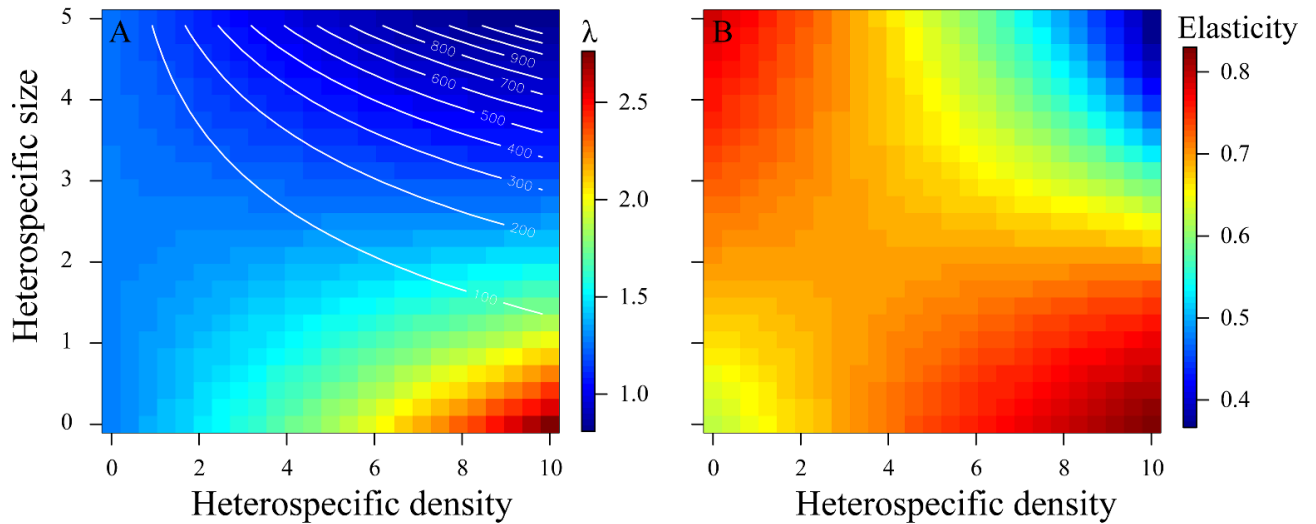


Figure 2. The effects of heterospecific (*Bugula*) size and density on: **A.** the projected population growth rate (λ) of the focal species (*Watersipora*). Note that values of λ greater than those in heterospecific-free conditions (when heterospecific density is 0) indicate facilitation, while values of λ less than those in heterospecific-free conditions indicate competition. Values of $\lambda < 1$ predicts competitive exclusion of the focal species. White contour lines show equivalencies in total heterospecific biomass (as zooids) – which are the product of heterospecific size and density (see also Fig. 1c). **B.** The summed elasticities for each demographic parameter (survival, growth and reproduction) for all *Watersipora* sizes in the first-age class. Larger values of elasticity indicate that smaller, younger colonies contribute relatively more to λ than smaller elasticity values.

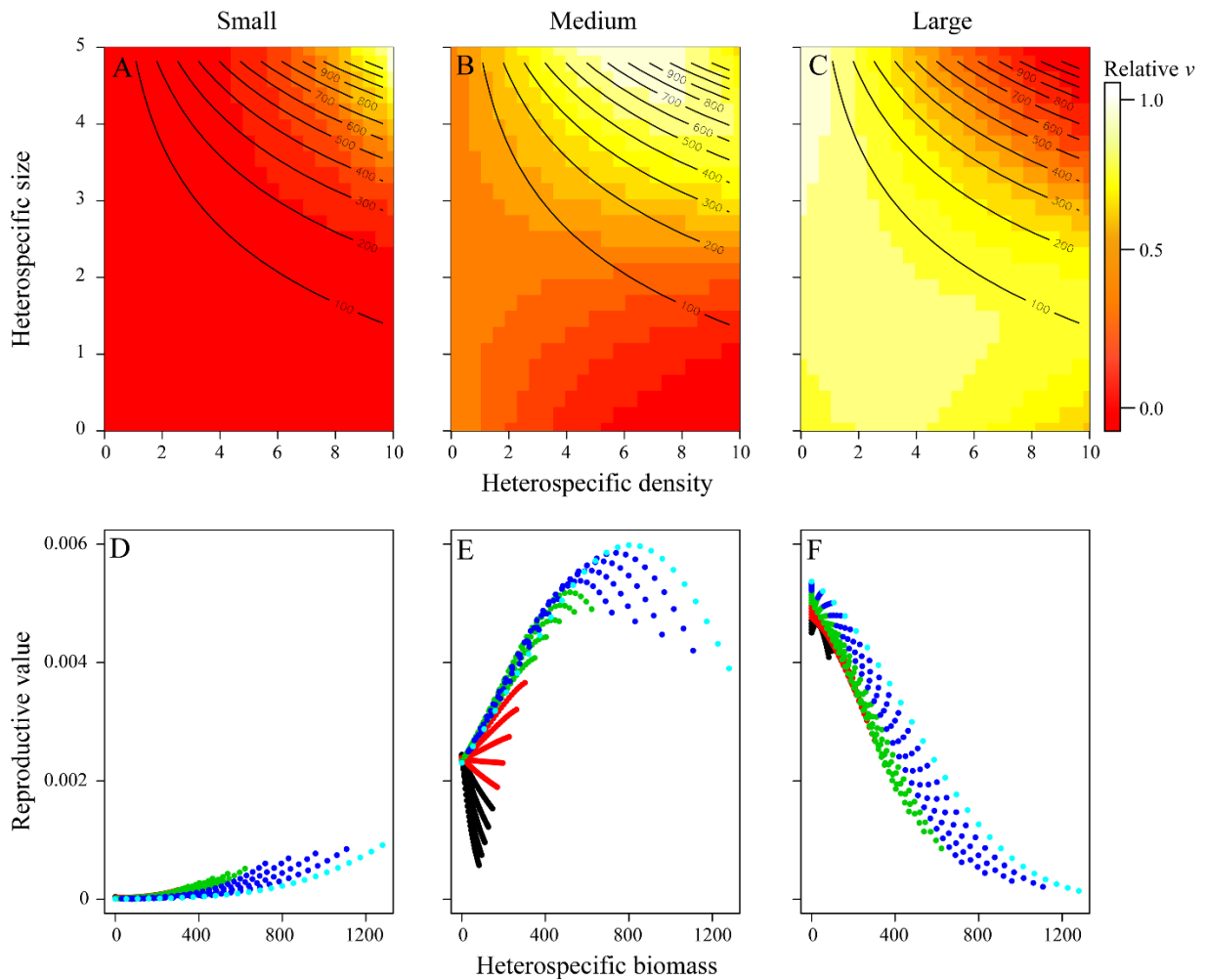


Figure 3. The reproductive values (v) of small (**A,D**), intermediate (**B,E**) and large (**C,F**) *Watersipora* colonies in response to the size, density and total biomass of heterospecifics (*Bugula*). Panels **A-C** show relative values of v within each *Watersipora* size class (legend) as a function of heterospecific size (as bifurcations) and density (individuals 121 cm^{-2}). Black contour lines represent equivalencies of heterospecific biomass (as zooids) – i.e. the product of heterospecific size and density (see Fig 1c). Panels **D-F** are absolute values of v plotted against total heterospecific biomass (zooids). Coloured dots represent the size-classes of the heterospecific (as bifurcations: black = 1, red = 2, green = 3, navy = 4, aqua = 5). The intercepts for these panels represent reproductive values for small (**D**), intermediate (**E**) and large (**F**) focal colonies under neighbour-free conditions. Note that in **D**, the reproductive values of smaller heterospecifics (black and red lines) are zero.

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Appendix 1: Field set up

We performed the experiment at the Blairgowrie Yacht Squadron, Victoria, Australia (38°21'20.2"S, 144°46'22.8"E) from December 2013 to April 2014. We obtained colonies of both species that varied in size using standard methods (Hart and Marshall 2009). Briefly, we grew colonies in the field on roughened acetate sheets (210 x 295 cm) that we attached to the undersides of PVC backing panels (400 x 400 cm) that hung 1 m below the water surface. We retrieved the acetate sheets bearing these colonies after 6 weeks in the field for use in our experiments. We created our experimental manipulations by cutting the colonies from the acetate sheets on which they had settled and gluing them onto PVC plates (11 x 11 cm; Fig. S1) according to our treatments (see experimental methods). We deployed the plates into the field by attaching them to the undersides of PVC backing panels (as described). Each backing panel received all eight replicate plates from two of the possible 30 heterospecific size and density combinations (see experimental methods; also Table S1). Thus, each backing panel received 16 plates (haphazardly arranged) and there were 15 backing panels. In our system, interactions occur at the scale of plates and competition beyond this is undetectable (Hart and Marshall 2009, Hart *et al.* 2012). Thus, individuals from adjacent plates were unlikely to interact. In total, our experiment included 1080 colonies of known size for both of our two species, arranged across 240 plates and 15 backing panels.

Table S1. The spatial arrangement of our experimental units (plates: $n = 240$) across PVC backing panels ($N = 15$) in the field. Each backing panel received eight replicate plates from two of the possible 30 heterospecific (*Bugula*) size and density combinations (16 plates total per panel). We also provide the size ranges (colony area; cm^2) of the focal species (*Watersipora*) that were allocated to each heterospecific size and density combination ($n = 8$).

| Panel | <i>Bugula</i> size; Bifurcations (zooids) | <i>Bugula</i> density; individuals 121 cm^{-2} | <i>Watersipora</i> size range; cm^2 |
|--------------|--|--|--|
| 1 | NA | 0 | 0.754 - 188.92 |
| 1 | 5 (128) | 10 | 0.907 - 192.163 |
| 2 | NA | 0 | 0.224 - 179.199 |
| 2 | 4 (64) | 10 | 0.565 - 128.168 |
| 3 | NA | 0 | 0.386 - 128.021 |
| 3 | 3 (32) | 10 | 0.932 - 76.292 |
| 4 | NA | 0 | 0.748 - 161.49 |
| 4 | 2 (16) | 10 | 0.976 - 105.194 |
| 5 | NA | 0 | 0.660 - 201.602 |
| 5 | 1 (8) | 10 | 0.551 - 96.912 |
| 6 | 1 (8) | 1 | 1.922 - 153.781 |
| 6 | 5 (128) | 5 | 1.567 - 102.664 |
| 7 | 2 (16) | 1 | 0.326 - 138.959 |
| 7 | 4 (64) | 5 | 0.45 - 107.636 |
| 8 | 3 (32) | 1 | 1.055 - 172.647 |
| 8 | 3 (32) | 5 | 0.240 - 305.282 |
| 9 | 4 (64) | 1 | 0.616 - 310.799 |
| 9 | 2 (16) | 5 | 0.185 - 189.815 |
| 10 | 5 (128) | 1 | 0.655 - 153.913 |
| 10 | 1 (8) | 5 | 0.761 - 147.589 |
| 11 | 1 (8) | 2 | 0.827 - 256.343 |
| 11 | 5 (128) | 3 | 1.09 - 127.76 |
| 12 | 2 (16) | 2 | 0.971 - 103.644 |
| 12 | 4 (64) | 3 | 2.314 - 162.291 |
| 13 | 3 (32) | 2 | 0.428 - 79.944 |
| 13 | 3 (32) | 3 | 0.305 - 109.267 |
| 14 | 4 (64) | 2 | 0.799 - 135.056 |
| 14 | 2 (16) | 3 | 0.655 - 414.204 |
| 15 | 5 (128) | 2 | 0.401 - 124.932 |
| 15 | 1 (8) | 3 | 1.112 - 209.536 |



Fig. S1. An example of our experimental manipulations used to test size- and density-mediated interactions between two colonial marine bryozoans: our focal species, the encrusting *Watersipora subtorquata* (**W**), and our heterospecific species, the arborescent *Bugula neritina* (**B**). Colonies were haphazardly glued onto PVC plates (11 x 11 cm) at various combinations of focal and heterospecific sizes, as well as different heterospecific densities (see methods).

Appendix 2: Heterospecific (*Bugula*) size and density affect conspecific survival

Intra- and interspecific interactions are obviously dynamical processes that occur simultaneously. We therefore monitored the survival of our heterospecific species (*Bugula*) at each census time to determine whether intraspecific interactions among the heterospecific species might affect the population dynamics of our focal species (*Watersipora*). *Bugula* mortality was characterised by the absence of the colonies from the plates, while all colonies that remained on the plates had living zooids – that is, *Bugula* only ever interacted with *Watersipora* as living colonies. We analysed the survival trajectories of these *Bugula* colonies across the experiment using a repeated measures analysis. Our response variable was the proportion of *Bugula* colonies that remained alive on the plates at each census, while our predictor variables were the initial sizes and densities of *Bugula* colonies on the plates at the beginning of the experiment, the panels on which they were deployed (all continuous, fixed), and time (in weeks; categorical, random).

Overall, the survival of *Bugula* decreased gradually over our eight-week experiment (Table S2; Fig. S2a). We also found that *Bugula* size and density interactively affected conspecific survival, but these effects tended to differ over time (Table S2). All *Bugula* size-classes showed a positive relationship between survival and density across the experiment (i.e. all sizes experienced intraspecific facilitation), but the relative strength of facilitation differed over time depending on *Bugula* size (Table S2). Early in the experiment (2 – 6 weeks), large *Bugula* colonies had consistently high survival across all conspecific densities; while smaller *Bugula* experienced the strongest intraspecific facilitation because their mortality was highest at low to intermediate densities (Fig. S2b). By the last census time (8 weeks), colonies of all sizes experienced the same strength of intraspecific facilitation –

although, the largest *Bugula* colonies has slightly (but not statistically) higher survival at higher conspecific densities than smaller colonies (Table S2; Fig. S2c).

The systematic differences in *Bugula* survival we observed across different conspecific sizes and densities provide some support for our hypothesis that these factors alter the local supply and demand of resources for our focal species (*Watersipora*; see discussion). Intraspecific facilitation largely occurred across heterospecific size classes, suggesting that increasing densities of *Bugula* disrupt flows to enhance the entrainment and(or) the supply of resources, to neighbouring conspecifics (Okamura 1990; Thompson *et al.* 2015; Cameron *et al.* 2016; Cameron and Marshall 2019). Early in the experiment, smaller colonies may benefit most from resource amelioration because they capture resources less efficiently in isolation (Okamura 1984, 1990; Cameron and Marshall 2019). In contrast, larger colonies were neutrally affected by conspecific density – presumably because they consume resources (food and oxygen) more efficiently (Okamura 1984; Barneche *et al.* 2017), such that they do not benefit from flow amelioration. In contrast, all *Bugula* colonies experienced the same strength of facilitation later in the experiment. This may be because as the colonies grew and total biomass on the plates increased, larger colonies may begin to benefit from increased resource supply by neighbours. We note that positive density dependence may also reduce predation risk in *Bugula* (Keough 1984). Certainly, predation from fish and nudibranchs is common in our study area (Keough 1984, Keough and Bone 2005), and may have contributed to the patterns of mortality we observe.

Regardless of the mechanisms generating intraspecific facilitation in *Bugula*, an important point to note is that our manipulations of heterospecific densities were consistent over the course of the experiment. High-density treatments remained relatively high, while low-density treatments remained low, suggesting the effects of heterospecifics on the focal species were not the result of self-thinning in our experimental heterospecific populations.

Table S2. Repeated measures analysis showing the effects of heterospecific (*Bugula*) size and density on conspecific survival across our eight-week field experiment.

| Source | df | MS | F | <i>p</i> | <i>G-G*</i> |
|-------------------------|----------|---------------|----------------|--------------------|-------------------|
| <i>Between subjects</i> | | | | | |
| Size | 1 | 11.357 | 80.510 | <0.0001 | |
| Density | 1 | 0.641 | 4.542 | 0.034 | |
| Size x density | 1 | 4.682 | 33.191 | <0.0001 | |
| Panel | 1 | 19.783 | 140.248 | <0.0001 | |
| Residual | 193 | 0.141 | | | |
| <i>Within subjects</i> | | | | | |
| Time | 3 | 0.262 | 6.562 | < 0.0001 | 0.0001 |
| Time x size | 3 | 0.110 | 2.751 | 0.042 | 0.051* |
| Time x density | 3 | 0.029 | 0.716 | 0.542 | 0.521 |
| Time x panel | 3 | 0.812 | 20.372 | <0.0001 | <0.0001 |
| Time x size x density | 3 | 0.062 | 1.550 | 0.200 | 0.207 |
| Residual | 579 | 0.040 | | | |

* *G-G*: Greenhouse-Geisser adjustment. Marginally non-significant interaction

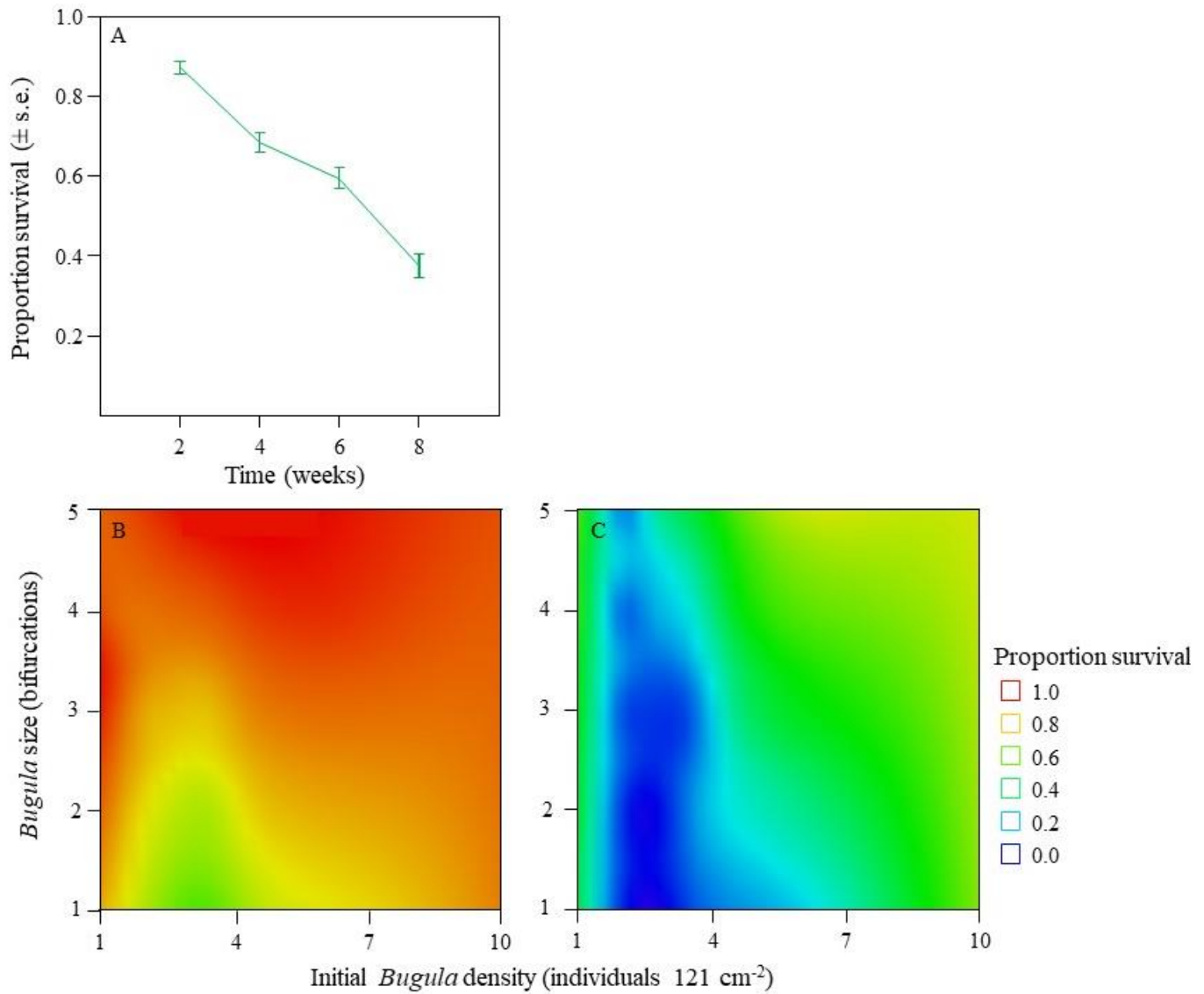


Figure S2. A. The overall trajectory of heterospecific (*Bugula*) survival over the course of our 8 week field experiment. Effects of the initial size and density of *Bugula* colonies on conspecific survival (side bar) after 2 (**B**) and 8 (**C**) weeks in the field. Note that size- and density-specific trajectories of survival after 4 and 6 week in the field largely reflect those shown in **B**.

Appendix 3: Model description

To test whether size-mediated competition affects the population dynamics of a focal species (*Watersipora*), we parameterised a single species, single-sex deterministic Integral Projection Model (IPM; Easterling *et al.* (2000)) with our experimental data. *Watersipora* colonies are simultaneous hermaphrodites, but we only consider female performance here. Our IPM is both a size- and age-structured model (Coulson *et al.* 2010).

$$n(z', 1, t + 1) = \sum_a \int [D(z'|z, a, y_b, N_b)M(z, a, y_b, N_b)B(z, a, y_b, N_b)n(z, a, t)]dz$$

$$n(z', a + 1, t + 1) = \int [G(z'|z, a, y_b, N_b)S(z, a, y_b, N_b)n(z, a, t)]dz$$

We model the continuous trait for size, z , as the square root of colony area (cm^2). This transformation produced a linear growth curve with time over the course of our study. In our model, the first equation deals with settlers, the second equation deals with the survival and growth of colonies.

$D(z'|z, y_b, a, N_b)$ is the conditional probability density function that describes the distribution of offspring sizes, z' , produced by parental colonies of size z at time $t + 1$. a is the experimental age class, y_b is the size of the competitor (as bifurcations), and N_b is the density of the competitor. For this function, we assumed a Gaussian distribution of offspring sizes (mean: $0.3 \text{ cm}^2 \pm 0.05 \text{ s.d.}$) that were independent of parental size (Marshall and Keough 2003). $M(z, a, y_b, N_b)$ is a continuous function describing the mean number of offspring produced by an individual with the colony area z , and of experimental age a , given the size and density of the competitor. $S(z, a, y_b, N_b)$ and $B(z, a, y_b, N_b)$ are continuous functions that describe the probability of an individual with colony area z , and experimental age a , at the beginning of the interval surviving and reproducing at the end of the interval, respectively, as

a function of competitor density and size. $G(z'|z, a, y_b, N_b)$ is the Gaussian probability density function describing transitions from colony area z at time t to colony area z' at time $t + 1$ among survivors as a function of experimental age, competitor size and density. $n(z, t)$ is the distribution of *Watersiporia* size at time t such that $N(t) = \int_x^y n(z, t) dz$ is the number of individuals between size z and y . We note that a , y_b and N_b are experimental factors that we manipulate and are not dynamic in the model.

To estimate the demographic functions required to parameterise the IPM, we used generalized linear models (GLMs) on our experimental data. Interrogation of the data revealed the following functional forms:

$$S(z, a, y_b, N_b) = \frac{1}{1 + e^{-V(z, \dots)}}$$

$$B(z, a, y_b, N_b) = \frac{1}{1 + e^{-V(z, \dots)}}$$

$$M(z, a, y_b, N_b) = e^{V(z, \dots)}$$

$$G(z'|z, a, y_b, N_b) = \theta(V(Z, \dots), W(Z, \dots))$$

$$D(z'|z, a, y_b, N_b) = \theta(V(Z, \dots), W(Z, \dots))$$

Where $V(Z, \dots)$ and $W(Z, \dots)$ are linear predictors that include the effects of experimental age, competitor size and density and (where appropriate) focal individual size. For the first three functions the dependent variable is size at time t , while for the second two functions it is size at time $t + 1$. Survival, growth and reproduction (but not fecundity) showed systematic covariation with the arrangement of panels in the field. For these functions, we assigned the panels to two groups that best captured this spatial variation and used these groups to model panel as a categorical predictor. For fecundity, we included all the panels (15 levels). For fecundity, we excluded colonies $> 1600 \text{ cm}^2$ (e.g. $Z > 40$) because otherwise

these colonies were treated as immortal in the model and provided nonsensical results. For each vital rate function we first fit full models, then reduced these models by removing non-significant interactions (assessed at $\alpha < 0.05$). We parameterised our IPM using the coefficients obtained from these reduced vital rate functions (Appendix 3; Table 1). Importantly, IPMs parameterised with the full models and those parameterised with simplified functions gave almost identical projections.

For numerical solutions of the IPM, we approximate the integro-difference equation describing the per time step dynamics with an age- and size-structured matrix, using the mid-point rule for numerical integration (Easterling *et al.* 2000; Ellner *et al.* 2016). *Watersipora* size was split into 100 classes that ranged from -2 to 50 cm, and model projections were checked to ensure there was no eviction from the model (Williams *et al.* 2012). Colonies can grow larger than 50 cm in our study population, but they did not do so in our experiment, and because we imposed a mortality threshold, they never did in the model.

Appendix 4: Heterospecific size and density affect the size-specific vital rates of a focal species

We found that the size and density of a heterospecific species (*Bugula*) have idiosyncratic effects on the size-specific vital rates of a focal species (*Watersipora*). We found that heterospecific size negatively affected the survival and (marginally) the growth of the focal species, while heterospecific density positively affected these vital rates (Table S3). The size of focal colonies at time t also positively affected survival and growth (Table S3). Overall then, focal survival and growth were greatest when heterospecifics were small and numerous – indicative of facilitation (Table S4).

In contrast, focal size, heterospecific size and heterospecific density interactively affected the probability that the focal colonies reproduced (Table S3). For example, at the final census, small focal colonies (0.25 mm^2) had a very low probability of reproducing ($< 4 \%$). Intermediate focal colonies (100 mm^2) displayed the most variation in reproductive success ($0 - 65 \%$), with highest values occurring when heterospecifics were large and numerous. For large focals (400 mm^2), the probability of reproduction was uniformly high across all heterospecific regimes ($90 - 100 \%$). For those colonies that did reproduce, focal size, heterospecific size and heterospecific density interactively affected reproductive output (i.e. fecundity; Table S3; Table S4). For example, at the last census, small focal colonies did not produce offspring across most of the parameter space, although some offspring were produced at the highest densities of large heterospecifics (< 20). Intermediate colonies also produced few offspring, but fecundity was slightly increased when heterospecifics were small and numerous. Large colonies had the highest variation in reproductive output ($0 - 60$ offspring) – they produced no offspring when heterospecifics were large and numerous, but produced the most offspring when heterospecifics were small and numerous.

Table S3. Generalised linear models (GLMs) testing the effects of heterospecific (*Bugula*) size and density on the size-specific vital rates of a focal species (*Watersipora*). Parameter estimates are those used to parameterise our integral projection model. Note we obtained these estimates from reduced GLMs that excluded non-significant interactions (assessed at $\alpha < 0.05$), but we include these tests (but not estimates) from the full models for completeness. Test statistics provided are χ^2 values for GLMs with binomial and Poisson errors (survival and fecundity, respectively) and F ratios for GLMs with Gaussian and quasi-binomial errors (growth and reproduction, respectively). Bolded values are significant at $\alpha < 0.05$.

| Source | Estimate | \pm SE | Df | Test statistic | <i>p</i> |
|--|----------|----------|----|----------------|--------------------|
| Survival | | | | | |
| Intercept | -0.395 | 0.299 | | | |
| Focal size | 0.488 | 0.055 | 1 | 492.79 | <0.0001 |
| Heterospecific size | -0.217 | 0.079 | 1 | 480.92 | <0.0001 |
| Heterospecific density | 0.054 | 0.038 | 1 | 473.05 | 0.005 |
| Focal size x heterospecific size | | | 1 | 401.8 | 0.572 |
| Focal size x heterospecific density | | | 1 | 401.73 | 0.797 |
| Heterospecific size x density | | | 1 | 401.73 | 0.949 |
| Focal size x heterospecific size x density | | | 1 | 400.09 | 0.2 |
| Panel | | | 1 | 406.14 | <0.0001 |
| Experimental age (census) | | | 3 | 402.12 | 0.259 |
| Census 2 | -0.205 | 0.307 | | | |
| Census 3 | -0.598 | 0.388 | | | |
| Census 4 | -0.824 | 0.498 | | | |
| Growth | | | | | |
| Intercept | 0.316 | 0.212 | | | |
| Focal size | 1.13 | 0.017 | 1 | 6068.33 | <0.0001 |
| Heterospecific size | -0.098 | 0.05 | 1 | 3.1815 | 0.075 |
| Heterospecific density | 0.04 | 0.024 | 1 | 7.0687 | 0.008 |
| Focal size x heterospecific size | | | 1 | 1.3683 | 0.243 |
| Focal size x heterospecific density | | | 1 | 0.7668 | 0.382 |
| Heterospecific size x density | | | 1 | 0.0149 | 0.903 |
| Focal size x heterospecific size x density | | | 1 | 1.1681 | 0.281 |
| Panel | | | 1 | 29.1188 | < 0.0001 |
| Experimental age (census) | | | 3 | 6.9597 | 0.0001 |
| Census 2 | 0.56 | 0.215 | | | |
| Census 3 | 0.774 | 0.234 | | | |
| Census 4 | 1.182 | 0.273 | | | |

| <i>Probability of Reproduction</i> | | | | | |
|--|--------|-------|----|---------|-------------------|
| Intercept | -22.81 | 1463 | | | |
| Focal size | 0.206 | 0.097 | 1 | 597.046 | <0.0001 |
| Heterospecific size | -2.772 | 1.242 | 1 | 12.157 | <0.001 |
| Heterospecific density | -0.034 | 0.337 | 1 | 35.717 | <0.0001 |
| Focal size x heterospecific size | 0.193 | 0.082 | 1 | 22.25 | <0.0001 |
| Focal size x heterospecific density | 0.008 | 0.027 | 1 | 1.092 | 0.297 |
| Heterospecific size x density | 0.247 | 0.166 | 1 | 5.75 | 0.017 |
| Focal size x heterospecific size x density | -0.015 | 0.013 | 1 | 5.505 | 0.02 |
| Panel | | | 1 | 41.102 | <0.0001 |
| Experimental age (census) | | | 3 | 95.23 | <0.0001 |
| Census 2 | -1.566 | 2600 | | | |
| Census 3 | 17.42 | 1463 | | | |
| Census 4 | 20.16 | 1463 | | | |
| <i>Fecundity</i> | | | | | |
| Intercept | 0.117 | 0.487 | | | |
| Focal size | 0.14 | 0.025 | 1 | 595.8 | <0.0001 |
| Heterospecific size | -0.071 | 0.26 | 1 | 568.13 | <0.0001 |
| Heterospecific density | -0.132 | 0.167 | 1 | 568.07 | 0.801 |
| Focal size x heterospecific size | 0.017 | 0.012 | 1 | 289.7 | 0.191 |
| Focal size x heterospecific density | 0.023 | 0.008 | 1 | 288.57 | 0.287 |
| Heterospecific size x density | 0.102 | 0.059 | 1 | 280.41 | 0.004 |
| Focal size x heterospecific size x density | -0.012 | 0.003 | 1 | 264.31 | <0.0001 |
| Panel | | | 11 | 298.83 | <0.0001 |
| Experimental age (census) | | | 1 | 291.41 | 0.006 |
| Census 4 | -0.317 | 0.11 | | | |

Table S4. Summary table of size (in cm²) and fecundity (in ovicells) of the focal species, *Watersipora*, after 8 weeks in the field across different heterospecific (*Bugula*) sizes and densities. Because the size of focal species affected subsequent size and reproduction, we report maximum observed values for each heterospecific size/density combination. Note that no focal colonies on Panels 13-15 survived to the final census.

| Panel | <i>Bugula</i> size; Bifurcations (zooids) | <i>Bugula</i> density; (individuals 121 cm⁻²) | <i>Watersipora</i> size (cm²) | <i>Watersipora</i> fecundity (ovicells) |
|--------------|--|---|---|--|
| 1 | NA | 0 | 4.25 | 20 |
| 1 | 5 (128) | 10 | 2.56 | 8 |
| 2 | NA | 0 | 4.23 | 28 |
| 2 | 4 (64) | 10 | 0.47 | 6 |
| 3 | NA | 0 | 8.28 | 26 |
| 3 | 3 (32) | 10 | 5.05 | 11 |
| 4 | NA | 0 | 6.66 | 40 |
| 4 | 2 (16) | 10 | 4.51 | 9 |
| 5 | NA | 0 | 6.43 | 25 |
| 5 | 1 (8) | 10 | 5.29 | 27 |
| 6 | 1 (8) | 1 | 3.45 | 2 |
| 6 | 5 (128) | 5 | 4.98 | 7 |
| 7 | 2 (16) | 1 | 0.79 | 0 |
| 7 | 4 (64) | 5 | 5.55 | 9 |
| 8 | 3 (32) | 1 | 4.40 | 3 |
| 8 | 3 (32) | 5 | 6.14 | 11 |
| 9 | 4 (64) | 1 | 3.69 | 6 |
| 9 | 2 (16) | 5 | 6.64 | 45 |
| 10 | 5 (128) | 1 | 5.94 | 54 |
| 10 | 1 (8) | 5 | 5.75 | 74 |
| 11 | 1 (8) | 2 | 2.32 | 0 |
| 11 | 5 (128) | 3 | 5.27 | 29 |
| 12 | 2 (16) | 2 | 7.95 | 25 |
| 12 | 4 (64) | 3 | 4.97 | 15 |

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