MONASH University

Integrating life-history theory and metabolic theory

Amanda Kate Pettersen

BSc Environmental Science

A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2017

School of Biological Sciences

Copyright notice

Abstract

Energy is essential to all life. Understanding the ways in which energy is acquired and allocated extends across all scales in ecology – from individuals to ecosystems. Biologists often seek either ultimate or proximate explanations in order to understand variation in the acquisition and allocation of energy, but the two approaches are not incompatible. Life-history theory and metabolic theory offer powerful tools for understanding processes driving phenotypic variance, yet traditionally these fields have done so independently of the other. This thesis provides some of the first steps towards integrating two fundamental fields in ecology: life-history theory and metabolic theory. Through integrating two mostly separate fields, I can address how variation in key fitness traits arise, how they might be maintained, and our expectations of how these traits will evolve across environments. I combine empirical work, both within and among species, in the laboratory and field, with meta-analyses to address questions central to life history and metabolic theory. First, I use metabolic theory to address life history patterns to provide general explanations for why larger offspring perform better than smaller offspring, and why colder mothers produce larger offspring. Second, I emphasise the importance of using formal microevolutionary approaches to explain how variation in metabolic rates persist, and measure the covariance between metabolic rates and fitness in the field. This thesis demonstrates the potential insights gained by integrating life-history theory and metabolic theory by explaining key patterns in offspring size and metabolic rates, and highlights the need for further work at the intersection of these two fields.

Publications during enrolment

Pettersen, A.K., C.R. White, and D.J. Marshall. 2015. Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proceedings of the Royal Society B-Biological Sciences 282.

Pettersen, A.K., C.R. White, and D.J. Marshall. 2016. Metabolic rate covaries with fitness and the pace of the life history in the field. Proceedings of the Royal Society B-Biological Sciences 283.

Torres, G., L. Giménez, A.K. Pettersen, M. Bue, M.T. Burrows, and S.R. Jenkins. 2016. Persistent and context-dependent effects of the larval feeding environment on post-metamorphic performance through the adult stage. Marine Ecology Progress Series 545:147-160.

Giménez, L., G. Torres, A.K. Pettersen, M.T. Burrows, A. Estevez, and S.R. Jenkins. 2017. Scale-dependent natural variation in larval nutritional reserves in a marine invertebrate: implications for recruitment and cross-ecosystem coupling. Marine Ecology Progress Series 570:141-155.

Pettersen, A.K., C.R. White, R.J. Bryson-Richardson, and D.J. Marshall. 2017. Does the cost of development scale allometrically with offspring size? Functional Ecology.

Pettersen, A.K., D.J. Marshall, and C.R. White. In press. Understanding variation in metabolic rate. Journal of Experimental Biology.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been 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 four original papers published in peer reviewed journals and no submitted publications. The core theme of the thesis is evolutionary ecology. 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 supervision of Prof Dustin Marshall and Prof Craig White.

(The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.)

In the case of Chapters 1, 2, 3, and 4 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
1	Why does offspring size affect performance? Integrating metabolic scaling with life-history theory	Published	Design, data collection, data analysis, and writing of manuscript 85%	1) Dustin Marshall, design, analysis, 10% 2) Craig White, design, analysis, 5%	No
2	Does the cost of development scale allometrically with offspring size?	Published	Design, data collection, data analysis, and writing of manuscript 85%	1) Dustin Marshall, design, analysis, 10% 2) Craig White, design, analysis, 4% 3) Robert Bryson-Richardson, design, analysis, 1%	No
3	Why do colder mothers produce larger offspring?	Not submitted	Design, data collection, data analysis, and writing of manuscript 85%	1) Dustin Marshall, design, analysis, 10% 2) Craig White, design, analysis, 4% 3) Robert Bryson-Richardson, design, analysis, 1%	No
4	Metabolic rate covaries with fitness and the pace of the life history in the field	Published	Design, data collection, data analysis, and writing of manuscript 85%	1) Dustin Marshall, design, analysis, 10% 2) Craig White, design, analysis, 5%	No

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

Student signature: [redacted] **Date:** 27th November, 2017

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: [redacted] **Date:** 27th November, 2017

Acknowledgements

This research was supported by an Australian Government Research Training Program (RTP) Scholarship, a Faculty of Science Dean's Postgraduate Research 'Top-Up' Scholarship, and an Endeavour Research Fellowship [grant number 5801-2017].

The last four and a half years have certainly been a journey. And there are many people I am indebted to for getting me through it in one piece. I always thought finishing a PhD with my sanity intact would be considered a success in itself. Well, it probably depends how you define sanity, but to my family, friends, and colleagues I couldn't have made it to the end without you! How to adequately acknowledge everyone who got me through the journey that has been "The PhD"? I'm not sure I can do it justice, but here goes…

Firstly, thank you to my supervisor, Dustin. Working with you has certainly tested my lab, field, and writing abilities (as well as my resilience), and pushed all of these to limits I never knew I had in me. I should have read the warning signs when I was thinking about applying for a PhD with you, where you responded to my questions in an email using all capital letters. There have been many ups and downs, but you always push me to work and think harder than ever before, and ultimately produce some good science (Many science. So research. Such PhD. Wow!). Before I started, I knew very little about life-history theory and had almost no appreciation for *Bugula*! Now that it's done, I can say it has been a great PhD, and I'd like to think we've found out some cool things along the way (including how amazing cronuts are!). I love studying life-histories, particularly in marine critters, and I owe a huge amount of this to the intensity of your mentoring, your passion for science, and our countless morning coffee walks. I know submitting this PhD is never going to convince you that I will actually ever leave Monash, but I think this is a good first step. This is fine.

To Craig, my co-supervisor, and "cool PhD uncle", I am so glad you moved your lab to Monash (though, given you don't actually have a physical lab yet, you might not feel the same!). Your encyclopaedic knowledge of physiology and metabolic theory blows me away at every meeting and reading group. It has been such a great experience having your insights close-by – whether it's helping me to build a tree, providing career advice, or explaining why the naked mole rat is an ectotherm. You have made learning about metabolic scaling a thoroughly enjoyable venture.

I would never have been able to survive some of the PhD tortures without my MEEG lab family. Thank you to my PhD big brothers, Ted and Marcelo who were there from the beginning. Ted, thank you for your patience with helping me resolve basic computer, statistical, and life issues, and for being an all-around great guy (and fellow, avid 'The Life Aquatic' fan). Marcelo, thank you for inviting me to be part of Team Bugula (we still need to get that tattoo!), for training me to use the SDR equipment, and for keeping me endlessly entertained with your throat singing, and unique, metal-head, Marcelo-ness. To my Scandi-sister, Karin – you are an absolute gem. Your infectious smile and happiness (and passion for berries and sal-mon) made every day at uni a little brighter (especially when Harvey was illegally brought into the office). Hayley, you have been my biggest ally throughout this whole thing, both as an RA and as a PhD sister. You always make me challenge myself, but have backed me every step of the way, and have helped me to be a better scientist. Your warped sense of humour matches my own, and I will never get sick of mocking your constant fomo! To Annie-G, thank you for being the most random lab mate ever. Your work ethic was inspiring, and your hyperactive nature kept me going during some of the toughest times. Evatt, you will always be the baby of the lab. Thank you for being such a pleasure to work with (and make fun of!), and for showing me all your amazing secret snorkel spots. To all the other MEEG lab members over the years – Amy, Matt, Robin, Colin, Rolanda, Keyne, Henry, Victor, Diego, Martino, Giulia, Lukas, James,

Lucy and Belinda - there are too many of you to dedicate a whole paragraph to here (I would if I could!), but thank you for your friendship and your support.

I have been fortunate in publishing some of my thesis chapters during my PhD, and by doing so, gained some excellent feedback and insight into life-history theory and metabolic theory from experts in these fields. Thank you to Douglas Glazier and Neil Metcalfe in particular, who went through many rounds of review providing invaluable feedback which ultimately made my thesis much more polished and coherent than I could have achieved on my own. Also, thank you to Gary Carvalho, Charles Fox, Craig Franklin, Christian Jørgsensen, Pat Krug, Loeske Kruuk, Juha Merilä, Kwang Pum Lee, Art Woods, as well as several anonymous reviewers, for serving as reviewers and handling editors. I would also like to thank my supervisors during my Endeavour Research Fellowship in the United States this year – Barry Sinervo, David Reznick, and their lab groups for hosting me, and for giving me a new-found appreciation for vertebrates!

There are many people at the School of Biological Sciences who have made the last four and a half years more than just about the PhD. In particular, I'd like to thank the following academic, teaching, and professional staff for being an inspiring group, just by doing what you do, and for all the support: Fiona Hibbert, Susie Ho, Damian Dowling, Chris Johnstone, Beth McGraw, and Rob-Bryson Richardson. Thank you to my milestone panel for their excellent feedback and insightful (and challenging) questions over the years - Steven Chown, Martin Burd, Tim Connallon, and Matt Hall. Being a part of the Postgrad Committee allowed me to get to know my postgrad cohort better. I could not have got through it without this amazing bunch of friends to share Friday beers with, to celebrate the PhD highs, and commiserate the lows, in particular thanks to: Sean, Will, Leo, Tom, Ash, Rowan, Lotte, Marcus, and Sonia. Special thanks to Marie for being my other Scandi-sister, R-guru, and fellow PhD pea-in-the-pod. Candice, you are a treasure - thanks for all of the office lols. Keyne, thank you for your

mentorship, and for sharing all your evolutionary biology pearls of wisdom over school-night beers and laughs in Balaclava, and to Rolanda - thanks for being there from the start and for being your quirky, inspirational self.

While lately my life has largely been dominated by the PhD, it would have been impossible to reach this point without the love and support of James, my family, and my closest friends. James, you kept our lives together and kept me sane with your patience, understanding and love. I owe you around a thousand cooked dinners! Thanks to the strong women in my life – my Mum, and Grandma. You are the reason I am here, and the reason I was too stubborn to ever quit. Thanks to my sisters, Anne and Jess - while you were unable to witness the PhD madness first-hand, your long-distance support and belief in me kept me going, nonetheless. Thanks to Dad for making me feel like I was the only person special enough to be able to do a PhD. Melinda, Natasha, Lauren, Laura, Kate, Mani, Bowen, Liz and Sarah – thank you for all of your unconditional support, I'm looking forward to rekindling our friendships this summer!

Table of Contents

Note on the thesis

This thesis is comprised of two main sections – each with its own approach to integrating life-history theory and metabolic theory. Section 1 consists of a thesis introduction and Chapters 1 – 3, and uses processes in metabolic theory to understand patterns in life-history theory, with particular reference to offspring size variation. Section 2 consists of a general introduction, adapted from a recently accepted Commentary article (Pettersen et al. In press) and Chapter 4, and explores how natural selection shapes variation in metabolic rates. The thesis ends with a general discussion of Chapters 1 to 4.

General introduction - Section 1

The optimal animal, born with some amount of energy, proceeds through its life gaining and expending energy according to some schedule that maximises its total reproductive output.

- Schoener (1971)

Life-history theory seeks to understand the causes and consequences of key relationships in biology, by linking common patterns observed in nature to strategies that optimise individual reproductive success. One fundamental life-history trait that is ubiquitous across the metazoan is offspring size (Bernardo 1996). Offspring size is defined as the per propagule structure provisioned by the mother, and provides an indication of the per offspring unit of maternal investment. Hence, offspring size reflects both the offspring and maternal phenotype, and variation in this trait has intrigued biologists over the past century (Fox et al. 1997; Lloyd 1987; Sinervo 1990; Thorson 1936). In 1947, Lack first intuited a trade-off between clutch size and offspring quality. Since then, offspring size optimality models have pervaded life-history theory. Vance's fecundity-time hypothesis model (1973) predicted the benefit of producing many, small offspring against its associated cost of increased time to development. Smith and Fretwell (1974) proposed that, according to the shape of the relationship between offspring size and offspring fitness, there is a single optimal level of per offspring investment that will maximise parental reproductive success (Levitan 1996). Based on these pioneering works, subsequent theories developed for a broad-range of taxa similarly assume the existence of an offspring size-number trade-off (Brockelman 1975; Lloyd 1987; McGinley et al. 1987; Pianka 1974; Pianka 1976; Sakai and Harada 2001). Despite over 70 years since Lack's initial discovery, our current understanding of the per unit offspring investment, the mechanisms by which offspring size evolves, and the implications of provisioning for offspring is still unclear (Wolf and Wade 2009).

Offspring size and performance

Within species, larger offspring often outperform their smaller conspecifics, and this relationship is ubiquitous across taxa (Rollinson and Hutchings 2013). For example, increases in offspring size are concomitant with higher survival, growth, reproductive output, and resistance to predation and starvation (Einum and Fleming 2000; Kaplan 1989; Marshall and Bolton 2007; Marshall and Keough 2006; Moran and Emlet 2001; Reznick 1991). While the benefits of larger offspring size are often highly context-dependent, the offspring-size performance relationship has become a central tenet of life-history theory (Stearns 1992). While increasing per offspring size results in decreased fecundity for the mother, the observation that variation in offspring size exists suggests that benefits must outweigh the costs in many cases. What remains unclear however, is *why* the offspring size-performance relationship exists at all. Several explanations have been proposed, for example, larger offspring are often better competitors, better able to avoid predation, better able to withstand starvation, and able to consume a larger, and therefore greater variety of prey items than smaller offspring (Aubret 2012; Janzen et al. 2000; Leishman et al. 2000; Martin and Pfennig 2010; Reznick et al. 1996; Rivest 1983; Sinervo 1990). Yet few studies offer a taxon-wide approach that can adequately address the ubiquity of the offspring size-performance relationship more broadly. One potentially universal mechanism, relevant to all organisms, is how energy use scales with offspring size.

Integrating metabolic scaling with the costs of development

Metabolic theory describes how the flow of energy through a living system, drives patterns and processes in biology (Glazier 2005). The most fundamental relationship in metabolic theory, is how metabolic rate (MR) scales with body mass (M), and is expressed by the power function,

$MR = aM^b$ (Kleiber 1932; Rubner 1908). While the exact value of the scaling exponent, *b* and its underlying mechanisms remain contentious, it is generally accepted that the relationship between metabolic rate and body mass is allometric, where *b* is less than 1 (Brown et al. 2004; Glazier 2010; Kooijman 2010). Hence, larger bodied organisms have a lower metabolic rate, per unit mass than smaller bodied organisms. Historically, metabolic theory has focussed on among species scaling relationships with adult body size, but more recent studies have sought to account for phylogeny and ontogeny (Caruso et al. 2010; Chown et al. 2007; Glazier 2005; Kearney and White 2012; Kozlowski and Konarzewski 2004; White et al. 2011). Static metabolic scaling measures the relationship between metabolic rate with mass within a single development stage, accounting for ontogenetic effects on metabolic rate throughout the life history (Pelabon et al. 2013). Measuring static metabolic scaling throughout the early life history can provide insights into the energy efficiency of small and large offspring. This is particularly crucial for understanding selection on offspring size, as all organisms have some part of the life history that is entirely dependent on maternal provisioning – such as throughout brooding, development or metamorphosis (from here on termed the 'dependent phase'). It is likely that offspring that end the dependent phase higher amounts of energy will show higher survival (Berkeley et al. 2004). Hence, the energy efficiency of offspring is important, and any factor that alters energy acquisition (i.e. offspring size) relative to energy use during the dependent phase is likely to shape the evolution of offspring size. If energy use during the dependent phase (such as during development or metamorphosis) scales allometrically with offspring size, then there may be hidden maternal benefits to producing larger offspring, and these benefits have previously been unrecognised by life-history theory.

Context-dependent selection on offspring size

Variation in offspring size may reflect a trade-off in maternal fitness between producing fewer, more energy efficient large offspring, relative to the benefits of increased fecundity by producing smaller offspring. Offspring size may also be constrained, where minimum offspring size is proportional to the minimum energy requirements needed to survive the dependent phase. While larger offspring generally outperform their smaller conspecifics, selection for larger offspring size is also often found to be context-dependent. For example, larger offspring have been found to be particularly favoured under low resource availability (Fox 2000; Hutchings 1991), intermediate competition (Allen et al. 2008) and as greater targets for fertilisation under low sperm availability (Crean and Marshall 2008). Conversely, larger offspring may experience higher mortality under low oxygen conditions (Einum et al. 2002); high predation (Reznick et al. 1990), or when settlement is delayed (Svanfeldt et al. 2016) relative to smaller offspring. In colder environments, mothers produce larger offspring – a pattern that is also found across latitudinal, and seasonal gradients (Barnes and Barnes 1965; Wootton and Smith 2014) and in experimental manipulations (Atkinson et al. 2001; Yampolsky and Scheiner 1996). The offspring size-temperature relationship is likely to be an adaptive response – survival of larger offspring relative to smaller offspring is more pronounced under colder environments than in warmer environments (Bownds et al. 2010; Burgess and Marshall 2011). While these patterns are well documented, the underlying adaptive mechanisms driving the offspring size-temperature relationship so far remain elusive. One potentially general explanation is how temperature alters the costs of development, as a function of metabolic rate and development time, and hence selection on offspring size.

Thesis aims and outline – Section 1

Life-history theory has often sought general explanations to address key patterns in traits that closely align with fitness, such as maternal investment and offspring size. Despite the ubiquity of metabolic scaling relationships across all scales of ecology, there has been surprisingly little influence of metabolic theory in explaining common life-history patterns, such as how size and temperature influence the costs of development during critical life stages. To address this knowledge gap, this thesis aims to unify two previously disparate fields of ecology by using key processes in metabolic theory to explain key life-history patterns, and point towards some potentially universal explanations in ecology (Chapters 1 to 3). In Chapters 1 and 2, I test whether allometric scaling with body size also applies to offspring of varying size, and whether this can provide a general explanation for the offspring size-performance relationship. The dependent phase consists of the stage during the life history from when offspring are released from maternal energy provisioning, through to development of feeding structures ("independence"). In order to test whether allometric scaling with offspring size is a general phenomenon, I measured energy use in two developmental modes, where the dependent phase consisted of either metamorphosis (release of offspring to completion of feeding structures) in two marine bryozoan species, *Bugula neritina* and *Watersipora subtorquata* (Chapter 1), and development (fertilisation to development of feeding structures) in the freshwater fish, *Danio rerio* (Chapter 2). In Chapter 3, I test underlying mechanisms that are driving the offspring size-temperature relationship by measuring the temperature sensitivity of the costs of development in two species, *Bugula neritina* and *Danio rerio*. I combine empirical tests with two phylogenetically-controlled meta-analyses to test the generality of my findings.

References

Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. American Naturalist 171:225-237.

Atkinson, D., S. A. Morley, D. Wheetman, and R. N. Hughes. 2001. Offspring size responses to maternal temperature in ectotherms. Environment and animal development:269-285.

Aubret, F. 2012. Body-size evolution on islands: are adult size variations in tiger snakes a nonadaptive consequence of selection on birth size? American Naturalist 179:756-767.

Barnes, H., and M. Barnes. 1965. Egg size, nauplius size, and their variation with local, geographical, and specific factors in some common cirripedes. Journal of Animal Ecology 34:391-402.

Berkeley, S. A., C. Chapman, and S. M. Sogard. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258-1264.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: Patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Bownds, C., R. Wilson, and D. J. Marshall. 2010. Why do colder mothers produce larger eggs? An optimality approach. Journal of Experimental Biology 213:3796-3801.

Brockelman, W. Y. 1975. Competition, fitness of offspring, and optimal clutch size. American Naturalist 109:677-699.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771-1789.

Burgess, S. C., and D. J. Marshall. 2011. Temperature-induced maternal effects and environmental predictability. Journal of Experimental Biology 214:2329-2336.

Caruso, T., D. Garlaschelli, R. Bargagli, and P. Convey. 2010. Testing metabolic scaling theory using intraspecific allometries in Antarctic microarthropods. Oikos 119:935-945.

Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. R. B. Lighton, and T. M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Functional Ecology 21:282-290.

Crean, A. J., and D. J. Marshall. 2008. Gamete plasticity in a broadcast spawning marine invertebrate. Proceedings of the National Academy of Sciences 105:13508-13513.

Einum, S., and I. A. Fleming. 2000. Selection against late emergence and small offspring in Atlantic salmon (Salmo salar). Evolution 54:628-639.

Einum, S., A. P. Hendry, and I. A. Fleming. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B-Biological Sciences 269:2325-2330.

Fox, C. W. 2000. Natural selection on seed-beetle egg size in nature and the laboratory: Variation among environments. Ecology 81:3029-3035.

Fox, C. W., M. S. Thakar, and T. A. Mousseau. 1997. Egg size plasticity in a seed beetle: An adaptive maternal effect. American Naturalist 149:149-163.

Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662.

Glazier, D. S. 2010. A unifying explanation for diverse metabolic scaling in animals and plants. Biological Reviews 85:111-138.

Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salelinus fontinalis*. Evolution 45:1162-1168.

Janzen, F. J., J. F. Tucker, and G. L. Paukstis. 2000. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. Journal of Evolutionary Biology 13:947-954.

Kaplan, R. H. 1989. Ovum size plasticity and maternal effects on the early development of the frog, *Bombina orientalis* Boulenger, in a field population in Korea. Functional Ecology 3:597-604.

Kearney, M. R., and C. R. White. 2012. Testing metabolic theories. American Naturalist 180:546-565.

Kleiber, M. 1932. Body size and metabolism, Pages 315-353.

Kooijman, S. A. L. M. 2010, Dynamic energy budget theory for metabolic organisation. Cambridge, Cambridge University Press.

Kozlowski, J., and M. Konarzewski. 2004. Is West, Brown and Enquist's model of allometric scaling mathematically correct and biologically relevant? Functional Ecology 18:283-289.

Lack, D. 1947. The significance of clutch size. Ibis 89:302-352.

Leishman, M. R., I. J. Wright, A. T. Moles, and M. Westoby. 2000. The evolutionary ecology of seed size. Seeds: the ecology of regeneration in plant communities 2:31-57.

Levitan, D. R. 1996. Predicting optimal and unique egg sizes in free-spawning marine invertebrates. American Naturalist 148:174-188.

Lloyd, D. G. 1987. Selection of offspring size at independence and other size-versus-number strategies. American Naturalist 129:800-817.

Marshall, D. J., and T. F. Bolton. 2007. Effects of egg size on the development time of non-feeding larvae. Biological Bulletin 212:6-11.

Marshall, D. J., and M. J. Keough. 2006. Complex life cycles and offspring provisioning in marine invertebrates. Integrative and Comparative Biology 46:643-651.

Martin, R. A., and D. W. Pfennig. 2010. Maternal investment influences expression of resource polymorphism in amphibians: implications for the evolution of novel resource-use phenotypes. Plos One 5.

McGinley, M. A., D. H. Temme, and M. A. Geber. 1987. Parental investment in offspring in variable environments - theoretical and empirical considerations. American Naturalist 130:370-398.

Moran, A. L., and R. B. Emlet. 2001. Offspring size and performance in variable environments: Field studies on a marine snail. Ecology 82:1597-1612.

Pelabon, C., G. H. Bolstad, C. K. Egset, J. M. Cheverud, M. Pavlicev, and G. Rosenqvist. 2013. On the Relationship between Ontogenetic and Static Allometry. American Naturalist 181:195-212.

Pettersen, A. K., D. J. Marshall, and C. R. White. In press. Understanding variation in metabolic rate. Journal of Experimental Biology.

Pianka. 1974, Evolutionary Ecology. New York, Harper & Row.

Pianka, E. R. 1976. Natural selection of optimal reproductive tactics. American Zoologist 16:775-784.

Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357-359.

Reznick, D. N. 1991, Maternal effects in fish life histories: Unity of Evolutionary Biology, Vols 1 and 2.

Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996. Life-history evolution in guppies (*Poecilia reticulata*) .6. Differential mortality as a mechanism for natural selection. Evolution 50:1651-1660.

Rivest, B. R. 1983. Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia, Buccinidae). Journal of Experimental Marine Biology and Ecology 69:217-241.
Rollinson, N., and J. A. Hutchings. 2013. The relationship between offspring size and fitness: integrating theory and empiricism. Ecology 94:315-324.
Rubner, M. 1908, Das Problem der Lebensdaur und seine Beziehungen zu Wachstum und Ernährung. Munich: Oldenberg.
Sakai, S., and Y. Harada. 2001. Why do large mothers produce large offspring? Theory and a test. American Naturalist 157:348-359.
Schoener, T. W. 1971. Theory of Feeding Strategies. Annual Review of Ecology and Systematics 2:369-404.
Sinervo, B. 1990. Experimental and comparative analyses of egg size in lizards: constraints on the adaptive evolution of maternal investment per offspring, Pages 725-734 *in* E. C. Dudley, ed., The Unity of Evolutionary Biology. Portland, OR, Dioscordies Press.
Smith, C. C., and S. D. Fretwell. 1974. Optimal balance between size and number of offspring. American Naturalist 108:499-506.
Stearns, S. C. 1992, The Evolution of Life Histories. New York, Oxford University Press.
Svanfeldt, K., K. Monro, and D. J. Marshall. 2016. Dispersal duration mediates selection on offspring size. Oikos.
Thorson, G. 1936. The larval development, growth and metabolism of arctic marine bottom invertebrates compared with those of other seas. Meddelelser Om Grønland 100:155.
Vance, R. R. 1973. On reproductive strategies in marine benthic invertebrates. American Naturalist 107:339-352.
White, C. R., M. R. Kearney, P. G. D. Matthews, S. A. L. M. Kooijman, and D. J. Marshall. 2011. A manipulative test of competing theories for metabolic scaling. American Naturalist 178:746-754.
Wolf, J. B., and M. J. Wade. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B-Biological Sciences 364:1107-1115.
Wootton, R. J., and C. Smith. 2014. Reproduction and life-history evolution, Pages 323-356, Reproductive Biology of Teleost Fishes. United States, John Wiley & Sons, Ltd.
Yampolsky, L. Y., and S. M. Scheiner. 1996. Why larger offspring at lower temperatures? A demographic approach. American Naturalist 147:86-100.

Chapter 1

Why does offspring size affect performance? Integrating metabolic scaling with life-history theory

Amanda K. Pettersen[1], Craig R. White[2], Dustin J. Marshall[1]

[1] School of Biological Sciences, Monash University, Victoria, 3800, Australia.

[2] School of Biological Sciences, University of Queensland, Queensland, 4072, Australia

Abstract

Within species, larger offspring typically outperform smaller offspring. While the relationship between offspring size and performance is ubiquitous, the cause of this relationship remains elusive. By linking metabolic and life-history theory, we provide a general explanation for why larger offspring perform better than smaller offspring. Using high-throughput respirometry arrays, we link metabolic rate to offspring size in two species of marine bryozoan. We found that metabolism scales allometrically with offspring size in both species: while larger offspring utilise absolutely more energy than smaller offspring, larger offspring use proportionally less of their maternally-derived energy throughout the dependent, non-feeding phase. The increased metabolic efficiency of larger offspring while dependent on maternal investment may explain offspring size effects –larger offspring reach nutritional independence (feed for themselves) with a higher proportion of energy relative to structure than smaller offspring. These findings offer a potentially universal explanation for why larger offspring tend to perform better than smaller offspring but studies on other taxa are needed.

Introduction

Offspring size is a key life-history trait that can affect all aspects of performance (Stearns 1992). Within many taxa, larger offspring perform better than their smaller conspecifics; they survive, grow and reproduce more than smaller offspring (Fox and Czesak 2000; Krist 2011; Marshall and Keough 2008b). Larger offspring can also be more resistant to predation and starvation, and are often better competitors than smaller offspring (Allen et al. 2008; Chen et al. 2014; Hutchings 1991; Janzen et al. 2000). Benefits of increased offspring size are not inevitable however, and they are not universal (Krist 2011). For example, in some instances, increased offspring size can confer a fitness disadvantage, where bigger offspring have higher mortality than smaller offspring (Kaplan 1992). Generally, offspring size effects manifest in early development, but they can persist throughout the life history affecting reproduction and even the performance of the subsequent generation (Plaistow et al. 2006). Understanding the relationship between offspring size and performance is of fundamental importance to life-history theorists because this relationship should drive the evolution of offspring size and explain the massive variation in offspring size we observe among species (Smith and Fretwell 1974). While many studies have documented the offspring size-performance relationship, surprisingly few have identified why this relationship occurs.

There are several viable explanations for why larger offspring often perform better than smaller offspring. A general consensus is that bigger offspring tend to have more mass and so it is often inferred that larger offspring have more energy to devote to fitness enhancing processes such as growth (Sinervo 1990). While it seems intuitive that larger offspring have more maternally-derived energy in total, presumably the associated costs of maintaining a larger size must also be higher. It then follows that in order for larger offspring to have proportionally more energy, the ratio of energy to the combined costs of maintenance and

construction must also be higher in larger offspring than in smaller offspring. So far, there is no evidence to support the hypothesis that larger offspring have proportionally more energy than small individuals. In fact, some studies suggest that larger offspring have lower levels of mass relative to volume and should have relatively less surplus energy (Chambers et al. 1989; McEdward and Coulter 1987). Another potential explanation for the offspring size-performance relationship is the scaling of offspring size with structural components, such as feeding structures, which may allow larger offspring to better assimilate resources (Aubret 2012; Hart 1995). Several studies have also shown that larger hatchling size reduces susceptibility to predation (Janzen et al. 2000; Kaplan 1992; Rivest 1983), however there are exceptions (Reznick et al. 1996; Roff 1992). While these explanations are all plausible, they are unlikely to provide any common explanation for the effects of offspring size on performance across taxa; instead their relevance will vary according to trophic mode and life history (Litvak and Leggett 1992). One explanation that has received little attention, although common to all organisms, is the interaction between offspring size and metabolic scaling.

Metabolic scaling with body size is one of the most ubiquitous and contentious relationships in biology. While there are many competing explanations for metabolic scaling with size and much controversy (see e.g. White and Kearney 2014 for a recent review), there is consensus that increases in mass rarely result in perfectly proportional increases in metabolism, rather the scaling exponent relating mass to metabolism is often less than 1 (Brown et al. 2004; Glazier 2005; Kooijman 2010). Because metabolic scaling with size is allometric, larger organisms have lower mass-specific metabolic rates than smaller organisms both within and among species (Glazier 2010). The extent to which metabolic rate scales with size changes considerably among individuals and throughout ontogeny, and the causes for these changes are unclear (White et al. 2011). To date, most intraspecific studies have examined

ontogenetic scaling, and measured how metabolic rate scales with size as animals grow, usually by comparing large and small individuals of different ages, or by experimentally manipulating individuals to create size gradients (Giguere et al. 1988; Hoegh-Guldberg and Manahan 1995; Moran and Allen 2007; Post and Lee 1996; Svetlichny et al. 2004). Many of these types of studies have found isometric or near-isometric scaling in the early life history (Riisgard 1998; Zeuthen 1953), especially for pelagic species and life stages (Glazier 2006) but they confound size for age and developmental stage. An alternative and preferable approach to ontogenetic scaling is the examination of static scaling (e.g. Rogowitz and Chappell 2000; Schimpf et al. 2013; Vogt and Appel 1999), where the relationship between metabolic rate and body size is examined for animals at the same age and developmental stage, by comparing individual metabolic rates across a naturally varying size range (Cheverud 1982; Pelabon et al. 2013). The general implications of static allometry remain the same for life-history theory; smaller individuals should have greater energy expenditure for a given mass than larger individuals (Glazier 2005).

Despite the likely implications of metabolic scaling for offspring size evolution, we are aware of only one study that has explored the interaction between natural offspring size variation and static metabolic scaling. Kinoshita et al. (1997) found an allometric relationship between body mass and metabolic rates of ephyra larvae where mass variation was obtained through measurements of one to multiple individuals per respiration container. Technological innovations now provide higher-throughput arrays with more sensitive equipment such that much higher levels of replication using individual offspring as replicates is now possible, and should provide more precise estimates of the relationship between offspring size and metabolic rate. This is the first study to measure static metabolic scaling for individual offspring. Here, we determine the static metabolic scaling exponents across natural variation in offspring size

for two marine invertebrates, *Bugula neritina* and *Watersipora subtorquata*, repeating this at multiple stages of development until individuals reach the stage of independence (where offspring commence feeding and no longer rely on maternally-supplied energy provisioning). Offspring size effects in these species have been well studied; larger offspring produce colonies that have higher survival, growth rates and reproductive output (Marshall et al. 2003; Marshall and Monro 2013). We then estimate the total energy use of maternal energy provisioning for different sizes of offspring in order to determine how energy use to independence scales with offspring size.

Materials and methods

Experimental overview

To understand how metabolic theory can be used as a potential explanation for the offspring size-fitness relationships central to life-history theory, it is important to understand the extent to which metabolism scales with offspring size. In order to do this we 1) used measurements of volume and density of individual larvae to obtain estimates of offspring mass (details in Appendix A), 2) measured the rate of oxygen consumption (VO_2) as a proxy for metabolic rate for individual offspring within a naturally occurring size range. We then 3) calculated the total energy use as a proportion of the supplied energy throughout the dependent phase in order to determine the magnitude to which offspring size dictates energy consumption. Non-feeding offspring, which rely completely on a mother's allocation of energy supplies (which we assume is proportional to offspring mass), offer us the best study organism to examine the scaling of maternally-provisioned energy use in offspring, as sources of external energy supply do not need to be considered. Thus, depending on the environment post-release, non-feeding offspring often reach a stage of independence with significantly depleted energy. Energy use is therefore of key importance to the fitness of both offspring and mother.

Study species, collection and measurement of offspring size

Two marine bryozoans, the arborescent *Bugula neritina* and encrusting *Watersipora subtorquata*, represent two subtidal species abundant in shallow temperate and tropical waters. Colonies brood larvae in either specialised chambers called ovicells (*B. neritina*) or on the body wall (*W. subtorquata*) for approximately 1 and 2 weeks respectively (Allen et al. 2008; Marshall and Keough 2008b). The released, non-feeding larvae are competent to settle and begin metamorphosis immediately but in the field, settlement can be delayed for more than 24 hours (Burgess and Marshall 2011). We define the dependent phase as commencing with

release of larvae, up until the post-settlement period once metamorphosis is complete and the feeding structure (the lophophore) is fully developed. During the dependent phase offspring are completely reliant on maternally provided energy, although it has been suggested that they may uptake dissolved organic matter (DOM; Jaeckle and Manahan 1989). However, for *B. neritina* at least it has been shown that larvae do not utilise significant amounts of DOM and studies on the effect of extending the larval duration support this (Burgess et al. 2013; Wendt 2000). The duration of post-settlement metamorphosis is independent of larval size and lasts approximately two – five days, depending on temperature (A.K Pettersen, unpublished data). Sexually mature colonies of *B. neritina* and *W. subtorquata* were collected at Royal Brighton Yacht Club in Victoria, Australia (37°54'18.9"S, 144°58'48.3"E) and Blairgowrie Yacht Squadron, Victoria, Australia (38°21'20.4"S, 144°46'24.8"E) respectively, during January to February 2014. Five colonies were then spawned using standard procedures (Marshall et al. 2003): following the maintenance of colonies in an aerated seawater system for three to five days at 17.5°C, colonies were exposed to bright light to stimulate larval release.

Upon release, larvae were haphazardly sampled for measurement of body area then either introduced directly into individual respiration vials, or allowed to settle onto roughened acetate sheets that were cut out and placed into vials. However, for *W. subtorquata* settlers, body area of 24-h-old settlers was used as it has been shown to provide a good predictor of larval size (Marshall and Monro 2013). Body areas of larvae were determined for both larval and settler experiments using standard techniques developed previously (Marshall et al. 2003). Larvae and settlers were photographed with a Moticam 5 digital camera (Motic, Hong Kong, China) mounted on a dissecting microscope and body area was estimated using image analysis software (Image J, 1.47v). Larvae were positioned such that the cillial groove was facing directly upwards, and length of the ciliated groove and the body area was measured to the nearest μm. Pilot studies of this method indicated that measurement error in larval size is small;

measurement error accounted for 0.8% and 4.4% of all variation in offspring size in *B. neritina* and *W. subtorquata* respectively, and this low error was also observed in the experimental measurements. In other words, the repeatability of our measures was 99.2% for *B. neritina* and 95.6% for *W. subtorquata*, reflecting an intraclass correlation coefficient (ICC) of 0.995 and 0.977 respectively, and suggesting that measurement error accounted for very little variation in our estimates of body size. For a detailed account of how offspring mass and energy content were determined, see Appendix A.

Metabolic scaling exponents: fluorescence-based oxygen measurements (VO_2) and conversion to metabolic rate (mJ per hour)

The rate of oxygen consumption (VO_2) was measured as a proxy for metabolic rate for larvae and two post-settlement stages of *B. neritina* and *W. subtorquata*. Oxygen consumption was measured using a 24-channel PreSens sensor dish reader (Sensor Dish Reader SDR2, PreSens), with 24-chamber glass micro plate (200µl) (Loligo Systems Aps, Tjele, Denmark) according to standard techniques (Alton et al. 2012; Köster et al. 2008). Individual larvae or settlers were placed in a glass vial containing 0.2 µm filtered seawater and a nonconsumptive O_2 sensor spot and VO_2 was calculated from the rate of change of O_2 saturation (m_a; $\%h^{-1}$) as

$VO_2 = -1\ (m_a - m_b / 100)\ V\beta O_2$ (as per White et al. 2011),

where m_b is the rate of change of O_2 saturation for blank vials containing no larvae or in the case of settlers, only acetate ($\%\ h^{-1}$), βO_2 is the oxygen capacitance of air-saturated seawater at 17.5°C (5.8 mL L^{-1}; Cameron 1986), and V is water volume (chambers were 2.0 x 10^{-4}L, and water volume was calculated by subtracting the volume of acetate and animals). Four blank vials were recorded simultaneously to account for microbial oxygen consumption, and sensor spots were calibrated with air-saturated (AS) seawater (100% AS) and water containing 2% sodium sulfite (0% AS). All VO_2 measurements were conducted in a dark, constant-temperature room at 17.5˚C. For VO_2 measurements for larvae, oxygen concentration in the

vials was recorded over 30 minutes (we used a short period to ensure no larvae began settlement while in the chambers). For settlers, oxygen concentration was recorded over three hours at two different stages of development prior to completing metamorphosis and development of the lophophore (from here on, designated early and late). Pilot studies showed no systematic differences in the duration of metamorphosis associated with offspring size. Hence, VO_2 for all metamorphosing individuals was measured at two discrete times; 0 h and 24 h for *B. neritina* and *W.subtorquata* was measured at 0 h and 54 h post-settlement to represent the start and mid-point of the post-settlement dependent phase. Each experimental run consisted of simultaneously VO_2 measurements for 20 individuals recorded at three development stages (larval, early and late stage). To determine the rate of energy expenditure by different sized offspring, VO_2 ($\mu l\ h^{-1}$) was converted to metabolic rate ($mJ\ h^{-1}$) using the calorific conversion factor of 20.08 $J\ ml^{-1}\ O_2$ (Crisp 1971).

All data were analysed in a log-log framework. Due to various logistical limitations (for a detailed description of the analytical approach, see Appendix A), scaling exponents and coefficients for larvae were analysed independently to those of the post-settlement stages (early and late) for both species. A repeated-measures ANCOVA approach was taken for measuring the same individual settlers throughout the early and late stages. As different individuals were measured for the larval stage, a linear mixed effects ANCOVA was used to determine whether differences existed between larval and settler stage metabolic rates. All analyses included the random effect of Experimental Run and all possible interactions with Stage and Log_{10}Mass. A standard ANCOVA framework rather than RMA approach was used as the error structure of our data was not suited to RMA (see Appendix A).

Predicting proportional energy use from offspring size

In order to calculate the proportional energy use for offspring of different sizes, we parameterised the power relationship between mass (M) and metabolic rate (MR), where

$MR=aM^b$. Using the coefficients (a) and scaling exponents (b) estimated by our experiments, combined with the approximate length of time spent in each stage, we were able to predict total energy consumption throughout the dependent or non-feeding stage of development.

To determine whether the energy difference between large and small offspring was substantial, such that it may affect settlement success or post-settlement survival, we compared the proportional energy use for offspring at the extremes of our observed size distribution. We calculated energy use by offspring across an approximately three-fold difference in size (see Appendix A), which reflects the size range of offspring in natural populations of these species (Marshall et al. 2003; Marshall and Monro 2013).

Results

Metabolic scaling exponents

Allometric scaling relationships of metabolic rate were observed for both species and across all developmental stages (Table 1), where the scaling exponents were significantly different from both 0 and 1 ($P<0.05$). For offspring in both species, there was a significant development-stage effect where overall metabolic rate was highest during the larval phase in *B. neritina* and lowest in the early settler stage (Table 2, Table 3). Conversely, metabolic rate overall increased through ontogeny in *W. subtorquata*, (Table 2, Table 3). For *W. subtorquata,* no interaction between stage and mass was detected; therefore a single scaling exponent was obtained for all three stages. For *B. neritina*, the slopes among the early and late settler stages were found to be significantly different (Table 2) and therefore separate scaling exponents were obtained for each stage. For each analysis, there were significant effects of experimental run for the pooled larvae and early-stage settlers of *B. neritina* (Table 1) and for late-stage settlers of *B. neritina* (Table 2). There was also a significant effect of experimental run for the pooled larvae, early-stage and late-stage settlers of *W. subtorquata* (Table 3). We found no support for fitting a random-slopes model (i.e. no significant run x mass effect was detected), but there was significant among-run variation in the intercept of the relationship between size and metabolic rate (Table 2, Table 3). This run-associated variation could stem from multiple sources (phenotypic and genetic differences among colonies, temporal effects). Crucially however, the relationship between mass and metabolic rate was constant among all runs, so our principle findings hold across colonies and times.

Predicting proportional energy use from offspring size

The coefficient and scaling exponent values obtained from the linear model of $\log_{10}$ transformed data for each developmental stage were then used to predict metabolic rate according to the power function $MR=aM^b$ (Figure 1). The predicted metabolic rate was then multiplied by the average amount of time spent at each developmental stage based on previous observations (*B. neritina*; larval stage 0.5 h, early stage 24 h, late stage 30 h, *W.subtorquata*; larval stage 0.5 h, 54h, 52h) to obtain total energy consumption from release as larvae until the completion of metamorphosis and development of the feeding structure. The predicted total energy use by the smallest observed *B. neritina* offspring of 6.7 μg was 11.2 mJ, while the largest offspring with a mass of 24.5 μg uses only 19.4 mJ. For *W.subtorquata*, the smallest offspring of 9.3 μg uses 39.9 mJ, and the largest offspring of 25.3 μg uses 77.2 mJ by the end of the dependent phase. Thus, where a large offspring is twice the mass of a small offspring, it consumes only 1.3 times the energy in *B. neritina* and 1.5 times the energy in *W. subtorquata* than that of a small offspring in reaching a stage of independence.

Using carbon content values reported by Wendt (2000), the energy density for *B. neritina* was found to be 3.85×10^{-6} μg μm^{-3}. As a proportion of known energy content extrapolated from Wendt (2000) we can compare proportional energy use for the natural size range we found in this species. The smallest observed *B. neritina* larvae (6.7 μg) will use 46.7% of maternal energy provisioning while the largest observed offspring (24.5 μg) will use only 22.1 % of its supplied energy to reach independence. For *W. subtorquata*, oxygen consumption rate increased two-fold through development and total energy use was over 3.5 times that of *B. neritina* such that energy density must be higher. Based on our estimate of offspring energy density of 7.78×10^{-6} μg μm^{-3}, as a proportion of total energy supply, the smallest offspring (9.3 μg) would use 60.6% total energy compared with 43.2% of total energy for the largest offspring (25.3 μg) to reach independence.

Discussion

Allometric scaling of metabolism and the benefits of increased offspring size

We found that when offspring depend entirely on maternal resources to complete metamorphosis and burn around 30-50% of their total energy content, larger offspring have much lower relative metabolic rates than their smaller conspecifics. Both *B. neritina* and *W. subtorquata* show strong relationships between offspring size and post-metamorphic performance – bigger larvae survive better and reproduce more as colonies (Marshall et al. 2003; Marshall and Monro 2013). The allometric scaling of metabolic rate with offspring size may explain this relationship. We found that the metabolic dynamics of small and large offspring are very different: on an absolute scale, larger offspring use more energy sourced from the mother than smaller offspring; however, on a relative scale (i.e., per unit of body mass), larger offspring use less energy than smaller offspring. In effect, larger offspring are more metabolically efficient during the key phase of dependence on maternal energy, while smaller offspring could be regarded as more wasteful. Hence, larger offspring not only reach nutritional independence with absolutely more energy, but also with relatively more energy because, relative to structural components of size, they use fewer resources during the phase in which they depend on maternal resources. Note that this increased efficiency of larger offspring will occur regardless of the initial composition of smaller and larger larvae. For example, different sized larvae could have different proportions of lipid (although this is unlikely given larval sizes were all of similar density in each species), which would affect the final energy content of different sized offspring, but would not alter our finding that larger larvae burn proportionally less of their reserves.

In a previous study, Sinervo (1990) alluded to a potential metabolic mechanism for the observed relationship between offspring size and performance in lizards, where "juvenile size

and growth rate are functionally related because some underlying determinants of growth rate (e.g., metabolism) are allometrically related to size". Despite this prescient suggestion, there has been little evidence for static (within developmental stage) allometric scaling relationships between naturally occurring offspring size variation and metabolism until now. In contrast, static allometric scaling of metabolic rate has been observed in adult insects (e.g. Rogowitz and Chappell 2000; Schimpf et al. 2013; Vogt and Appel 1999), mammals (e.g. Heymsfield et al. 2002; Labocha et al. 2004), and chickens (Damme et al. 1987), but the static scaling exponent of metabolic rate is almost isometric in adult pied flycatchers *Ficedula hypoleuca* (Bushuev et al. 2012), and metabolic rate and body mass are independent in some (e.g. Van Voorhies et al. 2004), but not all (e.g. Arnold et al. 2013), studies of adult *Drosophila melanogaster*. Thus, although static allometric scaling of metabolic rate is not ubiquitous, it may be widespread within life stages throughout development (e.g. Greenlee and Harrison 2004; Snelling et al. 2011), such that it may provide a general explanation for the often-observed positive relationship between offspring size and performance across a wide range of taxa. We believe the metabolic benefits of producing larger offspring offer several exciting new lines of inquiry for understanding variation in offspring size.

Hidden metabolic costs of smaller offspring

Life-history theory has long assumed that the fitness benefits of producing smaller offspring come from smaller offspring being 'cheaper' to make, allowing mothers to make many more offspring. While smaller offspring almost certainly require fewer maternal resources to make, our findings show that they use these resources much less efficiently than larger offspring. Per unit of body mass, metamorphosis costs more for smaller offspring than larger offspring. Interestingly, the size-fecundity relationship exacerbates these metabolic costs of producing smaller offspring. To illustrate this, we compare two mothers with equal reproductive investment, but which produce offspring of very different mean size. As shown in Figure 2,

mothers producing smaller offspring will lose 11 mJ per offspring investment to the metabolic costs of offspring development, while a mother producing larger offspring will lose 19 mJ (Figure 2a). Because fecundity is inversely proportional to offspring size, mothers producing smaller offspring will lose 47% of their total investment to metabolic costs of development while mothers producing larger offspring will lose only 22% (Figure 2b). While smaller offspring are much cheaper to make initially, they are much more expensive to provision through to nutritional independence – a finding that current life-history theory fails to consider explicitly. For species with post-release care (e.g., mammals and birds), this suggests that any decrease in initial offspring size via egg size or birth weight must be overcompensated for via parentally-supplied resources. A simple trade-off between pre- and post-release investment will not yield identical outcomes due to the reduced efficiency of smaller offspring during development. Instead, a small decrease in offspring size must be accompanied by a larger increase in post-release parental investment. For species with no post-release care, the increased inefficiencies of smaller offspring size will simply exacerbate the costs of smaller initial parental investment, extending the phase that smaller offspring need to feed simply to attain equivalent sizes to larger offspring. These subtle costs of producing smaller offspring should be included in future models of offspring size.

Modifiers of the offspring size-energy consumption relationship

Factors that increase the length of the dependent phase should increase the benefits of larger offspring sizes (and also increase the costs of smaller offspring sizes). For example, our results may explain the well-known relationship between temperature and offspring size, whereby mothers often produce larger offspring in cooler temperatures (Van Voorhies 1996). If temperature affects developmental rates more than metabolic rates, then mothers may make smaller offspring at higher temperatures because the relative costs of development are offset by faster development under those conditions. Conversely, in cooler temperatures where

development time is increased to a higher extent than metabolic rate is decreased, offspring will require larger energy stores to reach independence (Niklas and Hammond 2014). In species with extended periods of time that offspring depend on maternal resources (e.g., altricial species of birds), we would expect the benefits of increased egg size to be enhanced, and indeed, altricial species do tend to produce larger eggs than precocial species (Dyke and Kaiser 2010). Similarly, those species with longer incubation periods also have larger egg sizes (Clutton-Brock 1991). In our system, larval period and temperature are key modifiers of the length of the dependent phase. For our study species, the larval phase varies in nature between a few minutes and up to 24 hours (Burgess and Marshall 2011). A relatively long larval period of 12 hours (and the same size-specific metabolic rate) would therefore yield an almost two-fold increase in the differential of efficiency between big and small larvae in *B. neritina* and *W. subtorquata* (1.8 and 1.6 times respectively). Our results may provide an explanation for the finding that larger larvae cope better with prolonged swimming periods in *B. neritina*. Burgess *et al.* (2013) found that extending the larval period reduces post-metamorphic performance, but that smaller larvae showed the greatest reductions in performance relative to larger larvae. Furthermore, larger larvae in both *B. neritina* and *W. subtorquata* tend to reject low-quality settlement sites for longer than smaller larvae, thereby increasing their chances of colonising higher-quality environments (Burgess et al. 2013). Our results suggest that larger larvae can afford to be more selective of their settlement environment for longer, because the costs of extending the larval period are less for larger larvae relative to smaller larvae. Our results may therefore explain the well-known but poorly-understood relationship between offspring size and larval duration in marine invertebrates with non-feeding larvae (Marshall and Keough 2008a). If the patterns found here also apply to feeding larvae, the effects of egg size on metabolic efficiency could be even more profound. In such species, whose larval periods can extend for weeks to months depending on food and habitat availability (as reviewed

in Strathmann 1985), even slight differences in relative metabolic rates will be magnified over such extended periods of time. An important next step will be to repeat our study in species with feeding larvae.

It is important to note that our results are subject to a number of caveats. First, we assume that carbon content scales isometrically and with the same intercepts across our offspring size range for all of our experimental runs. Any divergence from these assumptions may explain the run effects observed in both species. If carbon content is affected by variables other than offspring size, then this may alter our estimates of energy use. However, our main finding that larger larvae use relatively less energy is unaffected by whether carbon content is consistent across runs. Second, we found allometric relationships between offspring size and metabolic rate for two very different species from the same phylum; however, it is too soon to generalise as to whether our findings hold more broadly. Instead, our proposed mechanism remains an attractive, but speculative, hypothesis as to why larger offspring perform better than smaller offspring, and more tests of static allometry in metabolic rate across offspring sizes in other taxa are needed.

Conclusions

We found that the scaling exponent of metabolic rate was less than 1 throughout the dependent phase in two marine bryozoans, *Bugula neritina and Watersipora subtorquata*. We propose that the allometric scaling of metabolic rate during early development has important life-history consequences but, at this stage, must restrict our conclusions to the two species tested. Larger offspring are provisioned with more energy than smaller offspring but, because metabolic rate scales allometrically with offspring size, larger offspring use energy at a relatively lower rate than smaller individuals. Therefore, all else being equal, larger offspring should reach independence with a higher proportion of maternal investment than smaller offspring. Our results may provide a general explanation for why larger offspring do perform better than smaller offspring, given that most animals show allometry with respect to metabolism, and that we expect the increased relative efficiencies of larger offspring to be widespread. However, this hypothesis requires further testing. Furthermore, our results show that there are intrinsic benefits to producing larger offspring (i.e., they are more efficient users of maternal resources). Hence, for these species at least, conditions that favour the production of smaller offspring must overcome the intrinsic metabolic benefits of increased size. Despite the extensive theoretical treatment of offspring size, we are unaware of extant theory that explicitly considers the metabolic benefits of increased offspring size. Development of such theory is an important next step.

References

Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. American Naturalist 171:225-237.

Alton, L. A., C. R. White, R. S. Wilson, and C. E. Franklin. 2012. The energetic cost of exposure to UV radiation for tadpoles is greater when they live with predators. Functional Ecology 26:94-103.

Arnold, P. A., K. N. Johnson, and C. R. White. 2013. Physiological and metabolic consequences of viral infection in *Drosophila melanogaster*. Journal of Experimental Biology 216:3350-3357.

Aubret, F. 2012. Body-Size Evolution on Islands: Are Adult Size Variations in Tiger Snakes a Nonadaptive Consequence of Selection on Birth Size? American Naturalist 179:756-767.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771-1789.

Burgess, S. C., M. Bode, and D. J. Marshall. 2013. Costs of dispersal alter optimal offspring size in patchy habitats: combining theory and data for a marine invertebrate. Functional Ecology 27:757-765.

Burgess, S. C., and D. J. Marshall. 2011. Field estimates of planktonic larval duration in a marine invertebrate. Marine Ecology Progress Series 440:151-161.

Bushuev, A. V., A. Husby, H. Sternberg, and V. G. Grinkov. 2012. Quantitative genetics of basal metabolic rate and body mass in free-living pied flycatchers. Journal of Zoology 288:245-251.

Cameron, J. N. 1986. The solubility of carbon dioxide as a function of temperature and salinity (Appendix table), Pages 254-259 *in* J. N. Cameron, ed. Principles of physiological measurement. United Kingdom, Academic Press.

Chambers, R. C., W. C. Leggett, and J. A. Brown. 1989. Egg size, female effects, and the correlations between early life-history traits of capelin, *Mallotis villosus*: an appraisal at the individual level. Fishery Bulletin 87:515-523.

Chen, B. J. W., H. J. During, P. J. Vermeulen, and N. P. R. Anten. 2014. The presence of a below-ground neighbour alters within-plant seed size distribution in *Phaseolus vulgaris*. Annals of Botany 114:937-943.

Cheverud, J. M. 1982. Relationships among ontogenetic, static, and evolutionary allometry. American Journal of Physical Anthropology 59:139-149.

Clutton-Brock, T. H. 1991, The evolution of parental care. Princeton, NJ, Princeton Univ. Press.

Crisp, D. J. 1971. Energy flow measurements, Pages 197-279 *in* N. A. Holme, and A. D. McIntyre, eds. Methods for the study of marine benthos. Oxford, Blackwell Sci.Pub.

Damme, K., F. Pirchner, H. Willeke, and H. Eichinger. 1987. Fasting metabolic rate in hens: 1. Effects of body weight, feather loss, and activity. Poultry Science 66:881-890.

Dyke, G. J., and G. W. Kaiser. 2010. Cracking a Developmental Constraint: Egg Size and Bird Evolution. Records of the Australian Museum 62:207-216.

Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. Annual Review of Entomology 45:341-369.

Giguere, L. A., B. Cote, and J. F. Stpierre. 1988. Metabolic rates scale isometrically in larval fishes. Marine Ecology Progress Series 50:13-19.

Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662.
Glazier, D. S. 2006. The 3/4-Power Law Is Not Universal: Evolution of Isometric, Ontogenetic Metabolic Scaling in Pelagic Animals. BioScience 56:325-332.
Glazier, D. S. 2010. A unifying explanation for diverse metabolic scaling in animals and plants. Biological Reviews 85:111-138.
Greenlee, K. J., and J. F. Harrison. 2004. Development of respiratory function in the American locust *Schistocerca americana* II. Within-instar effects. Journal of Experimental Biology 207:509-517.
Hart, M. W. 1995. What are the costs of small egg size for a marine invertebrate with feeding planktonic larvae. American Naturalist 146:415-426.
Heymsfield, S. B., D. Gallagher, D. P. Kotler, Z. M. Wang, D. B. Allison, and S. Heshka. 2002. Body-size dependence of resting energy expenditure can be attributed to nonenergetic homogeneity of fat-free mass. American Journal of Physiology-Endocrinology and Metabolism 282:E132-E138.
Hoegh-Guldberg, O., and D. T. Manahan. 1995. Coulometric measurement of oxygen consumption during development of marine invertebrate embryos and larvae. Journal of Experimental Biology 198:19-30.
Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salelinus fontinalis*. Evolution 45:1162-1168.
Jaeckle, W. B., and D. T. Manahan. 1989. Feeding by "non-feeding" larva: uptake of dissolved amino-acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. Marine Biology 103:87-94.
Janzen, F. J., J. F. Tucker, and G. L. Paukstis. 2000. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. Journal of Evolutionary Biology 13:947-954.
Kaplan, R. H. 1992. Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. Ecology 73:280-288.
Kinoshita, J., J. Hiromi, and S. Kadota. 1997. Do respiratory metabolic rates of the scyphomedusa *Aurelia aurita* scale isometrically throughout ontogeny in a sexual generation? Hydrobiologia 347:51-55.
Kooijman, S. A. L. M. 2010, Dynamic energy budget theory for metabolic organisation. Cambridge, Cambridge University Press.
Köster, M., C. Krause, and G. A. Paffenhofer. 2008. Time-series measurements of oxygen consumption of copepod nauplii. Marine Ecology Progress Series 353:157-164.
Krist, M. 2011. Egg size and offspring quality: a meta-analysis in birds. Biological Reviews 86:692-716.
Labocha, M. K., E. T. Sadowska, K. Baliga, A. K. Semer, and P. Koteja. 2004. Individual variation and repeatability of basal metabolism in the bank vole, *Clethrionomys glareolus*. Proceedings of the Royal Society B-Biological Sciences 271:367-372.
Litvak, M. K., and W. C. Leggett. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Marine Ecology Progress Series 81:13-24.
Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131-3137.
Marshall, D. J., and M. J. Keough. 2008a. The evolutionary ecology of offspring size in marine invertebrates, Pages 1-60 *in* D. W. Sims, ed., Advances in Marine Biology. Advances in Marine Biology. United Kingdom.
—. 2008b. The relationship between offspring size and performance in the sea. American Naturalist 171:214-224.

Marshall, D. J., and K. Monro. 2013. Interspecific competition alters nonlinear selection on offspring size in the field. Evolution 67:328-337.
McEdward, L. A., and L. K. Coulter. 1987. Egg volume and energetic content are not correlated among sibling offspring of starfish: implications for life history theory. Evolution 41:917-917.
Moran, A. L., and J. D. Allen. 2007. How does metabolic rate scale with egg size? An experimental test with sea urchin embryos. Biological Bulletin 212:143-150.
Niklas, K. J., and S. T. Hammond. 2014. Assessing scaling relationships: uses, abuses, and alternatives. International Journal of Plant Sciences 175:754-763.
Pelabon, C., G. H. Bolstad, C. K. Egset, J. M. Cheverud, M. Pavlicev, and G. Rosenqvist. 2013. On the Relationship between Ontogenetic and Static Allometry. American Naturalist 181:195-212.
Plaistow, S. J., C. T. Lapsley, and T. G. Benton. 2006. Context-dependent intergenerational effects: The interaction between past and present environments and its effect on population dynamics. American Naturalist 167:206-215.
Post, J. R., and J. A. Lee. 1996. Metabolic ontogeny of teleost fishes. Canadian Journal of Fisheries and Aquatic Sciences 53:910-923.
Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996. Life-history evolution in guppies (Poecilia reticulata) .6. Differential mortality as a mechanism for natural selection. Evolution 50:1651-1660.
Riisgard, H. U. 1998. No foundation of a "3/4 power scaling law" for respiration in biology. Ecology Letters 1:71-73.
Rivest, B. R. 1983. Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia, Buccinidae). Journal of Experimental Marine Biology and Ecology 69:217-241.
Roff, D. A. 1992, The Evolution of Life Histories: Theory and Analysis. New York, Chapman and Hall.
Rogowitz, G. L., and M. A. Chappell. 2000. Energy metabolism of eucalyptus-boring beetles at rest and during locomotion: Gender makes a difference. Journal of Experimental Biology 203:1131-1139.
Schimpf, N. G., P. G. D. Matthews, and C. R. White. 2013. Discontinuous gas exchange exhibition is a heritable trait in speckled cockroaches *Nauphoeta cinerea*. Journal of Evolutionary Biology 26:1588-1597.
Sinervo, B. 1990. Experimental and comparative analyses of egg size in lizards: constraints on the adaptive evolution of maternal investment per offspring, Pages 725-734 *in* E. C. Dudley, ed., The Unity of Evolutionary Biology. Portland, OR, Dioscordies Press.
Smith, C. C., and S. D. Fretwell. 1974. Optimal balance between size and number of offspring. American Naturalist 108:499-506.
Snelling, E. P., R. S. Seymour, P. G. D. Matthews, S. Runciman, and C. R. White. 2011. Scaling of resting and maximum hopping metabolic rate throughout the life cycle of the locust *Locusta migratoria*. Journal of Experimental Biology 214:3218-3224.
Stearns, S. C. 1992, The Evolution of Life Histories. New York, Oxford University Press.
Strathmann, R. R. 1985. Feeding and non-feeding larval development and life history evolution in marine invertebrates. Annual Review of Ecology and Systematics 16:339-361.
Svetlichny, L. S., G. Abolmasova, E. S. Hubareva, G. A. Finenko, L. Bat, and A. E. Kideys. 2004. Respiration rates of *Beroe ovata* in the Black Sea. Marine Biology 145:585-593.
Van Voorhies, W. A. 1996. Bergmann size clines: A simple explanation for their occurrence in ectotherms. Evolution 50:1259-1264.

Van Voorhies, W. A., A. A. Khazaeli, and J. W. Curtsinger. 2004. Lack of correlation between body mass and metabolic rate in *Drosophila melanogaster*. Journal of Insect Physiology 50:445-453.

Vogt, J. T., and A. G. Appel. 1999. Standard metabolic rate of the fire ant, *Solenopsis invicta* Buren: effects of temperature, mass, and caste. Journal of Insect Physiology 45:655-666.

Wendt, D. E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biological Bulletin 198:346-356.

White, C. R., and M. R. Kearney. 2014. Metabolic Scaling in Animals: Methods, Empirical Results, and Theoretical Explanations. Comprehensive Physiology 4:231-256.

White, C. R., M. R. Kearney, P. G. D. Matthews, S. A. L. M. Kooijman, and D. J. Marshall. 2011. A Manipulative Test of Competing Theories for Metabolic Scaling. American Naturalist 178:746-754.

Zeuthen, E. 1953. Oxygen uptake as related to body size in organisms. Quarterly Review of Biology 28:1-12.

Tables

Table 1. Summary of scaling exponents and coefficients (±SE) for metabolic rate and mass of the development stages of *B. neritina* and *W. subtorquata*, using a log-log transformed linear relationship where Log_{10}Metabolic rate = *b* x Log_{10}Mass + *a*. Wald tests were used to determine whether the scaling exponent (*b*) was significantly different from both 0 and 1.

Species	***B. neritina***					***W. subtorquata***				
	Coefficient (*a*)	Scaling exponent (*b*)	P-value b > 0	P-value b < 1	R^2	Coefficient (*a*)	Scaling exponent (*b*)	P-value b > 0	P-value b < 1	R^2
Developmental stage										
Larvae	-1.15 (±0.14)	0.76 (+/- 0.11)	<0.01	<0.05	0.75	-1.08 (±0.15)	0.66 (±0.11)	<0.01	<0.01	0.62
Early	-1.66 (±0.13)	0.76 (+/- 0.11)	<0.01	<0.01	0.75	-1.34 (±0.15)	0.66 (±0.11)	<0.01	<0.01	0.62
Late	-0.77 (±0.11)	0.29 (+/- 0.10)	<0.01	<0.01	0.29	-0.89 (±0.15)	0.66 (±0.11)	<0.01	<0.01	0.62

Early stage = 0 hr post-settlement. Late stage for *B. neritina* = 24 hr post-settlement, for *W. subtorquata* = 54 hr post-settlement.

Table 2. Repeated-Measures analysis for the longitudinal study between Log_{10} Metabolic rate and Log_{10} Offspring Mass. Metabolic rate for the same individuals of *B. neritina* and *W. subtorquata* was measured at two distinct post-settlement development stages; early and late (d.f presented as num d.f., den d.f.)

Species	***B. neritina***				***W. subtorquata***			
	d.f	Mean Squares	F-ratio	P-value	d.f	Mean Squares	F-ratio	P-value
Between subjects								
Log_{10} Mass	1,184	1.04	37.38	<0.01	1,50	0.35	21.31	<0.01
Experimental Run	11,184	0.08	2.76	<0.01	3,50	0.20	12.02	<0.01
Log_{10} Mass x Experimental Run	11,173	0.03	0.94	0.50	3,47	0.01	0.28	0.84
Within subjects								
Stage	1, 184	0.49	31.19	<0.01	1,47	0.09	4.78	0.03
Stage x Log_{10} Mass	1, 184	0.17	11.05	<0.01	1,47	0.02	1.21	0.28
Stage x Experimental Run	11, 184	0.01	0.70	0.73	3,47	0.01	0.66	0.58
Stage x Log_{10} Mass x Experimental Run	11, 173	0.01	0.81	0.63	3,47	0.01	0.54	0.66

Table 3. Linear mixed-effects model for cross-sectional study between Log_{10} Metabolic rate and Log_{10} Offspring Mass. Metabolic rate was measured for different individuals of *B. neritina* and *W. subtorquata* at two development stages; larval and early post-settlement stage.

Species	*B. neritina* (larval and early stages)			*B. neritina* (late stage)			*W. subtorquata* (larval, early and late stages)		
	d.f	χ^2	P-value	d.f	χ^2	P-value	d.f	χ^2	P-value
Log_{10} Mass	1	39.21	<0.01	1	6.57	<0.01	1	24.50	<0.01
Experimental Run	1	19.41	<0.01	1	27.25	<0.01	1	22.65	<0.01
Stage	1	37.07	<0.01				1	11.93	<0.01
Log_{10} Mass x Experimental Run	1	0.21	0.65	1	0.05	0.83	1	0	1
Log_{10} Mass x Stage	1	0.21	0.65				1	0	1
Stage x Experimental Run	1	0	1				1	0	1
Log_{10} Mass x Stage x Experimental Run	1	0	1				1	0	1

Figures

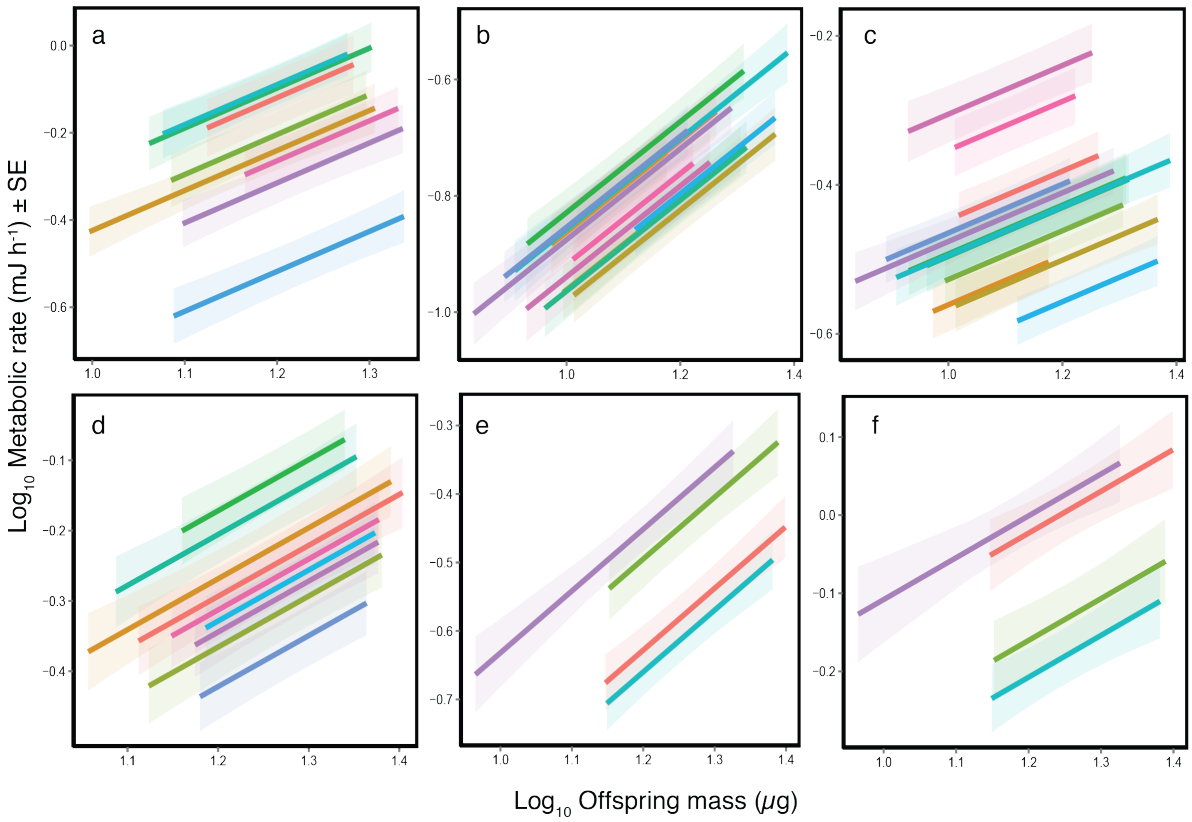


Figure 1. Predicted lines of best fit per experimental run from linear mixed-effects model (± standard error) for the relationship between Log_{10} offspring mass (µg) and Log_{10} metabolic rate (mJ h^{-1}) for *B. neritina*: (a) larval stage, (b) early post-settlement stage, (c) late post-settlement stage and *W. subtorquata*: (d) larval stage, (e) early post-settlement stage, (f) late post-settlement stage. Each line represents experimental run with a common slope and its own unique intercept per development stage.

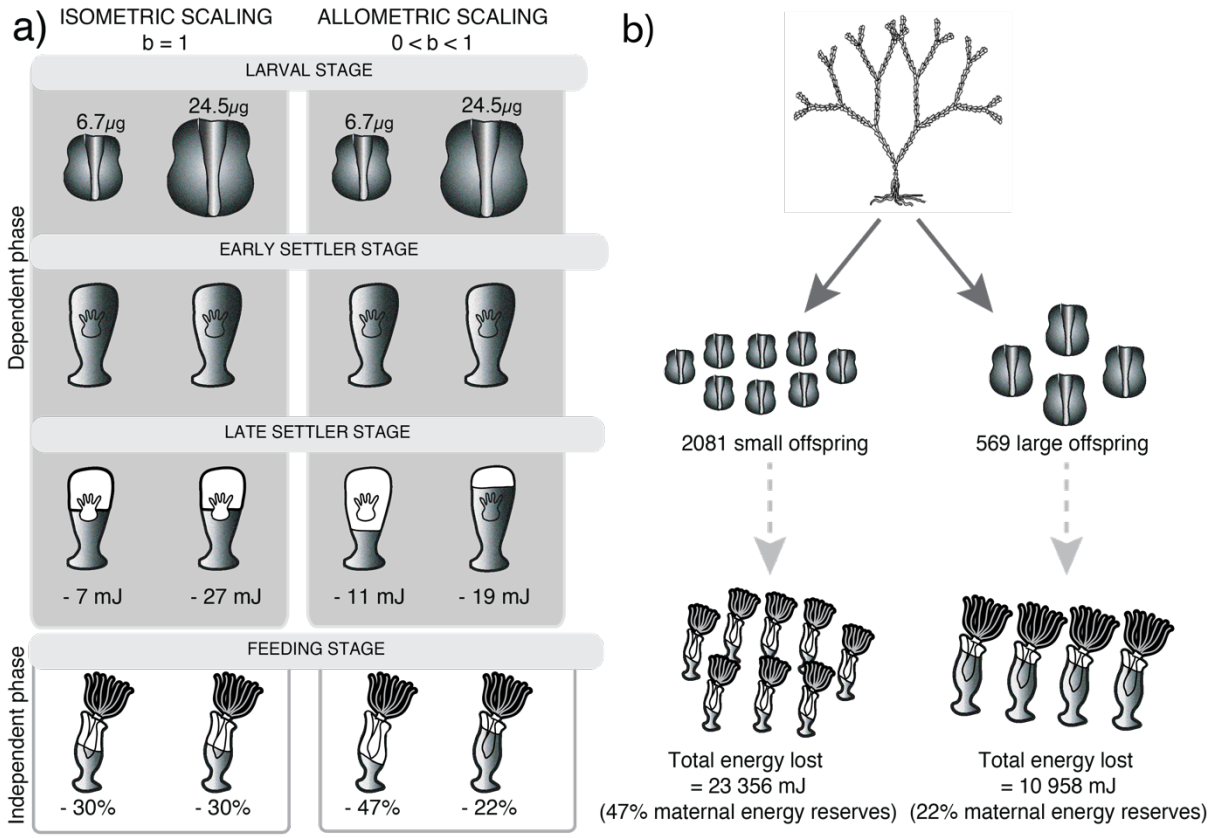


Figure 2. Schematic showing proportion of energy used by the smallest (6.7 µg) and largest (24.5 µg) *B. neritina* larvae observed in our study. Panel a) shows hypothetical isometric relationship (scaling exponent, b = 1; as assumed by life-history theory) versus an allometric relationship between size and metabolic rate up until the independent phase based on scaling exponents obtained from our results (larval stage; b=0.76, early stage; b=0.76, late stage; b=0.29). According to the power function, $MR = aM^b$, energy use is directly proportional to body mass in an isometric relationship (where b = 1), while in an allometric relationship, smaller offspring use relatively more energy per unit body mass than larger offspring (b < 1). Panel b) shows the relative amount of energy that is consumed in the dependent phase for

mothers with identical total reproductive investments (fecundities based on field observations at our field site) but where one mother produces the largest observed offspring and the other produces the smallest observed offspring. As a proportion of total supplied energy, the fewer, larger offspring in total will use less than half of the maternally supplied energy relative to the many smaller offspring (i.e Mother producing larger offspring: Total energy [50000 mJ] / per offspring energy [87.9 mJ] = offspring number [569] * individual energy burned [19.3] = total metabolic cost [10958 mJ]).

Appendix A

Materials and methods

Estimates of larval mass and energy content

In order to determine the relationship between metabolic rate and larval mass, accurate estimates of mass based on previously collected measurements of body area were needed. Due to time constraints associated with measuring the volume of live larvae and the requirement of destructive sampling to quantify mass, these were determined post-hoc. To calculate estimates of larval mass from body area, volume dimensions and density were obtained (Mass = Volume x Density).

a. Calculations of larval volume

100 larvae (from 5 colonies) each of *B. neritina* and *W. subtorquata* were photographed and measured to provide dimensions of body and basal area through length (distance parallel to the cillial groove), width (perpendicular to cillial groove) and depth (perpendicular to width) as per techniques described previously. Up until now, it has been assumed that volume is proportional to body area, where the larval body dimensions of *B. neritina* are cylindrical (Kosman and Pernet 2009; Marshall et al. 2003; Wendt 2000) However, this has not been formally tested and for *W. subtorquata*, these dimensions have yet to be ascertained at all. McEdward and Carson (1987) highlight the need for measurements of three perpendicular diameters in order to avoid systematic error of offspring volume estimates. Assuming a linear relationship, the correlation between volume and body area for both species was found using ordinary least squares analysis (SYSTAT 13).

b. Measurements of larval density

To find the correlation between larval volume and mass of the bryozoan species, density measures for larvae of known size were obtained. The body area of 100 larvae for each species was measured using standard techniques mentioned previously. For larval density measurements, each larvae was preserved in 8% formalin in seawater and pipetted into a 15ml centrifuge tube (BD, BD Biosciences, Belford, USA) containing a density gradient and centrifuged (Beckman GS-6 Centrifuge) at 500g for 5 minutes. The density gradient was created using a gradient former (model 385; Bio-Rad, Hercules, California, USA) with seawater, and 100% Percoll (Sigma, St. Louis, Missouri, USA) mixed with sodium chloride such that it was isotonic with the larvae. Centrifugation caused the larvae to sink until they reached a point in the tube equivalent to their own density. The larvae were then carefully pipetted out of the tube and density of the Percoll-seawater solution surrounding the larvae was determined using a refractometer.

The combined estimates of volume and density were then used to obtain mass (Mass = Volume x Density). If density did not change with body area, then the mean density found was used for all individuals, and mass was assumed to be proportional to volume. However, where significant differences in density with body area were found, a general linear model was used such that mass of individuals could be calculated based on the relationship between body area and density gained from the model.

c. Conversion of larval mass to energy content

To convert mass of larvae to total energy content, we used values of carbon content of *B. neritina* as reported by (Wendt 2000). For larvae with a mean body length 271 ± 2 μm, elemental carbon content in newly released (<1 h old) larvae was reported to be 1313 ± 03 ng. This was then converted to 51.86 mJ, or 3.85 x 10^{-6} mJ μm^{-3} larvae based on conversion factors shown by Gosselin and Qian (1998). We are unaware of any available published data for *W.*

subtorquata larval energy content. Based on our findings of larval density and metabolic rate, it appears that *W. subtorquata* requires a larger energy density than that of *B. neritina*, similar to reported values for echinoderms (McEdward and Chia 1991). Hence, to estimate *W. subtorquata* energy density, we took an average value based on two sources; those calculated for *B. neritina* larvae (3.85×10^{-6} mJ μm^{-3}), along with mean energy densities of echinoderm eggs as summarised by (McEdward and Morgan 2001). Egg volume and total egg energy content for 24 species of echinoderms with lecithotropic development were used to calculate an average egg energy density of 1.17×10^{-5} mJ μm^{-3}. We excluded *Notasterias armata* and *Perknaster fucus* from calculations as these were reported as highly variable or extreme outliers by (Pernet and Jaeckle 2004). From this we could then obtain energy content based on the volume of each larva. Using the predicted energy consumption throughout the development stage for larvae of different size, we could compare the proportion of total energy use for small and large offspring.

Analytical approach to estimating coefficients

Throughout our analyses, we used logged body masses and logged metabolic rates. Log transformation was used in order to reduce observed increased variation with the mean, and thereby satisfy the assumption of homoscedasticity, it also provides a more readily interpretable measure of scaling exponents (in a log-log framework, the estimated coefficient is the scaling exponent in the untransformed power function). Most importantly, a linear framework allows for more tractable and reliable mixed-effects models that incorporate experimental runs.

Our analytical approach was necessarily tortuous due to the logistics of estimating metabolic rate of individual larvae. Because we could not measure the metabolic rates of free swimming larvae and then allow those same larvae to settle and metamorphose, we had to conduct two experiments, one that estimated larval metabolic rates and one that estimated

settler metabolic rates twice (early and later in development). As such, our measures constitute one cross-sectional and two longitudinal estimates of metabolic rate and these approaches require different statistics. For the longitudinal data, we used a repeated-measures approach to account for individual identity effects for consecutive samples (see Quinn and Keough (2002) pg. 305 for details) that included run as a random effect). The repeated measures approach is most appropriate for these data because the early and late estimates for each settler are not independent measures. A formal test of a stage by mass interaction provides an estimate of whether the scaling exponent differs significantly across stages. For *B. neritina* we found that the exponent did differ significantly between early and late stages but found no difference in exponents among stages for *W. subtorquata*. To determine whether the metabolic scaling exponent for larvae differed significantly to that of settlers, a repeated measures approach was not appropriate because the individuals used for the larval stage were not the same individuals that were used for the settler stage. Consequently, for these cross-sectional data, we instead used a mixed-effects model where stage was a fixed effect and we tested whether the scaling exponent changed significantly between the larval stage and the early settler stage. In all of our analyses, we also incorporated the random effect of Experimental Run and all of its interaction with Stage and Log_{10}Mass. We first tested whether random slopes models provided a significantly better fit (they never did, see Table A2) and then reduced the model as is appropriate (Quinn and Keough 2002). In all of our analyses, Experimental Run explained significant levels of variation (in other words, a random intercepts model provided a better fit) and so Run was retained in the final model.

We used this statistical approach in preference to reduced major axis (RMA) regression, because the error rate in our measurements of size is likely to be less than a third of the error rate in our measurements of VO_2, making OLS preferable over RMA regression (McArdle 1988; Niklas and Hammond 2014). RMA assumes equal amounts of biological noise in both

variables (Mathot et al. 2013), that is, a symmetrical relationship between body mass and BMR. Our data clearly show that this assumption does not hold in our study. Perhaps more importantly RMA approaches do not easily admit mixed-effects models, nor is there an equivalent repeated-measures framework that uses RMA. Given the relative error structure of our data, we believe this our approach is therefore the most reliable and best partitions the variation we observed among runs. We then used Wald tests to formally test whether the predicted scaling exponent was significantly different from both 0 and 1.

Importantly, the range of body masses for both species did not span an order of magnitude – this range reflects the range observed in nature and therefore is biologically relevant. Meta-analyses have reported that studies with smaller body mass ranges produce less precise estimates of scaling exponents (e.g. White et al. 2007). While this is broadly true, formal tests of scaling exponent values account for this decreased precision such that these tests of significant differences from 0 or 1 are equally reliable regardless of body mass ranges.

Results

Estimates of larval mass and energy content

There was a strong significant correlation between larval body area and volume for both *B. neritina* (ANOVA, $F_{1,98} = 21.4$, $P<0.0001$)

$$Volume = Area \times ((Length \times 0.25) + 130.3) \quad (1)$$

and *W. subtorquata* (ANOVA, $F_{2,582} = 1016.5$, $P<.0001$)

$$Volume = (Area \times 216.4) - 8615.4 \quad (2)$$

(where volume is in μm^3 and length in μm)

No significant relationship between volume and density was found for *B. neritina* (Figure A1; ANOVA, $F_{1,111} = 3.6$, $P=0.06$) so the mean density obtained from all individuals (1.07×10^{-6} $\mu g\ \mu m^{-3}$) was used such that mass was directly proportional to body volume. For *W. subtorquata* larvae, volume was significantly positively correlated with density (Figure A1; ANOVA, $F_{1,117} = 14.6$, $P<0.0001$) where the regression coefficients were:

$$Density = (0.4 \times Volume) + 1096.9 \quad (3)$$

(where volume is in m^3 and density in kg m^{-3})

The mass for individuals used in the experiment runs was then calculated from volume and density estimates for use in analysis of scaling exponents. The mass range for *B. neritina* was found to be 6.7 – 24.5 µg and for *W. subtorquata* it was 9.3 – 25.3 µg.

Tables

Table A1. Larval size traits (μg) for individuals used in measurements of offspring size and metabolic rate for *B. neritina* and *W. subtorquata*.

Species	*B. neritina*	*W. subtorquata*
Mean larval size ± std dev (μg)	14.3 ± 3.4	18.7 ± 2.9
Larval size range (μg)	6.7 – 24.5	9.3 – 25.3

Table A2. Chi-square distribution tests comparing random-slopes, random-intercept models to random-intercepts models for the relationship between Log_{10} Metabolic rate and Log_{10} Mass for *B. neritina* and *W. subtorquata*.

Species	Log_{10} Mass x Experimental Run		
	d.f	χ^2	P-value
B. neritina (larval and early stages)	1	0.21	0.65
B. neritina (late stage)	1	0.05	0.83
W. subtorquata (larval, early and late stages)	1	0	1

Figures

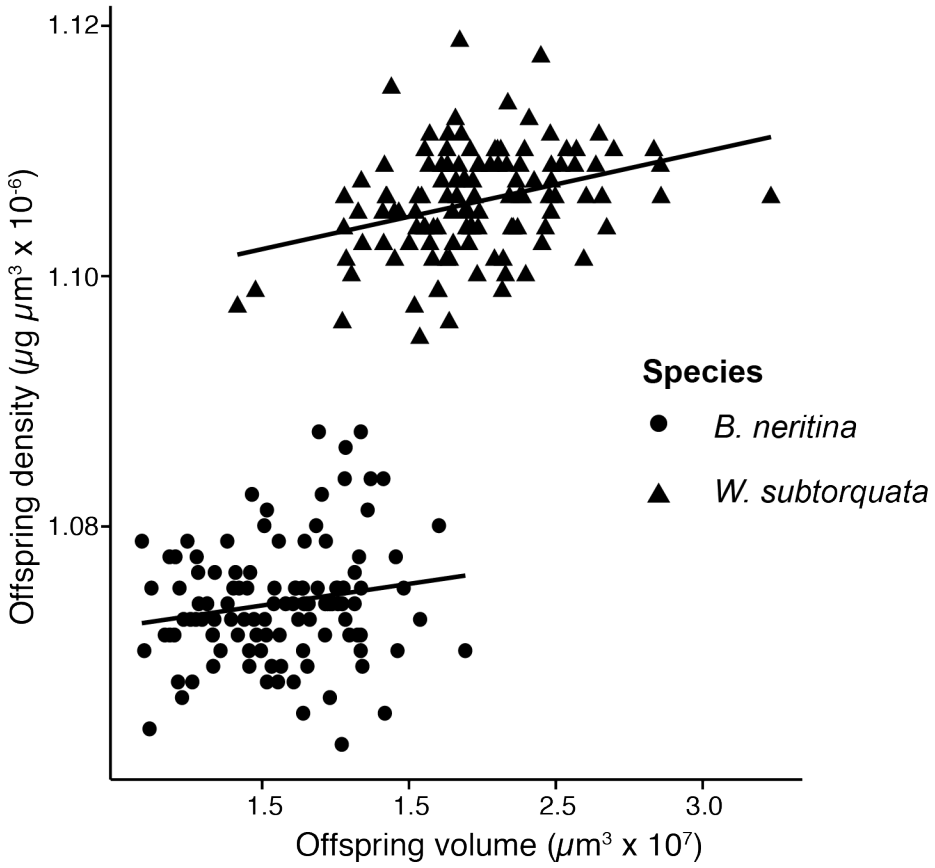


Figure A1. Relationship between offspring body density (μg μm^{-3} x 10^{-6}) and offspring volume (μm^3 x 10^7) for *B. neritina* and *W. subtorquata*. Volume was calculated from measurements of three perpendicular diameters and density was estimated using a gradient former method.

References

Gosselin, L. A., and P. Y. Qian. 1998. Analysing energy content: a new micro-assay and an assessment of the applicability of acid dichromate assays. Hydrobiologia 390:141-151.

Kosman, E. T., and B. Pernet. 2009. Diel Variation in the Sizes of Larvae of *Bugula neritina* in Field Populations. Biological Bulletin 216:85-93.

Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131-3137.

Mathot, K. J., K. Martin, B. Kempenaers, and W. Forstmeier. 2013. Basal metabolic rate can evolve independently of morphological and behavioural traits. Heredity 111:175-181.

McArdle, B. H. 1988. The structural relationship: regression in biology. Canadian Journal of Zoology-Revue Canadienne De Zoologie 66:2329-2339.

McEdward, L. R., and S. F. Carson. 1987. Variation in egg organic content and its relationship with egg size in the starfish *Solaster stimpsoni*. Marine Ecology Progress Series 37:159-169.

McEdward, L. R., and F. S. Chia. 1991. Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. Journal of Experimental Marine Biology and Ecology 147:95-102.

McEdward, L. R., and K. H. Morgan. 2001. Interspecific relationships between egg size and the level of parental investment per offspring in echinoderms. Biological Bulletin 200:33-50.

Niklas, K. J., and S. T. Hammond. 2014. Assessing scaling relationships: uses, abuses, and alternatives. International Journal of Plant Sciences 175:754-763.

Pernet, B., and W. B. Jaeckle. 2004. Size and organic content of eggs of marine annelids, and the underestimation of egg energy content by dichromate oxidation. Biological Bulletin 207:67-71.

Quinn, G. P., and M. J. Keough. 2002, Experimental design and data analysis for biologists. Cambridge, UK, Cambridge University Press.

Wendt, D. E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biological Bulletin 198:346-356.

White, C. R., P. Cassey, and T. M. Blackburn. 2007. Allometric exponents do not support a universal metabolic allometry. Ecology 88:315-323.

Chapter 2

Does the cost of development scale allometrically with offspring size?

Amanda K. Pettersen[1], Craig R. White[1], Robert J. Bryson-Richardson[2], Dustin J. Marshall[1]

[1] School of Biological Sciences/Centre for Geometric Biology, Monash University, Melbourne, Victoria, Australia

[2] School of Biological Sciences, Monash University, Melbourne, Victoria, Australia

Abstract

Within many species, larger offspring have higher fitness. While the presence of an offspring size-fitness relationship is canonical in life-history theory, the mechanisms that determine why this relationship exists are unclear. Linking metabolic theory to life-history theory could provide a general explanation for why larger offspring often perform better than smaller offspring. In many species, energy reserves at the completion of development drive differences in offspring fitness. Development is costly so any factor that decreases energy expenditure during development should result in higher energy reserves and thus subsequently offspring fitness. Metabolic theory predicts that larger offspring should have relatively lower metabolic rates and thus emerge with a higher level of energy reserves (assuming developmental times are constant). The increased efficiency of development in larger offspring may therefore be an underlying driver of the relationship between offspring size and offspring fitness, but this has not been tested within species. To determine how the costs of development scale with offspring size, we measured energy expenditure throughout development in the model organism *Danio rerio* across a range of natural offspring sizes. We also measured how offspring size affects the length of the developmental period. We then examined how hatchling size and condition scale with offspring size. We find that larger offspring have lower mass-specific metabolic rates during development, but develop at the same rate as smaller offspring. Larger offspring also hatch relatively heavier and in better condition than smaller offspring. That the relative costs of development decrease with offspring size may provide a widely applicable explanation for why larger offspring often perform better than smaller offspring.

Introduction

Offspring size is a fundamental, yet highly variable trait that reflects both a maternal and offspring phenotype (Bernardo 1996). Offspring size (which we refer to as the per propagule structure and yolk provisioned by a mother) affects fitness– larger offspring generally have higher fitness than smaller conspecifics (Stearns 1992). Increased maternal investment in each offspring can enhance their survival, reproductive output and growth, or may reduce their susceptibility to predation and starvation (Cipollini and Stiles 1991; Hutchings 1991; Janzen et al. 2000; Moran and Emlet 2001; Uller and Olsson 2010). The benefits of increased offspring size are pervasive in life-history theory, however there are exceptions, and the size-fitness relationship is often context-dependent (Mousseau and Fox 1998; Reznick et al. 1990). Although an increase in offspring size often confers a fitness benefit for the offspring, mothers must trade-off per-offspring investment with fecundity (Lack 1947; Smith and Fretwell 1974). Thus, the strength and direction of the offspring size-fitness relationship, together with size-number trade-off should drive the evolution of offspring size. Although life-history theory has long considered offspring size-fitness relationships within a wide range of taxa, the proximate mechanisms driving this relationship remain less well explored.

Various approaches have been taken to explain why offspring size often affects fitness, but the effects of offspring size are idiosyncratic. Larger offspring can feed better, can pass through vulnerable life stages faster (or slower), and be more (or less) susceptible to predators (Fox and Mousseau 1996; Kosman and Pernet 2011; Marshall and Keough 2008; Rivest 1983). What is lacking is a general mechanistic explanation for why offspring size affects fitness at all. It is often implicit in life-history theory that larger offspring have more 'energy' to dedicate to fitness-enhancing processes (Sinervo 1990; Stearns 1992; Wootton and Smith 2014). That larger offspring have more energy is a reasonable and potentially general explanation, but this

idea is incomplete because larger bodies are more costly to maintain. For larger offspring to have more energy to dedicate to fitness-enhancing processes, they need access to more energy reserves than smaller offspring, relative to their size (and therefore energy demands). Otherwise, any size-related increases in energy reserves will simply be offset by concomitant size-related increases in energy demands. Importantly, studies often find that the level of resources that an offspring has available once development is complete is a strong predictor of subsequent fitness. For example, experimental reductions of energy reserves at the end of development tend to reduce subsequent offspring fitness (Emlet and Hoegh-Guldberg 1997; Marshall and Keough 2006; Sinervo and McEdward 1988), even when offspring size *per se* is held constant. Similarly, offspring with naturally higher energy reserves at the end of development tend to have greater fitness than offspring with lower energy reserves (Berkeley et al. 2004). Thus, any factor that affects the level of energy reserves at the end of development is likely to affect subsequent offspring fitness.

Development is costly. If development becomes too costly during crucial developmental stages such as from the zygote to larvae, then this can lead to significant consequences for offspring survival or fitness in later life stages (Gagliano and McCormick 2007; Goulden et al. 1987). For species with complex life cycles that undergo complete reorganisation of tissue during development (e.g. fish, amphibians, insects, and marine invertebrates), energy loss during development and metamorphosis accounts for approximately 30 - 60% of initial energy reserves (Merkey et al. 2011; Seymour et al. 1991; Wendt 2000). Factors that exacerbate energy costs throughout this developmental period such as extended larval periods or delayed metamorphosis can be detrimental to survival and post-metamorphic fitness (Marshall et al. 2003; Mitchell and Seymour 2000). For species that do not undergo metamorphosis during development through embryogenesis to hatching (i.e. 'direct' developers such as reptiles and birds), offspring can use around 25-35% of their maternal

derived energy reserves (Deeming and Birchard 2007; Vleck and Hoyt 1991). Although the use of endogenous energy reserves (such as the yolk) during development for nonfeeding direct developers have been widely measured, how these costs scale with offspring size throughout this critical period so far remains unresolved.

Applying metabolic scaling principles to offspring size may provide a general explanation for the offspring size-fitness relationship, when it is observed. Both within and among species, the scaling exponent relating mass to metabolic rate is typically less than 1 (Glazier 2010). For species undergoing costly development, an allometric relationship between offspring mass and metabolic rate could provide a general explanation for the offspring size-fitness relationship, assuming that it outweighs any potential size-specific disadvantages (Pettersen et al. 2015). Because larger offspring are predicted to use relatively less energy per unit mass during development (assuming increases in offspring size do not extend the developmental period – an assumption that must be tested), larger offspring should reach the end of their developmental phase with a higher proportion of endogenous reserves. Importantly, this higher proportion of energy reserves may therefore allow larger offspring to perform better – they have relatively more energy available for fitness-enhancing functions. Indeed, a recent study in bryozoans showed that metamorphosis was less costly for larger offspring relative to smaller offspring (Pettersen et al. 2015). Similarly, Goulden et al. (1987) explored macroevolutionary patterns among species of daphniid Cladocera neonates, where developmental energy efficiency increased with offspring size throughout development – offspring hatching from larger eggs possessed more post-embryonic yolk than offspring from smaller eggs. They suggested that this may be because the rate of energy reserve loss scaled allometrically with body mass among species. Whether such benefits first proposed by Goulden, Henry and Berrigan (1987) extend to the entire developmental period within species remains unclear.

If larger offspring use relatively less energy than smaller offspring throughout development, then we would predict that larger offspring hatch with a higher proportion of maternal energy investment remaining than smaller offspring. A corollary of this prediction would be that post-development mass scales hyperallometrically with initial offspring size – that is, hatchlings from larger eggs should not only weigh more than hatchlings from smaller eggs, they should also lose *relatively* less mass. Hatchlings from larger eggs should also emerge in better condition because, relative to their size, they burn less resources throughout development than individuals hatched from smaller eggs. Here, using the model organism *Danio rerio*, we test first whether metabolic rate scales allometrically with offspring size (the per propagule combined structure and yolk provisioned by a mother) throughout development. Because some theory predicts that larger offspring should have longer developmental periods (Gillooly et al. 2001, but see Vance 1973), thereby offsetting any metabolic efficiency of larger offspring, we also examined how offspring size affects the rate of development. We found strong evidence for allometric scaling of metabolism throughout development and no evidence for an effect of offspring size on the length of the development (see Results), so we then tested whether larger offspring hatch with a higher proportion of their initial mass and with relatively larger yolk reserves than smaller offspring. In order for larger offspring to be considered more energy "efficient", they should also reach the same developmental stage with a higher proportion of hatchling mass relative to energy expended; that is, the conversion of energy to tissue should scale hyperallometrically with offspring size. To determine if larger offspring are more efficient, we calculated the key ratio for estimating energy efficiency during development: the ratio of (a) the energy that is expended during development to (b) the amount of hatchling tissue created (energy use: hatchling size). This ratio should decrease with offspring size if hypoallometric scaling of metabolic rate is to adequately explain the offspring size-fitness relationship.

Materials and methods

Experimental overview, collection and measurement of embryo mass

To determine how the energy costs of development scale with offspring size we: a) measured developmental time from fertilisation until hatching b) measured rate of oxygen consumption ($\dot{V}O_2$ a proxy for metabolic rate) across three developmental stages; c) estimated the relationship between offspring size and hatchling size; d) estimated the relationship between offspring size and hatchling condition (hatchling yolk area relative to hatchling size) across a range of offspring sizes through development and from this e) calculated energy efficiency with offspring size, that is, the scaling of offspring size with the ratio of total energy used to hatchling mass. As per Parichy et al. (2009), we use the term "embryo" to include both the developing hatchling and the yolk that it uses through development. Furthermore, from a life history perspective, total offspring size is the most relevant description of the total per offspring unit of investment by mothers.

Danio rerio (Hamilton 1822; henceforth *Danio*) is a tropical freshwater teleost used extensively as a model organism. *Danio* embryos undergo rapid development – the zygote consists largely of yolk that is absorbed throughout development and hatching until construction of feeding structures at approximately 72 h post-fertilisation, with the larva retaining some residual yolk (Jardine and Litvak 2003). All experiments were conducted during November 2015 – April 2016, using wild type strains maintained under standard operating procedures approved by the Monash Animal Services Animal Ethics Committee. *Danio* are oviparous and reproduce by external fertilisation, spawning gametes in response to a combination of light, visual and olfactory cues (see Westerfield 2007 for details). Hence, all embryo collections were carried out in the morning with eight 1 L tanks containing single male and female pairs separated by a plastic barrier to prepare individuals for gamete production.

Once the barrier was removed, naturally-released pheromones stimulated ovulation and oviposition in females and spawning by males (Vandenhurk and Lambert 1983). The 1 L tanks contained slits in the base of the tank that separated adults from the embryos, and thereby prevented cannibalism by adults. Each experimental run ('Experiment') consisted of fertilised embryos from parental pairs ('Parent ID') collected on different days. Embryos from parent pairs with highest fertilisation success were collected within one hour of barrier removal, transferred onto a mesh strainer and washed to remove debris. Embryos were then transferred into a petri dish and placed into an incubator at 28.5°C as per standard rearing techniques. After 4 h, embryos were pipetted into individual plate wells with 1 mL filtered freshwater and photographed at the 'sphere' stage (Olympus 1X73; x40). Embryonic developmental stages were identified based on Kimmel et al. (1995) where the equatorial diameter during the period directly preceding the gastrula stage (the 'sphere stage') has shown to provide a good indication of embryo size (Bownds et al. 2010). All measurements of equatorial diameter were taken using Olympus cellSens Dimension software. Embryo area (μm^2) was calculated from embryo radius (μm) and embryo volume (μl) was calculated as ($\frac{4}{3} \cdot \pi \cdot \text{embryo radius} \cdot 10^{-3}$). In order to allow for direct comparison of our scaling relationships with other studies, we used embryo mass as our measure of offspring size. Importantly, because the relationship between volume and mass was best fit by a linear function, using either measure of offspring size gave equivalent the scaling relationships with metabolic rate (see Appendix B for details). Due to the destructive nature of embryo mass sampling, double sampling was required in order to obtain estimates for projected embryo mass (here on referred to as 'embryo mass'). We therefore weighed a separate sample of 'sphere' stage embryos and calculated the relationship between embryo volume and mass (see Appendix B for details).

a. Scaling of developmental time and embryo mass

To determine whether embryo mass affects developmental time, 144 embryos were measured following the above methods and pipetted into a 24-well plate with 2 mL of pasteurised 'egg water' (60 μg ml^{-1} stock salts in 1 L distilled water, as per Westerfield 2007) and placed in a controlled temperature room at 28°C until hatching (where fertilisation until hatching was used to represent the "dependent phase"). Embryos were then photographed every 0.5 h until hatching using time-lapse software (Olympus 1X73; x10, Olympus cellSens Dimension software). We ran a general linear model to test for an effect of embryo mass on developmental time (R Development Core Team v3.2.5) and significance tested using maximum likelihood using the package lme4. We then ran a power analysis (G*Power 3.1.9.2) and calculated to a 95% confidence level what extent development time would need to scale with embryo mass in order to offset the efficiency of larger offspring through allometric scaling.

b. Scaling of metabolic rate and embryo mass

We measured the rate of oxygen consumption ($\dot{V}O_2$) as a proxy for metabolic rate, across three developmental stages of *Danio* in November 2015 – January 2016. $\dot{V}O_2$ was measured for 20 individuals of known size in individual vials simultaneously using a 24-channel PreSens sensor dish reader (Sensor Dish Reader SDR2, PreSens), with 24-chamber glass microplate (vial volume: 750 μl) (Loligo Systems Aps, Tjele, Denmark) at 28°C ±1°C as per standard techniques (Pettersen et al. 2015). Prior to the experimental runs, the non-consumptive O_2 sensor spots were calibrated using air-saturated (100% AS) egg water and egg water containing 2% sodium sulphite (0% AS). For each individual embryo within each experimental run, oxygen consumption was recorded at three stages over a 3h period to signify the beginning (gastrula stage), middle (prim-5 stage) and end (high-pec stage) of the dependent phase: 6 h, 24 h and 44 h post-fertilisation (Kimmel et al. 1995). At each development stage the same 20 embryos were placed individually into glass vials containing pasteurised egg water while the

remaining four vials were used as controls, containing only pasteurised egg water. Air saturation for each individual embryo was recorded every 2 minutes and $\dot{V}O_2$ was calculated from the rate of change of O_2 saturation (m_a; % h^{-1}) as $\dot{V}O_2 = -1\ (m_a - m_b / 100)\ V\beta O_2$ (as per White et al. 2011) where m_b is the rate of change of O_2 saturation for blank vials containing no embryos (% h^{-1}), βO_2 is the oxygen capacitance of air-saturated egg water at 28°C (5.48 ppt; Cameron 1986), and V is water volume in the vial (volume of individual embryos were subtracted from volume of 7.5 x 10^{-4} L chambers). To convert $\dot{V}O_2$ (µl h^{-1}) to metabolic rate (mJ h^{-1}), the calorific conversion factor of 20.08 J ml^{-1} O_2 was used (Crisp 1971). This procedure consisting of three $\dot{V}O_2$ measures for twenty individuals was repeated five times so we accumulated measures for a total of 100 individuals across three developmental stages.

Embryo mass and metabolic rates were log transformed and analysed in a linear framework in order to reduce increased variation with the mean and thereby satisfy the assumption of homoscedasticity (Niklas and Hammond 2014). Repeated measures ANCOVA (using the package lme4) was used to test for significance of the random effects of parent pair ('Parent ID') nested within experimental run ('Experiment'), and its interactions with Log_{10} mass ('Log_{10} Embryo mass') across the repeated measure factor of time i.e developmental stage ('Stage'). While there was a significant effect of Parent (nested within Experiment) and Stage x Experiment on Log_{10} Metabolic Rate, we found no support for fitting a random-slopes model (no significant Stage x Log_{10} Embryo mass effect was found), therefore Parent within Experiment, Stage and Log_{10} Embryo mass were retained in the final model. A formal test of Stage x Log_{10} Embryo mass interaction provides an estimate of whether the scaling exponent differs significantly across stages – for *Danio* embryos this interaction was found to be non-significant, therefore a single scaling exponent was used for all three stages using aggregated data (Table 1).

c. Scaling of developmental time and hatchling mass with embryo mass

In order to determine how initial offspring size affected the amount of mass lost during development through to hatching, we weighed individuals of known initial embryo size (measured as per methods section 'a') upon hatching. Larvae were photographed at the long-pec stage within one hour of hatching, using methods described earlier for embryos, and transferred into pre-weighed tin foil cartridges in 100 μL of distilled water. Samples were dried at 60°C for 48 h, and weighed with a microbalance (Mettler Toledo XP2U) to the nearest 0.1 μg as per Hachicho et al. (2015). As there was no effect of embryo size on developmental time (see results), we sampled hatchlings for mass measurements over 48 – 60 h post-fertilisation (any individuals that hatched after this time were excluded from the study). Hatchling masses increased over the experimental period, so Parent ID (timing of experimental run), was treated as a continuous factor. To test for an interaction between Log_{10} Embryo mass and Parent ID on Log_{10} Hatchling mass a general linear model was used, and significance tested using maximum likelihood. No interaction between Log_{10} Hatchling mass x Parent ID was found (regardless of whether Parent ID was treated as a continuous or categorical variable), so it was excluded. Parent ID was retained in the model as a covariate. In order to fit untransformed data in a power function, we then used nonlinear multiple regression to directly estimate parameters of interest. Parameter estimates were tested as significantly different from 0 and 1 using Wald tests.

d. Scaling of the ratio between hatchling yolk area and hatchling area with embryo area

To quantify the relative yolk consumption among embryos of different initial size throughout development, we measured the size of the yolk sac area retained at the end of the dependent phase (as per Jardine and Litvak 2003). 'Embryo area' (μm^2) and 'Hatchling area' (μm^2) were estimated as above. Photographs from the same hatched individuals taken from a lateral view were used to measure 'Yolk Area' in μm^2. We then ran a linear model to test how offspring size affected the ratio between hatchling yolk area and hatchling area. Due to a lack of overlap

among parent pairs for Embryo Area, we could not include 'Parent ID' in the models (Quinn and Keough 2002). Furthermore, because we found that embryo metabolism scaled allometrically with size, we were interested in whether this was driven by larger embryos possessing relatively higher amounts metabolically inert yolk relative to smaller embryos. We therefore measured a subsample of embryos photographed at the sphere stage and measured and calculated the relationship between embryo yolk area and total embryo area (see Appendix B for details).

e. Estimating efficiency

To determine whether the scaling of yolk sac area upon hatching was due to a more efficient conversion to tissue and not simply an artefact of larger offspring possessing larger yolk mass relative to embryo size, we calculated the size-dependent energy expenditure relative to the mass of new tissue synthesised throughout development (i.e. proportion of embryo mass converted to hatchling mass not including leftover yolk). Size-dependent total energy use was calculated from the scaling exponents and coefficients obtained above, multiplied by the average developmental time of 54h (since development time is unrelated to embryo mass, an average time was taken, see results). To convert our measures of hatchling length to hatchling mass independent of remaining yolk, we compiled estimates for the within-species relationship between length and weight of larval fish using FishBase (www.fishbase.org). Within species, available data suggested that the length-weight relationship is higher for larval fish than for post-larval fish (Le Cren 1951; Osse 1990; Vilizzi 1998). For the 12139 length-weight relationships in FishBase, the scaling exponent (d, where weight is proportional to length^d) was never less than 1.5 and 95% of the scaling exponents were between 2.44 and 3.54 (range 1.51 – 4.5, median: 3.01), however in order to provide conservative estimates of energy efficiencies

we present both the minimum (1.5) and median (3) scaling exponents of hatchling mass to hatchling length.

Results

a. Scaling of developmental time and embryo mass

We found no effect of embryo mass on developmental time in *D. rerio* reared under standard laboratory conditions ($F_{1,102} = 0.054$, $P > 0.05$; Figure B1). We calculated that in order for the advantages of allometric scaling with embryo mass to be offset, the slope of the relationship between developmental time and embryo mass would need to be 0.64 or greater (where Developmental time = 0.64 x Embryo mass + constant). Our power to detect such a relationship, were one to exist, exceeds 0.95, such that we can reasonably rule out the possibility that larger eggs take longer to develop and therefore can also rule out that the mass-specific metabolic benefits of increased offspring size are offset by a lengthened developmental period.

b. Scaling of metabolic rate and embryo mass

The relationship between Log_{10} Embryo mass and Log_{10} Metabolic rate was found to be allometric throughout development (Figure 1), where the scaling exponent was found to be significantly different from both 0 and 1 (Estimate ± CI: 0.32 ± 0.16, $p < 0.05$; Table 1) and significantly lower than common theoretical slopes of 0.66 ($p = 0.02$) and 0.75 (<0.001). We found a strong developmental stage effect on energy use – metabolic rate was lowest early in development during the gastrula period and increased over the three days until hatching as larvae (Table 1).

c. Scaling of developmental time and hatchling mass with embryo mass

Heavier embryos hatched as proportionally heavier larvae ($F_{1,200} = 273.54$, $P < 0.001$; Figure 2). The scaling exponent for the relationship between Embryo mass and Hatchling mass was found to be significantly greater than 1 ($p < 0.05$; Table 2). We found no significant interaction

between Parent ID and Log_{10} Embryo mass ($F_{1,199} = 2.02$, $P > 0.05$), however Parent ID showed a significant effect on Log_{10} Hatchling mass ($F_{1,200} = 74.77$, $P < 0.001$) and was therefore retained in the final model. Possible sources of variation among Parent ID may include temporal differences across experimental run, or genetic differences between parent pairs. The relationship between Hatchling mass and Embryo mass was described by the following nonlinear power function

$$\text{Hatchling mass} = 0.230 \times \text{Embryo mass}^{1.296} + 0.573$$

d. Scaling of the ratio between hatchling yolk area and hatchling area with embryo area

Hatchlings from larger eggs hatched as larvae with a relatively larger yolk area for their size (Figure 3). Embryo Area had a significant effect on Yolk Area relative to Hatchling Area ($F_{1,59} = 25.89$, $P < 0.001$) where the slope of the linear relationship was found to be significantly >0 ($p < 0.05$; Table 3). We found an isometric relationship between initial embryo area and yolk area (Appendix B), hence our finding that larger embryos hatch with relatively higher amounts of residual yolk are likely to be due to our findings of allometric scaling, rather than due to larger embryos possessing a higher proportion of initial yolk relative to smaller embryos.

e. Estimating efficiency

By combining our estimates of energy use and hatchling length with embryo mass we calculated that the ratio of energy expenditure relative to hatchling mass (independent of residual yolk mass) decreases with embryo mass (Figure 4). The direction of this relationship holds regardless of whether hatchling mass scales as a cubic function of hatchling length or even a more conservative estimate of mass α hatchling length$^{1.5}$. Total energy used throughout development was measured as the rate of energy use (mJ h^{-1}) multiplied by developmental time (h).

Discussion

We found an allometric relationship between metabolic rate during development and offspring size in *Danio*, a relationship that is likely to be widespread in other taxa. We found strong evidence larger offspring use their endogenous reserves more efficiently, and hatch with a higher proportion of their initial energy reserves than smaller offspring. Allometric scaling between metabolic rate and mass is one of the most fundamental relationships studied in metabolic theory - static allometric scaling (within developmental stages) has been well documented in adults across a range of taxa (Damme et al. 1987; Greenlee and Harrison 2004; Labocha et al. 2004). We now show that the same scaling relationship occurs across a range of offspring sizes for *Danio*. The presence of allometric scaling during development implies that larger offspring should hatch with proportionally more energy than smaller offspring upon reaching the independent phase. We now find support for this prediction - relative to their size, larger embryos retained a higher proportion of their initial yolk reserves than smaller embryos, and hatched relatively heavier than their smaller conspecifics. Our findings show that the relative costs of development decrease with offspring size and that larger offspring end their developmental phase with a higher proportion of energy reserves – this greater proportion of energy reserves may provide a general explanation for why larger offspring perform often better than smaller offspring.

Across a range of taxa, condition at the end of development is a good predictor of subsequent survival and growth (e.g. Naef-Daenzer *et al.* 2001, Baker & Fowler 1992, Janzen 1993, Jarrett & Pechenik 1997). In fish, various proxies of hatchling condition (including yolk area, and oil globule size and hatchling size), correlate with key fitness traits such as survival, starvation resistance and dispersal potential (Busch 1996; Probst et al. 2006; Semmens and Swearer 2011). The emergence of hatchlings with relatively more mass and larger yolk reserves

will influence subsequent survival and fitness throughout the life history (Houde 2002). For species that undergo organogenesis (i.e. animals), the transition from embryo to actively feeding larvae is regarded as the most critical event during early life (Hjort 1914). For early life stage development in *Danio* we calculated that for a standard 54 h developmental period at 28°C, the largest embryos size (73.5 µg) would use 18.7% of total reserves, while the smallest embryo (37.4 µg) would use 29.7% of its total energy reserves. The costs of development are not equivalent to embryo size – despite a two-fold decrease in size, the smallest measured embryo uses approximately 1.6 times its energy reserves relative to that of the largest measured embryo. These estimates are similar to those showing that the costs of metamorphosis in bryozoans decrease relative to offspring size (Pettersen et al. 2015). Our expectation is that allometric scaling renders the development of larger offspring more efficient within a range of taxa but this requires further testing.

In addition to our discovery of allometric scaling with offspring size in *Danio*, we found that larger embryos also hatch relatively heavier and in better condition than offspring hatched from smaller embryos. That larger offspring use relatively less energy throughout development and hatch with a higher proportion of their initial energy provides further evidence to support allometric scaling as a potentially universal mechanism for the offspring size-fitness relationship. Our findings reflect broader interspecific patterns of hatchling quality with offspring size. Among daphniid Cladocera, neonates of larger species metabolise proportionally less post-embryonic yolk, and are born with a larger relative amount of yolk than species with smaller neonates (Goulden et al. 1987). In a review of endogenous feeding in fish, Kamler (2008) showed that yolk absorption was related to egg size, but the rate of yolk absorption relative to total endogenous energy reserves was considerably lower in species with the largest eggs (chum salmon; 1.4% day^{-1}) and highest in that with the smallest eggs (bluegill sunfish; 50.2% day^{-1}). Through direct measurement of the condition of hatchlings across a

range of embryo sizes, our study also confirms predictions made in Pettersen et al. (2015) that larger offspring should hatch with a higher proportion of their initial maternal provisioning. Importantly, our findings may explain a long-standing puzzle in bird life histories. Williams (1994) found that in a range of species, "…chicks from larger eggs are heavier at hatching rather than structurally larger, i.e. that they hatch with more nutrient (yolk) reserves". These are exactly the effects we would expect if development is more metabolically efficient for larger eggs. Given that metabolic rate also scales allometrically with mass in some birds, our explanation for why egg size affects hatching mass and reserves is likely to apply (Williams and Ricklefs 1984).

Our data do not provide a mechanistic explanation for the low (b = 0.32) scaling exponent for the relationship between metabolic rate and total embryo mass. While scaling exponents between metabolic rate and adult body mass often fall between 0.66 and 0.75, values more extreme than this are not uncommon, and b can deviate as a result of metabolic characteristics associated with particular life-history stages (as discussed in Glazier 2014). A number of previous studies have also identified shallow scaling exponents between metabolic rate and offspring size (<0.5; Pettersen, White & Marshall 2015; Bishop & Torres 1999; Wieser and Oberhauser 1984). This shallow scaling may arise for a multitude of reasons, including a relative lack of resource transport networks in developing embryos, differential allocation of energy to biological functions, or shifts in rates of cell proliferation and expansion, compared with adults (Gaitan-Espitia et al. 2013; Glazier 2005; Kozlowski et al. 2003). Another possible mechanism for our observation is that mothers provision larger offspring with a higher proportion of yolk relative to total offspring (embryo and yolk) size. If we assume that embryo tissue is more metabolically active than yolk (which seems reasonable (Kooijman 2009)), and if larger eggs have a greater proportion of yolk relative to embryo size, then offspring from larger eggs should have lower metabolic rates during development. That the relative amount

of yolk increases with egg size seems likely based on the geometry of the developing egg (the embryo grows essentially as a two-dimensional sheet on the top portion of the egg so embryo size will scale with egg size at a lower power than yolk). Alternatively, if initial embryo size is directly proportional to total offspring size then metabolic rate should be isometric with offspring size and this mechanism therefore does not hold. Testing whether a hyperallometric relationship between yolk and egg size exists is beyond the scope of this study, however we advocate this as an important next step in identifying the underlying mechanism driving the allometric scaling of metabolic rate with offspring size.

Relative to smaller embryos, we found larger embryos use energy at a lower rate and hatch with a higher mass and in better condition, hence we expect the consequences of allometric scaling to alter the size-number trade-off. Despite this intuition, mothers often produce small to intermediate offspring sizes, and the most energy efficient, largest possible offspring sizes are rarely observed (Bernardo 1996). What then, are the benefits of producing smaller offspring and why do we continue to observe small offspring size? The benefits of increased fecundity may outweigh the costs of producing smaller, less fit, and less efficient offspring; if so theory predicts that mothers should produce many, small offspring. Producing smaller, more numerous offspring might be particularly advantageous when resources are abundant, such that larger offspring have little fitness advantage over smaller offspring and relative efficiencies of development are less important (Goulden et al. 1987; Monro et al. 2010). Likewise, in extremely stressful environments, or when resources are patchy, and the offspring size-fitness relationship is absent, life-history theory predicts that there is no benefit to producing larger offspring at the expense of fecundity (Allen et al. 2008; Einum and Fleming 2000; Venable and Brown 1988). Rather, increased fecundity which in turn enhances the opportunity for dispersal of offspring away from the stressful maternal environment, is likely to be selected upon (Winemiller and Rose 1993).

Conclusions

The offspring size-fitness relationship is often context dependent. The relative benefits of increased offspring size, such as enhanced metabolic efficiency may be widespread, however if other factors reduce or even override the benefits of allometric scaling for larger offspring, then these effects may be masked. For example, in low oxygen conditions, larger offspring may be less able to acquire oxygen via diffusion, leading to a constraint on offspring size (Einum et al. 2002). Similarly, larger offspring may be exposed to higher size-selective predation, or experience increased mortality when settlement is delayed, relative to smaller offspring (Reznick et al. 1990; Svanfeldt et al. 2016). The metabolic theory of ecology predicts that developmental time should scale to one-quarter power of mass (Gillooly et al. 2001), and this is supported by some interspecific comparisons (Pauly and Pullin 1988; Clarke 1982). If this is the case, then the benefits of allometric scaling for larger offspring may be offset by extended developmental time, such that the costs of development become independent, or increase with offspring size. Within species, this trend is less well resolved with studies indicating positive (Marshall and Bolton 2007), negative (Hinegardner 1975; Sinervo and McEdward 1988); and absent (Emlet 1995; Hoegh-Guldberg and Pearse 1995) relationships between offspring size and development rate. While we did not detect any effect of embryo size on developmental time, further tests are needed to elucidate whether interspecific patterns are reflected on a microevolutionary scale, and how these patterns may change across the life history. If the benefits of allometric scaling can overcome the disadvantages of offspring size-dependent factors, such as extended developmental time, then the costs of development should decrease with offspring size.

References

Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. American Naturalist 171:225-237.

Baker, J. D., and C. W. Fowler. 1992. Pup weight and survival of northern fur seals *Callorhinus ursinus*. Journal of Zoology 227:231-238.

Berkeley, S. A., C. Chapman, and S. M. Sogard. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258-1264.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: Patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Bishop, R. E., and J. J. Torres. 1999. Leptocephalus energetics: metabolism and excretion. Journal of Experimental Biology 202:2485-2493.

Bownds, C., R. Wilson, and D. J. Marshall. 2010. Why do colder mothers produce larger eggs? An optimality approach. Journal of Experimental Biology 213:3796-3801.

Busch, A. 1996. Transition from endogenous to exogenous nutrition: Larval size parameters determining the start of external feeding and size of prey ingested by Ruegen spring herring *Clupea harengus*. Marine Ecology Progress Series 130:39-46.

Cameron, J. N. 1986. The solubility of carbon dioxide as a function of temperature and salinity (Appendix table), Pages 254-259 *in* J. N. Cameron, ed. Principles of physiological measurement. United Kingdom, Academic Press.

Cipollini, M. L., and E. W. Stiles. 1991. Seed predation by the bean weevil *Acanthoscelides obtectus* on Phaseolus species: consequences for seed size, early growth and reproduction. Oikos 60:205-214.

Crisp, D. J. 1971. Energy flow measurements, Pages 197-279 *in* N. A. Holme, and A. D. McIntyre, eds. Methods for the study of marine benthos. Oxford, Blackwell Sci.Pub.

Damme, K., F. Pirchner, H. Willeke, and H. Eichinger. 1987. Fasting metabolic rate in hens: 1. Effects of body weight, feather loss, and activity. Poultry Science 66:881-890.

Deeming, D. C., and G. F. Birchard. 2007. Allometry of egg and hatchling mass in birds and reptiles: roles of developmental maturity, eggshell structure and phylogeny. Journal of Zoology 271:78-87.

Einum, S., and I. A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567.

Einum, S., A. P. Hendry, and I. A. Fleming. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B-Biological Sciences 269:2325-2330.

Emlet, R. B., and O. Hoegh-Guldberg. 1997. Effects of egg size on postlarval performance: experimental evidence from a sea urchin. Evolution 51:141-152.

Fox, C. W., and T. A. Mousseau. 1996. Larval host plant affects fitness consequences of egg size variation in the seed beetle *Stator limbatus*. Oecologia 107:541-548.

Gagliano, M., and M. I. McCormick. 2007. Maternal condition influences phenotypic selection on offspring. Journal of Animal Ecology 76:174-182.

Gaitan-Espitia, J. D., A. Bruning, F. Mondaca, and R. F. Nespolo. 2013. Intraspecific variation in the metabolic scaling exponent in ectotherms: Testing the effect of latitudinal dine, ontogeny and transgenerational change in the land snail Cornu aspersum. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 165:169-177.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, and J. H. Brown. 2002. Effects of size and temperature on developmental time. Nature 417:70-73.

Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662.
Glazier, D. S. 2010. A unifying explanation for diverse metabolic scaling in animals and plants. Biological Reviews 85:111-138.
Goulden, C. E., L. Henry, and D. Berrigan. 1987. Egg size, postembryonic yolk, and survival ability. Oecologia 72:28-31.
Greenlee, K. J., and J. F. Harrison. 2004. Development of respiratory function in the American locust *Schistocerca americana* II. Within-instar effects. Journal of Experimental Biology 207:509-517.
Hachicho, N., S. Reithel, A. Miltner, H. J. Heipieper, E. Kuester, and T. Luckenbach. 2015. Body mass parameters, lipid profiles and protein contents of zebrafish embryos and effects of 2,4-dinitrophenol exposure. Plos One 10.
Hinegardner, R. T. 1975. Morphology and genetics of sea-urchin development. American Zoologist 15:679-689.
Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe, viewed in the light of biological research. Rapports et Proce`s-Verbaux des Re´unions du Conseil Permanent International Pour L'Exploration de la Mer 20:1-228.
Houde, E. D. 2002. Chapter 3. Mortality *in* L. A. Fuiman, and R. G. Werner, eds. Fishery science: The unique contribution of early life stages. Oxford, Blackwell Scientific Publishing.
Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salelinus fontinalis*. Evolution 45:1162-1168.
Janzen, F. J. 1993. An experimental analysis of natural selection on body size of hatchling turtles. Ecology 74:332-341.
Janzen, F. J., J. F. Tucker, and G. L. Paukstis. 2000. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. Journal of Evolutionary Biology 13:947-954.
Jardine, D., and M. K. Litvak. 2003. Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. Journal of Fish Biology 63:388-397.
Jarrett, J. N., and J. A. Pechenik. 1997. Temporal variation in cyprid quality and juvenile growth capacity for an intertidal barnacle. Ecology 78:1262-1265.
Kamler, E. 2008. Resource allocation in yolk-feeding fish. Reviews in Fish Biology and Fisheries 18:143-200.
Kimmel, C. B., W. W. Ballard, S. R. Kimmel, B. Ullmann, and T. F. Schilling. 1995. Stages of embryonic development of the zebrafish. Developmental Dynamics 203:253-310.
Kooijman, S. A. L. M. 2009, Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge, Cambridge University Press.
Kosman, E. T., and B. Pernet. 2011. Intraspecific variation in larval size and its effects on juvenile lophophore size in four bryozoans. Marine Ecology Progress Series 429:67-73.
Kozlowski, J., M. Konarzewski, and A. T. Gawelczyk. 2003. Cell size as a link between noncoding DNA and metabolic rate scaling. Proceedings of the National Academy of Sciences of the United States of America 100:14080-14085.
Labocha, M. K., E. T. Sadowska, K. Baliga, A. K. Semer, and P. Koteja. 2004. Individual variation and repeatability of basal metabolism in the bank vole, *Clethrionomys glareolus*. Proceedings of the Royal Society B-Biological Sciences 271:367-372.
Lack, D. 1947. The significance of clutch size. Ibis 89:302-352.

Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology 20:201-219.
Marshall, D. J., and M. J. Keough. 2006. Complex life cycles and offspring provisioning in marine invertebrates. Integrative and Comparative Biology 46:643-651.
—. 2008. The evolutionary ecology of offspring size in marine invertebrates, Pages 1-60 *in* D. W. Sims, ed. Advances in Marine Biology. Advances in Marine Biology.
Marshall, D. J., J. A. Pechenik, and M. J. Keough. 2003. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial, ascidian *Diplosoma listerianum*. Marine Ecology Progress Series 246:153-162.
Merkey, A. B., C. K. Wong, D. K. Hoshizaki, and A. G. Gibbs. 2011. Energetics of metamorphosis in *Drosophila melanogaster*. Journal of Insect Physiology 57:1437-1445.
Mitchell, N. J., and R. S. Seymour. 2000. Effects of temperature on energy cost and timing of embryonic and larval development of the terrestrially breeding moss frog, *Bryobatrachus nimbus*. Physiological and Biochemical Zoology 73:829-840.
Monro, K., T. Sinclair-Taylor, and D. J. Marshall. 2010. Selection on offspring size among environments: the roles of environmental quality and variability. Functional Ecology 24:676-684.
Moran, A. L., and R. B. Emlet. 2001. Offspring size and performance in variable environments: Field studies on a marine snail. Ecology 82:1597-1612.
Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. Trends in Ecology & Evolution 13:403-407.
Naef-Daenzer, B., F. Widmer, and M. Nuber. 2001. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. Journal of Animal Ecology 70:730-738.
Niklas, K. J., and S. T. Hammond. 2014. Assessing scaling relationships: uses, abuses, and alternatives. International Journal of Plant Sciences 175:754-763.
Osse, J. W. M. 1990. Form changes in fish larvae in relation to changing demands of function. Netherlands Journal of Zoology 40:362-385.
Parichy, D. M., M. R. Elizondo, M. G. Mills, T. N. Gordon, and R. E. Engeszer. 2009. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. Developmental Dynamics 238:2975-3015.
Pettersen, A. K., C. R. White, and D. J. Marshall. 2015. Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proceedings. Biological sciences / The Royal Society 282.
Probst, W. N., G. Kraus, R. M. Rideout, and E. A. Trippel. 2006. Parental effects on early life history traits of haddock *Melanogrammus aeglefinus*. Ices Journal of Marine Science 63:224-234.
Quinn, G. P., and M. J. Keough. 2002, Experimental design and data analysis for biologists. Cambridge, UK, Cambridge University Press.
R Development Core Team. 2016.R: A language and environment for statistical computing, Vienna, Austria.
Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357-359.
Rivest, B. R. 1983. Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia, Buccinidae). Journal of Experimental Marine Biology and Ecology 69:217-241.

Semmens, D., and S. E. Swearer. 2011. Extended incubation affects larval morphology, hatching success and starvation resistance in a terrestrially spawning fish, *Galaxias maculatus* (Jenyns 1842). Journal of Fish Biology 79:980-990.
Seymour, R. S., F. Geiser, and D. F. Bradford. 1991. Metabolic cost of development in terrestrial frog eggs (*Pseudophryne bibronii*). Physiological Zoology 64:688-696.
Sinervo, B. 1990. Experimental and comparative analyses of egg size in lizards: constraints on the adaptive evolution of maternal investment per offspring, Pages 725-734 *in* E. C. Dudley, ed., The Unity of Evolutionary Biology. Portland, OR, Dioscordies Press.
Sinervo, B., and L. R. McEdward. 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. Evolution 42:885-899.
Smith, C. C., and S. D. Fretwell. 1974. Optimal balance between size and number of offspring. American Naturalist 108:499-506.
Stearns, S. C. 1992, The Evolution of Life Histories. New York, Oxford University Press.
Svanfeldt, K., K. Monro, and D. J. Marshall. 2016. Dispersal duration mediates selection on offspring size. Oikos:n/a-n/a.
Uller, T., and M. Olsson. 2010. Offspring size and timing of hatching determine survival and reproductive output in a lizard. Oecologia 162:663-671.
Vance, R. R. 1973. On reproductive strategies in marine benthic invertebrates. American Naturalist 107:339-352.
Vandenhurk, R., and J. G. D. Lambert. 1983. Ovarian steroid glucuronides function as sex pheromones for male zebrafish, *Brachydanio rerio*. Canadian Journal of Zoology-Revue Canadienne De Zoologie 61:2381-2387.
Venable, D. L., and J. S. Brown. 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. American Naturalist 131:360-384.
Vilizzi, L. 1998. Age, growth and cohort composition of 0+ carp in the River Murray, Australia. Journal of Fish Biology 52:997-1013.
Vleck, C. M., and D. F. Hoyt. 1991. Metabolism and energetics of reptilian and avian embryos, Pages 285-306 *in* D. C. Deeming, and M. W. J. Ferguson, eds. Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles, Cambridge University Press.
Wendt, D. E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biological Bulletin 198:346-356.
Westerfield, M. 2000, The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). Eugene, University of Oregon Press.
White, C. R., M. R. Kearney, P. G. D. Matthews, S. A. L. M. Kooijman, and D. J. Marshall. 2011. A Manipulative Test of Competing Theories for Metabolic Scaling. American Naturalist 178:746-754.
Wieser, W., and C. Oberhauser. 1984. Ammonia production and oxygen consumption during the life cycle of *Porcellio scaber* (Isopoda, Crustacea). Pedobiologia 26:415-419.
Williams, J. B., and R. E. Ricklefs. 1984. Egg temperature and embryo metabolism in some high-latitude procellariiform birds. Physiological Zoology 57:118-127.
Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews of the Cambridge Philosophical Society 69:35-59.
Winemiller, K. O., and K. A. Rose. 1993. Why do most fish produce so many tiny offspring? American Naturalist 142:585-603.
Wootton, R. J., and C. Smith. 2014. Reproduction and life-history evolution, Pages 323-356 Reproductive Biology of Teleost Fishes, John Wiley & Sons, Ltd.

Tables

Table 1. Repeated measures analysis for the longitudinal study between Log_{10} Metabolic Rate and Log_{10} Embryo Mass and scaling exponents and coefficients (± CI) for metabolic rate and mass across developmental stages of *Danio rerio* throughout the dependent phase until hatching using a log-log transformed linear relationship, where Log_{10} Metabolic Rate = b x Log_{10} Embryo Mass + a. Developmental stage 1 = gastrula stage (6 h p.f.), stage 2 = prim-5 stage (24 h p.f.) and stage 3 = high-pec stage (44 h p.f.). (d.f. presented as num d.f., den d.f.).

Parameter	**d.f**	**F-ratio**	**P-value**	**Developmental Stage**	**Coefficient (a)**	**Scaling exponent (b)**	**P-value b > 0**	**P-value b < 1**
Between subjects								
Log_{10} Embryo Mass	1,72	4.76	<0.05	1	-0.52 (± 0.30)	0.32 (± 0.16)	<0.05	<0.001
Experiment	4,72	1.52	0.21	2	-0.14 (± 0.30)	0.32 (± 0.16)	<0.05	<0.001
Parent ID (Experiment)	5,72	0.57	0.73	3	-0.05 (± 0.27)	0.32 (± 0.16)	<0.05	<0.001
Log_{10} Embryo Mass x Experiment	4,68	0.50	0.73					
Within subjects								
Stage	2,144	3.37	<0.05					
Stage x Log_{10} Embryo Mass	8,144	1.93	0.15					
Stage x Experiment	8,144	5.25	<0.01					
Stage x Parent ID (Experiment)	10,144	1.31	0.23					
Stage x Experiment x Log_{10} Embryo Mass	8,136	0.09	1.00					

Table 2. General linear model for Hatchling Mass in relation to Embryo Mass and scaling exponents and coefficients (± CI) for the nonlinear relationship, where Hatchling mass = a x Embryo Mass^{b} + c x Parent ID (d.f. presented as num d.f., den d.f.).

Parameter	**d.f**	**F-ratio**	**P-value**	**Parameter**	**Estimate**	**P-value b > 1**
Log_{10} Embryo Mass	1,200	273.54	<0.001	a	0.23 (± 0.10)	
Parent ID	1,200	74.77	<0.001	b	1.30 (± 0.10)	<0.001
Parent ID x Log_{10} Embryo Mass	1,199	2.02	0.16	c	0.76 (± 0.10)	

Table 3. General linear model for Hatchling Yolk Area:Hatchling Area in relation to total Embryo Area and estimate of the slope (b) where Embryo Area = b x Hatchling Yolk Area:Hatchling Area + c. Wald tests were used to determine whether slopes were significantly different from both 0 and 1 (d.f. presented as num d.f., den d.f.).

Parameter	d.f	F-ratio	P-value	Estimate	P-value b > 0
Embryo Area	1,59	12.31	<0.005	0.73 (± 0.21)	<0.005

Figures

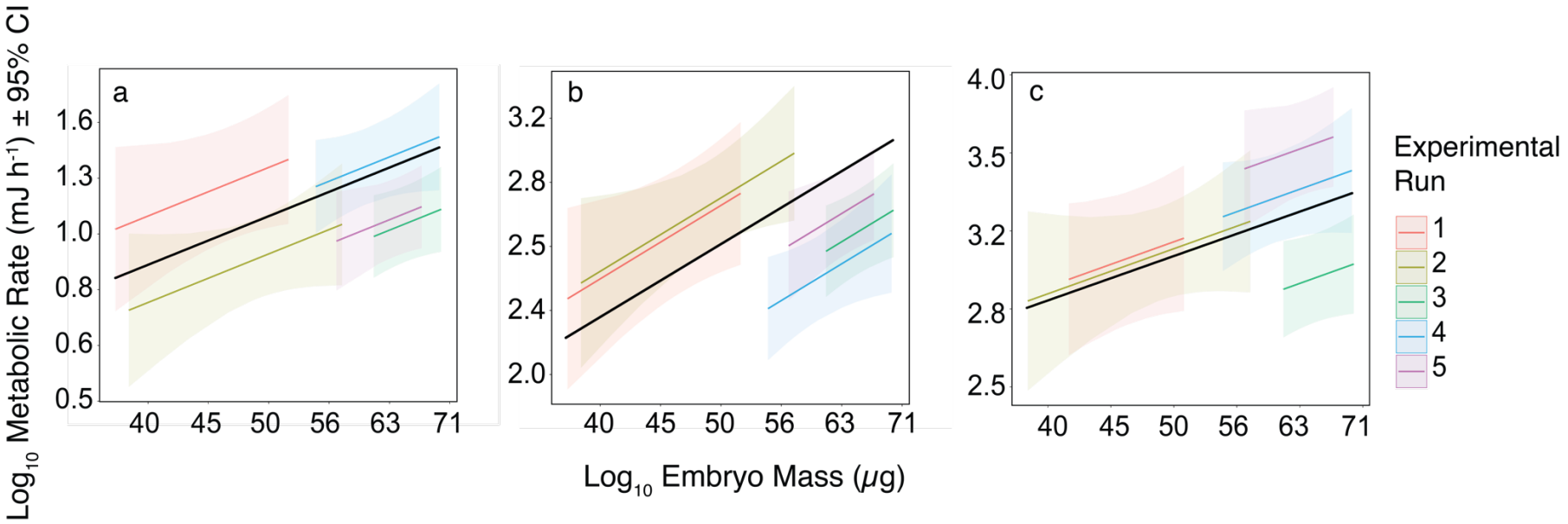


Figure 1. Mixed-effects model (± 95% confidence interval) for the relationship between Log_{10} Metabolic Rate (mJ h^{-1}) and projected embryo mass (Log_{10} Embryo Mass; µg) during *Danio rerio* developmental stages; (a) Stage 1; 6h p.f., (b) Stage 2; 24h p.f., (c) Stage 3; 44h p.f. Each coloured line represents an experimental run with a common slope and its own intercept per developmental stage. Bold line represents overall line of best fit. Shaded areas represent 95% confidence intervals for each experimental run. All axes labels log-untransformed.

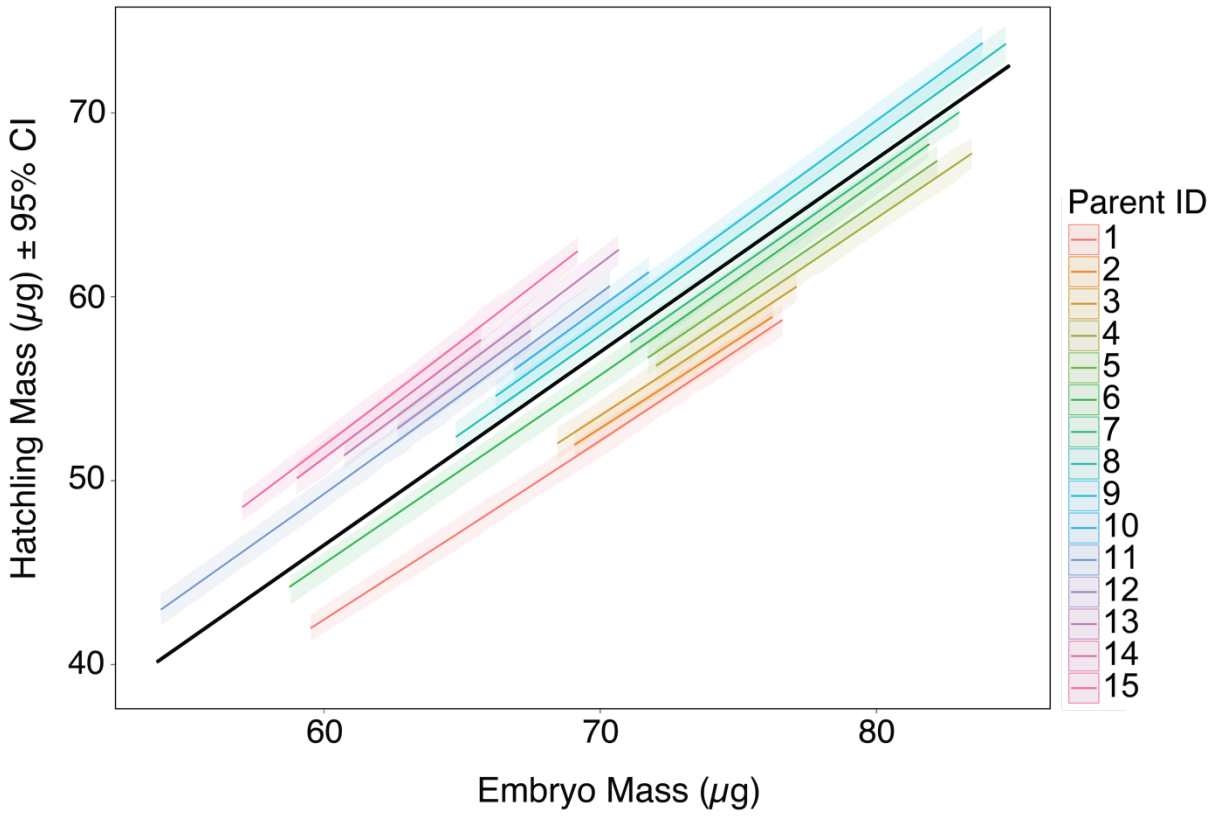


Figure 2. Multiple nonlinear regression (± 95% confidence interval) for the relationship between Hatchling Mass (µg) and projected embryo mass (Embryo Mass; µg) in *Danio rerio*. Each coloured line represents Parent ID with a common slope and its own intercept. Bold line represents overall line of best fit (Slope estimate: 1.23 ± 0.10). Shaded areas represent 95% confidence intervals for each Parent ID. Note: original data analyses were performed on $\log_{10}$ transformed data for Hatchling Mass and Embryo Mass, however we plot this relationship on arithmetic axes to aid with interpretation.

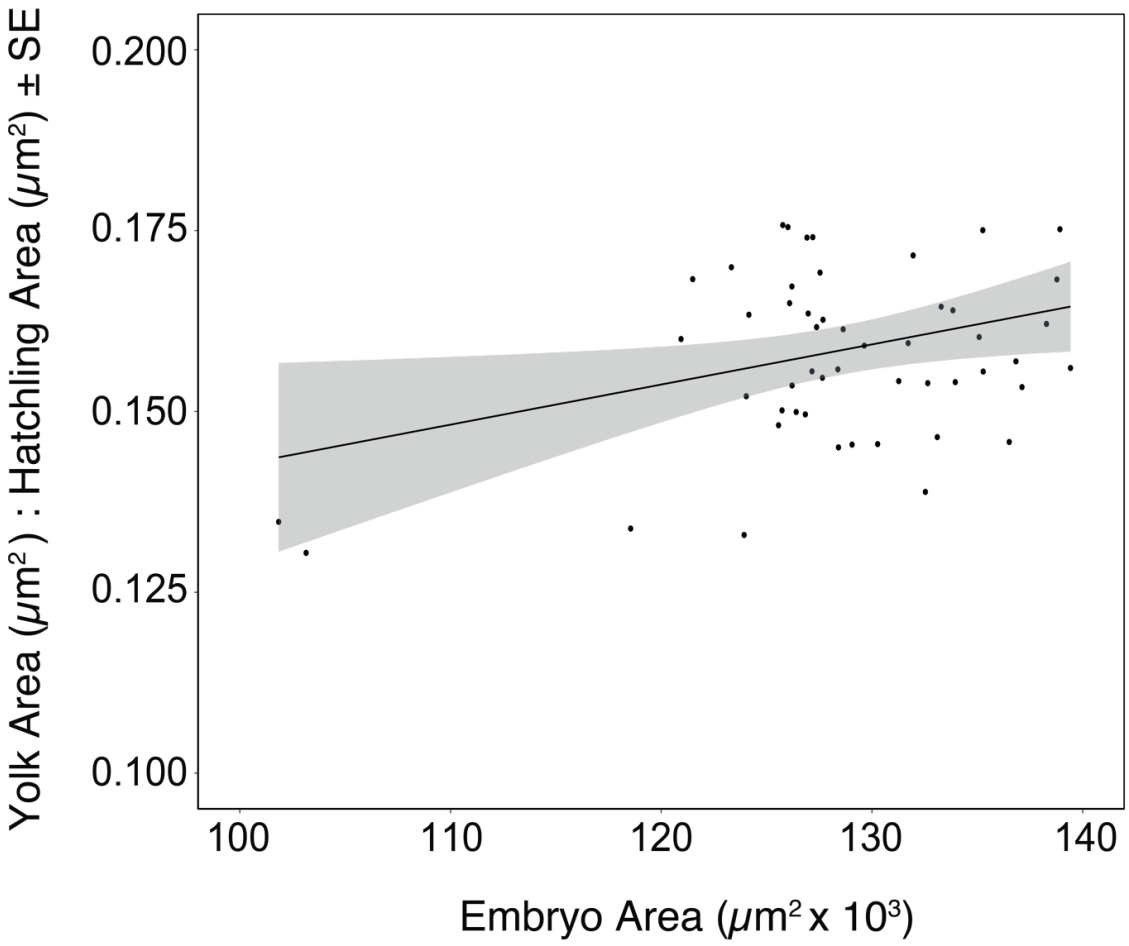


Figure 3. General linear model (± standard error) for the relationship between Yolk Area (μm^2):Hatchling Area (μm^2) relative to Embryo Area (μm^2) in *Danio rerio* (Slope estimate: 0.73 ± 0.21, R^2 adj. = 0.29). Dots represent raw data points. Shaded area represents standard error.

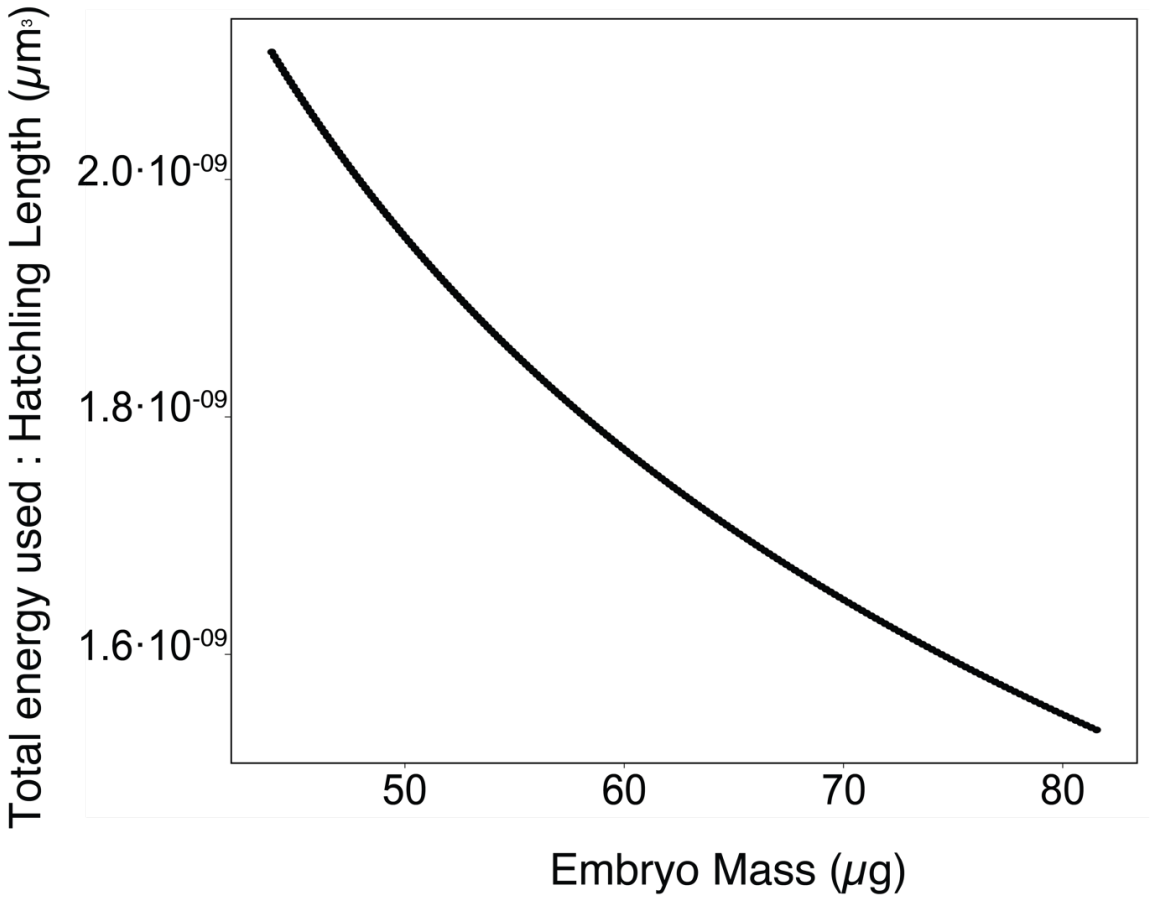


Figure 4. Predicted relationship between projected embryo mass (Embryo Mass; µg) and energy efficiency, calculated as the ratio of total energy used relative to Hatchling Mass (calculated as Hatchling Length; μm^3) in *Danio rerio*.

Appendix B

Materials and Methods

Relationship between embryo size and mass

In order to provide comparable scaling relationships between offspring size and metabolic rate, we used embryo mass as our measure of offspring size. Due to the destructive nature of weighing eggs, an indirect approach was used to determine embryo mass. *Danio rerio* embryos were harvested and volume ($V = \frac{4}{3} r^3$ where r is half the equatorial diameter of the 'sphere' stage) measured using the protocol outlined in the methods section of the main text (see '*Experimental overview, collection and measurement of embryo mass*'). To determine dry weight, the embryos were then transferred into pre-weighed aluminium foil cartridges and dried at 60°C for 48 h and weighed with a microbalance (Mettler Toledo XP2U) to the nearest 0.1µg as per Hachicho et al. (2015). To determine whether embryo volume provides a good proxy for embryo mass, and assuming a linear relationship, ordinary least squares analysis was used to find the strength of the correlation between embryo size and mass.

Relationship between embryo area and yolk area

To determine whether our findings that hatchlings from larger embryos retain with relatively higher energy reserves (see Results; '*Scaling of the ratio between yolk sac area and hatchling length with embryo area*') were as a direct result of allometric scaling with offspring size, rather than larger offspring possessing relatively higher metabolically inert yolk reserves, we measured a subsample of embryos (n = 30 for each of the 5 experimental runs in section b '*Scaling of metabolic rate and embryo mass'*). Individual embryos were photographed at the sphere stage as per main text ('*Experimental overview, collection and measurement of embryo mass'*). Due to the transparency of *Danio* embryos we were able to obtain precise measures of total embryo area and yolk area in μm^2 from these images. We found no effect of the random

effect of experimental run 'Experiment' on the relationship between Log_{10} Yolk Area and Log_{10} Embryo Area, hence it was removed, and a final linear model was run.

Results

Relationship between embryo size and mass

There was a significant correlation between embryo volume and embryo mass (ANOVA, $F_{1,94}$ = 32.755, P <0.001). We found no support for fitting an intercept to the relationship, so the relationship was best fit by the model, Embryo mass = Volume · 27.179 (where mass is in µg and volume is in µl). Embryo volume was found to be a good predictor of embryo mass (R^2 = 0.26). Parent pair identity had no effect on the relationship between embryo volume and mass (ANOVA, $F_{1,93}$ = 0.374, P = 0.542), nor did the interaction between parent pair identity and embryo volume (ANOVA, $F_{1,92}$ = 0.767, P = 0.384). Based on the equation between embryo diameter and mass, the calculated mass ranges were 37.4 µg – 69.6 µg and 43.9 µg - 74.7 µg for the metabolic rate and hatchling size experimental runs, respectively.

Relationship between embryo area and yolk area

The relationship between Log_{10} Yolk area and Log_{10} Embryo area was found to be isometric (ANOVA, $F_{1,60}$ = 380.6, P < 0.0001), where the scaling exponent was not significantly different from 1 (Estimate ± CI: 0.93 ± 0.09, p = 0.440).

Figures

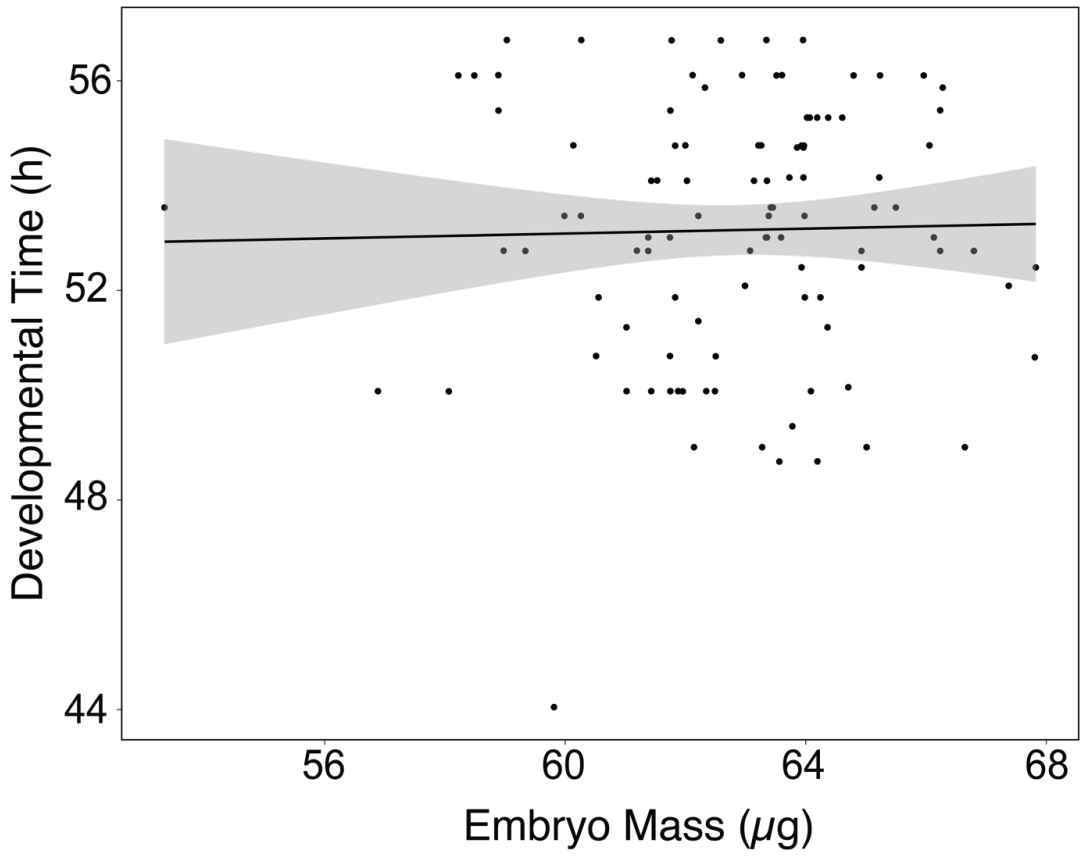


Figure B1. General linear model for the relationship between projected embryo mass (Embryo Mass; µg) and Developmental Time (h) in *Danio rerio* (± 95% confidence interval). Dots represent raw data points. Bold line represents overall line of best fit. Shaded area represents 95% confidence interval.

Chapter 3

Why do colder mothers produce larger offspring?

Amanda K. Pettersen[1*], Craig R. White[1], Robert J. Bryson-Richardson[2], Dustin J. Marshall[1]

[1] School of Biological Sciences/Centre for Geometric Biology, Monash University, Melbourne, Victoria, Australia

[2] School of Biological Sciences, Monash University, Melbourne, Victoria, Australia

Abstract

Temperature alters the size of offspring that mothers produce. Among and within species, mothers in colder environments produce larger offspring than mothers in warmer environments. Such patterns have been recognised for almost a century, but broadly applicable explanations for the relationship between temperature and offspring size are lacking, and the mechanistic drivers of these patterns remain unresolved. Here, we formally evaluated the ubiquity of the temperature-offspring size relationship in a meta-analysis, and found strong support for a negative relationship between temperature and offspring size across a variety of taxa. We then tested a potentially universal explanation for this relationship by linking life-history theory and metabolic theory. We measured the temperature-dependence of the costs of development as a function of development time and metabolic rate in an invertebrate, *Bugula neritina* and a vertebrate, *Danio rerio*. We found that both metabolic and developmental rates increase with temperature, but development rate is more temperature-sensitive than metabolic rate, such that the overall costs of development decrease with temperature. To determine whether this finding holds more generally, we then ran a second meta-analysis and show that development rate is more sensitive to temperature than metabolic rate across 52 species of ectotherms spanning four phyla. Thus, we find it generally more costly to develop in colder conditions than warmer conditions, and colder mothers may need to provision their offspring with higher energy reserves accordingly.

Introduction

The size of offspring when they begin life away from their parents is a key life-history trait that can determine both maternal and offspring fitness (Bernardo 1996). Larger offspring often exhibit higher survival and reproductive output, or lower susceptibility to predation and starvation (Einum and Fleming 2000; Hutchings 1991; Janzen et al. 2000; Marshall et al. 2006; Tessier and Consolatti 1989). The effects of offspring size can persist throughout ontogeny, and across generations (Marshall et al. 2003; Plaistow et al. 2006). The benefits of larger offspring sizes are offset by fecundity costs for the mother; given a fixed amount of reproductive resources, a mother can either produce a few large or many small offspring (Lack 1947; Smith and Fretwell 1974). Furthermore, the offspring size-performance relationship is highly context-dependent – for example, in low-stress environments smaller offspring may perform equally well or even better than larger offspring (Litvak and Leggett 1992; Reznick et al. 1996). Hence, because environmental variation can alter selection on offspring size, we often observe plasticity in offspring size in response to this variation (Fox 1997).

Offspring size covaries with environmental temperature across a remarkable range of taxa and systems; where temperatures are warmer, mothers produce smaller offspring (Fox and Czesak 2000; Marshall et al. 2012; Yampolsky and Scheiner 1996). Among species, offspring size covaries strongly with temperature in both terrestrial and aquatic systems (Blanckenhorn 2000; Chown and Gaston 1999; Laptikhovsky 2006; Marshall et al. 2012). Within species, the same patterns occur across both time and space – mothers increase the size of their offspring in cooler seasons, and at higher latitudes (Barnes and Barnes 1965; Harvey 1983; Kerfoot 1974; Wootton and Smith 2014). Experimental manipulations of temperature show the same effect (see Atkinson et al. 2001, and Figure 1 here for a formal meta-analysis). While covariation between offspring size and temperature (here we will use the acronym OST) is ubiquitous in

ectotherms (for exceptions, see Baur and Raboud (1988); Fleming and Gross (1990)), the drivers of this relationship are unclear.

Several theoretical considerations of OST relationships imply such patterns are merely a physiological by-product of the thermodynamics of development (Sinervo and Licht 1991; van der Have and de Jong 1996). Nevertheless, the presence of a physiological constraint does not preclude an adaptive underpinning for OST. Several lines of evidence suggest that offspring size responses to the effects of temperature are maintained by selection (Partridge and Coyne 1997, see review by Yampolsky and Scheiner 1996). For example, transgenerational plasticity experiments demonstrate that temperature-mediated changes in offspring size are adaptive (Bownds et al. 2010; Burgess and Marshall 2011). Similarly, experimental evolution studies in insects show an adaptive change in offspring size in response to different temperature regimes – larger eggs from mothers reared at cooler temperatures had higher hatching success and were more likely to produce larger hatchlings with higher survival compared with smaller eggs from the same mothers (Blanckenhorn 2000; Fischer et al. 2003).

Broadly applicable adaptive explanations for why offspring size co-varies with temperature are lacking (Fox and Czesak 2000). Here we consider one largely overlooked but potentially universal explanation for why mothers might produce larger offspring in cooler temperatures: the differential costs of development to nutritional independence under different temperatures. In a recent paper discussing the relationship between temperature and adult body size, Zuo et al. (2012) speculate that if metabolic rates are less sensitive to temperature than developmental rates, then the total costs of development in ectotherms should decrease with temperature. Under this scenario, higher temperatures will increase metabolic rates, increasing the energy used by developing offspring per unit time. However, as developmental rates will increase faster than metabolic rates, embryos will spend much less time undergoing costly development such that total energy use declines with increasing temperatures (see schematic,

Figure 2). If this theory holds true more generally, it could provide a broad explanation for why colder mothers produce larger offspring. Below we develop a simple model that links life-history theory with metabolic theory.

Following foundational theory by Vance (1973), if we assume that an important function of maternal investment (and hence offspring size) is to provide offspring with the resources necessary to reach a developmental stage where they can feed for themselves (what we will call nutritional independence), and that offspring size is positively correlated with energetic status, then any factor that affects the costs of reaching independence should alter selection on offspring size (Pettersen et al. 2017). If we assume that offspring size (*OS*) is shaped in part by the cost of reaching nutritional independence (*C*) then the minimum offspring size will scale with the cost of development [1],

[1] $$OS_{\min} \propto C$$

and mothers must therefore produce larger offspring when *C* increases.

From an energy perspective, *C* is simply a product of both the time spent in the dependent phase, defined by total development time (*D*), which is inversely proportional to developmental rate ($D = 1/DR$), and the rate of energy expenditure during the dependent phase, metabolic rate (*MR*). How *C* scales with temperature will depend on the relative temperature sensitivities of *D* and *MR* [2].

[2] $$C_{(T)} = D_{(T)} \times MR_{(T)}$$

The relationship between temperature and the rate of these processes can be described by an exponential function as per Gillooly et al. (2001); Gillooly et al. (2002), where *a* and *b* represent the temperature dependence of *D* and *MR*, ∂ and γ are coefficients, α and β are the offspring mass scaling exponents for $D_{(T)}$ and $MR_{(T)}$ respectively, and *T* is absolute temperature. $C_{(T)}$ can therefore be described by the temperature sensitivity of development time [3],

[3] $$D_{(T)} = \partial \cdot e^{(-aD \cdot T)} \cdot (\text{Offspring mass}^{\alpha})$$

and metabolic rate [4],

[4] $$MR_{(T)} = \gamma \cdot e^{(bMR \cdot T)} \cdot (\text{Offspring mass}^{\beta})$$

Both development time and metabolic rate are highly temperature-dependent, and both are also affected by offspring size, albeit in complex ways (Clarke and Fraser 2004; Gillooly et al. 2002; Marshall and Keough 2008; Pettersen et al. 2015). Unless development time and metabolic rate have the same temperature dependence ($a = b$), then the costs of development must change with temperature. If increases in temperature increase development rate more than they increase metabolic rate (i.e. $a > b$), then we would predict that the overall costs of development to independence decrease with increasing temperature. Based on classic optimality theory, we would therefore expect warmer temperatures to result in decreases in offspring size because less energy is required for offspring to reach independence. Alternatively, if metabolic rate is more sensitive to temperature than development rate (i.e. $b > a$), the reverse would be expected: increases in temperature will increase the costs of development and mothers should produce larger offspring in warmer temperatures.

Despite the potential for a metabolic life-history theory to provide a broad explanation for why warmer mothers produce smaller offspring, we are not aware of any specific attempts to estimate the temperature dependence of metabolic rate and development rate simultaneously for the same species. Here we took four steps: **1)** We performed a phylogenetically-controlled meta-analysis to determine the relationship between the temperature that mothers experience, and the size of their offspring within 33 species across six phyla. **2)** We then experimentally manipulated temperature to examine the relative temperature dependencies of developmental rates and metabolic rates during the dependent phase in two model systems, *Bugula neritina* and *Danio rerio,* throughout metamorphosis and embryogenesis respectively. Importantly, in both species, warmer mothers produce smaller offspring, and this relationship appears to be adaptive – larger offspring showed higher survival in cooler environments than smaller

offspring (Bownds et al. 2010; Burgess and Marshall 2011). **3)** We then fit exponential equations to predict the total costs of development across offspring size and temperature. We found that development rates are indeed more sensitive to temperature than metabolic rates in both species, and therefore find strong support for the intuitions of Zuo et al. (2012), and the predictions of our model. **4)** In order to test whether our model is likely to apply more generally, we then combed the literature to compile estimates of the temperature dependence of development rate and metabolic rate during development for a wide range of ectotherm species while controlling for phylogeny. We found that the temperature dependence of development rate is generally higher than the temperature dependence of metabolic rate (however there were exceptions), thus the increased costs of development at cooler temperatures may provide an adaptive mechanism for the ubiquitous influence of temperature on offspring size.

Materials and methods

1. Meta-analysis of temperature and offspring size

The methods used to produce a phylogenetically-controlled meta-analysis were followed as per the guidelines presented in the 'Preferred Reporting Items for Systematic Reviews and Met-Analyses' (PRISMA) statement (Nakagawa and Poulin 2012) – for details see Appendix C. We only included empirical studies on ectotherms where the mother had been reared under different experimentally-controlled temperature treatments in order to avoid confounding effects of variation in maternal condition, see Table C1. For each study, effect size for mean offspring/egg/larval diameter or length with temperature was calculated using Cohen's D: the difference in offspring size between the greatest temperature differential measured was divided by standard deviation. A phylogenetic tree was then 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 offspring size with temperature (ΔT) due to the shared evolutionary history among the study species sampled, relative to that independent of phylogeny. We then fit a mixed-effects (fixed slope, random intercepts) model including ΔT 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 effect size (Cohen's D) of temperature on offspring size. Likelihood ratio tests were then 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. This proportion of variance attributable to phylogeny is equivalent to the more widely-used metric of Pagel's lambda (λ) - the proportion of variance associated with the random effect of phylogeny (Hadfield and Nakagawa 2010).

2. *Empirical estimates of costs of development with temperature*

Study species

In a series of experiments, offspring from two species, a marine invertebrate, *Bugula neritina* (Bryozoa: Cheilostomata) and a freshwater vertebrate, *Danio rerio* (Chordata: Cypriniformes) were used to measure the temperature sensitivity of development time and metabolic rate (hereon referred to by genus). While offspring of *Bugula* and *Danio* exhibit very different life histories, they both possess a 'dependent phase' where early development is characterised by complete reliance on maternal energy investment until feeding structures are formed. Both species reproduce across a range of naturally varying temperatures, where mothers exposed to higher temperatures produce smaller offspring – and these shifts appears to be adaptive (Bownds et al. 2010; Burgess and Marshall 2011). Recent studies have shown the costs of development to be substantial in these species with a decline of up to 47% of initial energy reserves in *Bugula* and 23% of dry weight in *Danio* (Hachicho et al. 2015; Pettersen et al. 2015). Thus, factors which exacerbate energy costs throughout the dependent phase are likely to pose important fitness consequences.

Bugula is an arborescent bryozoan with global distribution, colonising sheltered, subtidal structures. Reproductively mature colonies undergo internal fertilisation and brood single larvae on individual maternal zooids. Settlers then undergo metamorphosis over approximately three days, throughout this time, offspring are entirely dependent on maternally-derived energy provisions. The completion of metamorphosis and the development of a feeding structure (the lophophore) thus represents the end of the 'dependent' phase where offspring commence feeding and are able to obtain external energy from the environment (Wendt 2000).

Danio is a commonly used laboratory model organism that naturally occupies slow-moving, shallow water bodies in the Indian subcontinent (Spence et al. 2008). *Danio* reproduces sexually by spawning gametes into the water column where fertilised eggs undergo

several stages of development. During this dependent phase, embryos do not uptake external nutrients, but rely exclusively on yolk supplies in the egg to meet energy requirements for growth and development until post-hatching, where feeding structures form after approximately one week post-fertilisation under normal laboratory conditions (Bryson-Richardson et al. 2011; Hachicho et al. 2015; Kimmel et al. 1995).

Offspring sampling and size measurements

Offspring size may produce either temperature-dependent or -independent effects on development time and metabolic rate, therefore the effect of offspring size was included in our estimates. Due to the destructive sampling of embryo mass, we used double sampling: one set of samples were used for measures of metabolic rate or developmental rate (outlined below) and the other to obtain estimates of offspring mass. Larvae and eggs were spawned and measured in *Bugula* and *Danio* respectively, using standard techniques (for further detail, see Appendix C).

a) Development rate under different temperature regimes

To determine the effect of environmental temperature on development time in *Bugula* and *Danio*, 96 larvae and 144 embryos were placed into each of four temperature treatments respectively, such that a total of 384 larvae and 576 embryos (over six experimental runs) were used. Treatments were representative of natural temperature ranges experienced by these species (Bownds et al. 2010; Scott and Johnston 2012), and parents were acclimated at temperatures within these ranges (18°C ±2°C in *Bugula* and 28°C ±1°C in *Danio*). *Bugula* larvae were maintained at one of: 12°C, 16°C, 20°C and 24°C (for details, see Appendix C). *Danio* embryos were reared at 20°C, 24°C, 28°C and 32°C. For *Danio,* feeding commences approximately two days post-hatching (at 28˚C), but because development of feeding structures is difficult to confirm noninvasively, time to hatching of larvae was used as a proxy for the end of the dependent phase, and was monitored using a time-lapse (Olympus 1X73; x10, Olympus

cellSens Dimension software) where individuals were photographed every 0.5h. Importantly, there is no evidence to suggest that the post-hatching/pre-feeding phase within either species shows differential temperature dependence to earlier phases.

b) Metabolic rate under different temperature regimes

Fluorescence-based oxygen measurements were taken throughout development of the 'dependent' phase to determine rate of oxygen consumption, or $\dot{V}O_2$, as a commonly used proxy for metabolic rate as per standard techniques by Pettersen et al. (2015). $\dot{V}O_2$ was measured at 24 h intervals (i.e. 'Time') from six hours post-settlement/post-fertilisation in *Bugula* and *Danio* respectively, throughout the dependent phase. For further details, see Appendix C.

3. Total energy expenditure across offspring size and temperature

The $MR_{(T)}$ estimates obtained from each nonlinear regression for each Time were then multiplied by the duration spent at each time (e.g. 24 h for Time 1 and where total time was calculated from the predicted $D_{(T)}$ and combined to calculate total cost of development ($C_{(T)}$) across the entire range of temperatures and offspring sizes measured, where $C_{(T)} = D_{(T)} \times MR_{(T)}$.

4. *Meta-analysis of temperature, development time and metabolic rate in other taxa*

To determine whether the total costs of development decrease with temperature more generally, we compiled data on $D_{(T)}$ and $MR_{(T)}$ from previous studies of 52 species and analysed these data using a phylogenetically-controlled meta-analysis. Due to the paucity of data on these rates under varying temperature regimes, we could not rely on search terms as per the previous meta-analysis (Appendix C methods), rather we combed the literature haphazardly using *ISI Web of Science* using a range of search terms and following relevant citations. We collated mean values of $MR_{(T)}$ and $D_{(T)}$, as well as natural temperature ranges ('T range') experienced by each species during the early life history when offspring are non-feeding and entirely dependent on maternally derived energy reserves (i.e. throughout the 'dependent

phase'). Where T range was not specified for the studies that measured $MR_{(T)}$ and $DT_{(T)}$, we searched the literature for studies that reported temperature ranges in the wild for each species in similar locations. Total costs of development were then calculated by multiplying $D_{(T)}$ and $MR_{(T)}$ for each experimental temperature treatment. Most studies did not report sample size or error associated with measurements, therefore we used generalised least squares using the "gls" function within the "nlme" R package (Pinheiro et al. 2011) to determine whether sample size (for those studies that did report sample size) was related to a higher precision of estimates. This approach has been suggested by Nakagawa and Lagisz (2016) for use in meta-analyses which only measure the absolute magnitude (i.e. mean) of the effect size on the response variable (i.e. effect of temperature on mean *D* and *MR*). We used phylogenetic generalised least squares to fit models where residuals are correlated (i.e. correlation structure taken from phylogeny) using the "pgls" function in the R package "caper" (Orme 2013). We then calculated an estimate for the slope of a 10% change in temperature from each species natural mean temperature, on the change in costs of development, 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 (sample size) did not affect the magnitude of the relationship (χ^2 = 0.148, P-value = 1), therefore we used a phylogenetically-controlled approach as used above in Part 1 (*Meta-analysis of temperature and offspring size*). We calculated the effect of the phylogenetic signal on the relationship between temperature and the total costs of development using a correlation matrix (see detailed methods above). The effect size for the costs of development ($\Delta C_{(T)} = (\Delta\ D_{(T)}$ x $MR_{(T)})$) was calculated using Cohen's D and a phylogenetic tree was then constructed. We again fit a mixed-effects model to test the fixed effect of ΔT and the random effect of the relatedness matrix from the phylogeny, on Δ*C* over a 10% change in mean temperature.

Results

1. *Meta-analysis of temperature and offspring size*

For 27 of the 33 species, offspring size decreased with increases in rearing temperature (Figure 1: coefficient = -0.232 ± 0.047, $F_{1,47}$ = 23.950, p <0.0001), and the proportion of variance attributable to phylogeny (conditioned on the fixed effects) was not significantly different from 0 (χ^2= $8.772 \cdot 10^{-6}$, p = 1).

2. *Total energy expenditure across offspring size and temperature*

a) *Development rate*

The temperature at which early-stage *Bugula* and *Danio* were reared significantly affected development time through the dependent phase – across the temperatures tested, development time decreased with temperature (Figure 3). For both species there was a main effect of Temperature (*Bugula;* $F_{1,275}$ = 1172.378, p < 0.0001, *Danio*; $F_{1,547}$ = 8811.577, p < 0.0001) but no effect of ln(Offspring Mass), hence α and β = 0. The relationship between development time and temperature; $DR_{(T)}$ for *Bugula* was therefore best described by the exponential function,

$D_{(T)Bugula} = 6054 \cdot e^{(-0.363 \cdot T)} \cdot (\text{Offspring mass}^0) + 32.41$

For *Danio*, the random effect of Experiment was significant (χ^2 = 7.108, p < 0.05), however its interactions with Temperature and ln(Offspring Mass) were not, therefore the slopes among experimental runs were not significantly different and Experiment could be excluded from the final model. The temperature dependence of development time for *Danio* was described by,

$D_{(T)Danio} = 12340 \cdot e^{(-0.224 \cdot T)} \cdot (\text{Offspring mass}^0) + 35.27$

b) *Metabolic rate*

We found a significant, positive relationship between Temperature and ln(MR) throughout the dependent phase across all time stages for both species (Figure 4). The random effect of Run

had a significant effect on ln(MR) for both species across almost all times measured (Table C4). While there was considerable variation in the intercept between ln(Offspring mass) and ln(MR) among runs for each temperature, the interaction between these was either non-significant or inconsistent among runs within each Time (see rationale in Methods) and hence, we found no support for fitting a random-slopes model overall. For each time, a single model was fit for the relationship between metabolic rate, mass and temperature (Table 1).

3. *Total energy consumption*

We found overall that total energy expenditure as a product of $D_{(T)}$ and $MR_{(T)}$ was inversely related to temperature for both *Bugula* and *Danio* offspring (Figure 5). Due to a relatively higher sensitivity of development time ($D_{(T)}$) relative to metabolic rate ($MR_{(T)}$), the costs of development are highest at coolest temperatures tested. For *Danio*, the costs of development decreased linearly over the temperatures tested (20°C – 32°C). However, for *Bugula,* while the cost of development decreased over 12°C - 20°C, we found that the costs increased slightly at 24°C (Table 2).

4. *Meta-analysis of temperature, development time and metabolic rate in other taxa*

We found that, in line with our empirical estimates for *Bugula* and *Danio*, at cooler temperatures the costs of development increased for the majority of the 52 species tested, where the intercept of the relationship was significantly >0 (Estimate = 0.037 ± 0.006; $p < 0.0001$). Phylogeny explained less than 0.01% of the variance between costs of development and temperature ($\lambda = 1.012 \cdot 10^{-7} \pm 5.737 \cdot 10^{-12}$; $\chi^2 = 0$, $p = 1$), therefore the final analysis was run as a linear model. We also found a quantitative relationship between the relative change in temperature (ΔT) and the relative costs of development (ΔT) – where the slope was significantly >0 (Estimate = 0.010 ± 0.003, $p < 0.0001$). We found that temperature sensitivity in the costs of development reflected natural temperature ranges. Species that experience more

narrow temperature ranges showed a greater increase in the costs of development with a 10% decrease in temperature, compared to species with a naturally-occurring, wide temperature range (Estimate = -0.001 ± 0.004, $p = 0.012$).

Discussion

Costs of development as an explanation for the offspring size-temperature relationship

Offspring size-temperature relationships are ubiquitous – we provide a potentially broad explanation for them (Atkinson et al. 2001; Marshall et al. 2012; Thorson 1936). Building on proximal physiological mechanisms suggested by Zuo et al. (2012), we propose an ultimate cause for the offspring size-temperature relationship – the temperature-dependent costs of development. Previous studies across a range of species have speculated that the costs of development decrease with temperature (Angilletta et al. 2000; Booth and Thompson 1991; DuRant et al. 2011; Gutzke et al. 1987; Irlich et al. 2009), and the results of our study confirm this speculation. We show empirically that for two (very different) species in which the temperature-offspring size relationship has been shown to be adaptive, *Bugula neritina* and *Danio rerio*, development rate is more temperature sensitive than metabolic rate ($a_{DR} > b_{MR}$), such that the overall costs of development decrease with temperature. For example, a temperature change from 24°C to 12°C increased the costs of development in *Bugula* by ~33%. We also find that this pattern applies more broadly – for a range of species across four phyla, the costs of development are higher at cooler temperatures. In the moss froglet *Crinia nimbus* for example, a 10°C decline in incubation temperature is predicted to incur a 41% increase in the costs of development. The magnitude of the effect of decreasing temperature on the costs of development depended on the species natural temperature range. Species that naturally experience narrow temperature fluctuations in their environment showed a greater increase in costs of development with a 10% decrease in temperature, compared to species with a naturally wider temperature range. If minimum offspring size (OS_{min}) must at least provide for the costs of completing development (which seems inevitable), then our results could serve as a general explanation for why colder mothers produce larger offspring (Vance 1973).

Mechanisms driving the temperature-dependence of the costs of development

While the effect of temperature on the costs of development may serve to explain the offspring size-temperature relationship, the underlying biophysical mechanisms driving this relationship are less clear. In line with our own conclusions, Zuo, *et al.* (2012) invoke lower activation energies of growth (biomass accumulation) rate relative to development (cell differentiation) rate, to explain why body size at maturity is smaller in warmer temperatures (i.e. the temperature-size rule; TSR). A simple reason for why development is more temperature sensitive than metabolism may be physical. Development and metamorphosis requires the division and differentiation of cells – there is some evidence that cell cleavage is extremely temperature-dependent because cell protoplasm viscosity mediates cell cleavage speeds (Marsland 1950). In a study on sea urchin eggs, Costello (1934) found an exponential decrease in cell viscosity with temperature. Furthermore, earlier studies in this species found that the Q_{10} for viscosity was higher than the temperature coefficient for oxygen consumption – but only at the lower temperature ranges tested (Loeb and Chamberlain 1915; Loeb and Wasteneys 1911). With increases in temperature, viscosity decreased (and therefore development rate increased) more rapidly than the rate of oxygen consumption (metabolic rate). In contrast, over the higher temperature range measured, the reverse was observed – with increases in temperature, the rate of oxygen consumption increased relatively higher than that of cell cleavage, which reflects our predictions for the costs of development at extreme high temperatures (see discussion below). Alternatively, the differential thermal sensitives of metabolic rate and development rate could be a consequence of the thermal sensitivities of the enzymes that underlie them. Within the universal temperature dependence (UTD) framework, activation energies between 0.2 and 1.2 eV are predicted for metabolic rate and development time, based on the average temperature sensitivity of biochemical pathways relevant to plants and animals (Gillooly et al. 2001; Gillooly et al. 2002). While the thermal sensitivity of enzymes involved in protein synthesis could be driving the temperature dependence of

development rate relative to metabolic rate, a direct comparison of the enzyme kinetics underlying these processes is needed (Clarke and Fraser 2004).

Alternative explanations for the relationship between offspring size and temperature

Our explanation of why warmer mothers reduce offspring size does not preclude other adaptive explanations. Oxygen diffusion during incubation can pose physical constraints on offspring size (Fleming and Gross 1990; Lee and Strathmann 1998; Seymour and Bradford 1995), however the size-dependent fitness consequences of oxygen limitation have shown mixed results (Einum et al. 2002; Woods 1999). While we did not detect any temperature-dependent mortality during development, oxygen limitation could be driving these effects for temperatures outside those of a species natural range. In a recent model, Martin et al. (2017) showed that laboratory-based measures of oxygen limited thermal stress in a range of fish species consistently underestimated field-based estimates of larval mortality. If size-dependent oxygen limitation is exacerbated at higher temperatures such that larger eggs are more sensitive to increases in developmental temperature, then this may also drive the offspring size-temperature relationship. Alternatively, because offspring size can be constrained by maternal body size, the offspring size-temperature relationship may be merely a consequence of TSR (Congdon and Gibbons 1987). If offspring size is constrained by maternal body size, then TSR may enable mothers to increase the size of their offspring in response to selection in colder environments. While this is a plausible explanation for field-based studies, laboratory manipulations of maternal rearing temperature can control for maternal size, and is therefore unlikely to serve as a general explanation for the offspring size-temperature relationship. It is feasible that the proximal constraints discussed here may not be mutually exclusive explanations, but working complimentarily with the costs of development with temperature to drive the offspring size-temperature relationship.

Conclusions

While studies measuring relative temperature dependencies of physiological processes (eg. growth and development) have largely been focused on adults as a possible mechanism for TSR (Atkinson 1994; Zuo et al. 2012), the implications of increased energy costs are likely to be even more profound during development because offspring are non-feeding. Offspring and maternal fitness during this vulnerable life history-stage rely largely on maternal provisioning (i.e. OS_{min}). The costs of development with temperature provides an adaptive underpinning which may serve as a universal explanation of macroevolutionary patterns in maternal investment, and hence offspring size.

References

Akbar, S. M., T. Pavani, T. Nagaraja, and H. C. Sharma. 2016. Influence of CO2 and Temperature on Metabolism and Development of Helicoverpa armigera (Noctuidae: Lepidoptera). Environmental Entomology 45:229-236.

Angilletta, M. J., R. S. Winters, and A. E. Dunham. 2000. Thermal effects on the energetics of lizard embryos: Implications for hatchling phenotypes. Ecology 81:2957-2968.

Atkinson, D. 1994. Temperature and organism size - A biological law for ectotherms. Advances in Ecological Research, Vol 25 25:1-58.

Atkinson, D., S. A. Morley, D. Wheetman, and R. N. Hughes. 2001. Offspring size responses to maternal temperature in ectotherms. Environment and animal development:269-285.

Barnes, H., and M. Barnes. 1965. Egg size, nauplius size, and their variation with local, geographical, and specific factors in some common cirripedes. Journal of Animal Ecology 34:391-402.

Baur, B., and C. Raboud. 1988. Life-history of the land snail *Arianta arbustorum* along an altitudinal gradient. Journal of Animal Ecology 57:71-87.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: Patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Blanckenhorn, W. U. 2000. Temperature effects on egg size and their fitness consequences in the yellow dung fly *Scathophaga stercoraria*. Evolutionary Ecology 14:627-643.

Booth, D. T. 1987. Effect of temperature on development of mallee fowl *Leipoa ocellata* eggs. Physiological Zoology 60:437-445.

Booth, D. T., and M. B. Thompson. 1991. A comparison of reptilian eggs with those of megapode birds, Pages 325-344 *in* D. C. Deeming, and G. W. Ferguson, eds. Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles, Cambridge University Press.

Bownds, C., R. Wilson, and D. J. Marshall. 2010. Why do colder mothers produce larger eggs? An optimality approach. Journal of Experimental Biology 213:3796-3801.

Bryson-Richardson, R., S. Berger, and P. Currie. 2011, Atlas of Zebrafish Development, Academic Press.

Burgess, S. C., and D. J. Marshall. 2011. Temperature-induced maternal effects and environmental predictability. Journal of Experimental Biology 214:2329-2336.

Caamal-Monsreal, C., I. Uriarte, A. Farias, F. Diaz, A. Sanchez, D. Re, and C. Rosas. 2016. Effects of temperature on embryo development and metabolism of *O. maya*. Aquaculture 451:156-162.

Chown, S. L., and K. J. Gaston. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. Biological Reviews of the Cambridge Philosophical Society 74:87-120.

Clarke, A., and K. P. P. Fraser. 2004. Why does metabolism scale with temperature? Functional Ecology 18:243-251.

Congdon, J. D., and J. W. Gibbons. 1987. Morphological constraint on egg size: a challenge to optimal egg size theory? Proceedings of the National Academy of Sciences of the United States of America 84:4145-4147.

Costello, D. P. 1934. The effects of temperature on the viscosity of Arbacia egg protoplasm. Journal of Cellular and Comparative Physiology 4:421-433.

DuRant, S. E., W. A. Hopkins, and G. R. Hepp. 2011. Embryonic Developmental Patterns and Energy Expenditure Are Affected by Incubation Temperature in Wood Ducks (Aix sponsa). Physiological and Biochemical Zoology 84:451-457.

Einum, S., and I. A. Fleming. 2000. Selection against late emergence and small offspring in Atlantic salmon (Salmo salar). Evolution 54:628-639.

Einum, S., A. P. Hendry, and I. A. Fleming. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B-Biological Sciences 269:2325-2330.

Fischer, K., P. M. Brakefield, and B. J. Zwaan. 2003. Plasticity in butterfly egg size: Why larger offspring at lower temperatures? Ecology 84:3138-3147.

Fleming, I. A., and M. R. Gross. 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology 71:1-11.

Fox, C. W. 1997. Egg-size manipulations in the seed beetle Stator limbatus: consequences for progeny growth. Canadian Journal of Zoology-Revue Canadienne De Zoologie 75:1465-1473.

Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. Annual Review of Entomology 45:341-369.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248-2251.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, and J. H. Brown. 2002. Effects of size and temperature on developmental time. Nature 417:70-73.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml User Guide Release 3.0. Hemel Hempstead, HP1 1ES, UK, VSN International Ltd.

Gophen, M. 1976. Temperature effect on lifespan, metabolism, and development time of *Mesocyclops leuckarti* (Claus). Oecologia 25:271-277.

Gutzke, W. H. N., G. C. Packard, M. J. Packard, and T. J. Boardman. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). Herpetologica 43:393-404.

Hachicho, N., S. Reithel, A. Miltner, H. J. Heipieper, E. Kuester, and T. Luckenbach. 2015. Body mass parameters, lipid profiles and protein contents of zebrafish embryos and effects of 2,4-dinitrophenol exposure. Plos One 10.

Hadfield, J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software 33:1-22.

Hadfield, J. D., and S. Nakagawa. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. Journal of Evolutionary Biology 23:494-508.

Harvey, G. T. 1983. A geographical cline in egg weights in *Choristoneura fumiferana* (Lepidoptera: Tortricidae) and its significance in population dynamics. The Canadian Entomologist 115:1103-1108.

Hinchliff, C. E., S. A. Smith, J. F. Allman, J. G. Burleigh, R. Chaudhary, L. M. Coghill, K. A. Crandall et al. 2015. Synthesis of phylogeny and taxonomy into a comprehensive tree of life. Proceedings of the National Academy of Sciences 112:12764-12769.

Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salelinus fontinalis*. Evolution 45:1162-1168.

Irlich, U. M., J. S. Terblanche, T. M. Blackburn, and S. L. Chown. 2009. Insect Rate-Temperature Relationships: Environmental Variation and the Metabolic Theory of Ecology. American Naturalist 174:819-835.

Janzen, F. J., J. F. Tucker, and G. L. Paukstis. 2000. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. Journal of Evolutionary Biology 13:947-954.
Kerfoot, W. C. 1974. Egg-cycle of a cladoceran. Ecology 55:1259-1270.
Kimmel, C. B., W. W. Ballard, S. R. Kimmel, B. Ullmann, and T. F. Schilling. 1995. Stages of embryonic development of the zebrafish. Developmental Dynamics 203:253-310.
Lack, D. 1947. The significance of clutch size. Ibis 89:302-352.
Laptikhovsky, V. 2006. Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. Marine Ecology 27:7-14.
Lee, C. E., and R. R. Strathmann. 1998. Scaling of gelatinous clutches: Effects of siblings' competition for oxygen on clutch size and parental investment per offspring. American Naturalist 151:293-310.
Litvak, M. K., and W. C. Leggett. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Marine Ecology Progress Series 81:13-24.
Loeb, J., and M. M. Chamberlain. 1915. An attempt at a physico-chemical explanation of certain groups of fluctuating variation. Journal of Experimental Zoology 19:559-568.
Loeb, J., and H. Wasteneys. 1911. Sind die Oxidationsvorgänge die unabhängige Variable in den Lebenserscheinungen? Biochem. Zeit. 36:345.
Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131-3137.
Marshall, D. J., C. N. Cook, and R. B. Emlet. 2006. Offspring size effects mediate competitive interactions in a colonial marine invertebrate. Ecology 87:214-225.
Marshall, D. J., and M. J. Keough. 2008. The evolutionary ecology of offspring size in marine invertebrates, Pages 1-60 *in* D. W. Sims, ed., Advances in Marine Biology. Advances in Marine Biology. United Kingdom.
Marshall, D. J., P. J. Krug, E. K. Kupriyanova, M. Byrne, and R. B. Emlet. 2012. The Biogeography of Marine Invertebrate Life Histories. Annual Review of Ecology, Evolution, and Systematics 43:97-114.
Marsland, D. 1950. The mechanisms of cell division: temperature-pressure experiments on the cleaving eggs of Arbacia punctulata. Journal of Cellular and Comparative Physiology 36:205-227.
Martin, B. T., A. Pike, S. N. John, N. Hamda, J. Roberts, S. T. Lindley, and E. M. Danner. 2017. Phenomenological vs. biophysical models of thermal stress in aquatic eggs. Ecology Letters 20:50-59.
Michonneau, F., J. W. Brown, and D. J. Winter. 2016. rotl: an R package to interact with the Open Tree of Life data. Methods in Ecology and Evolution 7:1476-1481.
Mueller, C. A., J. Eme, R. G. Manzon, C. M. Somers, D. R. Boreham, and J. Y. Wilson. 2015. Embryonic critical windows: changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 185:315-331.
Nakagawa, S., and M. Lagisz. 2016. Visualizing unbiased and biased unweighted meta-analyses. Journal of Evolutionary Biology 29:1914-1916.
Nakagawa, S., and R. Poulin. 2012. Meta-analytic insights into evolutionary ecology: an introduction and synthesis. Evolutionary Ecology 26:1085-1099.
Orme, D. 2013. The caper package: comparative analysis of phylogenetics and evolution in R. R package version 5.
Partridge, L., and J. A. Coyne. 1997. Bergmann's rule in ectotherms: Is it adaptive? Evolution 51:632-635.

Pettersen, A. K., C. R. White, and D. J. Marshall. 2015. Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proceedings. Biological Sciences / The Royal Society 282.

Pettersen, A. K., C. R. White, R. J. Bryson-Richardson, and D. J. Marshall. 2017. Does the cost of development scale allometrically with offspring size? Functional Ecology.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. D. C. Team. 2011. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-101.

Plaistow, S. J., C. T. Lapsley, and T. G. Benton. 2006. Context-dependent intergenerational effects: The interaction between past and present environments and its effect on population dynamics. American Naturalist 167:206-215.

Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996. Life-history evolution in guppies (Poecilia reticulata) .6. Differential mortality as a mechanism for natural selection. Evolution 50:1651-1660.

Scott, G. R., and I. A. Johnston. 2012. Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. Proceedings of the National Academy of Sciences of the United States of America 109:14247-14252.

Seymour, R. S., and D. F. Bradford. 1995. Respiration of amphibian eggs. Physiological Zoology 68:1-25.

Seymour, R. S., F. Geiser, and D. F. Bradford. 1991. Metabolic cost of development in terrestrial frog eggs (*Pseudophryne bibronii*). Physiological Zoology 64:688-696.

Sinervo, B., and P. Licht. 1991. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. Science 252:1300-1302.

Smith, C. C., and S. D. Fretwell. 1974. Optimal balance between size and number of offspring. American Naturalist 108:499-506.

Spence, R., G. Gerlach, C. Lawrence, and C. Smith. 2008. The behaviour and ecology of the zebrafish, Danio rerio. Biological Reviews 83:13-34.

Tessier, A. J., and N. L. Consolatti. 1989. Variation in offspring size in *Daphnia* and consequences for individual fitness. Oikos 56:269-276.

Thorson, G. 1936. The larval development, growth and metabolism of arctic marine bottom invertebrates compared with those of other seas. Meddelelser Om Grønland 100:155.

van der Have, T. M., and G. de Jong. 1996. Adult size in ectotherms: Temperature effects on growth and differentiation. Journal of Theoretical Biology 183:329-340.

Vance, R. R. 1973. On reproductive strategies in marine benthic invertebrates. American Naturalist 107:339-352.

Wendt, D. E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biological Bulletin 198:346-356.

Woods, H. A. 1999. Egg-mass size and cell size: effects of temperature on oxygen distribution. American Zoologist 39:244-252.

Wootton, R. J., and C. Smith. 2014. Reproduction and life-history evolution, Pages 323-356, Reproductive Biology of Teleost Fishes. United States, John Wiley & Sons, Ltd.

Yampolsky, L. Y., and S. M. Scheiner. 1996. Why larger offspring at lower temperatures? A demographic approach. American Naturalist 147:86-100.

Zuo, W., M. E. Moses, G. B. West, C. Hou, and J. H. Brown. 2012. A general model for effects of temperature on ectotherm ontogenetic growth and development. Proceedings of the Royal Society B-Biological Sciences 279:1840-1846.

Tables

Table 1. Summary of scaling exponents and coefficients (± SE) for temperature, mass and metabolic rate of *Bugula neritina* and *Danio rerio* where $MR(T) = \gamma \cdot e^{(bMR \cdot T)} \cdot (\text{Offspring mass}^{\beta})$. Time 1 = 0 hps/ 0hpf, Time 2 = 24 hps/24 hpf, Time 3 = 48 hps/48 hpf, Time 4 = 72 hps/72hpf, Time 5 = 120 hps/120 hpf (where hps = hours post-settlement in *Bugula* and hpf = hours post fertilisation in *Danio*).

Time	Temperatures measured (T)	Coefficient (ɣ)	Scaling exponent Temperature (*b*)	Scaling exponent Mass (β)
Bugula neritina				
1	24°C, 20°C, 16°C, 12°C	0.022 ± 0.002	0.065 ± 0.006	0.659 ± 0.153
2	24°C, 20°C, 16°C, 12°C	0.012 ± 0.001	0.096 ± 0.005	0.664 ± 0.114
3	16°C, 12°C	0.007 ± 0.001	0.196 ± 0.012	0.441 ± 0.175
4	12°C	0.068 ± 0.015		0.609 ± 0.245
5	12°C	0.101 ± 0.030		0.537 ± 0.270
Danio rerio				
1	32°C, 28°C, 24°C, 20°C	0.007 ± 0.001	0.085 ± 0.004	0.630 ± 0.213
2	32°C, 28°C, 24°C, 20°C	0.026 ± 0.005	0.092 ± 0.003	0.462 ± 0.160
3	28°C, 24°C, 20°C	0.049 ± 0.012	0.092 ± 0.004	0.411 ± 0.180
4	24°C, 20°C	0.065 ± 0.022	0.094 ± 0.008	0.362 ± 0.221
5	20°C	0.161 ± 0.109		0.629 ± 0.304

Table 2. Predicted costs of development, $C_{(T)}$ as a product of $D_{(T)}$ and $MR_{(T)}$ across the range of temperatures tested for an average larval and embryo mass for *Bugula neritina* (12µg) and *Danio rerio* (57µg).

Temperature (°C)	D (h)	MR1 (mJh^{-1})	MR2 (mJh^{-1})	MR3 (mJh^{-1})	MR4 (mJh^{-1})	MR5 (mJh^{-1})	Total cost (mJ)
Bugula neritina							
12	110.08	0.25	0.20	0.22	0.31	0.38	28.76
16	50.59	0.32	0.29	0.48			15.90
20	36.67	0.42	0.43				15.36
24	33.41	0.54	0.63				18.81
Danio rerio							
20	175.12	0.49	1.06	1.63	1.84	2.05	282.40
24	92.36	0.69	1.53	2.35	2.68		164.20
28	58.57	0.97	2.21	3.39			112.16
32	44.78	1.36	3.20				99.01

Figures

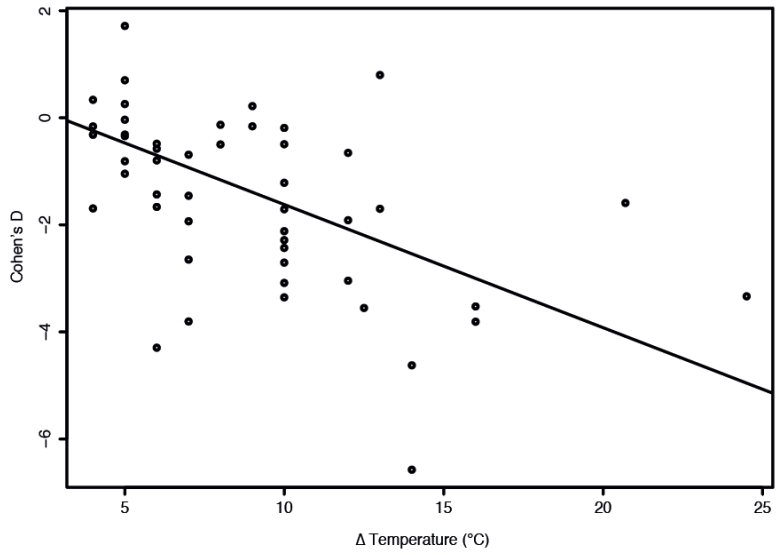


Figure 1. Relationship between Cohen's D (magnitude of change in offspring size with temperature, divided by standard deviation) and Δ Temperature (°C). Fitted line represents the final linear model for the correlation between change in experimental maternal brooding temperature (Δ Temperature) and change in offspring size for 33 species (six phyla). Each dot represents a single result (12 species with more than 1 result was accounted for in the mixed model, see Table C1 for list of species).

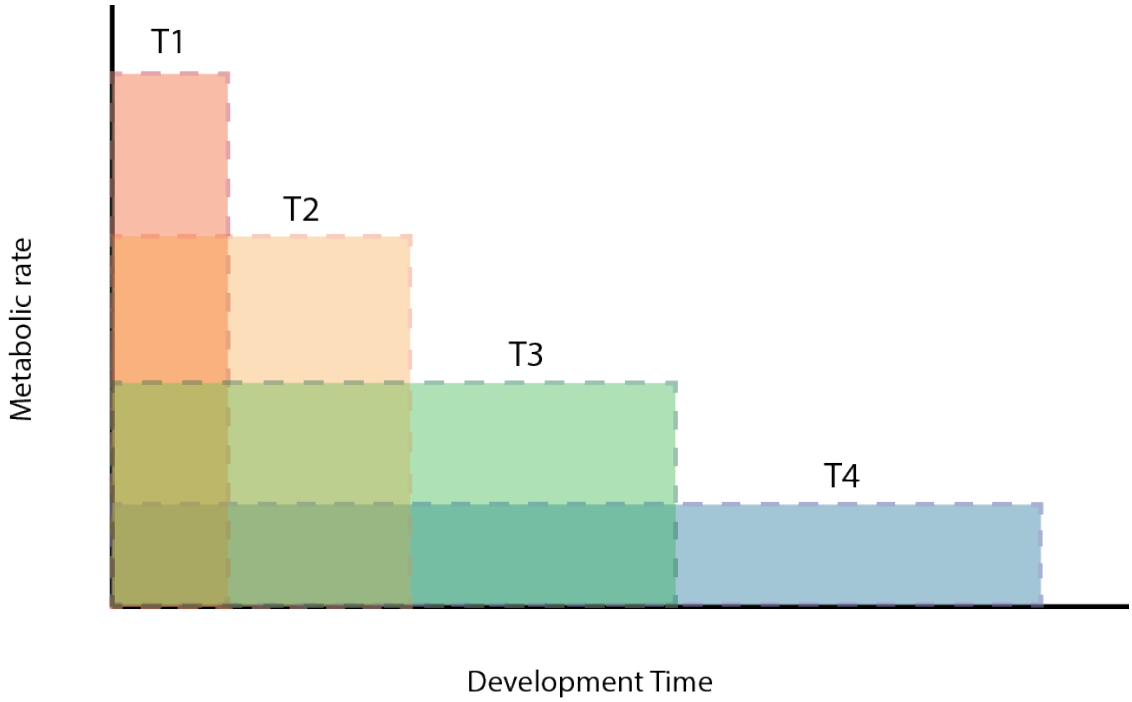


Figure 2. Schematic for the relationship between Development time (*D*) and Metabolic rate (*MR*) under four developmental temperatures (T1 = highest, to T4 = lowest). Shaded areas represent the predicted costs of development (*C*) at each temperature where $C_{(T)} = D_{(T)} \times MR_{(T)}$. As temperature increases from T4 to T1, development time is expected to decrease, and metabolic rate will increase.

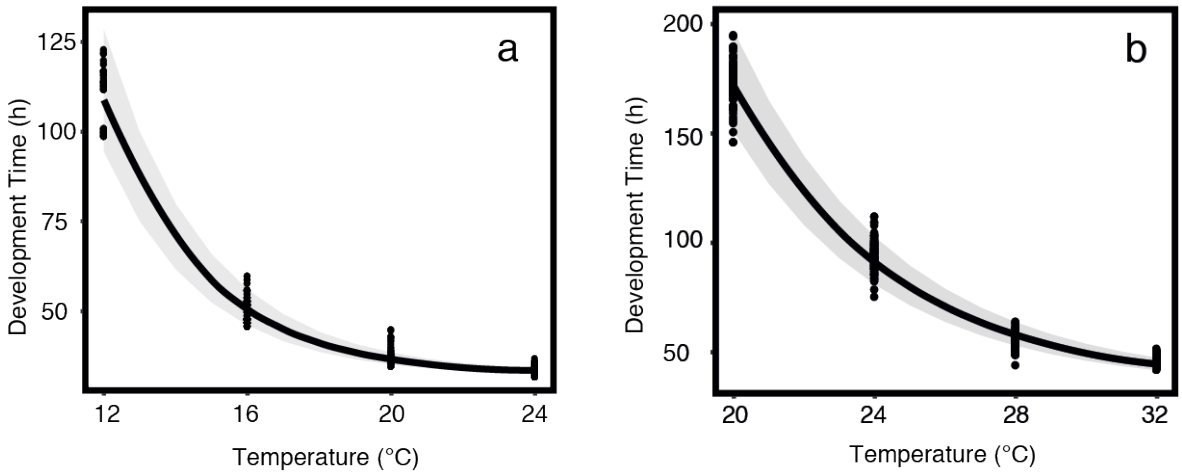


Figure 3. Predicted relationship between development time; $D_{(T)}$ (h) and Temperature (°C) for a) *Bugula neritina* and b) *Danio rerio*. Fitted lines represent the final exponential function for the relationship between Development time and Temperature ± SE (shaded area). Dot points are raw data.

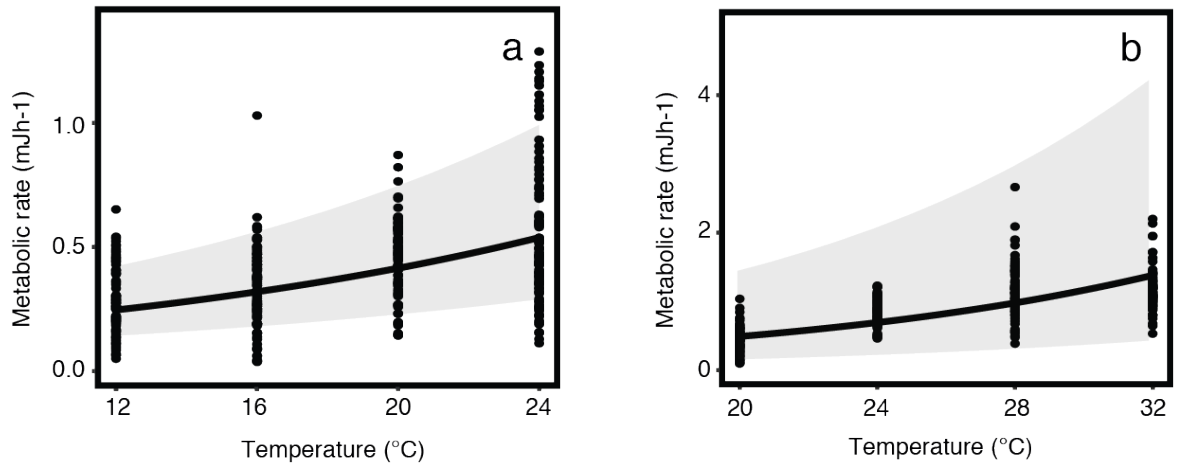


Figure 4. Predicted relationship between Metabolic rate (MR1; mJ h^{-1}) and Temperature (°C) for a) *Bugula neritina* and b) *Danio rerio*. Fitted lines represent the exponential function for the relationship between Metabolic rate (for the first measure of metabolic rate; MR1, where all temperatures were measured) and Temperature ± SE (shaded area) for an average larval and embryo mass for *Bugula neritina* (12µg) and *Danio rerio* (57µg) respectively. Dot points are raw data for all larval and embryo masses.

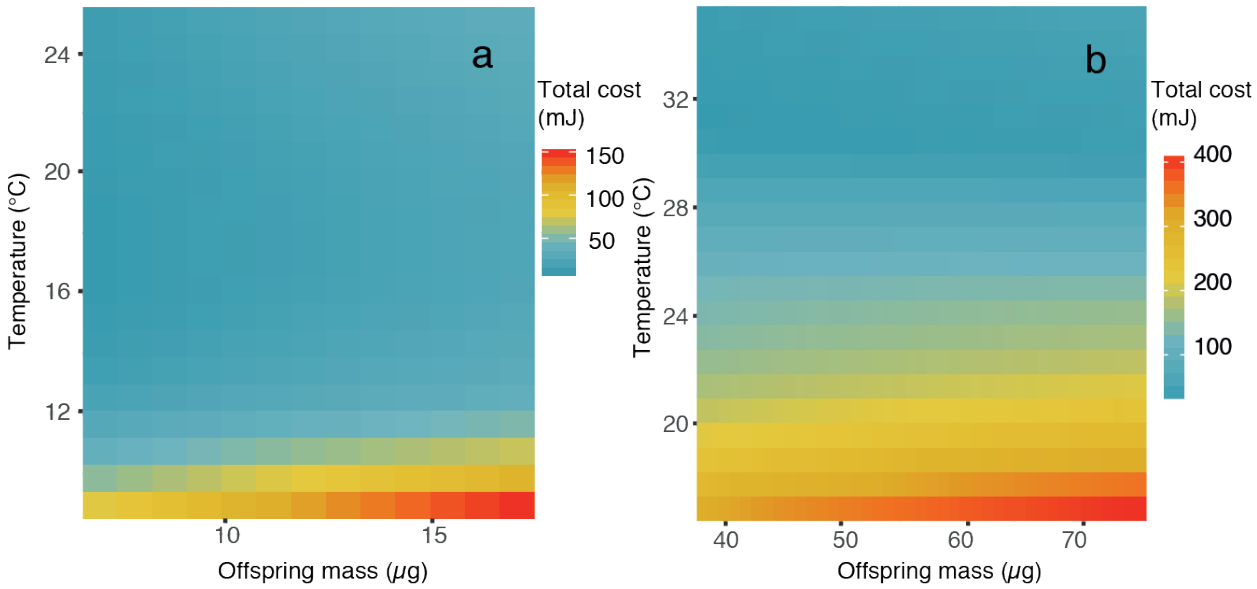


Figure 5. Predicted total costs of development (total mJ used) throughout the dependent phase in a) *Bugula neritina* from larval settlement through to emergence of the lophophore over 12°C - 24°C and in b) *Danio rerio* from fertilisation through to hatching as a larva over 20°C - 32°C.

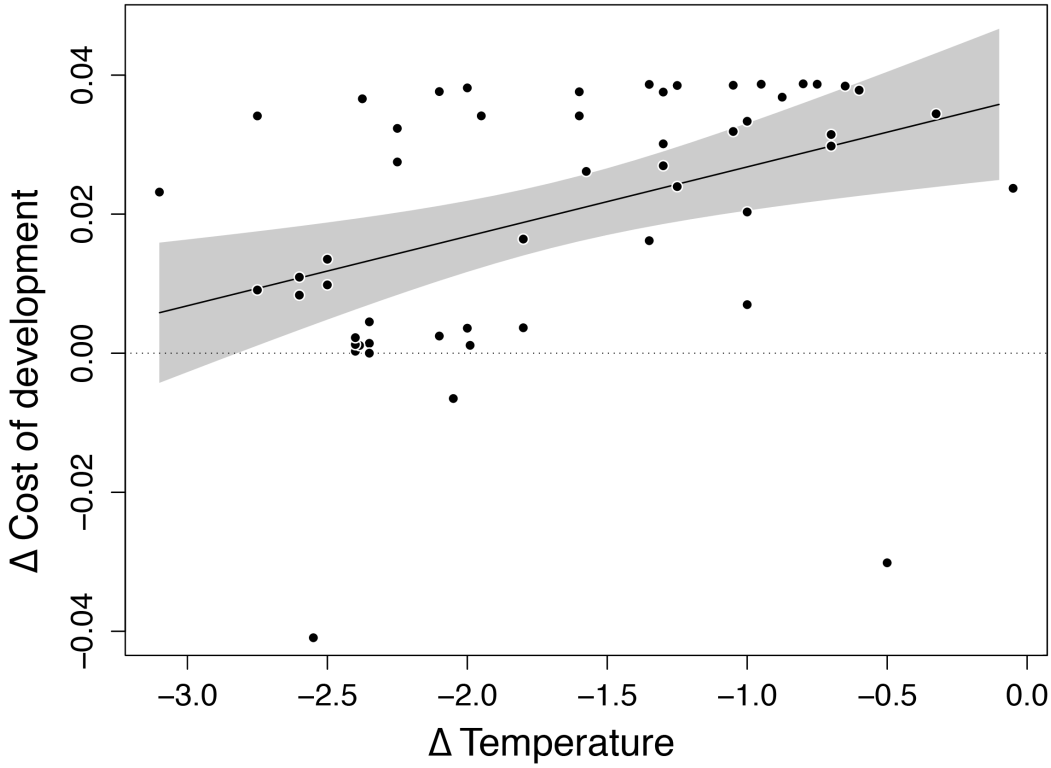


Figure 6. Relationship between the change in the costs of development (ΔC) with a 10% decrease in natural mean development temperature (ΔT) for 52 species across four phyla ± 95% confidence interval.

Appendix C

Methods

3. Meta-analysis of temperature and offspring size

Published articles presenting data on laboratory-controlled brooding temperature effects on offspring size were collected from *ISI Web of Science* using search terms "offspring size", "egg size"", "larv* size", "hatch* size" together with the terms "temperature" and "thermal". This method yielded 75 results, and of these 42 were rejected based on irrelevance. Studies were also only included if they reported sample size or a statistic from which sample size could be calculated (e.g., degrees of freedom) as well as a measure of error from which standard deviation could be determined in order to minimise sampling bias.

4. Empirical estimates of costs of development with temperature

Offspring sampling and size measurements

Offspring size is likely to affect the relationship of temperature on developmental rate and metabolic rate. While there are many studies that have examined the effects of temperature on developmental rate and metabolic rate, we are aware of none that examine the effects of offspring size and temperature on developmental rate and metabolic rate simultaneously such that equation [2] can be parameterised.

Reproductively mature colonies of *Bugula* were collected from the subtidal zone in Melbourne, Australia during March – May 2015 and transported to the laboratory where light-shock treatment was used to stimulate release of larvae as per standard techniques (Marshall et al. 2003; Pettersen et al. 2015). Larvae were then collected and measured as per (Pettersen et al. 2015) with a Motic 5 digital camera (Motic, Hong Kong, China) mounted on a dissecting microscope to obtain length of the cillial groove and body area to the nearest μm. A previous

study has shown this technique to be precise, with measurement error accounting for 0.8% of all variation in offspring size (Pettersen et al. 2015). Mass was calculated from the offspring size measurements based on the results of a previous study relating larval body area and length to measures of volume and density for *Bugula* (Pettersen et al. 2015). Once measured, individual larvae were immediately pipetted with a small volume of seawater onto roughened acetate sheets and covered in order to avoid desiccation and induce settlement via negative phototaxis (Marshall and Keough 2003; Wendt and Woollacott 1999).

Adult male and female *Danio* were reared in the laboratory at Monash University, Melbourne, Australia and induced to release gametes in November – December 2015 via standard procedures (Bryson-Richardson et al. 2011). Fertilised eggs were collected from separate parent pairs and cultured throughout development in 'egg water' (Westerfield 2000). In order to assess offspring mass non-destructively, embryos were photographed and measured at the 'sphere' stage (Olympus 1X73; x40, Olympus cellSens Dimension software) which has been shown to provide a reliable indication of embryo size (Bownds et al. 2010). Estimates of mass were gained from measurements of embryo diameter to the nearest μm, and from which calculated embryo volume has been shown to reliably predict mass ($R^2 = 0.26$; Pettersen et al. 2017). Once measured, embryos were maintained in 24-well plates throughout the duration of the dependent phase.

b) Development rate under different temperature regimes

We manipulated temperature with waterbaths using an aquarium heater (Shogun LED 200W) to maintain constant temperatures (±1°C). For development rate in *Danio*, a replicated control block design was used, where two temperatures were simultaneously tested in separate controlled temperature rooms. In order to minimise the effects of spatial and temporal variation among treatments, all combinations of the four temperatures were measured in each

experimental run. For *Bugula*, emergence of the lophophore for each individual was monitored every 0.5h using a dissecting microscope.

c) Metabolic rate under different temperature regimes

Fluorescence-based oxygen measurements ($\dot{V}O_2$) were used as a proxy for metabolic rate throughout development of the 'dependent' phase as previously described by Pettersen et al. (2015). The rate of oxygen consumption, $\dot{V}O_2$, was measured at 24 h intervals from six hours post-settlement/post-fertilisation in *Bugula* and *Danio* respectively, throughout the dependent phase. For each experimental run, oxygen consumption was measured over a 3 h period using a 24-channel PreSens sensor dish reader (Sensor Dish Reader SDR2, PreSens, Germany), with 24-chamber glass microplate (200ul; Loligo Systems Aps, Denmark) per temperature regime (Alton et al. 2012; Köster et al. 2008). For each experimental run, measurements at four temperatures were recorded simultaneously - 80 individual offspring (20 at each temperature) were placed into individual 200µL chambers containing 0.2µm filtered and pasteurised seawater (*Bugula*) or 'egg water' (*Danio*), as per Westerfield (2000). To maintain stable temperatures throughout $\dot{V}O_2$ measurements, each microplate was placed within a 1 L water bath (Loligo Systems APS, Denmark) connected to a 40 L holding tank with water at each experimental temperature (±1°C), continuously circulated around the micro-plate using aquaria pumps (Eheim Universal 300, Germany). A non-consumptive sensor spot on the base of each chamber recorded O_2 saturation at two minute intervals, and the rate of change over time ($\dot{V}O_2$) was calculated as

$\dot{V}O_2 = -1\ (m_a - m_b / 100)\ V\beta O_2$ (as per White et al. 2011) where m_a and m_b are the rates of change of O_2 saturation for experimental vials, and blank vials containing no offspring (% h^{-1}) respectively, βO_2 is the oxygen capacitance of air-saturated seawater/freshwater at each of the four temperature treatments (6.01 – 4.72 ppt and 6.40 – 5.10 ppt for seawater 12 – 24°C and freshwater 20 – 32°C respectively; Cameron 1986), and V is water volume (chambers were 2.0

x 10^{-4}L for *Bugula* and 7.5 x 10^{-4}L for *Danio*, and water volume was calculated by subtracting the volume of individual offspring). 20 vials per microplate contained individuals of known offspring size, while the remaining four vials were used as controls, containing only filtered seawater and acetate (*Bugula*) or filtered egg water (*Danio*). Prior to the experimental runs, sensor spots were calibrated using air-saturated (AS) seawater or freshwater (100% AS) and water containing 2% sodium sulphite (0% AS). As $\dot{V}O_2$ was measured for offspring every 24 h until the end of the dependent phase, and development was highly temperature dependent, the number of measurements of metabolic rate varied across temperature regimes. Based on the average development time, $\dot{V}O_2$ was measured for at least two and up to five periods depending on the temperature regime (higher temperatures had fewer measurement periods). To convert $\dot{V}O_2$ (µl h^{-1}) to metabolic rate (mJ h^{-1}), the calorific conversion factor of 20.08 J ml^{-1} O_2 was used (Crisp 1971). Each experimental was repeated five times for both species.

d) Statistical analysis

In order to determine the size-specific energy costs (*C*) for the development of offspring under varying temperatures for both *Bugula* and *Danio*, we combined the length of time spent in the dependent phase, i.e development time (*D*), with the rate of energy use or metabolic rate (*MR*) throughout the dependent phase. To find *D*, we fit models relating temperature to development time of individuals throughout the dependent stage (from either settlement through to development of the lophophore in *Bugula* or fertilisation to hatching in *Danio*). The effect of temperature on *D* was analysed using a general linear model for *Bugula* and linear mixed effects model for *Danio*. Natural log transformations of offspring mass and *D* were necessary in order to reduce observed variation with the mean, in order to satisfy the assumption of homoscedasticity. It also allowed for a more tractable and reliable mixed-effects model that could incorporate experimental run ('Run'). Temperature and ln(Offspring mass) were included as fixed factors while experimental run ('Run') was treated as random. For

Bugula, there was no effect of ln(Offspring mass) on ln(*D*) - contrary to studies on other marine invertebrates, offspring size did not alter the length of the post-settlement dependent phase, hence development time was calculated solely as a function of temperature. For *Danio* however, ln(*D*) was dependent on ln(Offspring mass).

To determine the relationship between *MR* and offspring size, the same individuals were measured every 24 h throughout development during the dependent phase. Since development time varied by approximately five-fold between the lowest and highest temperature treatments for each species, (individuals at higher temperatures reached independence before those at lower temperatures) fewer measures of *MR* were obtained at the higher temperature treatments. All temperature regimes had at least two measures of *MR* and could therefore be directly compared. Mass and *MR* were natural log transformed as per the rationale provided above. Repeated-measures analysis (using the R package lme4; Bates et al. 2015) was used to estimate the relationship between Temperature, ln(Offspring mass) and ln(MR) for Time 1 and Time 2 (24 h and 48 h post-settlement/fertilisation respectively, where MR at all temperatures were measured). Likelihood-ratio tests were used to test the significance of random effects (Supplementary Material, Table C2). For fixed effects, ln(MR) was dependent on the stage (i.e 'Time') that was measured in both species (*Bugula;* $F_{1,734} = 16.052$, $p < 0.0001$, *Danio*; $F_{1,674} = 1414.286$, $p < 0.001$) hence the relationship between temperature, mass and MR for each species was calculated using a fully-crossed orthogonal design where all combinations of Temperature and Time were tested. A significant effect of Time was also found when analysing individual temperatures across all times tested within that treatment (Table C3), thus separate Temperature and ln(Offspring mass) coefficients were determined for each Time. The relationship between Temperature, ln(Offspring mass), and ln(MR) were analysed at each Time using reduced maximum likelihood linear mixed models (REML) that could incorporate

the fixed effects of ln(Offspring mass) and ln(Temperature) with the random effect of Run (Table C4).

Tables

Table C1. The relationship between offspring size and temperature (T) under controlled laboratory conditions; data for 33 species used for Figure 1. NS = non-significant ($p > 0.05$).

Taxon	ΔT	Sign of relationship	Cohen's D	Reference
Chordata				
Actinopterygii (class):				
Cyprinodon nevadensis		Negative	-3.523	Shrode and Gerking (1977)
Danio rerio	10	Negative	-2.118	Bownds et al. (2010)
Engraulis japonica	13	Negative	-1.699	Imai and Tanaka (1987)
Solea solea	6	Negative	-4.294	Baynes and Howell (1996)
Amphibia (class):				
Bombina orientalis (high food)	8	Negative	-0.129	Kaplan (1987)
Bombina orientalis (low food)	8	Negative	-0.498	Kaplan (1987)
Reptilia:				
Sceloporus jarrovi	10	Negative	-0.494	Beuchat (1988)
Arthropoda				
Copepoda:				
Sinocalanus tenellus	20.7	Negative	-1.591	Kimoto et al. (1986)
Crustacea (subphylum):				
Betaeus emarginatus	7	Negative	-13.631	Wehrtmann and Lopez (2003)
Daphnia galeata	13	Positive	0.799	Machacek and Seda (2013)
Daphnia magna (control)	6	Negative	-1.431	Sakwinska (1998)
Daphnia magna (high predator kairomones)	6	Negative	-0.579	Sakwinska (1998)
Daphnia magna (low predator kairomones)	6	Negative	-1.664	Sakwinska (1998)

Daphnia pulex (diploid)	14	Negative	-6.573	Dufresne and Hebert (1998)
Daphnia pulex (polyploid)	14	Negative	-4.624	Dufresne and Hebert (1998)
Gammarus insensibilis	24.5	Negative	-3.333	Sheader (1996)
Simocephalus vetulus	12	Negative	-3.042	Perrin (1988)
Entognatha (class):				
Orchesella cincta	4	Negative	-1.692	Liefting et al. (2010)
Hexapoda (class):				
Folsomia candida (Brunoy clone)	9	NS	-0.159	Stam et al. (1996)
Folsomia candida (York clone)	9	Positive	0.219	Stam et al. (1996)
Insecta (class):				
Bicyclus anynana	7	Negative	-0.690	Steigenga and Fischer (2007)
Bicyclus anynana	7	Negative	-2.647	Fischer et al. (2006)
Bicyclus anynana	7	Negative	-1.456	Fischer et al. (2003b)
Bicyclus anynana	7	Negative	-1.932	Fischer et al. (2003a)
Chorthippus brunneus	5	Negative	-0.811	Willott and Hassall (1998)
Drosophila melanogaster	7	Negative	-3.805	Crill et al. (1996)
Drosophila melanogaster	10	Negative	-3.353	Imai (1934)
Drosophila melanogaster	12.5	Negative	-3.552	Azevedo et al. (1996)
Drosophila phalerata	10	Negative	-2.430	Avelar (1993)
Drosophila simulans	10	Negative	-2.705	Avelar (1993)
Drosophila subobscura	10	Negative	-2.286	Avelar (1993)
Notiophilus biguttatus	16	Negative	-3.808	Ernsting and Isaaks (2000)
Notiophilus biguttatus (eggs)	10	Negative	-1.215	Ernsting and Isaaks (1997)
Notiophilus biguttatus (larvae)	10	Negative	-1.709	Ernsting and Isaaks (1997)

Omocestus viridulus	5	NS	0.701	Willott and Hassall (1998)
Parnara guttata	5	Positive	1.717	Seko and Nakasuji (2006)
Scathophaga stercoraria (first clutch)	12	Negative	-0.654	Blanckenhorn (2000)
Scathophaga stercoraria (third clutch)	12	Negative	-1.912	Blanckenhorn (2000)
Bryozoa				
Bugula neritina (Run1)	6	Negative	-0.795	Burgess and Marshall (2011)
Bugula neritina (Run2)	6	Negative	-0.484	Burgess and Marshall (2011)
Mollusca				
Bivalvia:				
Caenorhabditis elegans	10	Negative	-3.083	Van Voorhies (1996)
Gastropoda:				
Crepidula atrasolea	5	Negative	-0.035	Collin and Salazar (2010)
Crepidula cf. marginalis	5	Negative	-17.588	Collin (2012)
Crepidula cf. onyx	5	Positive	0.259	Collin and Spangler (2012)
Crepidula incurva	5	Negative	-11.080	Collin and Spangler (2012)
Crepidula ustulatulina (Location 1)	5	Negative	-1.044	Collin and Salazar (2010)
Crepidula ustulatulina (Location 1)	5	Negative	-0.341	Collin and Salazar (2010)
Nematoda				
Caenorhabditis elegans	10	Negative	-3.083	Van Voorhies (1996)
Rotifera				
Brachionus calyciflorus	10	Negative	-0.188	Sun and Niu (2012)

Table C2. Log-likelihood ratio tests for significance tests of random effects in a repeated measures analysis of Temperature on ln(Metabolic rate) at Time 1 and Time 2 (all temperatures measured).

	Bugula neritina		*Danio rerio*	
	χ^2	P-value	χ^2	P-value
Between subjects				
Run	0.238	0.626	**8.214**	**<0.05**
Temperature * Run	0	1	0	1
ln(Offspring mass) * Run	3.011	0.082	0	1
Temperature * ln(Offspring mass) * Experiment	1.523	0.210	0	1
Within subjects				
Time * Run	0	1	0	1
Time * Temperature * Run	0	1	0	1
Time * ln(Offspring mass) * Run	0	1	0	1
Time * Temperature * ln(Offspring mass) * Run	0	1	0	1

Table C3. Log-likelihood ratio tests for significance tests for repeated measures analysis of Time on ln(Metabolic rate) at each temperature measured.

	Bugula neritina		*Danio rerio*	
	24°C (Time 1 and Time 2)		32°C (Time 1 and Time 2)	
Between subjects	χ^2	P-value	χ^2	P-value
ln(Offspring mass)	**12.727**	**<0.001**	0.073	0.787
Run	**244.904**	**<0.0001**	**5.566**	**0.018**
ln(Offspring mass) * Run	0	1	0	1
Within subjects				
Time	**23.690**	**<0.0001**	**159.497**	**<0.0001**
Time * ln(Offspring mass)	0	1	0	1
Time * Run	0	1	0	1
Time * ln(Offspring mass) * Run	0	1	0	1
	20°C (Time 1 and Time 2)		28°C (Time 1, Time 2 and Time 3)	
Between subjects	χ^2	P-value	χ^2	P-value
ln(Offspring mass)	**10.002**	**<0.05**	0.047	0.829
Run	1.012	0.314	**5.721**	**0.017**
ln(Offspring mass) * Run	0	1	0	1
Within subjects				
Time	0.411	0.521	**155.456**	**<0.0001**
Time * ln(Offspring mass)	0	1	0	1
Time * Run	0	1	0	1
Time * ln(Offspring mass) * Run	0	1	0	1
	16°C (Time 1, Time 2 and Time 3)		24°C (Time 1, Time 2, Time 3 and Time 4)	
Between subjects	χ^2	P-value	χ^2	P-value
ln(Offspring mass)	**21.554**	**<0.0001**	2.647	0.104
Run	**8.822**	**<0.05**	**7.689**	**0.021**
ln(Offspring mass) * Run	**359.635**	**<0.0001**	0.008	0.999
Within subjects				
Time	**17.609**	**<0.0001**	**199.595**	**<0.0001**

Time * ln(Offspring mass)	5.055	0.282	**0.850**	**<0.0001**
Time * Run	**5.781**	**0.016**	0.911	0.340
Time * ln(Offspring mass) * Run	0	1	0.850	0.356
	12°C (Time 1, Time 2, Time 3, Time 4 and Time 5)		20°C (Time 1, Time 2, Time 3, Time 4 and Time 5)	
Between subjects	χ^2	P-value	χ^2	P-value
ln(Offspring mass)	**22.385**	**<0.0001**	**245.467**	**<0.0001**
Run	**27.506**	**<0.0001**	**10.906**	**0.004**
ln(Offspring mass) * Run	0	1	0.044	0.998
Within subjects				
Time	**21.838**	**<0.0001**	**266.030**	**<0.0001**
Time * ln(Offspring mass)	1.466	0.226	0.664	0.415
Time * Run	**30.381**	**<0.0001**	**32.556**	**<0.0001**
Time * ln(Offspring mass) * Run	0.817	0.999	0.224	0.999

Table C4. Log-likelihood ratio tests for significance tests of random effects in linear mixed effects models for Temperature on ln(Metabolic rate) at each Time during the dependent phase of *Bugula neritina* (settlement – emergence of lophophore) and *Danio rerio* (fertilisation – hatching). All d.f. = 1.

	Bugula neritina		*Danio rerio*	
	Time 1 (24°C, 20°C, 16°C, and 12°C)		Time 1 (32°C, 28°C, 24°C and 20°C)	
	χ^2	P-value	χ^2	P-value
Run	**7.320**	**<0.05**	**6.392**	**<0.05**
ln(Temperature) * Run	0	1	0	1
ln(Offspring mass) * Run	0	1	0	1
ln(Temperature) * ln(Offspring mass) * Run	0	1	0	1
	Time 2 (24°C, 20°C, 16°C, and 12°C)		Time 2 (32°C, 28°C, 24°C and 20°C)	
	χ^2	P-value	χ^2	P-value
Run	**16.040**	**<0.0001**	2.250	0.134
ln(Temperature) * Run	0	1	0	1
ln(Offspring mass) * Run	0	1	0	1
ln(Temperature) * ln(Offspring mass) * Run	0	1	0	1
	Time 3 (16°C and 12°C)		Time 3 (28°C, 24°C and 20°C)	
	χ^2	P-value	χ^2	P-value
Run	**16.139**	**<0.0001**	1.315	0.251
ln(Temperature) * Run	0	1	0	1
ln(Offspring mass) * Run	0	1	0	1
ln(Temperature) * ln(Offspring mass) * Run	0	1	0	1
	Time 4 (12°C)		Time 4 (24°C and 20°C)	
	χ^2	P-value	χ^2	P-value
Run	**25.134**	**<0.0001**	**19.967**	**<0.0001**
ln(Temperature) * Run			0	1
ln(Offspring mass) * Run	0	1	0	1
ln(Temperature) * ln(Offspring mass) * Run			0	1
	Time 5 (12°C)		Time 5 (20°C)	
	χ^2	P-value	χ^2	P-value
Run	**7.311**	**<0.05**	**55.606**	**<0.0001**
ln(Offspring mass) * Run	0	1	0	1

Table C5. Studies included in meta-analysis for the temperature sensitivity of development time (D), metabolic rate (MR) and total costs (Δ Cost) of development with a 10% decrease from the mean of natural temperature range (TR) for 52 species.

Taxon	Temperature range (°C)	Temperatures tested (D; °C)	DT (h)	Temperatures tested (MR; °C)	MR (ml O_2 h^{-1})	Δ Cost	Reference
Chordata							
Actinopterygii (class):							
Clarias gariepinus	20-30	22.1	58.0	22.1	$2.10 \cdot 10^{-1}$	$8.35 \cdot 10^{-2}$	Kamler et al. (1994) (D, MR), Haylor and Mollah (1995) (TR)
		25	35.3	25	$2.70 \cdot 10^{-1}$		
		28.1	23.5	28.1	$3.50 \cdot 10^{-1}$		
Clupea harengus	8-12	5	672	8	$5.20 \cdot 10^{-4}$	$3.33 \cdot 10^{-2}$	Johnston et al. (1995) (D), (Almatar 1984) (MR, TR)
		8	384	13	$1.70 \cdot 10^{-3}$		
		12	216	18	$2.20 \cdot 10^{-4}$		
		15	192				
Coregonus clupeaformis	0.5-8	2	936	2	$2.17 \cdot 10^{-5}$	$3.44 \cdot 10^{-2}$	Mueller et al. (2015) (D, MR, TR)
		5	576	5	$2.76 \cdot 10^{-5}$		
		8	432	8	$4.14 \cdot 10^{-5}$		
Danio rerio	20-32	20	175.12	20	$2.44 \cdot 10^{-2}$	$1.09 \cdot 10^{-2}$	This study
		24	92.36	24	$3.42 \cdot 10^{-2}$		

		28	58.57	28	$4.81 \cdot 10^{-4}$		
		32	33.41	32	$6.76 \cdot 10^{-4}$		
Gadus morhua	4-10	6	393.3	4	$5.09 \cdot 10^{-4}$	$6.98 \cdot 10^{-3}$	Geffen et al. (2006) (D), Laurence (1978) (MR), Laurence and Rogers (1976) (TR)
		8	247.2	7	$5.91 \cdot 10^{-4}$		
		10	225.6	10	$7.41 \cdot 10^{-4}$		
		12	177.6				
Leuciscus cephalus	12-28	12.3	208.8	12	$1.21 \cdot 10^{-2}$	$1.13 \cdot 10^{-3}$	Kupren et al. (2008) (D,TR), Wieser and Forstner (1986)(MR)
		15.7	115.2	16	$1.90 \cdot 10^{-2}$		
		19	93.6	20	$3.02 \cdot 10^{-4}$		
		23	55.2	24	$3.80 \cdot 10^{-4}$		
		25	28.8				
Melanogrammus aeglefinus	4-10	2	487.2	4	$4.67 \cdot 10^{-3}$	$3.15 \cdot 10^{-2}$	Martell et al. (2005) (D), Laurence (1978)(MR), Laurence and Rogers (1976)(TR)
		4	391.2	7	$5.20 \cdot 10^{-3}$		
		6	312	9	$6.04 \cdot 10^{-3}$		
		8	254.4				
		10	218.4				
Oncorhynchus mykiss (*Salmo gairdneri*)	6-13	6	1480.8	6	$2.26 \cdot 10^{-3}$	$3.85 \cdot 10^{-2}$	Rombough (1988) (D,MR), Pankhurst et al. (1996)(TR)
		9	960	9	$3.57 \cdot 10^{-3}$		

		12	669.6	12	$4.96 \cdot 10^{-3}$		
		15	480	15	$4.15 \cdot 10^{-3}$		
Oncorhynchus tshawytscha	3-16	5	2707.2	5	$4.44 \cdot 10^{-3}$	$3.85 \cdot 10^{-2}$	Rombough (1994) (D, MR), Alderdice and Velsen (1978)(TR)
		7.3	1836	7.3	$5.15 \cdot 10^{-3}$		
		10	1296	10	$7.94 \cdot 10^{-3}$		
		12.5	962.4	12.5	$7.82 \cdot 10^{-3}$		
Pseudopleuronectes americanus	2-8	5	1920	5	$2.69 \cdot 10^{-3}$	$-3.01 \cdot 10^{-2}$	Laurence (1975) (D,MR,TR)
		8	1176	8	$3.91 \cdot 10^{-3}$		
Pleuronectes platessa	3-10	6	271.2	8	$7.43 \cdot 10^{-4}$	$3.84 \cdot 10^{-2}$	Fox et al. (2003) (D), Almatar (1984) (MR), Ryland and Nichols (1975) (TR)
		8	206.4	13	$1.12 \cdot 10^{-3}$		
		10	112.8	18	$1.57 \cdot 10^{-3}$		
		12	108				
Rutilus rutilus	8-24	7.8	781.82	12	$1.21 \cdot 10^{-2}$	$2.61 \cdot 10^{-2}$	Herzig and Winkler (1986) (D,TR), Wieser and Forstner (1986) (MR)
		15	214.72	16	$1.90 \cdot 10^{-2}$		
		22	102.01	20	$3.02 \cdot 10^{-2}$		
				24	$3.80 \cdot 10^{-2}$		
Salmo salar	0-16	6	1656	2.5	$4.50 \cdot 10^{-2}$	$3.87 \cdot 10^{-2}$	Brannas (1988) (D), McCarthy (2000)
		10	768	7	$1.56 \cdot 10^{-1}$		

		12	564	12.5	$2.33 \cdot 10^{-1}$		(MR), Elliott and Elliott (2010) (TR)
Salmo trutta fario	3-11	2.8	3960.40	2.8	$3.75 \cdot 10^{-3}$	$2.98 \cdot 10^{-2}$	Wood (1932) (D,MR), Réalis-Doyelle et al. (2016) (TR)
		5	2474.23	5	$4.47 \cdot 10^{-3}$		
		6.3	2135.23	6.3	$5.18 \cdot 10^{-3}$		
		7.3	1751.82	7.3	$5.98 \cdot 10^{-3}$		
		9	1343.78	9	$8.04 \cdot 10^{-3}$		
		12		12	$1.14 \cdot 10^{-3}$		
Salvelinus alpinus	4-8	4	444	4	$2.45 \cdot 10^{-3}$	$3.78 \cdot 10^{-2}$	Gruber and Wieser (1983) (D,MR,TR)
		8	228	8	$4.49 \cdot 10^{-3}$		
Scardinius erythrophthalmus	10-22	10	252	12	$1.21 \cdot 10^{-2}$	$3.76 \cdot 10^{-2}$	Teletchea et al. (2009) (D), Wieser and Forstner (1986) (MR), McDowell (2000) (TR)
		15	168	16	$1.90 \cdot 10^{-2}$		
		20	126	20	$3.02 \cdot 10^{-2}$		
				24	$3.80 \cdot 10^{-2}$		
Scomber scombrus	8-18	9	251.6	13	$4.51 \cdot 10^{-2}$	$2.70 \cdot 10^{-2}$	Mendiola et al. (2007) (D,TR), Giguere et al. (1988) (MR)
		11	166.2	16	$8.24 \cdot 10^{-2}$		
		13	125.6	19	$1.02 \cdot 10^{-1}$		
		15	101				
		18	77.4				

Xyrauchen texaus	10-22	10	449	10	$3.63 \cdot 10^{-4}$		
		15	256	15	$6.45 \cdot 10^{-4}$	$3.41 \cdot 10^{-2}$	Bozek et al. (1990) (D,MR,TR)
		20	158	20	$1.01 \cdot 10^{-3}$		
Amphibia (class):							
Crinia georgiana	12-25	12	456	12	$3.74 \cdot 10^{-4}$		
		15	288	15	$6.84 \cdot 10^{-4}$	$3.87 \cdot 10^{-2}$	Seymour and Roberts (1995) (D, MR, TR)
Crinia nimbus	0-15	5	4056	5	$2.22 \cdot 10^{-4}$		
		10	1800	10	$4.33 \cdot 10^{-4}$	$3.87 \cdot 10^{-2}$	Mitchell and Seymour (2000) (D, MR, TR)
		15	1056	15	$6.06 \cdot 10^{-4}$		
Neoceratodus forsteri	15-25	15	1344	15	$2.66 \cdot 10^{-4}$		
		20	504	20	$4.62 \cdot 10^{-4}$	$2.47 \cdot 10^{-3}$	Mueller et al. (2011) (D, MR, TR)
		25	336	25	$4.76 \cdot 10^{-4}$		
Pseudophryne bibronii	7-22	12	960	12	$6.99 \cdot 10^{-4}$		
		17	504	17	$1.15 \cdot 10^{-3}$	$1.62 \cdot 10^{-2}$	Seymour et al. (1991) (D, MR), Geiser and Seymour (1989) (TR)
		22	408	22	$1.64 \cdot 10^{-3}$		
Reptilia (class):							
Apalone spinifera	18-29	26.5	1848	26.5	$2.10 \cdot 10^{-1}$	$1.42 \cdot 10^{-3}$	

		28.5	1464	28.5	$2.64 \cdot 10^{-1}$		Ligon and Lovern (2012) (D,MR,TR)
		30.5	1272	30.5	$2.84 \cdot 10^{-1}$		
Bassiana duperreyi	17-24	25	984	25	$4.97 \cdot 10^{-2}$	$3.01 \cdot 10^{-2}$	Booth et al. (2000) (D,MR),Flatt et al. (2001) (TR)
		30	554.4	30	$9.16 \cdot 10^{-2}$		
Caretta caretta	24-31	27.6	1500.72	27.6	1.18	$2.00 \cdot 10^{-4}$	Reid et al. (2009) (D,MR), Patel et al. (2016) (TR)
		30	1177.92	30	1.43		
		31.8	1076.88	31.8	1.50		
Chelonia mydas	22-29	26	1896	26	1.64	$-4.09 \cdot 10^{-2}$	Booth and Astill (2001) (D,MR),Spotila et al. (1987) (TR)
		30	1272	30	2.33		
Crocodylus johnsoni	28-34	29	2421.6	29	2.97	$2.32 \cdot 10^{-2}$	Whitehead and Seymour (1990) (D,MR,TR)
		31	1958.4	31	3.32		
Lampropholis guichenoti	24-28	25	962.4	25	$2.96 \cdot 10^{-2}$	$3.82 \cdot 10^{-2}$	Booth et al. (2000) (D,MR), Shine (1983) (TR)
		30	664.8	30	$4.90 \cdot 10^{-2}$		
Macrochelys temminckii	18-29	26.5	2232	26.5	$4.94 \cdot 10^{-1}$	$9.10 \cdot 10^{-7}$	Ligon and Lovern (2012) (D,MR,TR)
		28.5	1968	28.5	$5.59 \cdot 10^{-1}$		
		30.5	1896	30.5	$5.02 \cdot 10^{-2}$		
Sphenodon punctatus	15-25	18	8535.26	18	$8.76 \cdot 10^{-2}$	$1.64 \cdot 10^{-2}$	

		20	6388.37	20	$1.29 \cdot 10^{-1}$		Booth and Thompson (1991) (DR,MR), Thompson (1990) (TR)
		22	4119.26	22	$1.53 \cdot 10^{-1}$		
Trachemys scripta	18-29	26.5	1704	26.5	$2.76 \cdot 10^{-1}$		
		28.5	1416	28.5	$3.48 \cdot 10^{-1}$	$4.50 \cdot 10^{-3}$	Ligon and Lovern (2012) (D,MR,TR)
		30.5	1248	30.5	$3.54 \cdot 10^{-1}$		
Arthropoda							
Insecta (class):							
Brevicoryne brassicae	10-30	14.5	20	10.6	$5.90 \cdot 10^{-1}$		
		20.2	12.5	15	$9.50 \cdot 10^{-1}$		
		22.1	12.05	19.7	1.23		
		24.1	11.63	24.2	1.79	$3.60 \cdot 10^{-3}$	Lamb (1961) (D,MR,TR)
				28.2	2.82		
				35.3	2.88		
				37.1	2.18		
Chironomus sp.	13-33	12.5	95.8	5	$4.00 \cdot 10^{-1}$		
		17.5	51.6	10	$8.70 \cdot 10^{-1}$	$3.66 \cdot 10^{-2}$	Stevens (1998) (D,TR), McFarlane and McLusky (1972) (TR)
		22.5	28.3	15	1.31		

		27.5	20.2				
		32.5	19.2				
		37.5	5.3				
Culex pipiens	15-33	10	600	10	$5.72 \cdot 10^{-1}$		
		15	124	15	1.006		
		20	68	20	1.469	$1.25 \cdot 10^{-3}$	Richards (1964) (D,MR), Vinogradova (2000) (TR)
		25	38.5	25	2.558		
		30	30	30	3.645		
		35	29	35	5.331		
Drosophila melanogaster	16-29	14.95	67.90	17.5	$9.10 \cdot 10^{-4}$		
		20.07	33.44	22.5	$1.48 \cdot 10^{-3}$		
		25.06	20.39	27.5	$1.80 \cdot 10^{-3}$	$9.82 \cdot 10^{-3}$	Powsner (1935) (D), De Moed et al. (1998) (MR), Economos and Lints (1986) (TR)
		30.05	16.77				
		32	18.17				
Hippodamia convergens	10-35	0	5210	0	$3.85 \cdot 10^{-4}$		
		10	1337.20	10	$8.67 \cdot 10^{-4}$		
		20	343.21	20	$2.84 \cdot 10^{-3}$	$3.23 \cdot 10^{-2}$	Katsarou et al. (2005) (D), Acar et al. (2001) (MR, TR)
		30	88.09	30	$3.33 \cdot 10^{-3}$		

		40	22.61	40	$4.41 \cdot 10^{-3}$		
Melanoplus differentialis	16-32	15	2898	15	$3.75 \cdot 10^{-1}$		
		17.5	1334	17.5	$5.00 \cdot 10^{-1}$		
		20	810	20	$7.07 \cdot 10^{-1}$	$1.11 \cdot 10^{-3}$	Richards (1964) (D,MR), Swenk and Bratt (1941) (TR)
		25	520	25	1.12		
		30	315	30	1.58		
Melanoplus sanguinipes	14-39	13	2263.87	13	$4.29 \cdot 10^{-4}$		
		18	1365.24	18	$7.33 \cdot 10^{-4}$		
		27	922.24	27	$1.66 \cdot 10^{-3}$	$3.41 \cdot 10^{-2}$	Fielding (2004) (D,TR), Chappell (1983) (MR)
		31	669.33	31	$2.90 \cdot 10^{-3}$		
		41	510.45	41	$3.90 \cdot 10^{-3}$		
Oncopeltus fasciatus	13-35	13	1575	13	$2.60 \cdot 10^{-5}$		
		15	807	15	$3.50 \cdot 10^{-5}$	$2.24 \cdot 10^{-2}$	Richards and Suanraksa (1962) (D,MR), Baldwin and Dingle (1986) (TR)
		17.5	492	17.5	$5.90 \cdot 10^{-5}$		
		25	168	25	$1.19 \cdot 10^{-4}$		
Ostrinia nubialis	15-30	15	435	15	$2.69 \cdot 10^{-1}$		
		20	202	20	$4.66 \cdot 10^{-1}$	$2.75 \cdot 10^{-2}$	Richards (1964) (D,MR), Matteson and Decker (1965) (TR)
		25	139	25	$8.83 \cdot 10^{-1}$		

		30	82	30	1.19		
		35	74	35	1.56		
Polypedilum sp.	5-15	10	24.99	5	$2.10 \cdot 10^{-1}$	$2.03 \cdot 10^{-2}$	Mackey (1977) (D), McFarlane and McLusky (1972) (MR,TR)
		15	13.38	10	$5.50 \cdot 10^{-1}$		
		20	9.14	15	1.29		
Solenopsis invicta	17-24	15	1169.95	15	$6.10 \cdot 10^{-7}$	$-6.52 \cdot 10^{-3}$	Porter (1988) (D,TR), Elzen (1986) (MR)
		20	812.18	20	$4.00 \cdot 10^{-6}$		
		25	563.81	25	$7.06 \cdot 10^{-6}$		
		30	391.40	30	$9.49 \cdot 10^{-6}$		
		35	271.71	35	$1.38 \cdot 10^{-5}$		
		40	188.62	40	$1.86 \cdot 10^{-5}$		
Tribolium confusum	18-38	17.5	729.6	10	$1.18 \cdot 10^{-2}$	$9.09 \cdot 10^{-3}$	Howe (1960) (D,TR), Richards (1964) (MR)
		22.5	276	14	$1.65 \cdot 10^{-2}$		
		32.5	96	17	$2.37 \cdot 10^{-2}$		
		37.5	98.4	25	$5.24 \cdot 10^{-2}$		
Malacostraca (class):							
	15-24	12	322.82	12	$4.00 \cdot 10^{-2}$	$3.41 \cdot 10^{-2}$	

Neohelice granulate (*Chasmagnathus granulata*)		15	208.69	15	$4.90 \cdot 10^{-2}$		Ismael et al. (1997) (D,MR,TR)
		18	146.12	18	$5.80 \cdot 10^{-2}$		
		21	108.10	21	$7.20 \cdot 10^{-2}$		
		24	83.26	24	$1.12 \cdot 10^{-1}$		
Euphausia superba	-1-2	-1	394.8	-1	5.93		
		0	232.6	0	6.64	$2.37 \cdot 10^{-2}$	Ross et al. (1988) (D,TR), Quetin and Ross (1989) (MR)
		1	200.4	2	8.87		
		2	194.2				
Hyas araneus	3-15	2	1540.8	3	$9.70 \cdot 10^{-2}$		
		6	674.4	6	$2.01 \cdot 10^{-1}$		
		12	288	9	$2.45 \cdot 10^{-1}$	$3.68 \cdot 10^{-2}$	Anger (1983) (D,TR), Jacobi and Anger (1985) (MR)
		18	194.4	12	$2.76 \cdot 10^{-1}$		
				15	$3.01 \cdot 10^{-1}$		
				18	$3.22 \cdot 10^{-1}$		
Bryozoa							
Bugula neritina	12-24	12	110.08	12	$1.23 \cdot 10^{-2}$	$3.66 \cdot 10^{-3}$	This study
		16	50.59	16	$1.59 \cdot 10^{-2}$		

		20	36.67	20	$2.07 \cdot 10^{-2}$		
		24	33.41	24	$2.68 \cdot 10^{-2}$		
Echinodermata							
Dendraster excentricus	8-16	12	57	12	$6.00 \cdot 10^{-3}$	$2.40 \cdot 10^{-2}$	McEdward (1985) (D,MR), Bingham et al. (1997) (TR)
		17	36	17	$7.37 \cdot 10^{-3}$		
		22	24	22	$8.62 \cdot 10^{-3}$		
Strongylocentrotus purpuratus	4-20	6	63.5	13	$7.95 \cdot 10^{-3}$	$3.87 \cdot 10^{-2}$	Fujisawa (1993) (D), Padilla-Gamiño et al. (2013) (MR), Hammond and Hofmann (2010) (TR)
		7.5	53	18	$9.75 \cdot 10^{-3}$		
		10	41.25				
		13	31.5				
		16	26.33				
Mollusca							
Chorus giganteus	10-16	9	2496	9	$4.30 \cdot 10^{-2}$	$3.76 \cdot 10^{-2}$	Cancino et al. (2010) (D,MR,TR)
		12	1848	12	$4.20 \cdot 10^{-2}$		
		15	1464	15	$5.20 \cdot 10^{-2}$		

Octopus maya	15-27	18	797.33	18	2.96	$3.76 \cdot 10^{-2}$	Caamal-Monsreal et al. (2016) (D,MR,TR)
		22	626.18	22	3.12		
		26	571.38	26	4.48		
		30	467.20	30	3.36		
Stramonita canaliculata (*Thais haemastoma canaliculata*)	22-28	22	288	22	4.21	$1.35 \cdot 10^{-2}$	Roller and Stickle (1989) (D,MR,TR)
		28	192	28	6.17		

References

Acar, E. B., B. N. Smith, L. D. Hansen, and G. M. Booth. 2001. Use of calorespirometry to determine effects of temperature on metabolic efficiency of an insect. Environmental Entomology 30:811-816.

Alderdice, D. F., and F. P. J. Velsen. 1978. Relation between temperafure and incubation time for eggs of Chinook salmon (*Oncorhynchus tshawytscha*). Journal of the Fisheries Research Board of Canada 35:69-75.

Almatar, S. M. 1984. Effects of acute changes in temperature and salinity on the oxygen uptake of larvae of herring (*Clupea harengus*) and plaice (*Pleuronectes platessa*). Marine Biology 80:117-124.

Alton, L. A., C. R. White, R. S. Wilson, and C. E. Franklin. 2012. The energetic cost of exposure to UV radiation for tadpoles is greater when they live with predators. Functional Ecology 26:94-103.

Anger, K. 1983. Temperature and the larval development of *Hyas araneus* L. (Decapoda : Majidae); extrapolation of laboratory data to field conditions. Journal of Experimental Marine Biology and Ecology 69:203-215.

Avelar, T. 1993. Egg size in *Drosophila:* standard unit of investment or variable response to environment? The effect of temperature. Journal of Insect Physiology 39:283-289.

Azevedo, R. B. R., V. French, and L. Partridge. 1996. Thermal evolution of egg size in *Drosophila melanogaster*. Evolution 50:2338-2345.

Baldwin, J. D., and H. Dingle. 1986. Geographic variation in the effects of temperature on life-history traits in the large milkweed bug *Oncopeltus fasciatus*. Oecologia 69:64-71.

Bates, D., M. Machler, B. M. Bolker, and S. C. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1-48.

Baynes, S. M., and B. R. Howell. 1996. The influence of egg size and incubation temperature on the condition of *Solea solea* (L) larvae at hatching and first feeding. Journal of Experimental Marine Biology and Ecology 199:59-77.

Beuchat, C. A. 1988. Temperature effects during gestation in a viviparous lizard. Journal of Thermal Biology 13:135-142.

Bingham, B. L., M. Bacigalupi, and L. G. Johnson. 1997. Temperature adaptations of embryos from intertidal and subtidal sand dollars (*Dendraster excentricus*, Eschscholtz). Northwest Science 71:108-114.

Blanckenhorn, W. U. 2000. Temperature effects on egg size and their fitness consequences in the yellow dung fly *Scathophaga stercoraria*. Evolutionary Ecology 14:627-643.

Booth, D. T., and K. Astill. 2001. Incubation temperature, energy expenditure and hatchling size in the green turtle (*Chelonia mydas*), a species with temperature-sensitive sex determination. Australian Journal of Zoology 49:389-396.

Booth, D. T., and M. B. Thompson. 1991. A comparison of reptilian eggs with those of megapode birds, Pages 325-344 *in* D. C. Deeming, and G. W. Ferguson, eds. Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles, Cambridge University Press.

Booth, D. T., M. B. Thompson, and S. Herring. 2000. How incubation temperature influences the physiology and growth of embryonic lizards. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 170:269-276.

Bownds, C., R. Wilson, and D. J. Marshall. 2010. Why do colder mothers produce larger eggs? An optimality approach. Journal of Experimental Biology 213:3796-3801.

Bozek, M. A., L. J. Paulson, and G. R. Wilde. 1990. Effects of ambient Lake Mohave temperatures on development, oxygen consumption, and hatching success of the razorback sucker. Environmental Biology of Fishes 27:255-263.

Brannas, E. 1988. Emergence of Baltic salmon, *Salmo salar* L., in relation to temperature: a laboratory study. Journal of Fish Biology 33:589-600.

Bryson-Richardson, R., S. Berger, and P. Currie. 2011, Atlas of Zebrafish Development, Academic Press.

Burgess, S. C., and D. J. Marshall. 2011. Temperature-induced maternal effects and environmental predictability. Journal of Experimental Biology 214:2329-2336.

Caamal-Monsreal, C., I. Uriarte, A. Farias, F. Diaz, A. Sanchez, D. Re, and C. Rosas. 2016. Effects of temperature on embryo development and metabolism of *O. maya*. Aquaculture 451:156-162.

Cameron, J. N. 1986. The solubility of carbon dioxide as a function of temperature and salinity (Appendix table), Pages 254-259 *in* J. N. Cameron, ed. Principles of physiological measurement. United Kingdom, Academic Press.

Cancino, J. M., J. A. Gallardo, and A. Brante. 2010. The relationship between temperature, oxygen condition and embryo encapsulation in the marine gastropod *Chorus giganteus*. Journal of the Marine Biological Association of the United Kingdom 91:727-733.

Chappell, M. A. 1983. Metabolism and thermoregulation in desert and montane grasshoppers. Oecologia 56:126-131.

Collin, R. 2012. Temperature-mediated trade-offs and changes in life-history integration in two slipper limpets (Gastropoda: Calyptraeidae) with planktotrophic development. Biological Journal of the Linnean Society 106:763-775.

Collin, R., and M. Z. Salazar. 2010. Temperature-mediated plasticity and genetic differentiation in egg size and hatching size among populations of *Crepidula* (Gastropoda: Calyptraeidae). Biological Journal of the Linnean Society 99:489-499.

Collin, R., and A. Spangler. 2012. Impacts of adelphophagic development on variation in offspring size, duration of development, and temperature-mediated plasticity. Biological Bulletin 223:268-277.

Crill, W. D., R. B. Huey, and G. W. Gilchrist. 1996. Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. Evolution 50:1205-1218.

Crisp, D. J. 1971. Energy flow measurements, Pages 197-279 *in* N. A. Holme, and A. D. McIntyre, eds. Methods for the study of marine benthos. Oxford, Blackwell Sci.Pub.

De Moed, G. H., G. De Jong, and W. Scharloo. 1998. The energetics of growth in *Drosophila melanogaster*: Effect of temperature and food conditions. Netherlands Journal of Zoology 48:169-188.

Dufresne, F., and P. D. N. Hebert. 1998. Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex. Ecoscience 5:433-437.

Economos, A. C., and F. A. Lints. 1986. Developmental temperature and life span in *Drosophila melanogaster*. I. Constant developmental temperature: evidence for physiological adaptation in a wide temperature range. Gerontology 32:18-27.

Elliott, J. M., and J. A. Elliott. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. Journal of Fish Biology 77:1793-1817.

Elzen, G. W. 1986. Oxygen consumption and water loss in the imported fire ant *Solenopsis invicta* Buren. Comparative Biochemistry and Physiology A 84:13-17.

Ernsting, G., and A. Isaaks. 2000. Ectotherms, temperature, and trade-offs: Size and number of eggs in a carabid beetle. American Naturalist 155:804-813.
Ernsting, G., and J. A. Isaaks. 1997. Effects of temperature and season on egg size, hatchling size and adult size in *Notiophilus biguttatus*. Ecological Entomology 22:32-40.
Fielding, D. J. 2004. Developmental time of *Melanoplus sanguinipes* (Orthoptera : Acrididae) at high latitudes. Environmental Entomology 33:1513-1522.
Fischer, K., S. S. Bauerfeind, and K. Fiedler. 2006. Temperature-mediated plasticity in egg and body size in egg size-selected lines of a butterfly. Journal of Thermal Biology 31:347-354.
Fischer, K., A. N. M. Bot, P. M. Brakefield, and B. J. Zwaan. 2003a. Fitness consequences of temperature-mediated egg size plasticity in a butterfly. Functional Ecology 17:803-810.
Fischer, K., E. Eenhoorn, A. N. M. Bot, P. M. Brakefield, and B. J. Zwaan. 2003b. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. Proceedings of the Royal Society B-Biological Sciences 270:2051-2056.
Flatt, T., R. Shine, P. A. Borges-Landaez, and S. J. Downes. 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. Biological Journal of the Linnean Society 74:339-350.
Fox, C. J., A. J. Geffen, R. Blyth, and R. D. M. Nash. 2003. Temperature-dependent development rates of plaice (*Pleuronectes platessa* L.) eggs from the Irish Sea. Journal of Plankton Research 25:1319-1329.
Fujisawa, H. 1993. Temperature sensitivity of a hybrid between two species of sea urchin differing in thermotolerance. Development, Growth & Differentiation 35:395-401.
Geffen, A. J., C. J. Fox, and R. D. M. Nash. 2006. Temperature-dependent development rates of cod *Gadus morhua* eggs. Journal of Fish Biology 69:1060-1080.
Geiser, F., and R. S. Seymour. 1989. Influence of Temperature and Water Potential on Survival of Hatched, Terrestrial Larvae of the Frog *Pseudophryne bibronii*. Copeia 1989:207-209.
Giguere, L. A., B. Cote, and J. F. Stpierre. 1988. Metabolic rates scale isometrically in larval fishes. Marine Ecology Progress Series 50:13-19.
Gruber, K., and W. Wieser. 1983. Energetics of development of the alpine charr, *Salvelinus alpinus*, in relation to temperature and oxygen. Journal of Comparative Physiology 149:485-493.
Hammond, L. M., and G. E. Hofmann. 2010. Thermal tolerance of *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced gene expression and biogeographic patterns. Marine Biology 157:2677-2687.
Haylor, G. S., and M. F. A. Mollah. 1995. Controlled hatchery production of African catfish, *Clarias gariepinus*: The influence of temperature on early development. Aquatic Living Resources 8:431-438.
Herzig, A., and H. Winkler. 1986. The influence of temperature on the embryonic development of three cyprinid fishes, *Abramis brama*, *Chalcalburnus chalcoides mento* and *Vimba vimba*. Journal of Fish Biology 28:171-181.
Howe, R. W. 1960. The effects of temperature and humidity on the rate of development and mortality of *Tribolium confusum* Duval (Coleoptera, Tenebrionidae). Annals of Applied Biology 48:363-376.
Imai, C., and S. Tanaka. 1987. Effect of sea water temperature on egg size of Japanese Anchovy. Nippon Suisan Gakkaishi 53:2169-2178.
Imai, T. 1934. The influence of temperature on egg size and variation in *Drosophila melanogaster*. Wilhelm Roux Archiv Fur Entwicklungsmechanik Der Organismen 132:206-219.

Ismael, D., K. Anger, and G. S. Moreira. 1997. Influence of temperature on larval survival, development, and respiration in *Chasmagnathus granulata* (Crustacea, Decapoda). Helgolander Meeresuntersuchungen 51:463-475.

Jacobi, C. C., and K. Anger. 1985. Effect of temperature on respiration of larval stages of *Hyas araneus* and *H. coarctatus* (Decapoda, Majidae). Marine Ecology Progress Series 26:181-186.

Johnston, I. A., V. L. A. Vieira, and M. Abercromby. 1995. Temperature and myogenesis in embryos of the Atlantic herring *Clupea harengus*. The Journal of Experimental Biology 198:1389-1403.

Kamler, E., M. Szlaminska, M. Kuczynski, J. Hamackova, J. Kouril, and R. Dabrowski. 1994. Temperature-induced changes of early development and yolk utilization in the African catfish *Clarias gariepinus*. Journal of Fish Biology 44:311-326.

Kaplan, R. H. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. Oecologia 71:273-279.

Katsarou, I., J. T. Margaritopoulos, J. A. Tsitsipis, D. C. Perdikis, and K. D. Zarpas. 2005. Effect of temperature on development, growth and feeding of *Coccinella septempunctata* and *Hippodamia convergens* reared on the tobacco aphid, *Myzus persicae nicotianae*. Biocontrol 50:565-588.

Kimoto, K., S. Uye, and T. Onbé. 1986. Egg production of a brackish-water calanoid copepod *Sinocalanus tenellus* in relation to food abundance and temperature. Bulletin of Plankton Society of Japan 33:133-145.

Köster, M., C. Krause, and G. A. Paffenhofer. 2008. Time-series measurements of oxygen consumption of copepod nauplii. Marine Ecology Progress Series 353:157-164.

Kupren, K., A. Mamcarz, D. Kucharczyk, and S. Krejszeff. 2008. Influence of water temperature on eggs incubation time and embryonic development of fish from genus *Leuciscus*. Polish Journal of Natural Science 23:461-481.

Lamb, K. P. 1961. Some effects of fluctuating temperatures on metabolism, development, and rate of population growth in the cabbage aphid, *Brevicoryne brassicae*. Ecology 42:740-745.

Laurence, G. C. 1975. Laboratory growth and metabolism of the winter flounder *Pseudopleuronectes americanus* from hatching through metamorphosis at three temperatures. Marine Biology 32:223-229.

—. 1978. Comparative growth, respiration and delayed feeding abilities of larval cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) as influenced by temperature during laboratory studies. Marine Biology 50:1-7.

Laurence, G. C., and C. A. Rogers. 1976. Effects of temperature and salinity on comparative embryo development and mortality of Atlantic cod (*Gadus morhua* L.) and haddock (*Melanogrammus aeglefinus* (L.)). ICES Journal of Marine Science 36:220-228.

Liefting, M., M. Weerenbeck, C. van Dooremalen, and J. Ellers. 2010. Temperature-induced plasticity in egg size and resistance of eggs to temperature stress in a soil arthropod. Functional Ecology 24:1291-1298.

Ligon, D. B., and M. B. Lovern. 2012. Interspecific variation in temperature effects on embryonic metabolism and development in turtles. ISRN Zoology 2012:13.

Machacek, J., and J. Seda. 2013. Over-wintering *Daphnia*: uncoupling the effects of temperature and food on offspring size and filtering screen morphology in *D. galeata*. Journal of Plankton Research 35:1069-1079.

Mackey, A. P. 1977. Growth and larval development of larval Chironomidae. Oikos 28:270-275.

Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131-3137.
Marshall, D. J., and M. J. Keough. 2003. Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. Marine Ecology Progress Series 255:145-153.
Martell, D. J., J. D. Kieffer, and E. A. Trippel. 2005. Effects of temperature during early life history on embryonic and larval development and growth in haddock. Journal of Fish Biology 66:1558-1575.
Matteson, J. W., and G. C. Decker. 1965. Development of the European corn borer at controlled constant and variable temperatures. Journal of Economic Entomology 58:344-349.
McCarthy, I. D. 2000. Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. Journal of Fish Biology 57:224-238.
McDowell, R. M. 2000, The Reed field guide to New Zealand freshwater fishes. Auckland.
McEdward, L. R. 1985. Effects of temperature on the body form, growth, electron transport system activity, and development rate of an echinopluteus. Journal of Experimental Marine Biology and Ecology 93:169-181.
McFarlane, A., and D. S. McLusky. 1972. The oxygen consumption of chironomid larvae from loch leven in relation to temperature. Comparative Biochemistry and Physiology Part A: Physiology 43:991-1001.
Mendiola, D., L. Ibaibarriaga, and P. Alvarez. 2007. Thermal effects on growth and time to starvation during the yolk-sac larval period of Atlantic mackerel *Scomber scombrus* L. Journal of Fish Biology 70:895-910.
Mitchell, N. J., and R. S. Seymour. 2000. Effects of temperature on energy cost and timing of embryonic and larval development of the terrestrially breeding moss frog, *Bryobatrachus nimbus*. Physiological and Biochemical Zoology 73:829-840.
Mueller, C. A., J. Eme, R. G. Manzon, C. M. Somers, D. R. Boreham, and J. Y. Wilson. 2015. Embryonic critical windows: changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 185:315-331.
Mueller, C. A., J. M. P. Joss, and R. S. Seymour. 2011. The energy cost of embryonic development in fishes and amphibians, with emphasis on new data from the Australian lungfish, *Neoceratodus forsteri*. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 181:43-52.
Padilla-Gamiño, J. L., M. W. Kelly, T. G. Evans, and G. E. Hofmann. 2013. Temperature and CO2 additively regulate physiology, morphology and genomic responses of larval sea urchins, *Strongylocentrotus purpuratus*. Proceedings of the Royal Society B: Biological Sciences 280.
Pankhurst, N. W., G. J. Purser, G. Van Der Kraak, P. M. Thomas, and G. N. R. Forteath. 1996. Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. Aquaculture 146:277-290.
Patel, S. H., S. J. Morreale, V. S. Saba, A. Panagopoulou, D. Margaritoulis, and J. R. Spotila. 2016. Climate impacts on sea turtle breeding phenology in Greece and associated foraging habitats in the wider Mediterranean region. Plos One 11.
Perrin, N. 1988. Why are offspring born larger when it is colder? Phenotypic plasticity for offspring size in the cladoceran *Simocephalus vetulus* (Muller). Functional Ecology 2:283-288.

Pettersen, A. K., C. R. White, R. J. Bryson-Richardson, and D. J. Marshall. 2017. Does the cost of development scale allometrically with offspring size? Functional Ecology:n/a-n/a.
Pettersen, A. K., C. R. White, and D. J. Marshall. 2015. Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proceedings. Biological Sciences / The Royal Society 282.
Porter, S. D. 1988. Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. Journal of Insect Physiology 34:1127-1133.
Powsner, L. 1935. The effects of temperature on the durations of the developmental stages of *Drosophila melanogaster*. Physiological Zoology 8:474-520.
Quetin, L. B., and R. M. Ross. 1989. Effects of oxygen, temperature and age on the metabolic rate of the embryos and early larval stages of the Antarctic krill *Euphausia superba* Dana. Journal of Experimental Marine Biology and Ecology 125:43-62.
Réalis-Doyelle, E., A. Pasquet, D. De Charleroy, P. Fontaine, and F. Teletchea. 2016. Strong effects of temperature on the early life stages of a cold stenothermal fish species, brown trout (*Salmo trutta* L.). PLoS ONE 11:e0155487.
Reid, K. A., D. Margaritoulis, and J. R. Speakman. 2009. Incubation temperature and energy expenditure during development in loggerhead sea turtle embryos. Journal of Experimental Marine Biology and Ecology 378:62-68.
Richards, A. G. 1964. The generality of temperature effects on developmental rate and on oxygen consumption in insect eggs. Physiological Zoology 37:199-211.
Richards, A. G., and S. Suanraksa. 1962. Energy expenditure during embryonic development under constant versus variable temperatures (*Oncopeltus fasciatus* (Dallas)). Entomologia Experimentalis et Applicata 5:167-178.
Roller, R. A., and W. B. Stickle. 1989. Temperature and salinity effects on the intracapsular development, metabolic rates, and survival to hatching of *Thais haemastoma canaliculata* (Gray) (Prosobranchia: Muricidae) under laboratory conditions. Journal of Experimental Marine Biology and Ecology 125:235-251.
Rombough, P. J. 1988. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. Canadian Journal of Zoology-Revue Canadienne De Zoologie 66:651-660.
—. 1994. Energy patitioning during fish development: additive or compensatory allocation of energy to support growth? Functional Ecology 8:178-186.
Ross, R. M., L. B. Quetin, and E. Kirsch. 1988. Effect of temperature on developmental times and survival of early larval stages of *Euphausia superba* Dana. Journal of Experimental Marine Biology and Ecology 121:55-71.
Ryland, J. S., and J. H. Nichols. 1975. Effect of temperature on the embryonic development of the plaice, *Pleuronectes Platessa* L. (teleostei). Journal of Experimental Marine Biology and Ecology 18:121-137.
Sakwinska, O. 1998. Plasticity of *Daphnia magna* life history traits in response to temperature and information about a predator. Freshwater Biology 39:681-687.
Seko, T., and F. Nakasuji. 2006. Adaptive significance of egg size plasticity in response to temperature in the migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperiidae). Population Ecology 48:159-166.
Seymour, R. S., F. Geiser, and D. F. Bradford. 1991. Metabolic cost of development in terrestrial frog eggs (*Pseudophryne bibronii*). Physiological Zoology 64:688-696.
Seymour, R. S., and J. D. Roberts. 1995. Oxygen uptake by the aquatic eggs of the Australian frog *Crinia georgiana*. Physiological Zoology 68:206-222.
Sheader, M. 1996. Factors influencing egg size in the gammarid amphipod *Gammarus insensibilis*. Marine Biology 124:519-526.

Shine, R. 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. Oecologia 57:397-405.
Shrode, J. B., and S. D. Gerking. 1977. Effects of constant and fluctuating temperatures on reproductive performance of a desert pupfish, *Cyprinodon n. nevadensis*. Physiological Zoology 50:1-10.
Spotila, J. R., E. A. Standora, S. J. Morreale, and G. J. Ruiz. 1987. Temperature-dependent sex determination in the green turtle (*Chelonia mydas*) - effects on the sex-ratio on a natural nesting beach. Herpetologica 43:74-81.
Stam, E. M., M. A. vandeLeemkule, and G. Ernsting. 1996. Trade-offs in the life history and energy budget of the parthenogenetic collembolan *Folsomia candida* (Willem). Oecologia 107:283-292.
Steigenga, M. J., and K. Fischer. 2007. Ovarian dynamics, egg size, and egg number in relation to temperature and mating status in a butterfly. Entomologia Experimentalis Et Applicata 125:195-203.
Stevens, M. 1998. Development and survival of *Chironomus tepperi* Skuse (Diptera: Chironomidae) at a range of constant temperatures. Aquatic Insects 20:181-188.
Sun, D., and C. J. Niu. 2012. Adaptive significance of temperature-induced egg size plasticity in a planktonic rotifer, *Brachionus calyciflorus*. Journal of Plankton Research 34:864-873.
Swenk, M. H., and C. H. Bratt. 1941. The relation of temperature to the embryonic and nymphal development of the differential grasshopper *Melanoplus differentialis* Thomas. Research Bulletin: Bulletin of the Agricultural Experiment Station of Nebraska 122.
Teletchea, F., J. N. Gardeur, E. Kamler, and P. Fontaine. 2009. The relationship of oocyte diameter and incubation temperature to incubation time in temperate freshwater fish species. Journal of Fish Biology 74:652-668.
Thompson, M. B. 1990. Incubation of eggs of tuatara, *Sphenodon punctatus*. Journal of Zoology 222:303-318.
Van Voorhies, W. A. 1996. Bergmann size clines: A simple explanation for their occurrence in ectotherms. Evolution 50:1259-1264.
Vinogradova, E. B. 2000, *Culex pipiens pipiens* Mosquitoes: Taxonomy, Distribution, Ecology, Physiology, Genetics, Applied Importance and Control, Coronet Books Incorporated.
Wehrtmann, I. S., and G. A. Lopez. 2003. Effects of temperature on the embryonic development and hatchling size of *Betaeus emarginatus* (Decapoda Caridea Alpheidae). Journal of Natural History 37:2165-2178.
Wendt, D. E., and R. M. Woollacott. 1999. Ontogenies of phototactic behavior and metamorphic competence in larvae of three species of *Bugula* (Bryozoa). Invertebrate Biology 118:75-84.
Westerfield, M. 2000, The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). Eugene, University of Oregon Press.
White, C. R., M. R. Kearney, P. G. D. Matthews, S. A. L. M. Kooijman, and D. J. Marshall. 2011. A Manipulative Test of Competing Theories for Metabolic Scaling. American Naturalist 178:746-754.
Whitehead, P. J., and R. S. Seymour. 1990. Patterns of metabolic rate in embryonic crocodilians *Crocodylus johnstoni* and *Crocodylus porosus*. Physiological Zoology 63:334-352.
Wieser, W., and H. Forstner. 1986. Effects of temperature and size on the routine rate of oxygen consumption and on the relative scope for activity in larval cyprinids. Journal of

Comparative Physiology B-Biochemical Systemic and Environmental Physiology 156:791-796.
Willott, S. J., and M. Hassall. 1998. Life-history responses of British grasshoppers (Orthoptera : Acrididae) to temperature change. Functional Ecology 12:232-241.
Wood, A. H. 1932. The effect of temperature on the growth and respiration of fish embryos (*Salmo Fario*). Journal of Experimental Biology 9:271-276.

General Introduction - Section 2

Understanding variation in metabolic rate

Amanda K. Pettersen[1], Dustin J. Marshall[1], Craig R. White[1]

[1] School of Biological Sciences/Centre for Geometric Biology, Monash University, Melbourne, Victoria, Australia

Abstract

Metabolic rate reflects an organism's capacity for growth, maintenance and reproduction and is likely to be a target of selection. Physiologists have long sought to understand the causes and consequences of within-individual to among-species variation in metabolic rates– how metabolic rates relate to performance and how they should evolve. Traditionally, this has been viewed from a mechanistic perspective, relying primarily on hypothesis-driven approaches. A more agnostic, but ultimately more powerful tool for understanding the dynamics of phenotypic variation is through use of the breeder's equation, because variation in metabolic rate is likely to be a consequence of underlying microevolutionary processes. Here we show that metabolic rates are often significantly heritable, and are therefore free to evolve under selection. We note, however, that "metabolic rate" is not a single trait: in addition to the obvious differences between metabolic levels (e.g. basal, resting, free-living, maximal), metabolic rate changes through ontogeny and in response to a range of extrinsic factors, and is therefore subject to multivariate constraint and selection. We emphasise three key advantages of studying metabolic rate within a quantitative genetics framework: its formalism, and its predictive and comparative power. We make several recommendations when applying a quantitative genetics framework: i) measuring selection based on actual fitness, rather than proxies for fitness; ii) considering the genetic covariances between metabolic rates throughout ontogeny; and iii) estimating genetic covariances between metabolic rates and other traits. A quantitative genetics framework provides the means for quantifying the evolutionary potential of metabolic rate and why variance in metabolic rates within populations might be maintained.

Introduction

Metabolic rate reflects the 'pace of life' and is one of the most widely measured physiological traits. Metabolic rate has been linked to key physiological and life-history traits, including survival, growth, immunity, predation and reproductive output. While metabolic rate is somewhat predictable – allometric scaling between mass and metabolic rate is widespread, for example – variation is still substantial. Among species, there is a several-fold magnitude of difference in basal metabolic rate among individuals of the same mass (White and Kearney 2013). At the level at which selection operates (that is, within species) basal metabolic rate can also vary considerably (Konarzewski and Ksiazek 2013). This variation has long intrigued physiologists and various hypotheses have been proposed to understand it (Glazier 2005). As such, the field has been dominated by studies that seek to understand the proximal causes of variation – the biochemical and physiological mechanisms underlying the response of metabolic rate to biotic and abiotic drivers (as reviewed by Glazier, 2005). For example, the rate-of-living hypothesis (Rubner 1908) proposes that metabolic rate inversely determines longevity, based on observations that species with higher metabolic rates have shorter lifespans, although this remains controversial (Glazier 2015; Speakman 2005). More recent mechanistic explanations that seek to link metabolic rate to the pace of life have been proposed (Nilsson 2002). The 'compensation hypothesis' (or 'allocation hypothesis') suggests a fitness advantage of lower basal or standard metabolic rates as a result of lower maintenance costs, and thereby greater allocation of energy to reproduction (Gadgil and Bossert 1970; Larivee et al. 2010; Steyermark 2002). Alternatively, higher basal or standard metabolic rates allow for greater energy turnover and synthesis and maintenance of larger organs, leading to greater reproductive yield, known as the 'increased-intake hypothesis' (Bennett and Ruben 1979; Hayes et al. 1992). While these approaches may assign causation to an immediate response,

exclusively mechanistic approaches have had limited success predicting how traits evolve. One key limitation with the mechanistic approach is that it lacks standardised methods to compare across studies. Meanwhile, phenomenological approaches such as those used in evolutionary biology are underutilised in studies of metabolic rate.

Evolutionary biology seeks to understand the ultimate causes of variation in traits – causes that are a consequence of many generations of selection (Mayr 1961). Darwin first observed that natural selection operating within populations ultimately shapes heritable differences among species. Estimates of the heritability of metabolic rate vary widely, but are often more than zero (see genetic variation in metabolic rates section below). Within-population studies elucidate the selective forces acting on individuals, and the underlying genetic processes that constrain their evolution. To understand patterns in metabolic rates, and predict how they are likely to evolve under selection, it is necessary to measure 'performance' as fitness – the lifetime reproductive output of an individual – and to determine how fitness covaries with metabolic rates throughout ontogeny. While metabolic rate is likely to evolve in response to selection, underlying genetic constraints may alter its evolution in ways that have yet to be considered in many physiological studies (Arnold 1988), with some notable exceptions (e.g. Garland and Carter 1994). We argue that quantitative genetics provides a powerful framework for understanding the inheritance and evolution of traits, including their responses to selection.

Quantitative genetics partitions the population-level phenotypic variation of quantitative traits into heritable and non-heritable components through measures of heritability and genetic correlation, and links those components to fitness via measures of selection. We emphasise three key advantages of studying metabolic rate within a quantitative genetics framework. 1) Formalism: evolutionary biologists have been thinking about the ultimate processes driving variation in traits since Darwin; microevolutionary theory and the powerful

statistical tools developed from this work have been widely applied in the evolution community for over 40 years, and can be leveraged by physiological studies. 2) Predictive: microevolutionary approaches allow us to quantify how traits are likely to evolve given specific selection and genetic parameters. 3) Comparative: quantitative genetics provides standardised estimates of selection and heritability that are directly comparable among populations, species and environments. Here we advocate for wider adoption of the quantitative genetics approach in physiological studies in order to gain insights into evolutionary causes and consequences of variation in metabolic rate.

The breeder's equation as a framework

The breeder's equation is a fundamental tool used in quantitative genetics for understanding phenotypic evolution in response to selection and has been used by evolutionary biologists for over 50 years. Quantitative traits have phenotypes that are continuously distributed in natural populations, and include morphological, physiological, behavioural and molecular phenotypes. Like other quantitative traits, metabolic rates are likely to be genetically complex and sensitive to environmental conditions. Quantitative genetic variation underlies phenotypic evolution – measuring the genetic basis of variation in quantitative traits is therefore essential to understanding variation in phenotypes, such as metabolic rates. The univariate breeder's equation predicts the amount of change in a single trait from one generation to the next in response to selection. The response of a quantitative trait to selection, R, is described by the breeder's equation $R = h^2S$, where h^2 is narrow-sense heritability (the ratio of additive genetic variance to total phenotypic variance; see section on genetic variation in metabolic rates below) and S is the selection differential (the change in population mean after selection). The breeder's equation serves as a simple, but powerful, tool for understanding variation in metabolic rate and other physiological traits.

Univariate selection on metabolic rate

Selection is the phenotypic covariance between a trait and fitness, where fitness of an individual is determined by the contribution of offspring to the next generation (Falconer and Mackay 1996). If fitness covaries with a trait, then that trait is said to be under selection. This relative difference in fitness among phenotypes (selection) forms one half of the breeder's equation and provides a standardised estimate of the strength and direction in which evolution is expected to occur, if the trait has adequate genetic variation. The slope of the relationship between relative fitness and a particular character, weighted by the phenotype distribution, represents standardised estimates of selection.

Two general forms of univariate selection can occur: linear and quadratic selection (**Box 1**). Linear selection occurs when fitness (w) consistently increases or decreases with the value of a trait (z), and is fit by a linear function, $w = \alpha + \beta z$, where α is the intercept of the fitness function and β is the coefficient giving the direction (positive or negative) and magnitude of selection. If a trait exhibits sufficient genetic variation (i.e. if it is heritable) and not constrained by other traits that are also correlated with fitness (see metabolic rate is more than a single trait section below), persistent directional selection *should* result in a shift in the mean trait of a population (Kingsolver and Pfennig 2007). Quadratic selection is characterised by a nonlinear fitness function that can also be positive (disruptive) or negative (stabilising), and is described by the quadratic fitness function

$$w = \alpha + \beta z + (1/2)\gamma z^2, \qquad (1)$$

where γ is the degree of curvature in the fitness function. Selection is stabilising when β is 0 and γ is negative, such that intermediate values of a trait possess highest fitness while extreme trait values have lowest fitness. Selection is disruptive when β is 0 and γ is positive. Where disruptive selection is maintained across generations, population variance will increase as

selection favours trait values on the tail ends of the trait distribution. Under constant stabilising selection, there is a single optimal value for a phenotype, hence variance in population traits would be expected to decrease over generations. Note that quadratic selection can occur when $\beta \neq 0$ – this is termed either concave (stabilising) or convex (disruptive) selection. By providing comparable estimates of selection on metabolic rate, selection analyses have the potential to leverage comparative data (i.e comparing values of β and γ) that vary across spatial and temporal scales, study systems and phenotypic characters (Kingsolver et al. 2001). Indeed, the idea that a single value of a trait is consistently beneficial under all circumstances seems unlikely, and the same is true of metabolic rate. Spatial and temporal variation in selection therefore seems likely to be a mechanism by which variance in metabolic rate is maintained. To some extent, selection analyses have already been implemented in the general mechanisms that have been proposed to explain variation in metabolic rates – that is, covariance between metabolic rate and some measure of performance (fitness). The increased-intake and compensation hypotheses point towards positive and negative directional selection on (basal or standard) metabolic rate, respectively, for example, while the 'context dependent' hypothesis (Burton et al. 2011) points toward selection gradients that vary in space and time. The approach we advocate is therefore not incompatible with proximate mechanistic approaches, rather selection analyses provide the formalism and standardised measures required to make comparable estimates for the relationship between metabolic rate and fitness.

In order to gain reliable estimates of selection, studies need to measure actual fitness. So far, selection studies on metabolic rate have relied almost exclusively on the use of fitness proxies, such as survival, growth, or reproductive traits such as clutch size, rather than the ultimate measure of fitness: lifetime reproductive output (**Box 2**). This view is illustrated by the compilations of Biro and Stamps (2010), Burton et al. (2011), and White and Kearney (2013). The tables summarising the known phenotypic correlations between metabolic rate and

fitness proxies in these papers do not provide any examples of a correlation between metabolic rate and actual fitness.

Using fitness proxies can create misleading or incomplete interpretations of the strength and direction of selection if these proxies trade-off with actual fitness. For example, Pettersen et al. (2016) show that metabolic rates through ontogeny covary with actual fitness (lifetime reproductive output) as well as several fitness proxies, but the direction and magnitude of the covariance differs among measurements of metabolic rate. Fitness was maximised when individuals had low metabolic rates early in ontogeny (MR_E) but high metabolic rates later (MR_L) (or vice versa). While we found evidence for correlational selection alone based on true fitness, estimates based on fitness proxies incorrectly implied that directional selection was operating. For example, individuals with higher MR_E reproduced sooner, but individuals with lower MR_L were longer lived, and growth rate was maximised when MR_E was high and MR_L was low. In this case, using any of the commonly used proxies for fitness (growth rate, longevity, age at the onset of reproduction) would lead to wildly different, and incorrect, conclusions about the expected evolutionary trajectory of metabolic rate.

Genetic variation in metabolic rates

As the breeder's equation elegantly illustrates, selection on a trait will not generate evolution of that trait unless the trait is heritable. The capacity for metabolic rates to evolve thus depends not only on covariation between metabolic rate and fitness, but also on the other half of the breeder's equation – the genetic basis of variation in metabolic rate. The total phenotypic variance of a trait (V_P) is the sum of the variances attributable to genetic (V_G) and environmental (V_E) influences (including maternal effects), and the variance associated with the interaction between genetic and environmental influences (V_{GE}). V_G can be further subdivided into three components: additive (V_A), dominance (V_D) and interaction (V_I)

variance, where collectively V_D and V_I are known as nonadditive genetic variance and are not easily disentangled using standard quantitative genetics designs. V_A quantifies deviations from the mean phenotype attributable to the additive contribution of particular alleles to the phenotype; V_D quantifies interactions between alleles (dominance) and V_I quantifies interactions between alleles (epistasis). Heritability in the broad sense (H^2) is calculated as V_G/V_P, whereas heritability in the narrow sense (h^2) – the metric of interest for the breeder's equation – quantifies the contribution of additive genetic variance to total phenotypic variance and is calculated as V_A/V_P.

The heritability of a trait can be estimated in multiple ways (**Box 3**), but a common feature of all approaches is that they require the measurement of usually hundreds or thousands of individuals of known pedigree. The requirement to measure so many individuals means that estimates of h^2 for metabolic rate are historically rare, but are becoming much more common: we are aware of only two estimates published prior to 2000 (Lacy and Lynch 1979; Lynch and Sulzbach 1984), and most (43) of the remaining 64 estimates we were able to locate have been published since 2010. The available estimates range from 0 to 0.72, h^2 is significantly higher for endotherms than for ectotherms, and h^2 is significantly higher for active metabolic levels than for resting metabolic levels, defined here as the rate of oxygen consumption of an inactive, non-reproductive, postabsorptive animal (**Box 4**). These heritability estimates suggest that metabolic rate is, in many cases and especially for endotherms and for active metabolic rates, likely to be free to evolve under selection. In support of this suggestion, artificial selection experiments have yielded responses to selection on basal metabolic rate (Ksiazek et al. 2004) and maximum metabolic rate in laboratory mice (Gebczynski and Konarzewski 2009; Wone et al. 2015), and maximum metabolic rate in bank voles *Clethrionomys glareolus* (Sadowska et al. 2015).

After accounting for genetic contributions to phenotypic variance, there remains a significant proportion of unexplained variation in metabolic rate that needs to be considered. Variation in metabolic rate may also be a consequence of environmental effects, which can affect metabolic rate either directly (e.g. temperature effects on metabolic rate in ectotherms; Angilletta et al. 2002), or indirectly (e.g. nutritional state on standard metabolic rate; Auer et al. 2015). Parental effects are also known to influence physiological traits (e.g. Bacigalupe et al. 2007; Sadowska et al. 2013). For example, brown trout may alter the routine metabolic rates of their offspring in order to control timing of emergence and therefore dispersal in larvae (Régnier et al. 2010). Addressing the relative importance of heritable versus non-heritable components of variation in metabolic rate will provide a more complete picture of how we expect variation in metabolic rate to evolve.

Multivariate breeder's equation

The univariate breeder's equation is a useful heuristic tool for understanding how microevolutionary processes work. Increasingly however it seems that a more complex approach to predicting microevolution is necessary. The univariate breeder's equation necessarily treats each trait in isolation but it has long been recognised that no trait is an island (Dobzhansky 1956). Traits covary with each other genetically such that evolution in one trait will necessarily cause evolution in another, and selection often acts on multiple traits simultaneously such that the fitness returns of one trait value depend on the value of other traits. The multivariate breeder's equation reflects this complexity and connectedness of traits in terms of both genetics and selection.

Consider the response to selection of a trait, we'll call trait 1 (z_1). As described by the univariate breeder's equation, the evolution of that trait will of course depend on the selection on that trait (β_1) and the genetic variation in that trait (which we will denote as $G_{1,1}$). But let us

suppose that another trait (trait 2) covaries genetically with trait 1, such a covariance would be denoted as $G_{1,2}$. Let us also suppose that trait 2 is under selection (β_2). The response of trait 1 (Δz_1) will therefore be the sum of the evolution due to direct selection on trait 1 and the *indirect* selection on trait 1 via the genetic covariance with trait 2 and selection on trait 2, or formally:

$$\Delta z_1 = (\beta_1 \text{ x } G_{1,1}) + (\beta_2 \text{ x } G_{1,2}) \qquad (2)$$

Furthermore, the covariance between traits 1 and 2 will also be affected by the correlational selection on these traits, formally represented as $\gamma_{1,2}$ (see below). Equation (2) can be extended to as many traits that genetically covary and experience selection:

$$\begin{bmatrix} \Delta z_1 \\ \Delta z_2 \\ \vdots \\ \Delta z_n \end{bmatrix} = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_n \end{bmatrix} \begin{bmatrix} G_{1,1} & G_{1,2} & \cdots & G_{1,n} \\ G_{1,2} & G_{2,2} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ G_{1,n} & \cdots & \cdots & G_{n,n} \end{bmatrix},$$

where the column vector of changes in phenotypic trait values for *n* traits, $\mathbf{\Delta z} = \{\Delta z_1, \Delta z_2, \ldots, \Delta z_n\}^T$, is a function of a column vector of selection gradients $\mathbf{\beta} = \{\beta_1, \beta_2, \ldots\}^T$ and a matrix of genetic variances and covariances (the **G** matrix). While more complicated, a quick consideration of a realistic but simple example reveals why the multivariate equation provides a more complete understanding of the microevolutionary forces acting on metabolic rate. Suppose for example that trait 1 is metabolic rate and trait 2 is running speed in a hypothetical lizard species. Further assume that metabolic rate is subjected to strong negative directional selection (i.e. β_1 is negative) and that the heritability of metabolic rate is high, because the trait has significant additive genetic variance ($G_{1,1} > 0$). The univariate breeder's equation and the first component of the multivariate breeder's equation (β_1 x $G_{1,1}$) would therefore predict that metabolic rate would decrease from one generation to the next. However, further suppose that metabolic rate covaries positively with running speed ($G_{1,2}$ is positive) and there is strong positive directional selection for faster running speeds (β_2 is positive). The second component of the multivariate breeder's equation (β_2 x $G_{1,2}$) would therefore be highly positive and might

'cancel out' the selection for lower metabolic rate in the first term. Thus, by considering more traits, we move from a misleading prediction of an evolutionary response to a more accurate one. Unfortunately, there is no magic number of traits that should be considered, instead we are left with the rather unsatisfying statement that more traits are likely to be more informative than fewer traits. A multivariate view of evolution is particularly important for considerations of metabolic rate specifically for at least two reasons. First, because metabolic rate is likely to be more than just a single trait and second, because metabolic rate is almost certainly under multivariate selection.

Metabolic rate is more than a single trait

What is metabolic rate? Measures of metabolic rate integrate the rates at which organisms expend energy to do metabolic work, and so incorporate energy expenditure for a wide range of processes including the maintenance of homeostasis, growth and reproduction, movement, and digestion. Metabolic rate is measured as the rate of heat production by direct calorimetry, or – more often – is estimated from rates of oxygen consumption or carbon dioxide production measured by indirect calorimetry (Lighton 2008). Metabolic rate can be measured for animals that are free-living in the field; for animals at rest; for animals experiencing elevated metabolic rates due to exercise, digestion, lactation, thermogenesis, or osmoregulation; or for animals exhibiting depressed metabolism due to hibernation or torpor, hypoxia or anoxia, desiccation, or aestivation (Suarez 2012). The major contributors to whole-organism metabolic rate will change as animals transition through these metabolic states, raising the important question of the extent to which they are constrained to always evolve together ("metabolic rate" is a single trait), or free to evolve independently ("metabolic rate" is many traits). In mammals, most metabolic activity during basal metabolism is associated with the internal organs including liver, kidney, gastrointestinal tract, heart, and brain. Whereas during exercise-induced maximal

metabolism, most (ca 90 %) metabolic activity is associated with work done by the locomotor muscles and the work done to deliver substrates and oxygen to these (reviewed by White and Kearney 2013). From a mechanistic perspective, it therefore seems reasonable to conclude that these metabolic states represent different traits. From a quantitative genetics perspective, however, what matters is the extent to which two putative traits covary genetically. Published mass-independent additive genetic correlations between basal and running-induced maximal metabolic rate range from 0.21 to 0.72 (Dohm et al. 2001; Wone et al. 2009; Wone et al. 2015). Thus, these traits – basal and maximal metabolic rate – are at least somewhat free to evolve independently, as has been demonstrated in selection experiments (Sadowska et al. 2015; Wone et al. 2015). What is less clear, however, is the extent to which measurements of a single metabolic state, but taken at different times, represent the same trait.

Two measurements of the same phenotype can be considered a single trait genetically only if they covary perfectly. Resting metabolic rate (as defined earlier), is perhaps the most widely measured physiological phenotype. Resting metabolic rate is repeatable (Auer et al. 2016a; Nespolo and Franco 2007; White and Kearney 2013) and heritable (see section on genetic variation in metabolic rates, above, and Box 4), but not perfectly so. It varies during ontogeny due to changes in size and growth (e.g. Moran et al., 2007; Rosenfeld et al., 2015), seasonally (e.g. Smit and McKechnie 2010), geographically (e.g. Broggi et al 2007), with food deprivation (e.g. Schimpf et al., 2013), due to changes mitochondrial coupling (Salin et al. 2015), and in response to a range of other biotic and abiotic variables (reviewed by Konarzewski and Książek, White and Kearney 2013). Furthermore, not only does metabolic rate vary over time in the same individuals, but individuals can vary in the flexibility of their metabolic rate – in other words the reaction norm of metabolic rate varies among individuals (Auer et al. 2016b; Auer et al. 2015). Thus, an organism has no single metabolic rate, even for a single well-defined metabolic state (e.g. resting metabolic rate), and metabolic rate is

therefore likely to be more than one single trait. Even if differences in metabolic rate throughout the life history were trivial, we know from a previous study that selection perceives metabolic rates (and their combinations) differently (Pettersen et al. 2016). In Pettersen et al. (2016), metabolic rate was only measured at two time points in the life history – both during early stages of development, which is unlikely to capture a complete picture of selection. We therefore suggest that the field should work towards gaining multiple measures of metabolic rate if we are to gain an accurate representation of net selection on metabolic rates. We acknowledge the considerable logistical challenges associated with doing so, but we nonetheless advocate treating metabolic rate at different times as separate traits as a useful heuristic for future studies.

Multivariate selection on metabolic rates

Selection acts on combinations of traits, rather than individual traits in isolation (Blows and McGuigan 2015; Lande and Arnold 1983). Multivariate (or nonlinear correlational) selection examines how selection affects, and is affected by correlations between traits (Phillips and Arnold 1989). Studies measuring selection on metabolic rate have largely focussed on relationships between fitness and single traits (although see Artacho et al., 2015), however univariate analyses provide limited scope for predicting change in phenotypic distribution (Phillips and Arnold 1989). This is because apparent selection on one trait may be due to selection on another unmeasured, correlated trait – resulting in misleading conclusions about selection on the initial trait. Genetically coupled traits will not evolve independently – selection on one trait is likely to cause evolutionary changes in the other trait. For example, selection on metabolic rate early in ontogeny (MR_E) may yield a correlated response in metabolic rate late in ontogeny (MR_L) if MR_E and MR_L are positively genetically correlated, even if there is no direct selection on MR_L. Metabolic rate is known to show additive genetically correlations with

a range of traits including body mass (Careau et al. 2011; Nilsson et al. 2009; Rønning et al. 2007; Schimpf et al. 2013; Tieleman et al. 2009), maximum metabolic rate (Sadowska et al. 2005; Wone et al. 2009; Wone et al. 2015), growth rate (Sadowska et al. 2009), the ability to cope with a poor diet (Sadowska et al. 2009), and exploratory behaviour (Careau et al. 2011). These and other additive genetic correlations may constrain the evolution of metabolic rate, but such constraints would not be identifiable in a univariate framework that considers metabolic rate in isolation. If several traits are measured however, a multivariate approach can determine relative direct and indirect selection acting on each trait through multiple regression.

Correlational selection favours certain combinations of traits, and is measured using second-order polynomial regression to produce a fitness surface that is a function of linear and squared (quadratic) trait values:

$$w = \alpha + \mathbf{z}\boldsymbol{\beta}^{T} + (1/2)\mathbf{z}^{T}\boldsymbol{\gamma}\mathbf{z}, \qquad (3)$$

where $\mathbf{z} = \{z_1, z_2, \ldots, z_n\}^T$ is a column vector of phenotypic values for n traits, $\boldsymbol{\beta} = \{\beta_1, \beta_2, \ldots\}^T$ is the column vector of directional selection gradients, and $\boldsymbol{\gamma}$ is the matrix of non-linear selection gradients:

$$\boldsymbol{\gamma} = \begin{bmatrix} \gamma_{1,1} & \gamma_{1,2} & \cdots & \gamma_{1,n} \\ \gamma_{1,2} & \gamma_{2,2} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ \gamma_{1,n} & \cdots & \cdots & \gamma_{n,n} \end{bmatrix},$$

where $\gamma_{i,i}$ is a stabilising or disruptive selection gradient for trait i, and $\gamma_{i,j}$ is a correlational selection gradient for traits i and j (Stinchcombe et al. 2008). Note that in the univariate case where correlational selection is not considered, equation (3) simplifies to $w = \alpha + z_i\beta_i + (1/2)\gamma_{i,i}z_i^2$ (i.e. equation 1). Despite the importance of estimating correlational selection for providing a more complete visualisation of the distribution of phenotypes, studies that measure correlational selection on physiological traits are rare.

In a study on a bryozoan, Pettersen et al. (2016) found significant negative correlational selection between metabolic rates across two life stages (early; MR_E and late; MR_L in juvenile development), but positive phenotypic covariance between these traits (individuals with high MR_E generally possessed high MR_L and vice versa). In other words, there is a positive covariance between the two metabolic rates but selection 'wants' to decrease this covariance. Furthermore, under persistent correlational selection across generations, we might expect the positive covariance among metabolic rates to be decrease and become negative over time. However, without an understanding of the degree of genetic covariance among traits (such as metabolic rates across ontogeny), our capacity to make such predictions remains limited.

Conclusions and future directions

Metabolic rate is perhaps the most widely measured physiological trait, and has long been argued to have important implications for life history, ecology, and evolution. We argue that more widespread adoption of a microevolutionary quantitative genetics framework is valuable for understanding variation in metabolic rate. In adopting such an approach, we should consider metabolic rate as a multivariate trait and measure actual fitness (lifetime reproductive output) in the field, in order to estimate the genetic covariance between metabolic rates and fitness throughout ontogeny. Such measurements are needed in order to understand the drivers of phenotypic variation in metabolic rate.

Glossary

Additive genetic variance (V_A): The magnitude of the total variance, due to the additive effects of each gene. The extent to which the average phenotype of the parent is reflected in the offspring, and the response to selection on a quantitative trait, is proportional to V_A.

Allometric scaling: The relationship between the mass of an organism and its metabolic rate, where the slope of the log-log scaled relationship is less than 1 (i.e non-isometric).

Breeder's equation: A tool developed to predict the amount of change in a single trait from one generation to the next: $R = h^2S$, where h^2 is narrow-sense heritability (the ratio of additive genetic variance to total phenotypic variance) and S is the selection differential (the change in population mean after selection).

Breeding values: The sum of the average effect of alleles carried by an individual

Compensation hypothesis ('allocation hypothesis'): Hypothesis whereby lower metabolic rates confer a fitness advantage as a result of lower maintenance costs, and thereby greater allocation of energy to reproduction.

Correlational selection: Form of nonlinear, multivariate selection where a combination of two or more traits interact non-additively to affect fitness.

Disruptive selection: Form of nonlinear, quadratic selection (see 'Quadratic selection') favouring individuals with extreme trait values. Under constant disruptive selection, the trait variance of a population will increase.

Directional selection: Form of univariate selection characterised by a linear fitness function, causing an increase or decrease in the population mean trait value.

Evolution: The change in heritable traits of a population across generations.

Fitness: The number of surviving offspring produced by an individual after a single generation.

G-matrix: Matrix of genetic variances and covariances, which summarises the inheritance of multiple, phenotypic traits.

Genetic correlation (r_A): A standardised version of genetic covariance (see definition below) that vary from -1 to 1.

Genetic covariance: The correlation between the breeding values for different traits.

Genetic drift: Changes in the frequency of alleles caused by chance.

Genetic variance (V_G): The value of the effect of all an individual's genes which affect the trait of interest. Genetic variance has three main components: additive genetic variance, dominance variance and interaction (epistatic) variance.

Heritability (H^2 or h^2): Proportion of variance in a phenotypic character in a population due to individual genetic differences that are inherited by offspring. Broad-sense heritability refers to the ratio of total genotypic variance to phenotypic variance ($H^2 = V_G/V_P$), while narrow-sense heritability refers to the ratio of additive genetic variance to phenotypic variance ($h^2 = V_A/V_P$).

Increased-intake hypothesis: Hypothesis relating performance with metabolic rates, where higher metabolic rates allow for greater energy turnover and synthesis of larger organs, leading to greater reproductive yield.

Indirect selection: Selection on one trait that arises from selection on another trait that is genetically correlated.

Linear selection: (see 'Directional selection').

Macroevolution: Among-species evolutionary change over long time scales.

Metabolic scaling: The relationship between the mass of an organism and its metabolic rate.

Metabolic theory: Patterns and processes that describe the flow of energy through a living system, from the cellular to global level.

Microevolution: Within-species evolutionary change over short time scales e.g. changes in gene frequencies within a population.

Nonlinear selection: Univariate (see 'Quadratic selection') or multivariate (see 'Correlational selection') selection that is nonlinear.

Quantitative genetics: The study of inheritance of genetically complex traits.

Quantitative trait: A trait that may be influenced by multiple genes, showing continuous variation in a population.

Rate-of-living hypothesis: Theory proposed by Rubner (1908) that lifespan is inversely related to metabolic rate, based on observations that larger animals with slower metabolic rates outlive smaller organisms with faster metabolic rates.

Selection: The differential survival and reproduction of individuals with varying phenotypes within a population. The covariance between fitness and a trait.

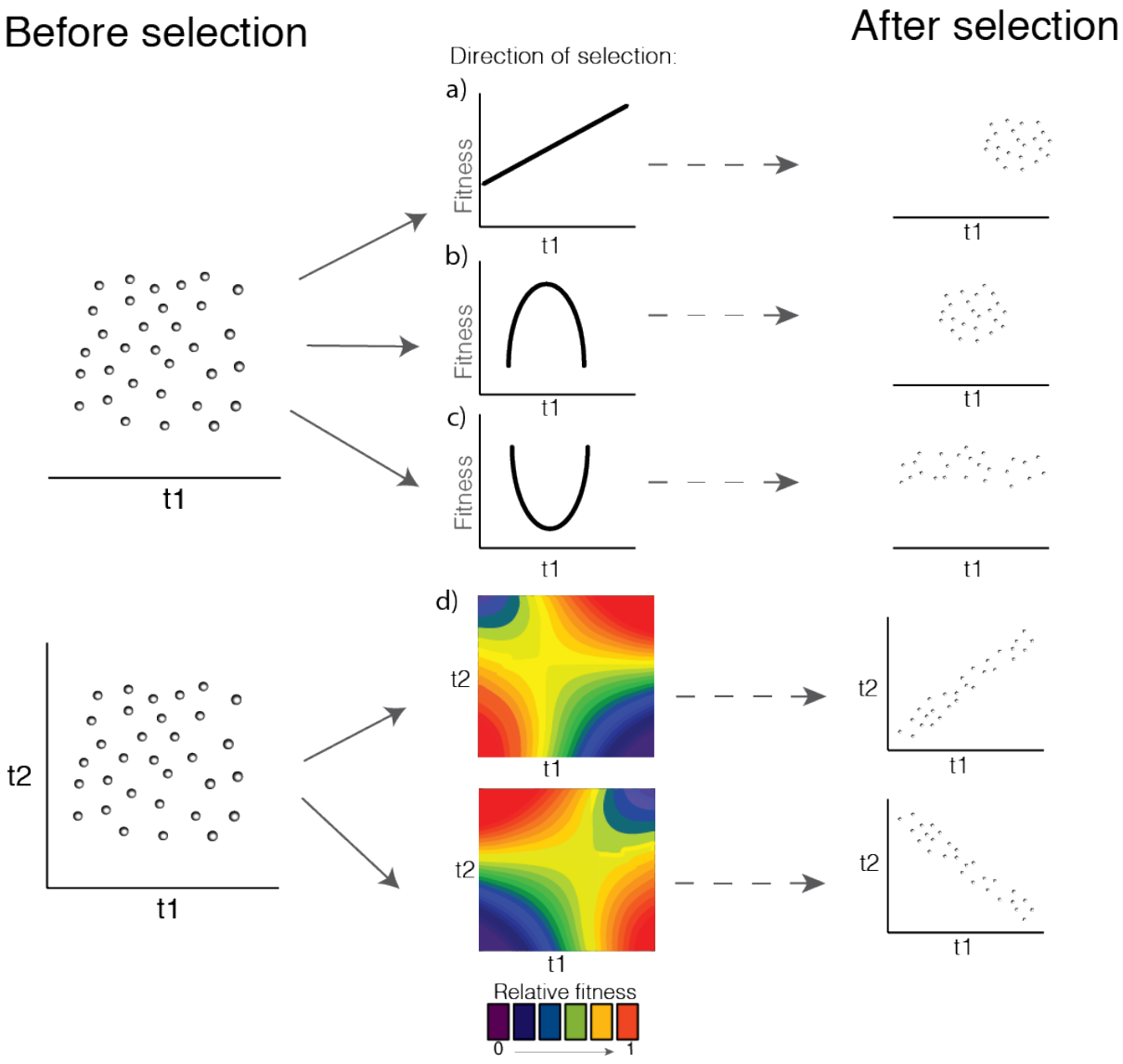
Selection coefficient (s): Difference in relative fitness

Selection differential (S): Difference between the mean trait value of the population before and after selection.

Selection gradient: The slope (linear; β and nonlinear; γ) of the regression of fitness on a trait value.

Stabilising selection: Form of nonlinear, quadratic selection (see 'Quadratic selection') favouring individuals with intermediate trait values. Under constant stabilising selection, the trait variance of a population will decrease.

Boxes



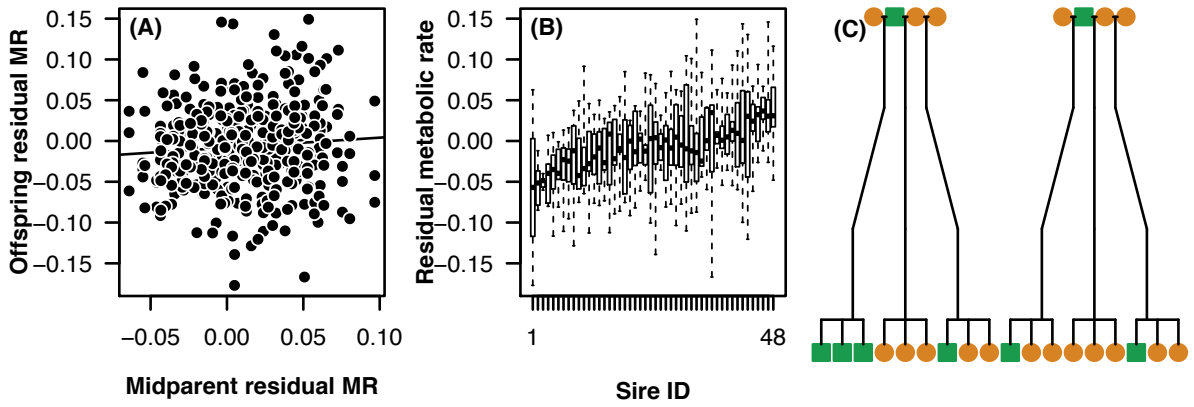
Box 1: Predicted population-level response to persistent univariate and multivariate selection. a) Directional selection, in this example the linear coefficient of selection, β is positive. Over generations, the population mean of t1 is expected to increase. b) Stabilising selection, where the quadratic coefficient, γ is negative. Over generations, the population variance will decrease,

forming a single optimum for t1. c) Disruptive selection, where the quadratic coefficient, γ is positive. Over generations, the population variance will decrease, forming two optima for t1. d) Positive correlational selection on t1 and t2 (where γ is positive) produces an increase in the covariance between t1 and t2. e) Negative correlational selection on t1 and t2 (where γ is negative) produces a decrease in the covariance between t1 and t2.

Species	MR measure	Fitness proxy	Reference
Laboratory studies			
Microgale dobsoni (Shrew tenrec)	RMR	Litter size, neonate mass, litter mass (+)	Stephenson and Racey (1993)
Mus musculus (Laboratory mouse)	RMR	Litter size (+), mean offspring mass (-)	Johnson et al. (2001)
Taeniopygia guttata	DEE	Clutch size (+), clutch mass (+), brood mass (+)	Vezina et al. (2006)
Field studies			
Bugula neritina (Marine bryozoan)	Unspecified	Reproductive output (negative correlational)	Pettersen et al. (2016)*
Cornu aspersum (Garden snail)	SMR	Survival (stabilising)	Bartheld et al. (2015)*
Cyanistes caeruleus (Blue tits)	BMR	Survival (+ and -)	Nilsson and Nilsson (2016)
Helix aspersa (Garden snail)	SMR	Juvenile survival (- and stabilising)	Artacho and Nespolo (2009)*
Microtus agrestis (Short-tailed field vole)	RMR	Over-winter survival (+)	Jackson et al. (2001)
Microtus oeconomus (Root vole)	RMR	Survival (+)	Zub et al. (2014)
Myodes glareolus (Bank vole)	BMR	Reproductive success (+)	Boratynski and Koteja (2010)*
Myodes glareolus (Bank vole)	BMR	Over-winter survival (-)	Boratynski et al. (2010)*
Salmo salar (Atlantic salmon)	MR	Survival (+, - and no relationship)	Robertsen et al. (2014)
Tamiasciurus hudsonicus (Red squirrel)	RMR	Over-winter survival (-)	Larivee et al. (2010)*

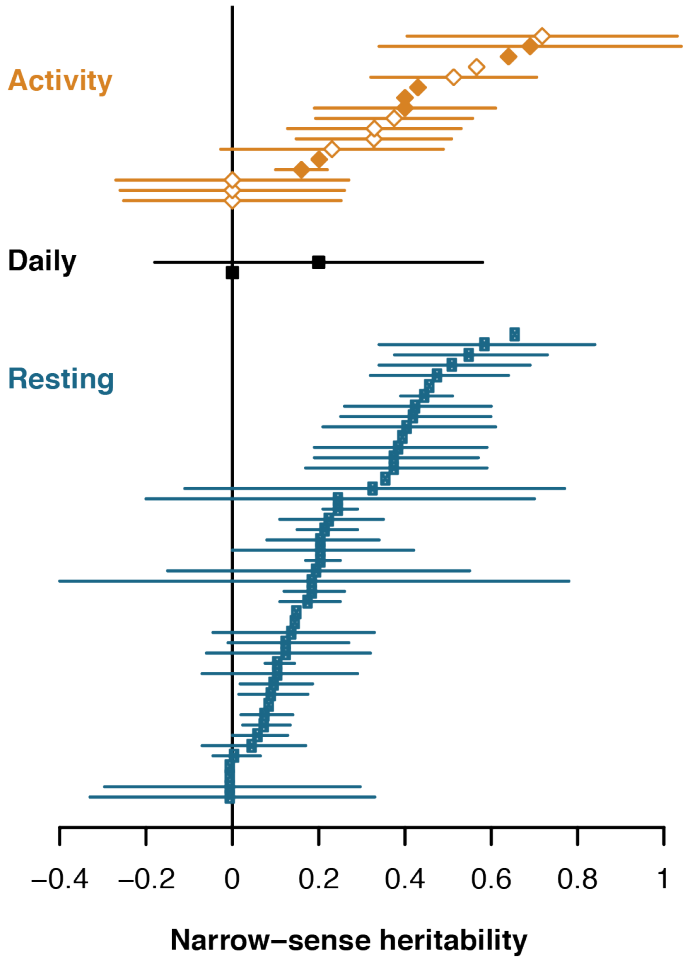
Tamiasciurus hudsonicus (Red squirrel)	DEE	Annual reproductive success (+)	Fletcher et al. (2015)*
Tamias striatus (Eastern chipmunks)	RMR	Juvenile survival (stabilising)	Careau et al. (2013) *
Zootoca vivipara (Common lizard)	RMR	Survival (-)	(Artacho et al. 2015) *

Box 2: Compilation of studies measuring the relationship between metabolic rates and survival or reproductive output as fitness proxies (values in parentheses are direction/form of significant selection on metabolic rates). BMR = basal metabolic rate, SMR = standard metabolic rate, DEE = daily energy expenditure, MR = maintenance metabolic rate. *These studies use a multiple regression framework, providing standardised and comparable estimates of selection (i.e the Lande and Arnold (1983) approach).



Box 3: Methods for estimation of the heritability of metabolic rate (parent-offspring regression, half sibling-full sibling breeding designs, and the 'animal model'), and a compilation of published estimates of the heritability of metabolic rate. (A) parent-offspring regression showing the relationship between parent and offspring metabolic rate for cockroaches *Nauphoeta cinerea* from a breeding design in which 48 sires were each mated to 3 dams, and the metabolic rates of all sires and dams, and 3 of the adult offspring from each clutch were measured (Schimpf et al. 2013). Narrow sense heritability is estimated as the slope of the line relating offspring and midparent trait values; here residual metabolic rates were calculated from a model describing variation in $\log_{10}$-tranformed metabolic rate as functions of $\log_{10}$-transformed body mass and sex ($h^2 = 0.12 \pm 0.07$ [SE]). (B) Among-sire differences in residual resting metabolic rate for cockroaches *Nauphoeta cinerea* from the same experiment (Schimpf et al. 2013). Half siblings have one quarter of their alleles in common, so in a half-sibling-full sibling breeding design, the among-sire variance (V_{SIRE}) is equal to ¼ of the additive genetic variance (V_A), and $h^2 = 4V_{SIRE}/V_P$, where V_P is total phenotypic variance. In the example in

(B), which utilises only the data for the adult offspring (i.e. those individuals with a known sire and dam; sires and dams of the parental generation are unknown), sire and dam variances were calculated for a model describing variation in $\log_{10}$-tranformed metabolic rate as functions of the fixed effects of sex and $\log_{10}$-transformed body mass, with random effects for sire and dam nested within sire estimated using restricted maximum likelihood (REML); $h^2 = 0.10 \pm 0.16$ (the model was implemented in ASReml-R v3.0 in R v3.0.2, with standard errors for variance ratios calculated using the delta method; Gilmour et al 2009, White 2013; R Development Core Team, 2016). For presentation, residual metabolic rates were calculated from a model describing variation in $\log_{10}$-tranformed metabolic rate as functions of $\log_{10}$-transformed body mass and sex, and data are shown ranked by the mean value of metabolic rate for each sire. (C) The 'animal model' is a form of mixed-effects model used to partition phenotypic variance into different genetic and environmental sources using knowledge of the relatedness of individuals in a population (Wilson et al. 2010), such as depicted here for the descendants of two sires in the cockroach half sibling-full sibling breeding design (males are green squares and females are orange circles). Calculated using the animal model, $h^2 = 0.12 \pm 0.07$ (the model was implemented in ASReml-R v3.0 in R v3.0.2, with standard errors for variance ratios calculated using the delta method).



Box 4. Forest plot summarising published estimates of the narrow-sense heritability (h^2 shown ± SE where possible) of metabolic rate for endotherms (filled symbols) and ectotherms (unfilled symbols), subdivided by activity level (Resting: resting, basal, or standard metabolic rate [blue circles]; Daily: daily rate of energy expenditure or sustained metabolic rate [black squares]; Activity: peak metabolic rate, flight metabolic rate, maximum metabolic rate, or maximum rate of oxygen consumption elicited by treadmill exercise or swimming [orange diamonds]). With the two values for daily metabolic rate excluded from analysis, there was no significant interaction between activity level (resting or active) and endothermy (endotherm or ectotherm) as predictors of h^2 in a mixed model including random effects of species and publication ($t_{56.4}$ = -0.61, p = 0.54; the model was implemented in the 'lme4' v1.1-13 package

of R v3.2.3, with the significance of fixed effects based on Satterthwaite approximation for denominator degrees of freedom from the 'lmerTest' package v2.0.33: Bates et al. (2015); Kuznetsova et al. (2016); R Development Core Team (2016)). With the non-significant interaction removed from the model, h^2 is significantly different from zero (intercept = 0.19 ± 0.06 [SE], $t_{13.2}$ = 3.00, p = 0.01), endotherms have significantly higher h^2 than ectotherms (parameter estimate = 0.19 ± 0.08, $t_{12.8}$ = 2.38, p = 0.03) and h^2 is higher for active metabolic levels than for resting metabolic levels (parameter estimate = 0.21 ± 0.05, $t_{54.6}$ = 4.3, p < 0.001); estimates of h^2 for mass-independent metabolic rates were not significantly different from estimates of h^2 for whole-animal metabolic rates (parameter estimate = -0.08 ± 0.05, $t_{56.0}$ = -1.67, p = 0.10); variance components: species = 0.0159, publication < 0.0001, residual = 0.0197.

References

Angilletta, M. J., P. H. Niewiarowski, and C. A. Navas. 2002. The evolution of thermal physiology in ectotherms. Journal of Thermal Biology 27:249-268.

Arnold, S. J. 1988. Genetic correlation and the evolution of physiology, Pages 189-212 *in* M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey, eds. New Directions in Ecological Physiology, Cambridge University Press.

Artacho, P., and R. F. Nespolo. 2009. Natural Selection Reduces Energy Metabolism in the Garden Snail, Helix aspersa (*Cornu aspersum*). Evolution 63:1044-1050.

Artacho, P., J. Saravia, B. D. Ferrandiere, S. Perret, and J.-F. Le Galliard. 2015. Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. Ecology and Evolution 5:3600-3609.

Auer, S. K., R. D. Bassar, K. Salin, and N. B. Metcalfe. 2016a. Repeatability of metabolic rate is lower for animals living under field versus laboratory conditions. Journal of Experimental Biology 219:631-634.

Auer, S. K., K. Salin, G. J. Anderson, and N. B. Metcalfe. 2016b. Flexibility in metabolic rate and activity level determines individual variation in overwinter performance. Oecologia 182:703-712.

Auer, S. K., K. Salin, A. M. Rudolf, G. J. Anderson, and N. B. Metcalfe. 2015. Flexibility in metabolic rate confers a growth advantage under changing food availability. Journal of Animal Ecology 84:1405-1411.

Bacigalupe, L. D., N. M. Araya, M. J. Carter, T. P. Catalána, M. A. Lardies, and F. Bozinovic. 2007. Maternal effects, maternal body size and offspring energetics: A study in the common woodlouse *Porcellio laevis*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 147:349-354.

Bartheld, J. L., J. D. Gaitan-Espitia, P. Artacho, C. Salgado-Luarte, E. Gianoli, and R. F. Nespolo. 2015. Energy expenditure and body size are targets of natural selection across a wide geographic range, in a terrestrial invertebrate. Functional Ecology 29:1463-1474.

Bates, D., M. Machler, B. M. Bolker, and S. C. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1-48.

Bennett, A. F., and J. A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649-654.

Biro, P. A., and J. A. Stamps. 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology & Evolution 25:653-659.

Blows, M. W., and K. McGuigan. 2015. The distribution of genetic variance across phenotypic space and the response to selection. Molecular Ecology 24:2056-2072.

Boratynski, Z., E. Koskela, T. Mappes, and T. A. Oksanen. 2010. Sex-specific selection on energy metabolism - selection coefficients for winter survival. Journal of Evolutionary Biology 23:1969-1978.

Boratynski, Z., and P. Koteja. 2010. Sexual and natural selection on body mass and metabolic rates in free-living bank voles. Functional Ecology 24:1252-1261.

Broggi, J., E. Hohtola, K. Koivula, M. Orell, R. L. Thomson, and J. A. Nilsson. 2007. Sources of variation in winter basal metabolic rate in the great tit. Functional Ecology 21:528-533.

Burton, T., S. S. Killen, J. D. Armstrong, and N. B. Metcalfe. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society B-Biological Sciences 278:3465-3473.

Careau, V., P. Bergeron, D. Garant, D. Reale, J. R. Speakman, and M. M. Humphries. 2013. The energetic and survival costs of growth in free-ranging chipmunks. Oecologia 171:11-23.

Careau, V., D. Thomas, F. Pelletier, L. Turki, F. Landry, D. Garant, and D. Reale. 2011. Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). Journal of Evolutionary Biology 24:2153-2163.

Dobzhansky, T. 1956. What is an adaptive trait? American Naturalist 90:337-347.

Dohm, M. R., J. P. Hayes, and T. Garland. 2001. The quantitative genetics of maximal and basal rates of oxygen consumption in mice. Genetics 159:267-277.

Falconer, D. S., and T. F. C. Mackay. 1996, Introduction to Quantitative Genetics, Longman.

Fletcher, Q. E., J. R. Speakman, S. Boutin, J. E. Lane, A. G. McAdam, J. C. Gorrell, D. W. Coltman et al. 2015. Daily energy expenditure during lactation is strongly selected in a free-living mammal. Functional Ecology 29:195-208.

Gadgil, M., and W. H. Bossert. 1970. Life Historical Consequences of Natural Selection. The American Naturalist 104:1-24.

Garland, T., and P. A. Carter. 1994. Evolutionary physiology. Annual Review of Physiology 56:579-621.

Gebczynski, A. K., and M. Konarzewski. 2009. Metabolic correlates of selection on aerobic capacity in laboratory mice: a test of the model for the evolution of endothermy. Journal of Experimental Biology 212:2872-2878.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml User Guide Release 3.0. Hemel Hempstead, HP1 1ES, UK, VSN International Ltd.

Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662.

Glazier, D. S. 2015. Is metabolic rate a universal 'pacemaker' for biological processes? Biological Reviews 90:377-407.

Hayes, J. P., T. Garland, and M. R. Dohm. 1992. Individual variation in metabolism and reproduction of Mus: are energetics and life history linked? Functional Ecology 6:5-14.

Jackson, D. M., P. Trayhurn, and J. R. Speakman. 2001. Associations between energetics and over-winter survival in the short-tailed field vole *Microtus agrestis*. Journal of Animal Ecology 70:633-640.

Johnson, M. S., S. C. Thomson, and J. R. Speakman. 2001. Limits to sustained energy intake II. Inter-relationships between resting metabolic rate, life-history traits and morphology in Mus musculus. Journal of Experimental Biology 204:1937-1946.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang et al. 2001. The strength of phenotypic selection in natural populations. Am Nat 157:245-261.

Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and power of phenotypic selection in nature. Bioscience 57:561-572.

Konarzewski, M., and A. Ksiazek. 2013. Determinants of intra-specific variation in basal metabolic rate. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 183:27-41.

Ksiazek, A., M. Konarzewski, and I. B. Lapo. 2004. Anatomic and energetic correlates of divergent selection for basal metabolic rate in laboratory mice. Physiological and Biochemical Zoology 77:890-899.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-33. http://cran.r-project.org/package=lmerTest.

Lacy, R. C., and C. B. Lynch. 1979. Quantitative genetic analysis of temperature regulation in *Mus musculus*. 1. Partitioning of variance. Genetics 91:743-753.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210-1226.

Larivee, M. L., S. Boutin, J. R. Speakman, A. G. McAdam, and M. M. Humphries. 2010. Associations between over-winter survival and resting metabolic rate in juvenile North American red squirrels. Functional Ecology 24:597-607.

Lighton, J. R. B. 2008, Measuring metabolic rates: a manual for scientists. Oxford, Oxford University Press.

Lynch, C. B., and D. S. Sulzbach. 1984. Quantitative genetic analysis of temperature regulation in *Mus musculus.* 2. Diallel analysis of individual traits. Evolution 38:527-540.

Mayr, E. 1961. Cause and effect in biology. Kinds of causes, predictability, and teleology are viewed by a practising biologist. Science 134:1501-&.

Moran, D., and R. M. G. Wells. 2007. Ontogenetic scaling of fish metabolism in the mouse-to-elephant mass magnitude range. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 148:611-620.

Nespolo, R. F., and M. Franco. 2007. Whole-animal metabolic rate is a repeatable trait: a meta-analysis. Journal of Experimental Biology 210:2000-2005.

Nilsson, J. A. 2002. Metabolic consequences of hard work. Proceedings of the Royal Society B-Biological Sciences 269:1735-1739.

Nilsson, J. A., M. Akesson, and J. F. Nilsson. 2009. Heritability of resting metabolic rate in a wild population of blue tits. Journal of Evolutionary Biology 22:1867-1874.

Nilsson, J. F., and J. A. Nilsson. 2016. Fluctuating selection on basal metabolic rate. Ecology and Evolution 6:1197-1202.

Pettersen, A. K., C. R. White, and D. J. Marshall. 2016. Metabolic rate covaries with fitness and the pace of the life history in the field. Proceedings of the Royal Society B-Biological Sciences 283.

Phillips, P. C., and S. J. Arnold. 1989. Visualising multivariate selection. Evolution 43:1209-1222.

R Development Core Team. 2016.R: A language and environment for statistical computing, Vienna, Austria.

Régnier, T., V. Bolliet, J. Labonne, and P. Gaudin. 2010. Assessing maternal effects on metabolic rate dynamics along early development in brown trout (*Salmo trutta*): an individual-based approach. Journal of Comparative Physiology B 180:25-31.

Robertsen, G., J. D. Armstrong, K. H. Nislow, I. Herfindal, S. McKelvey, and S. Einum. 2014. Spatial variation in the relationship between performance and metabolic rate in wild juvenile Atlantic salmon. Journal of Animal Ecology 83:791-799.

Rønning, B., H. Jensen, B. Moe, and C. Bech. 2007. Basal metabolic rate: heritability and genetic correlations with morphological traits in the zebra finch. Journal of Evolutionary Biology 20:1815-1822.

Rosenfeld, J., T. Van Leeuwen, J. Richards, and D. Allen. 2015. Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. Journal of Animal Ecology 84:4-20.

Rubner, M. 1908, Das Problem der Lebensdaur und seine Beziehungen zu Wachstum und Ernährung. Munich: Oldenberg.

Sadowska, E. T., K. Baliga-Klimczyk, M. K. Labocha, and P. Koteja. 2009. Genetic correlations in a wild rodent: grass-eaters and fast-growers evolve high basal metabolic rates. Evolution 63:1530-1539.

Sadowska, E. T., M. K. Labocha, K. Baliga, A. Stanisz, A. K. Wroblewska, W. Jagusiak, and P. Koteja. 2005. Genetic correlations between basal and maximum metabolic rates in a wild rodent: Consequences for evolution of endothermy. Evolution 59:672-681.

Sadowska, E. T., C. Stawski, A. Rudolf, G. Dheyongera, K. M. Chrzascik, K. Baliga-Klimczyk, and P. Koteja. 2015. Evolution of basal metabolic rate in bank voles from a multidirectional selection experiment. Proceedings of the Royal Society B-Biological Sciences 282.

Sadowska, J., A. K. Gębczyński, and M. Konarzewski. 2013. Basal metabolic rate is positively correlated with parental investment in laboratory mice. Proceedings of the Royal Society B: Biological Sciences 280:20122576.

Salin, K., S. K. Auer, B. Rey, C. Selman, and N. B. Metcalfe. 2015. Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. Proceedings of the Royal Society B-Biological Sciences 282:14-22.

Schimpf, N. G., P. G. D. Matthews, and C. R. White. 2012. Cockroaches that exchange respiratory gases discontinuously survive food and water restriction. Evolution 66:597-604.

Schimpf, N. G., P. G. D. Matthews, and C. R. White. 2013. Discontinuous gas exchange exhibition is a heritable trait in speckled cockroaches *Nauphoeta cinerea*. Journal of Evolutionary Biology 26:1588-1597.

Smit, B., and A. E. McKechnie. 2010. Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. Functional Ecology 24:330-339.

Speakman, J. R. 2005. Body size, energy metabolism and lifespan. Journal of Experimental Biology 208:1717-1730.

Stephenson, P. J., and P. A. Racey. 1993. Reproductive energetics of the Tenrecidae (Mammalia, Insectivora). 2. The shrew tenrecs, *Microgale* spp. Physiological Zoology 66:664-685.

Steyermark, A. C. 2002. A high standard metabolic rate constrains juvenile growth. Zoology 105:147-151.

Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62:2435-2440.

Suarez, R. K. 2012. Energy and Metabolism Comprehensive Physiology, John Wiley & Sons, Inc.

Tieleman, B. I., M. A. Versteegh, B. Helm, and N. J. Dingemanse. 2009. Quantitative genetics parameters show partial independent evolutionary potential for body mass and metabolism in stonechats from different populations. Journal of Zoology 279:129-136.

Vezina, F., J. R. Speakman, and T. D. Williams. 2006. Individually variable energy management strategies in relation to energetic costs of egg production. Ecology 87:2447-2458.

White, C. R., and M. R. Kearney. 2013. Determinants of inter-specific variation in basal metabolic rate. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 183:1-26.

Wilson, A. J., D. Réale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, L. E. B. Kruuk et al. 2010. An ecologist's guide to the animal model. Journal of Animal Ecology 79:13-26.

Wone, B., M. W. Sears, M. K. Labocha, E. R. Donovan, and J. P. Hayes. 2009. Genetic variances and covariances of aerobic metabolic rates in laboratory mice. Proceedings of the Royal Society B-Biological Sciences 276:3695-3704.

Wone, B. W. M., P. Madsen, E. R. Donovan, M. K. Labocha, M. W. Sears, C. J. Downs, D. A. Sorensen et al. 2015. A strong response to selection on mass-independent maximal metabolic rate without a correlated response in basal metabolic rate. Heredity 114:419-427.

Zub, K., Z. Borowski, P. A. Szafranska, M. Wieczorek, and M. Konarzewski. 2014. Lower body mass and higher metabolic rate enhance winter survival in root voles, *Microtus oeconomus*. Biological Journal of the Linnean Society 113:297-309.

Chapter 4

Metabolic rate covaries with fitness and the pace of the life history in the field

Amanda K. Pettersen[1], Craig R. White[1], Dustin J. Marshall[1]

[1] School of Biological Sciences/Centre for Geometric Biology, Monash University, Victoria, 3800, Australia.

Abstract

Metabolic rate reflects the 'pace of life' in every organism. Metabolic rate is related to an organism's capacity for essential maintenance, growth and reproduction – all of which interact to affect fitness. Although thousands of measurements of metabolic rate have been made, the microevolutionary forces that shape metabolic rate remain poorly resolved. The relationship between metabolic rate and components of fitness are often inconsistent, possibly because these fitness components incompletely map to actual fitness and often negatively covary with each other. Here we measure metabolic rate across ontogeny and monitor its effects on actual fitness (lifetime reproductive output) for a marine bryozoan in the field. We also measure key components of fitness throughout the entire life history including growth rate, longevity, and age at the onset of reproduction. We found that correlational selection favours individuals with higher metabolic rates in one stage and lower metabolic rates in the other – individuals with similar metabolic rates in each developmental stage displayed the lowest fitness. Furthermore individuals with the lowest metabolic rates lived for longer and reproduced more, but they also grew more slowly and took longer to reproduce initially. That metabolic rate is related to the pace of the life history in nature has long been suggested by macroevolutionary patterns but the present study reveals the microevolutionary processes that likely generated these patterns.

Introduction

Metabolic rate is associated with the 'pace of life' and is a fundamental trait relevant to all organisms. The rate at which organisms utilise, transform and expend energy essential for all biological functioning varies both among and within species (Konarzewski and Ksiazek 2013; White and Kearney 2013). Intuitively, one expects there to be an association between this key trait and fitness – the rate at which individuals use and process energy should inevitably have consequences for function and performance. Early work examining the consequences of variation in metabolic rate for organismal performance focussed on the 'rate-of-living' hypothesis, which proposes that an animal's pace of life (its metabolic rate) is inversely related to its lifespan (Rubner 1908). The rate-of-living hypothesis remains controversial (Glazier 2015; Speakman 2005) and recent studies have expanded the search for the performance consequences of variation in metabolic rate to a wider range of fitness proxies and components.

The strength and direction of selection on metabolic rate is predicted to vary among fitness proxies or components. Lower resting, routine or maximal metabolic rates may allow for the reallocation of energy towards growth, reproduction, and increased immune function, in what is known as the 'compensation hypothesis' (e.g. Downs et al. 2012; Larivee et al. 2010; Steyermark 2002). Alternatively, low metabolic rates may be unable to service essential physiological processes, and high metabolic rates may provide an increased capacity for functions that enhance fitness (the 'increased–intake' hypothesis; Bennett and Ruben 1979). For the increased-intake hypothesis, high maximal metabolic rates might improve aerobic performance, the ability for thermogenesis, and enable faster energy consumption and mobility (e.g. Downs et al. 2012; Hayes and O'Connor 1999; Nilsson 2002). Still other studies find no relationship between basal metabolic rate and rates of important physiological processes (e.g. Alvarez and Nicieza 2005; Derting and McClure 1989). One potential reason for the absence

of any clear pattern of association between metabolic rate and fitness proxies is that attempts at estimating selection on metabolic rate have often relied on fitness components that are likely to show complex and idiosyncratic relationships with each other, and more importantly, with actual fitness. An essential next step therefore is to estimate the relationship between metabolic rate and fitness using the appropriate evolutionary currency for actual fitness: lifetime reproductive output (Blackmer et al. 2005; Clutton-Brock 1988).

Estimating lifetime reproductive output in field populations of animals remains challenging and is often restricted to very large species that are easily tracked and where reproduction can be measured (e.g. Kruuk et al. 1999). Despite these challenges, field studies are likely to provide essential insights into selection on metabolic rate because selection is highly context-dependent: the strength and even direction of selection can change when comparing across laboratory and field populations. Recent studies have overcome these formidable challenges by measuring selection on fitness components under realistic field conditions (Artacho and Nespolo 2009; Artacho et al. 2015). Importantly, these studies have used the Lande and Arnold approach to formally estimate selection on metabolic rate so that comparisons can be made across studies (Lande and Arnold 1983). Here, we extend these studies by applying this classic multiple regression framework to estimate selection on metabolic rate at two life stages, where we use lifetime reproductive output as our measure of fitness in the field. We take advantage of the sessile nature of the adult stage of the marine bryozoan *Bugula neritina* to measure lifetime reproductive output. We also measured additional fitness components (early life-stage survival, growth, phenology and longevity) so as to understand the various correlations between metabolic rate and life-history traits that will ultimately affect fitness.

Materials and methods

Study species, site and larval mass measurements

Adult colonies of the aborescent bryozoan, *Bugula neritina* grow via asexual budding, by producing new pairs of zooids (individual subunits) at distal ends combined with regular bifurcations after approximately every four pairs of zooids to produce symmetrical branching (Keough 1989; Keough and Chernoff 1987). Once sexually mature, *B. neritina* zooids brood single embryos in clearly visible, calcified structures called ovicells which act as a placenta-like system and supply the offspring with maternally-derived nutrients (Woollacott and Zimmer 1975). Once embryogenesis is complete, the developed non-feeding larvae are released into the plankton where they are competent to settle almost immediately, yet remain dependent on maternally-derived energy reserves from release as larvae through post-settlement until the end of metamorphosis. This 'dependent phase' (sensu Pettersen et al. 2015) lasts approximately two days before the development of the first zooid with feeding structure (lophophore) is complete, and offspring feed for themselves.

All *B. neritina* colony collections and outplanting were conducted at Royal Brighton Yacht Club in Port Phillip Bay, Victoria, Australia (-37.909, 144.986), from March to November 2014. Sexually mature colonies were transported to the laboratory and maintained in darkened, aerated tanks at 17.5°C - a similar temperature to that of the bay at the time of the study. After two days, approximately ten colonies per experimental run were induced to spawn according to standard light-shock procedures: colonies were placed in beakers of filtered seawater and exposed to bright light (Marshall et al. 2003). The released larvae were then immediately photographed on a glass slide using a Moticam 5 digital camera (Motic, Hong Kong, China) mounted on a dissecting microscope as per standard techniques developed previously (Marshall et al. 2003). Measurements of larval body area and length of the ciliated

groove were estimated to the nearest μm using Image J software (1.47v) and larvae mass estimates based on calculations obtained in a previous study (Pettersen et al. 2015). Once photographed, larvae were then pipetted in a drop of seawater directly onto roughened acetate sheets to induce settlement. The range of larval mass measured in this study reflected the natural variation observed in larval size by this species (Marshall et al. 2003).

Metabolic rate measurements

Oxygen consumption rate ($\dot{V}O_2$; a commonly used proxy for metabolic rate) was measured for individual settlers of *B. neritina* at two developmental stages: 0h and 24h post-settlement (from here-on designated metabolic rate early (MR_E) and metabolic rate late (MR_L), respectively). Individual settlers were cut out on small sheets of acetate and placed into glass vials containing pasteurised, 0.2μm filtered seawater and a non-consumptive O_2 sensor spot. For each experimental run, $\dot{V}O_2$ was measured for 36 individuals at the same time along with 12 controls (blank vials containing only seawater and acetate) using 24-channel PreSens sensor dish readers (Sensor Dish Reader SDR2, PreSens), with 24-chamber 200μl glass micro plates (Loligo Systems Aps, Tjele, Denmark). $\dot{V}O_2$ was calculated from the rate of change of O_2 saturation over time (m_a; $\%h^{-1}$) as per White et al. (2011):

$\dot{V}O_2 = -1\ (m_a - m_b / 100)\ V\beta O_2$, where m_b is the rate of change of O_2 saturation for control vials ($\%h^{-1}$), βO_2 is the oxygen capacitance of air-saturated seawater at 17.5°C (5.8 mL L^{-1}; Cameron (1986)), and V is water volume (the volume of acetate and the animals was subtracted from the total chamber volume of 2.0×10^{-4}L). Prior to $\dot{V}O_2$ measurements sensor spots were calibrated with air-saturated (AS) seawater (100% AS) and water containing 2% sodium sulfite (0% AS). In order to obtain proxies for standard metabolic rate, all $\dot{V}O_2$ measurements were recorded in a darkened, constant-temperature room at 17.5°C over 3h, such that temperature in the vials became stable and individual settlers were not negatively affected by the procedure (i.e. all measurements were undertaken at O_2 saturation levels greater than the critical partial

pressure of O_2 for aerobic metabolism, below which $\dot{V}O_2$ declines). Each set of two $\dot{V}O_2$ measurements on 36 individuals represented a single 'experimental run', which was repeated six times. To convert oxygen consumption, $\dot{V}O_2$ ($\mu l\ h^{-1}$) to metabolic rate ($mJ\ h^{-1}$) the calorific conversion factor of 20.08 $J\ ml^{-1}$ O_2 was used (Crisp 1971). Ideally, in addition to measuring $\dot{V}O_2$ during two early-life stages, we would also measure $\dot{V}O_2$ later in ontogeny. However, measuring metabolic rates of large numbers of larger individuals would have required the return of individuals to the laboratory for several days and our primary goal was to gain as realistic measures of fitness as possible by leaving individuals in the field throughout their post-metamorphic lives.

Field deployment and measures of fitness traits

Following the final $\dot{V}O_2$ measurement, each piece of acetate containing a single settler was glued onto labelled PVC plates (55 x 55 x 3 mm), and maintained in tanks overnight with unfiltered seawater at 17.5°C before being outplanted into the field the following morning. For each experimental run (n=6), 36 plates were randomly assigned onto a single PVC backing panel (570 x 570 x 6 mm) such that a total of 216 settlers were deployed into the field. The backing panels were then suspended 1.5m below the water surface with the settlement plates facedown (for a detailed description of the field deployment, see Marshall and Keough (2009)). Several trait measurements were recorded for every individual over the entire life history, until all individuals had died (March – November 2014) to provide various components of fitness (Kingsolver and Pfennig 2007). Measures of early-stage survival (at 8 weeks post-outplant) and growth (number of bifurcations as an indication of colony size, see Keough and Chernoff (1987) for details) were recorded weekly. Mortality was noted for individual colonies when the individual was either absent from the plate or less than 10% of feeding zooids remained. Colonies were regularly checked for development of ovicells which were visible under a field microscope (x10). Age (number of days) at onset of reproduction was noted and occurred

approximately six weeks post-settlement. Reproductive output was measured as a count of the number of ovicells from thereon every two weeks to provide a total cumulative value for lifetime reproductive output. Longevity was recorded as the number of days from outplant of individuals up until mortality.

Statistical analyses

We used two statistical approaches to analyse the data. First, we used a classic multiple regression approach to formally estimate the direction and strength of selection on our three traits of interest (larval mass, and the metabolic rate of two post-settlement stages) for the fitness measure of lifetime reproductive output (Lande and Arnold 1983). Second, we modelled the remaining life-history traits of early-stage survival, growth, and at onset of reproduction as a function of larval mass and metabolic rate. Data were analysed using multivariate linear mixed models, fitted with maximum likelihood for longevity, logistic regression for age at onset of reproduction, and for size over time (growth), repeated measures within a general linear model framework. This approach allowed us to determine the relationship between metabolic rate and key life-history traits, and to determine whether trade-offs among fitness components may help to explain why we see mixed results in the literature. Metabolic rates at each stage were found to be significantly correlated (where mass was included as a covariate; $\chi^2 = 22.434$, df = 1, $p < 0.001$). However, this relationship was relatively weak ($R^2 = 0.19$) - all variance inflation factors were less than 5, and no evidence for multicollinearity was found. Larval mass, MR_E and MR_L were therefore treated as independent variables (see Figure D1).

Estimating selection gradients

Standardised estimates of linear (β) and nonlinear (γ) gradients of selection for total reproductive output were generated using a multiple regression approach (Lande and Arnold 1983; Phillips and Arnold 1989). The form of selection was tested with likelihood ratio tests

and the strength of selection gradients for total reproductive output (coefficient estimates) were calculated using linear regression.

Covariance between larval mass, metabolic rate and life-history traits

The relationship between continuous predictor variables of larval mass, MRE and MRL with key life-history response variables of growth, longevity and age at onset of reproduction were analysed separately. Biplots were produced to check for autocorrelation among response variables, and to ensure variation in one response variable was not explained by another measured response variable. Longevity and growth over 20 weeks were found to be significantly positively correlated ($\chi 2 = 26.794$, df = 1, $p < 0.001$). However, as the relationship between longevity and growth was not strong (R2 = 0.48), the variables were analysed separately. No significant relationships between age at onset of reproduction and longevity ($\chi 2 = 1.452$, df = 1, $p = 0.228$) or growth ($\chi 2 = 0.242$, df = 1, $p = 0.623$) were found (see Figure D2). For each model, experimental run was included as a random categorical factor - where run or its interactions were found to be non-significant, they were first removed from the model. Longevity was tested using a linear mixed model (maximum likelihood), using stepwise removal of non-significant terms. Age at onset of reproduction was treated as a binary response variable and tested with logistic regression. As development of ovicells on individual colonies occurred either much earlier or much later than 60 days post outplant, individuals that reproduced earlier than 60 days were assigned a value of 1 and individuals with late onset of reproduction (≥60 days) with a value of 0. The relationship between larval mass, metabolic rates and growth over the first 20 weeks of development (number of bifurcations over time) was tested using repeated measures analysis.

Results

Selection gradients

No significant linear selection on larval mass or metabolic rate was detected ($\chi^2 = 2.35$, df = 3, $p = 0.50$). We found significant nonlinear selection on metabolic rate ($\chi^2 = 12.67$, df = 6, $p = 0.04$). When we explored the two forms of nonlinear selection, we found no support for significant quadratic selection but we did find support for significant correlational selection (Table 1). Significant negative correlational selection showed that individuals that had higher metabolic rates in both stages or lower metabolic rates in both stages had the lowest fitness, whereas individuals that had higher metabolic rates in one stage but lower metabolic in the other stage had the highest fitness (Figure 1). The relatively strong correlational selection gradient of -0.194 indicated that correlational selection is acting to decrease the positive covariance between MR_E and MR_L (Blows and Brooks 2003).

Covariance among traits

Growth

Over the first 20 weeks post-settlement, individuals that developed from settlers with higher MR_E and lower MR_L grew larger than individuals from settlers with lower MR_E and higher MR_L (Figure 2). While MR_E was positively correlated with individual colony size (coefficient = 1.97, $F_{1,111} = 6.283$, P =0.014), MR_L showed a negative relationship with individual colony size over the first 20 weeks of development (coefficient = -0.9, $F_{1,111} = 4.415$, $p = 0.038$). Repeated measures analysis showed that the effects of larval mass, MR_E and MR_L did not change over time (no significant time x larval mass x MR_E x MR_L interaction was detected; $F_{6,648} = 0.252$, $p = 0.227$).

Longevity

Individuals originating from smaller larvae and with lower MR_L lived for longer than individuals that had originated as larger larvae with a higher MR_L (Figure 3). The final model showed a significant interaction between offspring mass and MR_L ($\chi^2 = 4.24$, df = 1, $p = 0.039$) where the two traits were negatively correlated with longevity of the settlers (Table 2).

Age at onset of reproduction

Individuals with higher MR_E reproduced sooner than individuals with lower MR_E (Figure 4). When fitting the model, MR_E, larval mass, MR_L, larval mass x MR_E and MR_E x MR_L all showed marginally significant effects and were therefore retained in the final model (Quinn and Keough 2002). However log-likelihood tests revealed MR_E ($\chi^2 = 5.064$, df = 1, $p = 0.002$) was the only trait to have significant effects on age at onset of reproduction (Table 3).

Discussion

Correlational selection for decreased covariance between metabolic rates

We found selection for decreased covariance between metabolic rates at each stage – individuals with high metabolic rates in one stage and low metabolic rates in another stage had higher lifetime reproductive output than individuals with either both high or both low metabolic rates in each stage. Assuming that our estimates of selection are persistent, and that metabolic rate is heritable, we would expect to see decreased covariance between metabolic rates at different developmental stages – leading to individuals with metabolic rates that are either high or low in both stages, being purged from the population. Until now, findings of correlational selection on metabolic rates had yet to be demonstrated - most other studies find benefits to either a higher or lower metabolic rate overall. In contrast, we found a benefit to having metabolic rates that are dissimilar to each other across developmental stages.

While we observed a slight, positive correlation for metabolic rate among developmental stages, selection favoured a negative correlation between these traits. That the strength and direction of selection on key life-history traits fluctuates across development has been previously demonstrated (Kingsolver et al. 2012; Monro and Marshall 2014). However, that selection should act to reduce covariance between two correlated traits appears to be counterintuitive. A lower metabolic rate early in development may need to be offset by a higher metabolic rate later in development in order to meet energy requirements for essential biological processes. Conversely, high energy expenditure early in development may be unsustainable, and a shift to a low metabolic rate in later development may be required to maintain energy reserves. If this is the case, then why consistently intermediate metabolic rates were not selected for throughout development remains unclear. We do not know what drives the negative correlational selection on two metabolic rates separated by only 24 hours, but that

the selection exists suggests that more studies should estimate selection on multiple metabolic rates across ontogeny.

Metabolic rate and the pace of life history

Metabolic rate was associated with other important life-history traits, and together these life-history traits drive the pace of the life history. We found no directional selection for higher or lower metabolic rates, but we did find strong evidence that certain metabolic rates may be associated with the timing of key life-history events. Overall, individuals with lower metabolic rates lived for longer, had slower growth rates and reproduced later in life than individuals with higher metabolic rate. While higher metabolic rates were generally correlated with higher growth rate, lower longevity and an earlier onset of reproduction. Across taxa, studies have shown that slow growth, late onset of reproduction and greater longevity that is often associated with low metabolic rate, can serve as an advantageous strategy in low stress environments, for instance, when competition and predation pressure are low and resources are abundant (Auer et al. 2010; Grime and Hunt 1975; Koons et al. 2008; Partridge and Fowler 1992; Rose et al. 1992). Conversely, a faster pace of life is likely to be advantageous in stressful environments, such as when food levels are low or predation is high, and thus higher metabolic rates are likely to be beneficial (Auer et al. 2015; Bochdansky et al. 2005; Reznick et al. 2004; Ricklefs 1998; Wilbur and Collins 1973). Contrary to this, in environments where reduced energetic requirements are advantageous such as during periods of starvation or temperature stress, then lower metabolic rates may be selected for (Harshman et al. 1999; Hoffmann and Parsons 1991). The environment is likely to influence the strength and direction of selection acting on metabolic rate (Burton et al. 2011). In our study, individuals were insulated from interspecific competition by our experimental design (though they were exposed to predation). Under environmental conditions where mortality rates are higher or size-dependent (e.g. faster growing individuals reach a size refuge sooner; (Arendt and Wilson 1997; Metcalfe and

Monaghan 2003)) then individuals with consistently higher metabolic rates may be favoured. In our species at least, it seems that metabolic rate is associated with key life-history traits that determine either a 'fast' (faster growth, earlier reproduction, shorter lifespan) or 'slow' (slower growth, later reproduction, longer life span) life history. For lifetime reproductive output under our experimental conditions, the environment favoured neither high nor low metabolic rates, rather individuals that had negatively correlated metabolic rates between developmental stages were favoured over those individuals with positively correlated metabolic rates.

Metabolic rate and its effects on performance change throughout development

Studies of selection on metabolic rate have been largely based on measures of metabolic rate at single time points in the life history (e.g. (Schimpf et al. 2012a; Schimpf et al. 2012b)). Our results suggest that metabolic rates at different stages throughout the life history can have different and interactive effects on performance. Metabolic rate can fluctuate throughout ontogeny, therefore repeatability is often low and it is unlikely that individuals will express a single metabolic phenotype throughout the life history (Criscuolo et al. 2008; White et al. 2013). In our study, MR_E and MR_L were not strongly correlated, rather their effects on fitness were contingent upon each other and the effects of each measure differed across life-history traits. For example, while individuals with lower MR_L were longer lived, no significant correlation with longevity and MR_E was detected. Conversely, MR_E showed a stronger effect on growth rate than MR_L - when individuals had lower MR_E, then the effects of MR_L were much less important for growth rate than when individuals had higher MR_E. Our findings showed that fitness was dependent on the interaction between MR_E and MR_L, and therefore raise the possibility that single measures of metabolic rate may not fully capture selection – rather, estimating multiple metabolic rates may increase inferential power. It seems that the fitness consequences of different metabolic rates are integrated across the life history. No single metabolic rate affects performance, rather multiple metabolic rates interact to affect

performance. Ideally, we would have taken additional measures of metabolic rate in later life stages, as our findings reflect broader arguments that including more traits is likely to yield a more complete view of selection (Blows 2007; Kingsolver and Pfennig 2007).

Conclusions

We detected a significant, though slight, positive correlation between metabolic rate at each developmental stage, yet we found strong negative correlational selection on metabolic rates such that the positive covariance should be reduced and ultimately made negative over time (assuming persistent selection across generations). If our estimate of correlational selection accurately reflects a persistent selection regime then the positive relationship between metabolic rates is unlikely to represent an adaptive response to selection. We suspect that genetic constraints maintain the positive relationship between metabolic rates, despite selection against this relationship. If metabolic rate in each stage is positively genetically correlated, then there is little genetic variation in the dimension in which selection acts and responses to correlation selection will be constrained. Estimates of the heritability of metabolic rate remain rare (see White and Kearney 2013, Table 5) and as far as we are aware, no study has examined genetic covariance between metabolic rates at different life stages. Thus, estimating the genetic covariance between metabolic traits measured at different stages is an important next step in the examination of the evolution of metabolic rate.

While we measured lifetime reproductive output, we insulated individuals from selection at two critical life stages: the larval and metamorphic phase. A necessary logistical constraint was to measure larvae and metamorphosing individuals in the laboratory. Individuals that expressed consistently high or low metabolic rate phenotypes across both developmental stages had poorest performance during the adult stage (in terms of reproductive output), however it is possible that these phenotypic combinations may yield highest performance in

the larval stage. For example, larvae with a higher metabolic rate may be better able to locate suitable settlement sites. Alternatively, individuals with lower metabolic rates may take longer to metamorphose and therefore suffer higher mortality during this key phase of the life history. Nevertheless, the benefits for those individuals with consistently high or low metabolic rates during the larval stage would have to be considerable in order to offset the fitness costs that are associated with consistent metabolic rates throughout the life history.

References

Alvarez, D., and A. G. Nicieza. 2005. Is metabolic rate a reliable predictor of growth and survival of brown trout (Salmo trutta) in the wild? Canadian Journal of Fisheries and Aquatic Sciences 62:643-649.

Arendt, J. D., and D. S. Wilson. 1997. Optimistic growth: Competition and an ontogenetic niche-shift select for rapid growth in pumpkinseed sunfish (Lepomis gibbosus). Evolution 51:1946-1954.

Artacho, P., and R. F. Nespolo. 2009. Natural Selection Reduces Energy Metabolism in the Garden Snail, Helix aspersa (*Cornu aspersum*). Evolution 63:1044-1050.

Artacho, P., J. Saravia, B. D. Ferrandiere, S. Perret, and J.-F. Le Galliard. 2015. Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. Ecology and Evolution 5:3600-3609.

Auer, S. K., J. D. Arendt, R. Chandramouli, and D. N. Reznick. 2010. Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (Poecilia reticulata). Ecology Letters 13:998-1007.

Auer, S. K., K. Salin, G. J. Anderson, and N. B. Metcalfe. 2015. Aerobic scope explains individual variation in feeding capacity. Biology letters 11.

Bennett, A. F., and J. A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649-654.

Blackmer, A. L., R. A. Mauck, J. T. Ackerman, C. E. Huntington, G. A. Nevitt, and J. B. Williams. 2005. Exploring individual quality: basal metabolic rate and reproductive performance in storm-petrels. Behavioral Ecology 16:906-913.

Blows, M. W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. Journal of Evolutionary Biology 20:1-8.

Blows, M. W., and R. Brooks. 2003. Measuring nonlinear selection. American Naturalist 162:815-820.

Bochdansky, A. B., P. Gronkjaer, T. P. Herra, and W. C. Leggett. 2005. Experimental evidence for selection against fish larvae with high metabolic rates in a food limited environment. Marine Biology 147:1413-1417.

Burton, T., S. S. Killen, J. D. Armstrong, and N. B. Metcalfe. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society B-Biological Sciences 278:3465-3473.

Cameron, J. N. 1986. The solubility of carbon dioxide as a function of temperature and salinity (Appendix table), Pages 254-259 *in* J. N. Cameron, ed. Principles of physiological measurement. United Kingdom, Academic Press.

Clutton-Brock, T. H. 1988, Reproductive success. Chicago, University Chicago Press.

Criscuolo, F., P. Monaghan, L. Nasir, and N. B. Metcalfe. 2008. Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. Proceedings of the Royal Society B-Biological Sciences 275:1565-1570.

Crisp, D. J. 1971. Energy flow measurements, Pages 197-279 *in* N. A. Holme, and A. D. McIntyre, eds. Methods for the study of marine benthos. Oxford, Blackwell Sci.Pub.

Derting, T. L., and P. A. McClure. 1989. Intraspecific variation in metabolic rate and its relationship with productivity in the cotton rat, *Sigmodon hispidus*. Journal of Mammalogy 70:520-531.

Downs, C. J., J. L. Brown, B. Wone, E. R. Donovan, K. Hunter, and J. P. Hayes. 2012. Selection for increased mass-independent maximal metabolic rate suppresses innate but not

adaptive immune function. Proceedings of the Royal Society B-Biological Sciences 280.
Glazier, D. S. 2015. Is metabolic rate a universal 'pacemaker' for biological processes? Biological Reviews 90:377-407.
Grime, J. P., and R. Hunt. 1975. Relative growth rate: its range and adaptive significance in a local flora. Journal of Ecology 63:393-422.
Harshman, L. G., A. A. Hoffmann, and A. G. Clark. 1999. Selection for starvation resistance in Drosophila melanogaster: physiological correlates, enzyme activities and multiple stress responses. Journal of Evolutionary Biology 12:370-379.
Hayes, J. P., and C. S. O'Connor. 1999. Natural selection on thermogenic capacity of high-altitude deer mice. Evolution 53:1280-1287.
Hoffmann, A. A., and P. A. Parsons. 1991, Evolutionary genetics and environmental stress, Oxford University Press.
Keough, M. J. 1989. Variation in growth rate and reproduction of the bryozoan *Bugula neritina*. Biological Bulletin 177:277-286.
Keough, M. J., and H. Chernoff. 1987. Dispersal and population variation in the bryozoan *Bugula neritina*. Ecology 68:199-210.
Kingsolver, J. G., S. E. Diamond, S. A. Seiter, and J. K. Higgins. 2012. Direct and indirect phenotypic selection on developmental trajectories in Manduca sexta. Functional Ecology 26:598-607.
Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and power of phenotypic selection in nature. Bioscience 57:561-572.
Konarzewski, M., and A. Ksiazek. 2013. Determinants of intra-specific variation in basal metabolic rate. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 183:27-41.
Koons, D. N., C. J. E. Metcalf, and S. Tuljapurkar. 2008. Evolution of Delayed Reproduction in Uncertain Environments: A Life-History Perspective. American Naturalist 172:797-805.
Kruuk, L. E. B., T. H. Clutton-Brock, K. E. Rose, and F. E. Guinness. 1999. Early determinants of lifetime reproductive success differ between the sexes in red deer. Proceedings of the Royal Society B-Biological Sciences 266:1655-1661.
Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210-1226.
Larivee, M. L., S. Boutin, J. R. Speakman, A. G. McAdam, and M. M. Humphries. 2010. Associations between over-winter survival and resting metabolic rate in juvenile North American red squirrels. Functional Ecology 24:597-607.
Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131-3137.
Marshall, D. J., and M. J. Keough. 2009. Does interspecific competition affect offspring provisioning? Ecology 90:487-495.
Metcalfe, N. B., and P. Monaghan. 2003. Growth versus lifespan: perspectives from evolutionary ecology. Experimental Gerontology 38:935-940.
Monro, K., and D. J. Marshall. 2014. Faster Is Not Always Better: Selection on Growth Rate Fluctuates across Life History and Environments. The American Naturalist 183:798-809.
Nilsson, J. A. 2002. Metabolic consequences of hard work. Proceedings of the Royal Society B-Biological Sciences 269:1735-1739.
Partridge, L., and K. Fowler. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46:76-91.

Pettersen, A. K., C. R. White, and D. J. Marshall. 2015. Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proceedings. Biological sciences / The Royal Society 282.

Phillips, P. C., and S. J. Arnold. 1989. Visualising multivariate selection. Evolution 43:1209-1222.

Quinn, G. P., and M. J. Keough. 2002, Experimental design and data analysis for biologists. Cambridge, UK, Cambridge University Press.

Reznick, D. N., M. J. Bryant, D. Roff, C. K. Ghalambor, and D. E. Ghalambor. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. Nature 431:1095-1099.

Ricklefs, R. E. 1998. Evolutionary theories of aging: Confirmation of a fundamental prediction, with implications for the genetic basis and evolution of life span. American Naturalist 152:24-44.

Rose, M. R., L. N. Vu, S. U. Park, and J. L. Graves. 1992. Selection on stress resistance increases longevity in *Drosophila melanogaster*. Experimental Gerontology 27:241-250.

Rubner, M. 1908, Das Problem der Lebensdaur und seine Beziehungen zu Wachstum und Ernährung. Munich: Oldenberg.

Schimpf, N. G., P. G. D. Matthews, and C. R. White. 2012a. Cockroaches that exchange respiratory gases discontinuously survive food and water restriction. Evolution 66:597-604.

Schimpf, N. G., P. G. D. Matthews, and C. R. White. 2012b. Standard metabolic rate is associated with gestation duration, but not clutch size, in speckled cockroaches *Nauphoeta cinerea*. Biology Open 1:1185-1191.

Speakman, J. R. 2005. Body size, energy metabolism and lifespan. Journal of Experimental Biology 208:1717-1730.

Steyermark, A. C. 2002. A high standard metabolic rate constrains juvenile growth. Zoology 105:147-151.

White, C. R., and M. R. Kearney. 2013. Determinants of inter-specific variation in basal metabolic rate. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 183:1-26.

White, C. R., M. R. Kearney, P. G. D. Matthews, S. A. L. M. Kooijman, and D. J. Marshall. 2011. A Manipulative Test of Competing Theories for Metabolic Scaling. American Naturalist 178:746-754.

White, C. R., N. G. Schimpf, and P. Cassey. 2013. The repeatability of metabolic rate declines with time. Journal of Experimental Biology 216:1763-1765.

Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305-1314.

Woollacott, R. M., and R. L. Zimmer. 1975. Simplified placenta-like system for transport of extraembryonic nutrients during embryogenesis of *Bugula-neritina* (Bryozoa). Journal of Morphology 147:355-377.

Tables

Table 1. Selection coefficients (±SEM) for larval mass (μg), metabolic rate early (MR_E; mJ h^{-1}), metabolic rate late (MR_L; mJ h^{-1}) with total lifetime reproductive output (cumulative number of offspring produced) for *B. neritina* colonies. β and ɣ represent linear and nonlinear selection gradients respectively.

		ɣ		
	β	Mass	MR_E	MR_L
Larval mass	-0.094	-0.067	-0.022	-0.092
	(0.070)	(0.054)	(0.064)	(0.085)
MR_E	0.070		-0.037	**-0.194***
	(0.070)		(0.040)	(0.083)
MR_L	-0.040			0.046
	(0.072)			(0.060)

*Significance level P-value <0.05

Table 2. Linear mixed model for the relationship between individual colony longevity (number of days until <10% colony alive) with larval mass (μg), metabolic rate early (MR_E; mJ h^{-1}) and metabolic rate late (MR_L; mJ h^{-1}). Model reduced using maximum log-likelihood to remove non-significant interactions (P-value >0.05). All degrees of freedom =1.

Trait	Estimate	χ^2	P-value
Fixed effects:			
Larval mass		0.386	0.534
MR_E		0.000	1
MR_L		0.343	0.558
Larval mass x MR_E		0.080	0.777
Larval mass x MR_L	**-126.375**	**4.244**	**0.039***
MR_E x MR_L		0.058	0.810
Larval mass x MR_E x MR_L		2.967	0.085
Random effects:			
Experimental run		0.001	0.975
Experimental run x Mass		0.000	1
Experimental run x MR_E		0.000	1
Experimental run x MR_L		0.620	0.431
Experimental run x Mass x MR_E		0.272	0.602
Experimental run x Mass x MR_L		0.272	0.602
Experimental run x MR_E x MR_L		0.000	1
Experimental run x Mass x MR_E x MR_L		0.000	1

*Significance level P-value <0.05

Table 3. Final logistic regression model for age at onset of reproduction (number of days) in relation to larval mass (µg), metabolic rate early (MR_E; mJ h^{-1}) and metabolic rate late (MR_L; mJ h^{-1}) for *B. neritina* where age at onset of reproduction (development of reproductive structures) was assigned either early = 0 (< 60 days old) or late = 1 (≥60 days old). Model reduced after testing for non-significant interactions (P-value >0.05). All degrees of freedom =1.

	Estimate	χ^2	P-value
Fixed effects:			
Larval mass		1.596	0.207
MR_E	**-47.822**	**5.064**	**0.024***
MR_L		1.526	0.217
MR_E x MR_L		2.604	0.107
Larval mass x MR_E		2.714	0.099
Larval mass x MR_L		0.029	0.865
Larval mass x MR_E x MR_L		0.008	0.929

*Significance level P-value <0.05

Figures

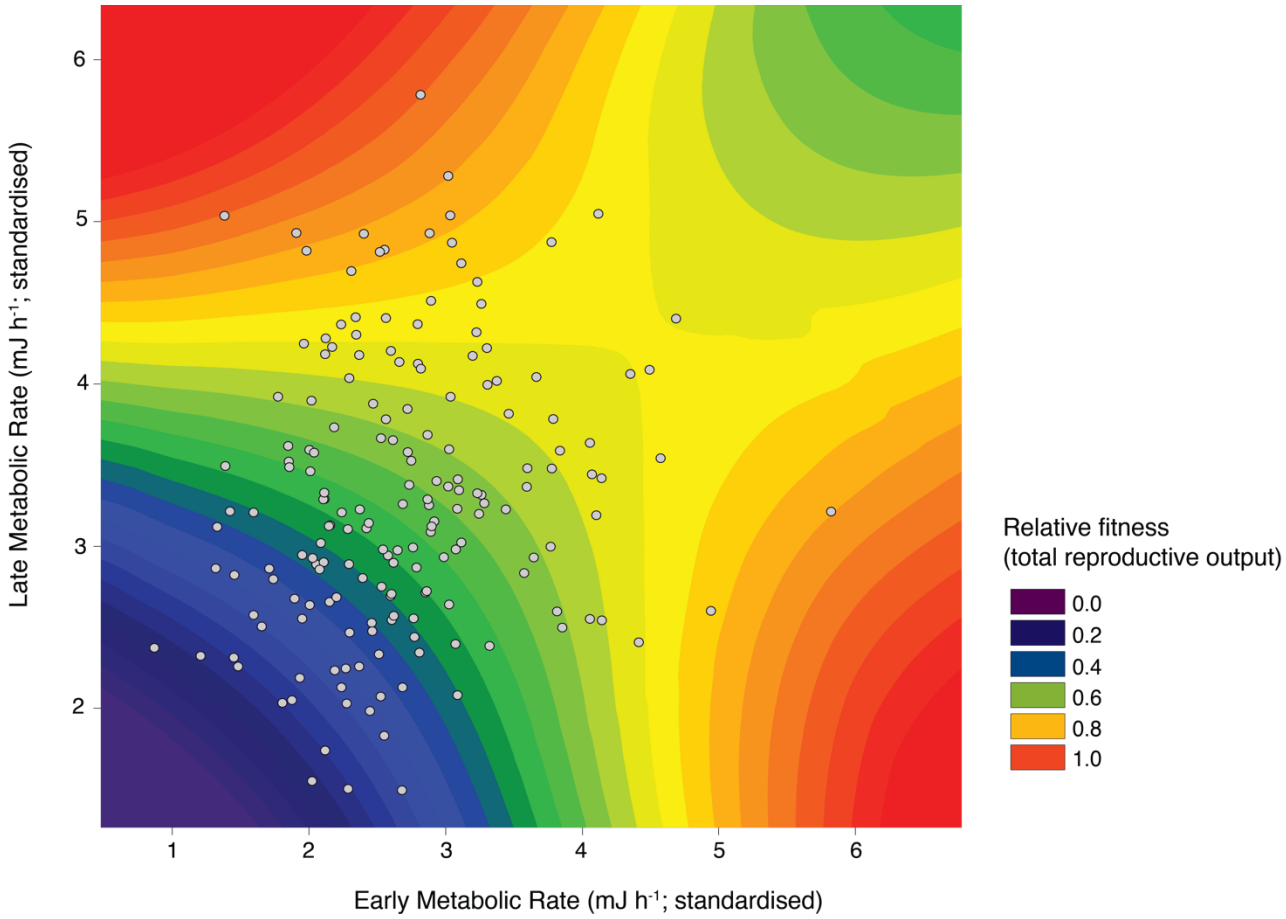


Figure 1. Predicted relative fitness (total reproductive output) plotted against metabolic rate early (MR_E; mJ h^{-1}) and metabolic rate late (MR_L; mJ h^{-1}) for *B. neritina* settlers. White dots represent raw data points between MR_E and MR_L (N = 179). Relative fitness of individuals is highest (red area) with either lower values of MR_E and higher values of MR_L, or higher values of MR_E and lower values of MR_L

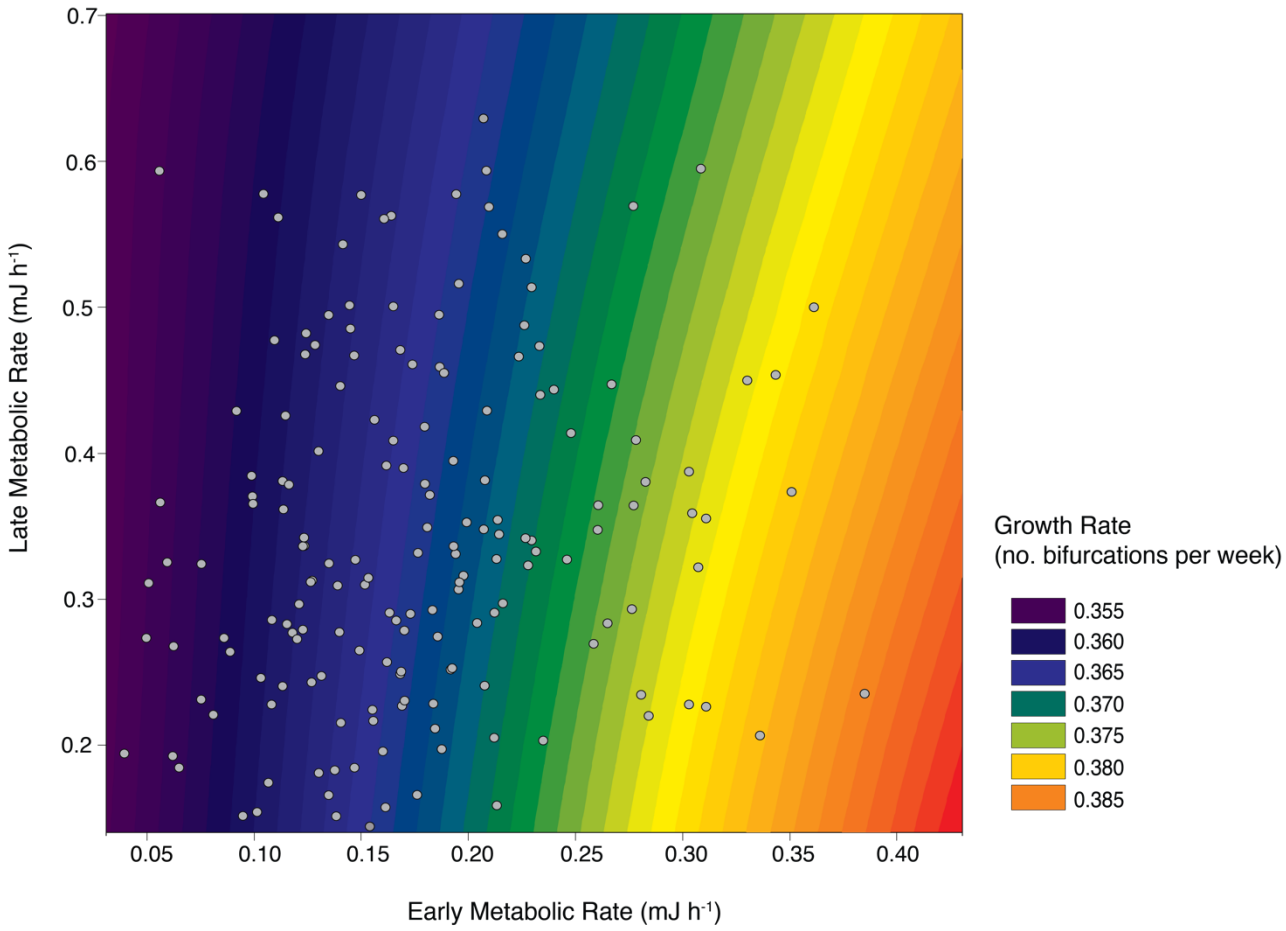


Figure 2. Predicted growth rate (number bifurcations per week for first 20 weeks of development) plotted against metabolic rate early (MR_E; mJ h^{-1}) and metabolic rate late (MR_L; mJ h^{-1}) for *B. neritina* settlers. Data points show raw data for measures of MR_E and MR_L (N = 179). Growth rate is highest for values of higher MR_E and lower MR_L.

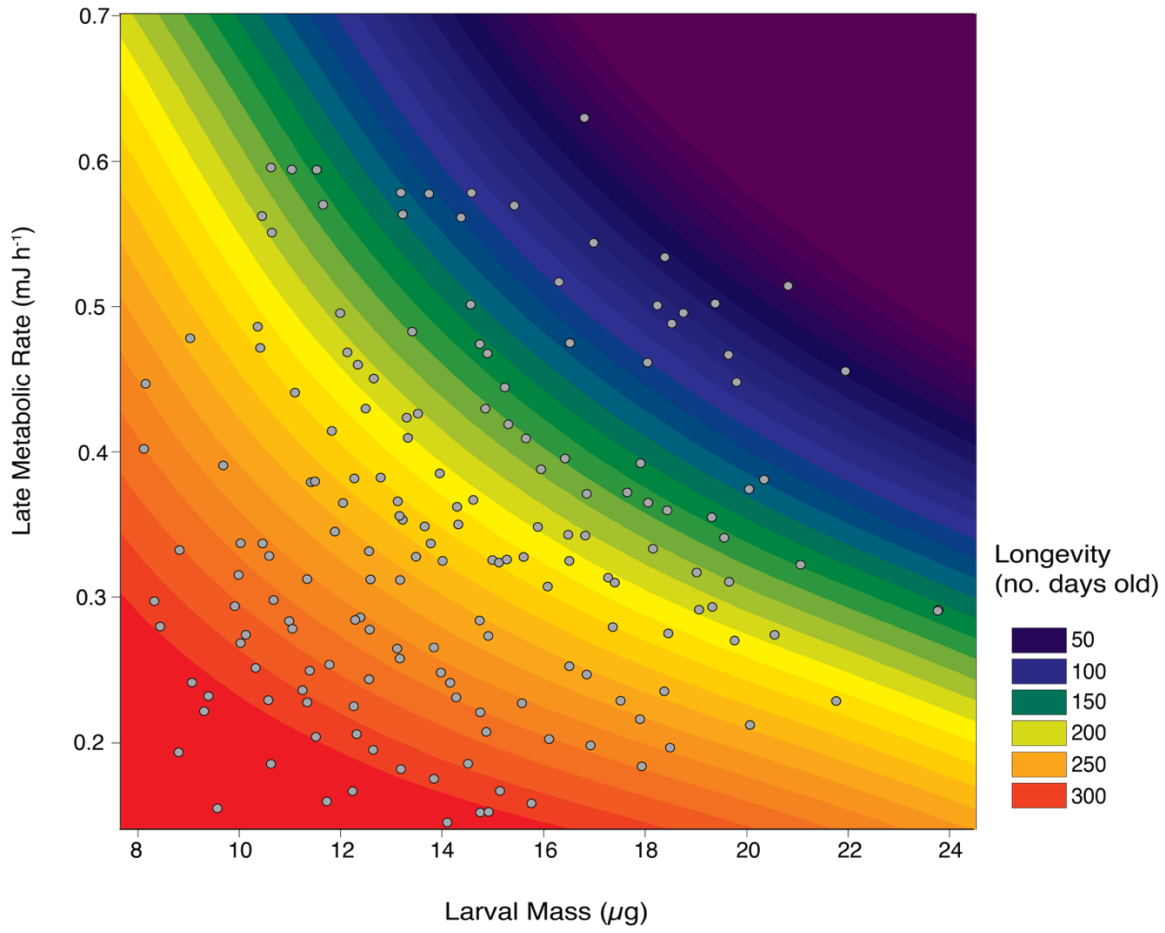


Figure 3. Predicted longevity (number of days until <10% colony alive) plotted against larval mass (µg) and metabolic rate late (MR_L; mJ h^{-1}) for *B. neritina* settlers. Data points show raw data for measures of larval mass and MR_L (N=179). Longevity of a colony is highest for individuals from smaller larvae with lower MR_L.

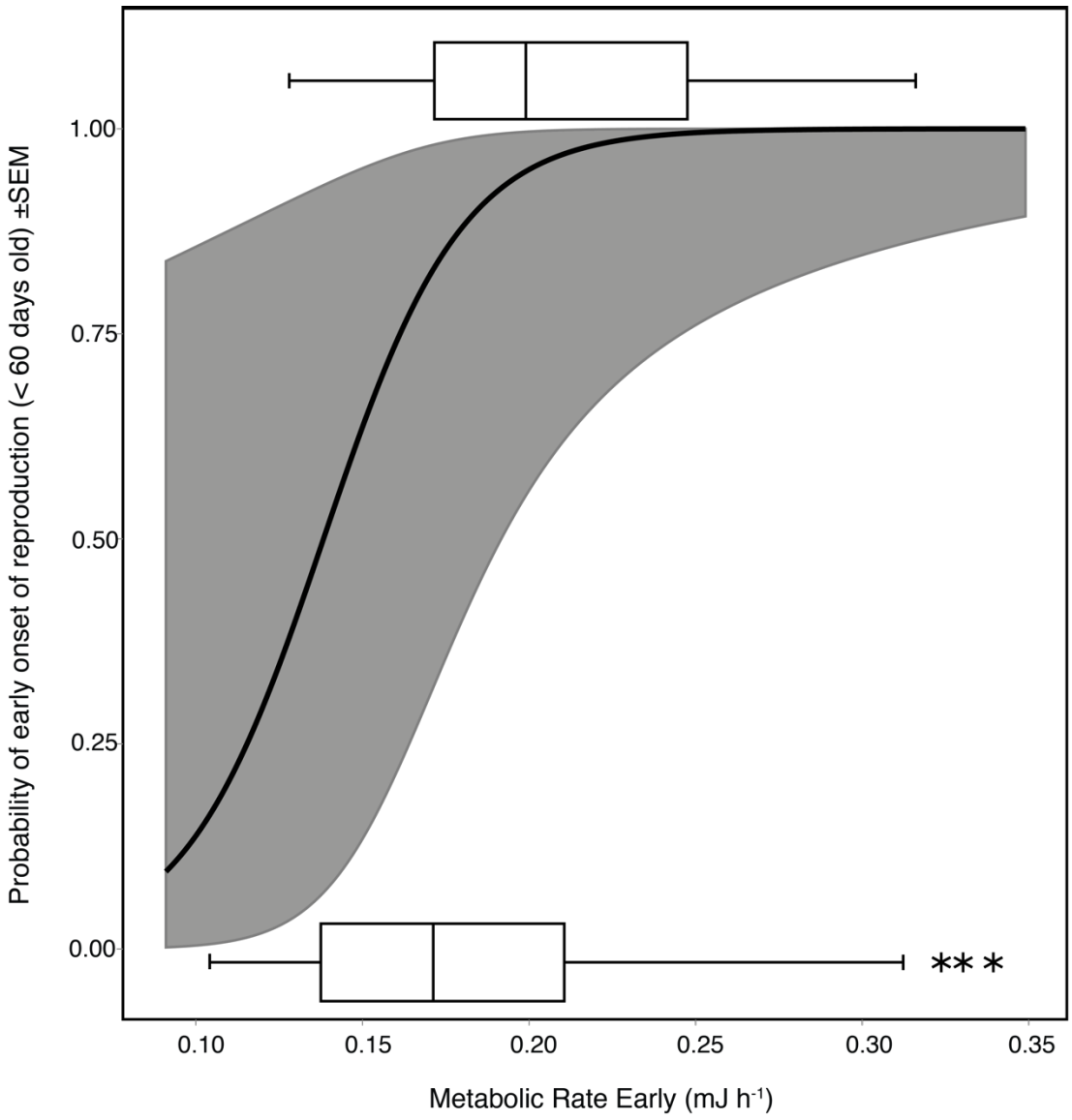


Figure 4. Predicted logistic regression between metabolic rate early (mJ h^{-1}) and probability of early onset of reproduction using logistic regression ± standard error. Early onset of reproduction (colony <60 days old) is assigned a value of 1 while late onset of reproduction (≥ 60 days old) is assigned a value of 0. Onset of reproduction occurs earliest for higher values of MR_E.

Appendix D

Figures

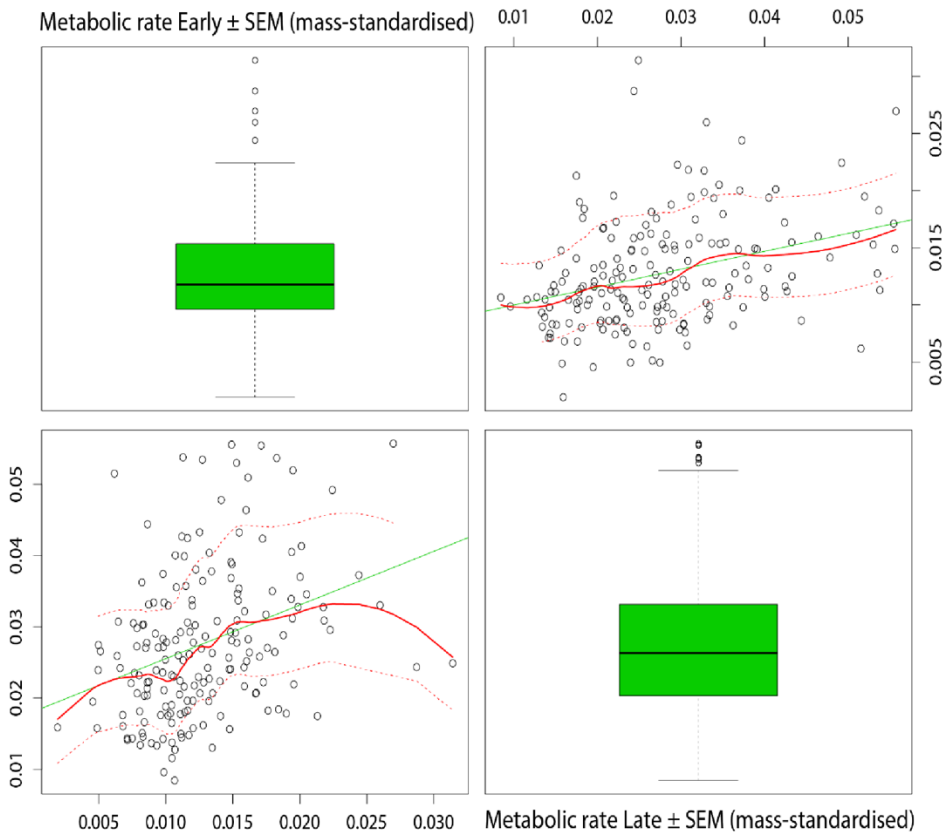


Figure D1. Relationship between metabolic rate early and metabolic rate late (standardised by offspring mass; N = 179). A significant, but weak association between offspring mass-specific metabolic rate early and metabolic rate late was found ($R^2 = 0.34$).

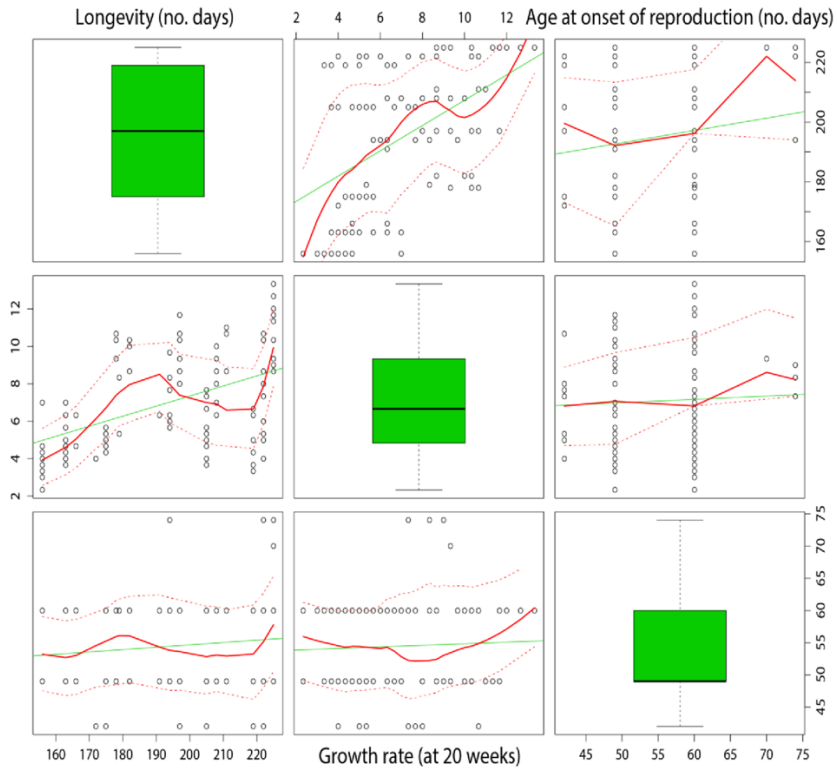


Figure D2. Scatterplot matrix for the relationships between response variables of growth, longevity and size at onset of reproduction (N = 179). A relatively weak, yet significant relationship between longevity and growth was found ($R^2 = 0.48$). No significant relationships between age at onset of reproduction and growth or longevity were found.

General discussion

Importance of integrating life-history theory and metabolic theory

Over the last century, life-history theory and metabolic theory have shaped the way we think about mechanisms by which organisms attain, transform and utilise energy from their environment to maximise fitness, but the two broad theories have developed largely in isolation from each other (Brown and Sibly 2006). Life-history theory explores ultimate causes in order to understand phenotypic variation (Stearns 1992), while metabolic theory links variation in metabolic rate with proximal constraints (Glazier 2005). Both life history and physiological patterns and processes operating at the level of the individual, can be extended to understand broader scale patterns at population, community, and ecosystem-levels. Life-history theory and metabolic theory offer two unique approaches to exploring similar questions in ecology, yet historically, there has been little overlap. While efforts have been made to integrate life-history theory and physiological ecology, with mention of links between energy trade-offs, physiological constraints and fitness (Sibly and Calow 1986; Stearns 1992), direct tests are lacking. Knowledge gaps remain within each field of life-history and metabolic theory, that can be addressed by a more formal consideration of the other. For example, life-history theory offers general patterns such as the offspring size-temperature association, which exists both among and within species, but fails to provide any general mechanism to explain this relationship. Physiologists often seek to assign mechanistic explanations for among-species patterns, such as the relationship between body size and temperature, yet these relationships may be adaptive, and shaped by selection (Atkinson and Sibly 1997; Clarke 2006). Assigning proximal constraints to explain phenotypic variation can only offer limited insight into how traits are likely to evolve under selection. Likewise, many key questions in life-history theory can be addressed by integrating key physiological processes relevant to all organisms.

Metabolic processes and selection are inextricably linked – integrating two fields that specialise in understanding these processes can help to advance our understanding of evolutionary ecology more generally. This thesis provides one of the first formal attempts to unify the fields of life-history theory and metabolic theory.

Using metabolic theory to understand patterns in life-history theory

Metabolic scaling relationships pervade ecology. Linking the flux of energy through living systems to patterns and processes in biology is relevant across scales from the individual level through to community and ecosystems (Brown et al. 2004; Kooijman 2000). Energy use is relevant to all life, during every stage of the life history. Despite this, the vast majority of metabolic scaling studies have been devoted to understanding energy use during the adult stage. Energy at independence is a critical bottleneck to reaching juvenile and adult stages, hence any factor that alters the costs of reaching independence is likely to impose strong fitness consequences (Houde 2002). Life-history theory has acknowledged the importance of early life stages as critical in determining fitness, for example, the offspring size-performance relationship (Marshall and Keough 2008). Yet life-history theory does not have the tools to understand the underlying processes by which these patterns occur. Rather than considering proximal and ultimate causes in isolation, measuring selection on metabolic scaling relationships, and how these change across space and time, can help to address key knowledge gaps in life-history theory. Below I provide examples of key life-history theory questions that I address through applying principles in metabolic theory.

A key life-history pattern is that within species, larger offspring often perform better than smaller offspring (Hutchings 1991; Marshall et al. 2003; Moran and Emlet 2001). I found a potentially widespread explanation for this pattern - that larger offspring are more efficient users of maternal energy investment than smaller offspring, during both metamorphosis

(Chapter 1) and development (Chapter 2). While larger offspring are more costly to provision initially, they use relatively less of their maternally-derived energy throughout the dependent phase, hence the return on a mother's investment of producing a few, large offspring is greater than that of producing many, small offspring of equivalent mass. Despite the ubiquity of allometric scaling of metabolic rate with adult body size, metabolic theory had yet to formally test how energy use relates to offspring size. Likewise, life-history theory had yet to consider the costs of development scaling as anything other than isometric with offspring size. Incorporating allometric scaling with offspring size can substantially alter our understanding of the offspring size-number trade-off, and how we think about the evolution of offspring size as a unit of maternal investment more generally.

Colder mothers often produce larger offspring (Atkinson et al. 2001). Broad-scale offspring size-temperature relationships among species, and across seasonal and latitudinal gradients reflect within-population level patterns of maternal rearing temperature and offspring size. There is good evidence to suggest that this relationship is adaptive – colder mothers make their offspring larger because particularly in colder environments, larger offspring perform better than smaller offspring (Bownds et al. 2010; Burgess and Marshall 2011). Here, I propose a potentially general adaptive explanation for why colder mothers produce larger offspring – the costs of development (as a product of metabolic rate and development time) are greatest at cooler temperatures. If offspring size reflects the total maternal investment required for an offspring to complete development, then colder mothers may need to provision their offspring with more resources (Chapter 3). The relationship between metabolic rate and temperature is central to metabolic theory (Gillooly et al. 2001; White et al. 2012), but is also dominated by studies on adult size, with only brief mention (and a lack of formal tests) for the mechanisms underlying the offspring size-temperature relationship (Zuo et al. 2012). I show that temperature can produce size-dependent fitness consequences for offspring, where the

temperature sensitivity of key physiological processes play a fundamental role in shaping the evolution of offspring size.

Using life-history theory to understand patterns in metabolic theory

As processes of metabolic theory, such as scaling relationships, can be used to inform life-history theory, so too can ultimate causes employed by life-history theory be used to help explain phenotypic variation in metabolic rates. Natural selection shapes phenotypic variation in natural populations, and is a key source of adaptive evolution. Yet, metabolic theory has primarily relied on mechanistic explanations to understand variation in metabolic rate. So far, there have been few examples where a microevolutionary framework has been implemented in studies of metabolic rate, and even fewer of these under natural field conditions (however see Artacho and Nespolo 2009; Artacho et al. 2015; Rønning et al. 2016; Nilsson et al. 2016). I show that metabolic rate is indeed under selection, and that in this study system at least, selection acts to decrease covariance between metabolic rates measured across ontogeny (Chapter 4). This has important implications for the evolution of metabolic rates – if our findings reflect selection on metabolic rates more generally, then under persistent negative correlational selection, we would expect decoupling of metabolic rates across the life history. Further, selection on metabolic rate during one life stage does not represent selection on metabolic rate later in ontogeny (even over small ontogenetic scales). Hence, it is important for the field to treat metabolic rates as such, and measure selection on multiple metabolic rates throughout the life history in order to adequately capture how metabolic rates relate to fitness.

Future directions for integrating life-history theory and metabolic theory

This thesis gives some insight into how life-history theory and metabolic theory can be integrated to explain key patterns in both fields, and variation in key life history and

physiological traits more generally. This is one of the first attempts to formally link two fundamental fields of ecology, which have until now provided independent perspectives on maternal investment and the pace of life. The results of this thesis offer some potentially widespread explanations for common patterns in life-history theory and metabolic theory, yet further tests are needed. Here, I outline several recommendations to further integrate life-history theory and metabolic theory, and to link other key patterns with general underlying processes.

The findings in this thesis generally lend support to current life-history theory, or the "bigger-is-better" hypothesis, with regards to offspring size. I found that larger offspring are more efficient users of maternal energy investment than smaller offspring, and that at cooler temperatures, increased costs of development are likely to drive selection for larger offspring size (Chapters 1-3). However, one clear question remains -if there are such clear advantages to producing larger, more energy efficient offspring, why do we continue to observe smaller offspring sizes? I propose that the fecundity costs will, in many cases, still offset any advantage to a mothers fitness for increasing offspring size. Despite the obvious fitness gains for offspring, maternal fitness is likely to drive the evolution of offspring size (Wolf and Wade 2001). For example, in Atlantic salmon, despite egg size being closely linked to offspring fitness, maternal fitness was maximised when producing offspring of intermediate size (Einum and Fleming 2000). Further studies relating the correlation between offspring and maternal fitness under natural conditions (and across environments) are needed, in order to elucidate the relationship between offspring size and maternal fitness (Venable and Brown 1988).

The offspring size-performance relationship is highly context-dependent (e.g. Reznick et al. 1990), which may simply be due to an absence of selection under benign environments (e.g. Monro et al. 2010), or conversely, size-independent mortality could occur under extremely stressful environments (e.g. Allen et al. 2008). Alternatively, disadvantages associated with

increases in offspring size may overcome the benefits of allometric scaling, and should be explored. For example, development time is positively correlated with size for nonfeeding offspring (Marshall and Bolton 2007; Moles and Westoby 2003; Vance 1973). Hence, increased development time can pose greater exposure to predation and starvation-disadvantages which could offset the advantages of allometric scaling. It is important to determine whether allometric scaling with offspring size (Chapters 1 and 2) occurs more generally – both in other species, and across spatial and temporal scales. Tests of whether development time scales more steeply (hyperallometrically) than metabolic rate scales hypoallometrically with offspring size, and the fitness consequences of this, are needed to inform the offspring size-number trade-off and variation in offspring size more generally.

Offspring size has traditionally been used as the measure of maternal investment. Offspring size is an easily measured trait that is ubiquitous across the metazoan and generally provides a good indication of offspring quality. Yet offspring size itself isn't everything – mothers provide much more than an egg. How maternal provisioning in its entirety scales with offspring size, will ultimately shape the offspring size-number trade-off, and the evolution of offspring size itself. This thesis only considers maternal energy allocation where all energy is allocated prior to development and metamorphosis. While this incorporates the majority of costs for species that either undergo metamorphosis (many fish, amphibians, insects and marine invertebrates), or direct development, such as reptiles and birds, it neglects to account for how maternal investment across other developmental modes might scale with offspring size. For example, in species with a high matrotrophic index, such as in placental mammals and fish, mothers provide constant provisioning of offspring from oogenesis through to independence (i.e at birth or hatching) - a nontrivial proportion of a mothers finite energy resources. Offspring size as a measure of the per unit offspring investment may become more, or less important with greater maternal investment. In order to determine whether allometric scaling with offspring

size is a general pattern, requires further investigation of scaling relationships across all reproductive strategies.

Findings from Chapter 3 predict that the costs of development cannot continue to decrease inevitably with temperature. At some point beyond a species natural temperature range, an increase in temperature will cause development time to plateau, while metabolic rate will continue to rise. This will result in an inflection point in the costs of development, where higher temperatures will induce greater costs of reaching independence, relative to cooler temperatures. Selection should act to reduce the costs of development, and given metabolic rate is generally somewhat heritable, we would expect it to evolve in response to selection. However, temperature-dependent selection on development time and metabolic rate has yet to be measured under natural conditions. Given the rate of climate change, a pertinent question is therefore, whether the temperature sensitivity of metabolic rate be able to evolve quickly enough to reverse or mediate this pattern, particularly in species with narrow temperature ranges that are likely to be more susceptible to temperature change.

Findings in Chapter 4 revealed the presence of selection acting on early life stage metabolic rates – these metabolic rates were also related to the pace of life. It would be interesting to explore whether the same selection pressures, and a positive covariance between higher metabolic rate and a faster pace of life exists for metabolic rates later in life. What is needed now, are measures of repeatability of metabolic rate throughout ontogeny under natural conditions. Further, selection may not be acting directly on metabolic rate, per se. Rather selection may be acting on metabolic rate indirectly, through selection on traits that are correlated with metabolic rate, such as growth rate or age at onset of reproduction. More measures of metabolic rate throughout the life history, traits correlated with the pace of life, and fitness (lifetime reproductive output) are needed in order to resolve relationships between the pace of life and metabolic rate, and how these should evolve.

Metabolic theory cannot explain all variation associated with metabolic rates. Chapter 4 showed that microevolutionary processes of selection can explain variation in metabolic rate, for example, in *Bugula neritina*, selection acts to decrease covariance between two metabolic rates. However, selection on almost any continuous trait is likely to be context-dependent (Johnson et al. 2013; Siepielski et al. 2009), and metabolic rate is no exception. While we found selection acting on metabolic rates, the fitness consequences of a fast or slow pace of life is likely to depend is also likely to be a product of its environment. For example, more stressful environments might select for faster metabolic rates, whereas under less stressful environments (such as in Chapter 4), selection on metabolic rates could be relaxed. Faster metabolic rates are also associated with faster growth rates and earlier onset of reproduction hence, selection for a faster 'pace of life' may be synonymous with selection for faster metabolic rates. Selection regimes change across environments, and may explain how variation in metabolic rate is maintained, even after accounting for key predictors of metabolic rate, such as body size and temperature. Measures of context-dependent selection on metabolic rate is an important next step for understanding the extent to which environmental heterogeneity predicts variation in metabolic rates more generally.

Thesis conclusions

Integrating life-history theory and metabolic theory can yield insights about key underlying processes that drive patterns relevant to both fields. This thesis serves as a basis upon which two previously distinct fields that deal with ultimate and proximate causes of variation, respectively, can be unified in order to understand the evolution of fundamental traits in ecology.

References

Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. American Naturalist 171:225-237.

Artacho, P., and R. F. Nespolo. 2009. Natural Selection Reduces Energy Metabolism in the Garden Snail, Helix aspersa (*Cornu aspersum*). Evolution 63:1044-1050.

Artacho, P., J. Saravia, B. D. Ferrandiere, S. Perret, and J.-F. Le Galliard. 2015. Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. Ecology and Evolution 5:3600-3609.

Atkinson, D., S. A. Morley, D. Wheetman, and R. N. Hughes. 2001. Offspring size responses to maternal temperature in ectotherms. Environment and animal development:269-285.

Atkinson, D., and R. M. Sibly. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends in Ecology & Evolution 12:235-239.

Bownds, C., R. Wilson, and D. J. Marshall. 2010. Why do colder mothers produce larger eggs? An optimality approach. Journal of Experimental Biology 213:3796-3801.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771-1789.

Brown, J. H., and R. M. Sibly. 2006. Life-history evolution under a production constraint. Proceedings of the National Academy of Sciences of the United States of America 103:17595-17599.

Burgess, S. C., and D. J. Marshall. 2011. Temperature-induced maternal effects and environmental predictability. Journal of Experimental Biology 214:2329-2336.

Clarke, A. 2006. Temperature and the metabolic theory of ecology. Functional Ecology 20:405-412.

Einum, S., and I. A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248-2251.

Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662.

Houde, E. D. 2002. Chapter 3. Mortality, Pages 64-87 *in* L. A. Fuiman, and R. G. Werner, eds. Fishery science: The unique contribution of early life stages. Oxford, Blackwell Scientific Publishing.

Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salelinus fontinalis*. Evolution 45:1162-1168.

Johnson, D. W., K. Monro, and D. J. Marshall. 2013. The maintenance of sperm variability: context-dependent selection on sperm morphology in a broadcast spawning invertebrate. Evolution 67:1383-1395.

Kooijman, S. A. L. M. 2000, Dynamic energy and mass budgets in biological systems Cambridge, Cambridge Univ. Press.

Marshall, D. J., and T. F. Bolton. 2007. Effects of egg size on the development time of non-feeding larvae. Biological Bulletin 212:6-11.

Marshall, D. J., and M. J. Keough. 2008. The relationship between offspring size and performance in the sea. American Naturalist 171:214-224.

Marshall, D. J., J. A. Pechenik, and M. J. Keough. 2003. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial, ascidian *Diplosoma listerianum*. Marine Ecology Progress Series 246:153-162.

Moles, A. T., and M. Westoby. 2003. Latitude, seed predation and seed mass. Journal of Biogeography 30:105-128.

Monro, K., T. Sinclair-Taylor, and D. J. Marshall. 2010. Selection on offspring size among environments: the roles of environmental quality and variability. Functional Ecology 24:676-684.

Moran, A. L., and R. B. Emlet. 2001. Offspring size and performance in variable environments: Field studies on a marine snail. Ecology 82:1597-1612.

Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357-359.

Rønning, B., J. Broggi, C. Bech, B. Moe, T. H. Ringsby, H. Pärn, I. J. Hagen et al. 2016. Is basal metabolic rate associated with recruit production and survival in free-living house sparrows? Functional Ecology 30:1140-1148.

Rønning, B., H. Jensen, B. Moe, and C. Bech. 2007. Basal metabolic rate: heritability and genetic correlations with morphological traits in the zebra finch. Journal of Evolutionary Biology 20:1815-1822.

Sibly, R. M., and P. Calow. 1986, Physiological ecology of animals: an evolutionary approach. Oxford, Blackwell Scientific Publications.

Siepielski, A. M., J. D. DiBattista, and S. M. Carlson. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecology Letters 12:1261-1276.

Stearns, S. C. 1992, The Evolution of Life Histories. New York, Oxford University Press.

Vance, R. R. 1973. On reproductive strategies in marine benthic invertebrates. American Naturalist 107:339-352.

Venable, D. L., and J. S. Brown. 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. American Naturalist 131:360-384.

White, C. R., P. B. Frappell, and S. L. Chown. 2012. An information-theoretic approach to evaluating the size and temperature dependence of metabolic rate. Proceedings of the Royal Society B-Biological Sciences 279:3616-3621.

Wolf, J. B., and M. J. Wade. 2001. On the assignment of fitness to parents and offspring: whose fitness is it and when does it matter? Journal of Evolutionary Biology 14:347-356.

Zuo, W., M. E. Moses, G. B. West, C. Hou, and J. H. Brown. 2012. A general model for effects of temperature on ectotherm ontogenetic growth and development. Proceedings of the Royal Society B-Biological Sciences 279:1840-1846.