THE ADAPTIVE SIGNIFICANCE OF EGG-SIZE VARIATION WITHIN AND AMONG POPULATIONS OF ATLANTIC SALMON

by

Njal Rollinson

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
July 2013

To Diane Greenfield, for dragging me (kicking and screaming)

to the front of the class.

Table of Contents

List of Tables

List of Figures

Abstract

This thesis focuses on the classic problem of investment per offspring. It is an attempt to (i) reconcile theoretical research with empirical methods that can be used to test theory, (ii) test a fundamental prediction that arises from classic theory, and (iii) test one of the more recent theoretical developments. We use Atlantic salmon (*Salmo salar*) as a model organism. Drawing from the classic Smith–Fretwell model, we provide defensible definitions of offspring fitness that can be used in empirical studies, and we show using simulation that the Weibull-1 statistical model provides the best estimates of optimal investment patterns. Next, we apply these methods to mark-recapture data collected for juvenile Atlantic salmon. This experiment supports the prediction that parental reproductive success is maximized by increasing investment per offspring when environmental conditions become unfavourable. Having verified this prediction, we test a general extension of classic theory which broadly suggests that large-bodied females decrease the quality of the offspring environment, such that larger females in a population ought to invest relatively heavily in investment per offspring. This might occur, for example, when larger females have a greater fecundity and if optimal investment per offspring increases with sibling competition among non-dispersive offspring. The results of this experiment generally do not support the idea that large females decrease the quality of the offspring environment in Atlantic salmon. Finally, we also provide evidence against a verbal hypothesis that attempts to explain inter-population variation in egg size of salmonids as an adaptation to population-specific spawning substrates. We conclude that the classic model of egg-size optimization can be a useful tool for understanding patterns of reproductive allocation in nature, but that investment per offspring is an extremely complex trait that cannot be fully understood by invoking a simple optimality model. Variation in investment per offspring, especially that which occurs within populations, is most parsimoniously attributed to the physiological factors (e.g., variation in testosterone levels), morphological constraints (e.g., the size of the pelvic aperture) and genetic factors (e.g., genetic correlations arising from pleiotropic genes) that affect this phenotype and that constrain adaptive evolution of this trait.

List of Abbreviations and Symbols Used

^{o}D	Degree Days
w_i	Akaike weight
Eco	Economy River
EWM	Egg Wet Mass
GI	Granulometric Index
GrV	Great Village River
HD	High Density
LD	Low Density
LG	Large Gravel
PC1	Principle Component 1
SG	Small Gravel
Stw	Stewiacke River
x-min	Minimum Viable Offspring Size

Acknowledgements

I flunked Grade 10 science. In fact, I also flunked Grade 10 math and Grade 12 physics. I almost flunked Grade 9 science, too. I think part of my problem was that I went to a French school, so all my courses were in French. I didn't have a clue what was going on most of the time. (Did I mention that I got a "D–" in Grade 12 French?). Other than sending me to a French school, my parents are the best, and their parenting and respective personalities are ultimately the reason I was able to write a PhD thesis. I suppose I should thank Kyla and Ayesha, too, for providing inspiration to stay fit, for being my crazy sisters, and for generally promoting 'favourable environmental conditions'.

My advisor, Jeff Hutchings, really came through when I needed him, and the opportunities Jeff has given me throughout the last five years can't be understated. Thanks Jeff, I really appreciate it. Both Mark Johnston and Bob Latta also gave me great advice. In fact, Chapter 2 arose in large part from Bob's concern about sampling error and Mark's suggestion to evaluate the performance of various statistical models. Many other people also provided help and feedback, including M. Kinnison, H. Kindsvater, T. Quinn, D. Marshall, T. Day, N. Metclafe, P. Debes, B. Lenentine and the DFO, J. Porter, K. Crosby, D. Keith, S. Mogensen, B. Farmer, J. Eddington, and J. Pezzullo. I owe a *huge* thanks to A. Houde, M. McBride and M. Yates for their help in the field and laboratory.

I met some great people over the last few years. I won't forget those lazy Friday afternoons with Devon, Igor, Bob, Susan, C-W-P, McElroy, Kate, and if we were lucky, Kozela. Nor will I forget Dave, Rebekah, Emily, Nancy and Steph, who were always willing to smile at my antics, and I at theirs. Kate Crosby kept it "hardcore on the 7th floor", and she helped me survive the LSC dungeon by dragging me outside for early afternoon Nolan Ryans. Lastly, I thank Paul Debes and Jackie Porter. The former, Paul, made the last few years fly by. The Germinator, my labmate, my flatmate, my partner in crime, the man who had my back in the Supreme Court of Nova Scotia, and my new and lifelong friend. The latter, Jackie, the love of my life, my adventurous new wife, destroyer of slum landlords, the one who continues to inspire me to be the best person that I can possibly be. Finally, I thank Natural Sciences and Engineering Research Council, the Killam Trust Foundation and Dalhousie University for funding this project.

Chapter 1: Introduction

"*Life history theory is an elaborate answer to the simple question of why having more offspring is not always selected for*." (Van Noordwijk and De Jong 1986, p. 137)

This thesis focuses on Smith and Fretwell's (1974) theory of egg-size evolution. In their paper, Smith and Fretwell draw from the ideas of Lack (1947) and Svärdson (1949), who recognized that parents must trade off the number of offspring they produce against the amount of energy invested in each offspring. Based on this principle, Smith and Fretwell argue that parents must balance the fitness gains accrued from increases in fecundity with the fitness losses that result from investment-related decreases in offspring viability. Natural selection on parental investment per offspring is therefore stabilizing, and it is the relationship between investment per offspring and offspring fitness in any given environment determines the optimal reproductive strategy for parents.

Smith and Fretwell's model has been tremendously influential, yet explicit and rigorous tests of the classic model (and its extensions) are very rare. Among the few studies in which optimal investment per offspring has been estimated, many use statistical methods or models that are not congruent with Smith and Fretwell's theory. Part of the problem is that no formal relationship between theoretical and empirical research has been established. The first objective of this thesis is to integrate Smith and Fretwell's (1974) classic theory of egg-size evolution with empirical methods that can be used to test theoretical predictions; this endeavour comprises Chapter 2.

One major prediction that arises from classic theory is that optimal investment per offspring should increase as the quality of the offspring rearing environment decreases. This idea is central to extensions of the Smith Fretwell model and to our understanding of spatial and temporal variation in investment strategies, but it has never been formally tested. The second objective of this thesis is to use the novel methods developed in Chapter 2 to test the hypothesis that optimal investment per offspring increases as environmental quality decreases. This study comprises Chapter 3. Chapters 2 and 3 therefore emphasize that very little empirical work has focused on fundamental aspects of

classic egg-size theory. These chapters fill a void in the literature and will hopefully promote an interest in the empirical study of offspring size – number strategies.

Having found reasonable (but not unequivocal) support for the environmental quality hypothesis in Chapter 3, we explore an extension of classic egg-size theory in Chapters 4 and 5. This extension relies on a negative relationship between optimal size and environmental quality, and it attempts to explain intraspecific correlations between maternal body size and investment per offspring in adaptive terms. No reasonable support is found for this extension of classic theory. Finally, Chapter 6 involves a direct test of a long-standing hypothesis which posits that mean egg size in populations of salmonid fishes evolves, at least in part, in response to population-specific spawning substrates. Little support is found for the original hypothesis. Therefore, Chapters 4, 5 and 6 suggest an underemphasized role of evolutionary constraints in generating variation in egg size both within and among populations. In the Conclusion of this thesis, I discuss the evolutionary constraints that limit the adaptive evolution of investment per offspring, and I emphasize how Smith and Fretwell's model is best used a guide to help one understand the causes and consequences of variation in investment per offspring.

Salmonids are indeed a good model organism for testing theories of parental care, and this group has a long history in the study of investment per offspring. One reason for this is because they provide little post-partum care to offspring, such that egg size (e.g., egg weight or egg diameter) is a good proxy for the amount of energy invested per offspring. Salmonid fish have been involved in tests of Smith and Fretwell's (1974) classic model (Hutchings 1991; Einum and Fleming 2000a), and in both developing and testing extensions of classic theory (Hendry et al. 2001; Einum and Fleming 2002; Einum et al. 2002; Hendry and Day 2003). For this reason, Atlantic salmon (*Salmo salar*) was chosen as a model organism for the experimental aspect of this thesis.

This is a publication-based thesis. All of the main chapters have been published in peer-reviewed journals, and each main chapter in this thesis is largely a stand-alone body of research with an Abstract, Introduction, a Methods sections and a Discussion. This thesis comprises five publications (Rollinson and Hutchings 2010, 2011a; b, 2013a; b) which are presented in a total of seven thesis chapters. All literature cited in this thesis has been integrated into a single literature cited section. A full citation for each

publication is given at the beginning of each chapter, and copyright permission letters for Rollinson and Hutchings (2011a, 2011b, 2013a) are found in the Appendices. Jeffrey Hutchings and I, Njal Rollinson, own the copyright for Rollinson and Hutchings (2010), as Evolutionary Ecology Research (the journal) vets copyright to its authors one year after publication. Similarly, Jeffrey Hutchings and I, Njal Rollinson, are allowed to reprint Rollinson and Hutchings (2013b) in this thesis, provided that we provide a full citation to this article, and that the copyright notice as printed in the journal is provided. Therefore, for Rollinson and Hutchings (2013b), which comprises Chapter 3 of this thesis, we have provided the full citation and the copyright notice at the beginning of Chapter 3. I affirm that I collected and analyzed the data in each publication, and I wrote each manuscript and thesis chapter. However, the pronoun "we" is used throughout the thesis because Jeffrey Hutchings is a contributing author for each publication. Further details are outlined in the "Student Contributions to Manuscripts in Thesis" form which accompanies this thesis. I have edited each publication such that the thesis chapters represent a flowing, cohesive body of research in which a central theme is emphasized and relationships among chapters are highlighted; in no case have I altered the principle arguments or conclusions underlying any publication. Some chapters have been edited to eliminate repetition, and citations have been updated to reflect the latest body of research on a subject. Text-based supplementary information published in the form of appendices has been integrated into the main text of this thesis, such that all publication-based appendices in this thesis comprise large data tables, not figures.

Chapter 2: The Relationship Between Offspring Size and Fitness: Integrating Theory and Empiricism

Published as: Rollinson, N. and J. A. Hutchings. 2013. The relationship between offspring size and fitness: integrating theory and empiricism. Ecology 94: 315-324.

2.1 Abstract

How parents divide the energy available for reproduction between size and number of offspring has a profound effect on parental reproductive success. Theory indicates that the relationship between offspring size and offspring fitness is of fundamental importance to the evolution of parental reproductive strategies: this relationship predicts the optimal division of resources between size and number of offspring, it describes the fitness consequences for parents that deviate from optimality, and its shape can predict the most viable type of investment strategy in a given environment (e.g., conservative *vs* diversified bet hedging). Many previous attempts to estimate this relationship and the corresponding value of optimal offspring size have been frustrated by a lack of integration between theory and empiricism. In the present study, we draw from Smith and Fretwell's classic model to explain how a sound estimate of the offspring–size fitness relationship can be derived with empirical data. We evaluate what measures of fitness can be used to model the offspring size–fitness curve and optimal size, as well as which statistical models should and should not be used to estimate offspring size–fitness relationships. To construct the fitness curve, we recommend offspring fitness be measured as survival up to the age at which the instantaneous rate of offspring mortality becomes random with respect to initial investment. Parental fitness is then expressed in ecologically meaningful, theoretically defensible, and broadly comparable units: *the number of offspring surviving to independence*. While logistic and asymptotic regression have been widely used to estimate offspring size–fitness relationships, the former provides relatively unreliable estimates of optimal size when offspring survival and sample sizes are low, and the latter is unreliable under all conditions. We recommend the Weibull-1 model be used to estimate this curve because it provides modest improvements in prediction accuracy under experimentally-relevant conditions.

2.2 Introduction

Natural selection on body size and size-related traits is ubiquitous and predominantly positive (Kingsolver and Diamond 2011). This is also true of selection during early life, where offspring emerging from larger eggs or seeds typically exhibit

greater survival (e.g., bryozoans: Marshall and Keough 2006; fish: Einum and Fleming 2000; amphibians: Altwegg and Reyer 2003; reptiles: Janzen et al. 2000; birds: Krist 2011; some plants: e.g., Charpentier et al. 2012). Yet, if selection usually favors large offspring, why do we not observe the evolution of increasingly large eggs and seeds? Current theory was fashioned by the ideas of Lack (1947) and Svardson (1949), who recognized that selection will act to maximize parental fitness, not offspring fitness, and that an increase in parental fecundity occurs at a cost to investment per offspring (offspring size). These and other concepts were synthesized by Smith and Fretwell (1974) in their classic model of offspring-size evolution. Smith and Fretwell proposed that there is an optimal level of investment per offspring that will maximize parental reproductive success in a given environment, but optimal size will differ among environments according to the shape of the relationship between offspring size and offspring fitness (Fig. 2.1).

Smith and Fretwell (1974) recognized that the relationship between offspring size and fitness is fundamental to the study of offspring size-number strategies (Fig. 2.1*A*). First, it will reveal the value of optimal offspring size in a given environment (Figs. 2.1, 2.2), such that quantitative tests of optimality can be performed with empirical data (Orzack and Sober 1994). Second, this relationship can be used to approximate the fitness consequences for parents that produce offspring that are larger or smaller than the optimal value, and this may lead to a better understanding of within or among individual variation in offspring size (see below, Fig. 2.2). Finally, subsequent development of the Smith-Fretwell model has illustrated that different types of offspring provisioning strategies, such as diversified bet hedging *versus* conservative bet hedging (Einum and Fleming 2004), usually require different functional relationships between offspring size and fitness, such that the shape of the fitness curve can also intimate the type of investment strategy that is viable in a given environment (for more details, see McGinley et al. 1987, Marshall et al. 2008).

While it is clear that the offspring size–fitness relationship is fundamental to understanding size-number strategies, few experimental studies have provided sound estimates of the offspring size-fitness curve and the corresponding value of optimal size. There are two reasons for this. First, empirical study of size-number strategies is still a

developing field. Experimental studies that estimate offspring size–fitness relationships began only recently (Hutchings 1991), and most have appeared since the turn of the 21st century (Table 2.1). The other reason is methodological. The recent proliferation of experimental research has occurred in the absence of a literature describing how to construct a sound, theoretically-defensible estimate of the offspring size–fitness relationship. It is telling that an exceedingly broad array of statistical models has been used to estimate the fitness curve from experimental data, including asymptotic regression (Charpentier et al. 2012), logarithmic regression (Dziminski et al. 2009), linear regression (Hutchings 1991), power regression (Heath et al. 2003), polynomial regression (Janzen and Warner 2009), logistic regression (Marshall and Keough 2008) and cubic splines (Rankin and Sponaugle 2011). Some of these models can on *a priori* grounds be deemed unlikely to accurately describe the fitness relationship. Furthermore, a sound estimate of this relationship requires a metric of offspring fitness that directly links parental reproductive success to investment per offspring, but many different metrics of offspring fitness are currently being used (e.g., Einum and Fleming 2000, Marshall and Keough 2008, Dziminski et al. 2009, Bownds et al. 2010).

The present synthesis has three objectives. The first is to explain why the offspring size–fitness curve and the concept of optimal offspring size are fundamental to understanding the ecological and evolutionary significance of size-number strategies. The second is to evaluate what measures of offspring fitness can be used to generate a sound estimate of the offspring size–fitness curve and the corresponding value of optimal size. Finally, we evaluate which statistical models should and should not be used to estimate offspring size-fitness relationships. Ultimately, we aim to promote an integration of theoretical and empirical research, and we hope that our recommendations will facilitate the comparison and communication of results, which may provide broad insight into the adaptive significance of size-number strategies.

2.2.1 Why Estimate Optimal Size?

The study of optimality focuses on evolutionarily stable phenotypes (Orzack and Sober 1994), and optimality models aim to predict these phenotypic values (Parker and Maynard Smith 1990). In general, a claim of optimality usually implies that strong

selection has overcome local constraints, such as drift or genetic limitations, and that a phenotype has evolved which confers the greatest fitness to the individual, compared to a range of plausible alternatives (see Orzack and Sober 1994 for an in-depth discussion). Under experimentation, an observed phenotype is said to be optimal if there is quantitative agreement between the value predicted by the optimality model and the observed value. If empirical observations do not quantitatively match model predictions but are in the same general direction, one can infer that selection likely played an important role in the evolution of the phenotype, but that other evolutionary forces were also important. In such cases, the phenotypic value is below the adaptive peak (Orzack and Sober 1994). Importantly, Smith and Fretwell's model is based on the premise that any observer can construct an offspring size-fitness relationship and ultimately estimate optimal offspring size, i.e., the phenotype that confers maximum reproductive success to the parent (Fig. 2.2).

One will probably concede, however, that our understanding of the direct and indirect demographic consequences of variation in most traits is incomplete. For this reason we should generally not expect optimality models to accurately predict observed phenotypes (Abrams 2001). But this should not discourage the use of optimality models in ecology and evolution: even when we do not expect optimality, comparing the predictions of an optimality model to observed phenotypes can provide valuable insight into ecological and evolutionary processes (Parker and Maynard Smith 1990). For example, a fitness curve that relates offspring size to parental fitness might inform an observer of the expected fitness consequences for parents that deviate from optimality (Fig. 2), and this may help explain why offspring size varies more in some groups and less in others (Mangel and Ludwig 1992). Optimality models, then, can be best appreciated as guides that help us understand evolutionary processes by providing a knowledge of the trait values that will and that will not maximize individual fitness (Abrams 2001).

2.2.2 Estimating Fitness of Parents and Offspring

Smith and Fretwell's model incorporates parental fecundity and offspring fitness components to estimate the level of investment per offspring that maximizes parental

reproductive success (Figs. 2.1, 2.2). In practice, a variety of fitness metrics have been used to estimate optimal size and the offspring-size fitness curve (e.g., Einum and Fleming 2000, Marshall and Keough 2008, Dziminski et al. 2009, Bownds et al. 2010), but some of these metrics are unlikely to allow an accurate assessment of optimal size. Smith and Fretwell (1974, p. 505) affirm that: "In most cases, [offspring] fitness will be measured by relative survival". While size-specific disparities in offspring survival following fertilization or parturition comprise the basis of Smith and Fretwell's thesis, these disparities will be eliminated over time through processes such as offspring growth and resource acquisition (Einum and Fleming 2000b; Nislow et al. 2004; Marshall and Keough 2009). We therefore recommend that size-number researchers measure offspring fitness as offspring survival up to the time at which the instantaneous rate of offspring mortality becomes random with respect to initial size. This provides a simple and reliable estimate of parental reproductive success that is consistent with Smith and Fretwell's thesis, given that parents maximizing the number of offspring surviving up to this point will (on average) leave the most offspring that survive to reproductive maturity. Moreover, it is simple to construct confidence intervals on the optimality estimate when a single metric comprises fitness; this is important given that confidence intervals are necessary for a quantitative test of optimality (Orzack and Sober 1994), even though confidence intervals are lacking for almost all estimates of optimal size (e.g., Hutchings 1991, Einum and Fleming 2000, Marshall and Keough 2006, 2008). Adopting this metric also means that, for some species, survival need only be measured over a small fraction of an organism's entire lifespan (e.g., the first 28 of the ~2000 days lived by Atlantic salmon; Einum and Fleming 2000). Ultimately, the parental fitness curve reflects the demographic consequences of variation in per-offspring investment, as fitness is expressed in theoretically defensible, ecologically meaningful, and broadly comparable units: the number of offspring surviving to independence.

Offspring fecundity can also be useful in size-number studies. Smith and Fretwell (1974, p. 505) acknowledge that "The competitive advantage during early growth resulting from a larger parental investment (e.g., larger seed size) may not be expressed until a seed has grown up to reproduce itself". Indeed, offspring survival and offspring fecundity are multiplicative components of parental reproductive success (Latta 2010),

such that parental fitness can be expressed as the predicted number of grandchildren (i.e., the number of offspring of size x that a parent can produce × offspring survival at size x × offspring fecundity at size x). However, while direct measures of offspring reproduction can be of interest in some systems (e.g., bryozoans: Dias and Marshall 2010), accurate assessments of reproduction can be difficult in other systems (e.g., estimating reproductive success of male offspring), or offspring reproduction may simply be random with respect to initial size. Incorporating offspring reproduction into expressions of parental fitness may therefore complicate matters unnecessarily in many systems.

In this vein, a common practice when estimating optimal size is to treat traits other than offspring survival and reproduction as multiplicative components of parental fitness (e.g., Marshall et al. 2006, Monro et al. 2010, Bownds et al. 2010). Depending on the goals of the study, this might present serious challenges. If the goal of the study is to estimate optimal size sensu Smith and Fretwell (1974), then fitness components must always be defined so that their product gives a direct estimate of parental reproductive success (Arnold and Wade 1984a; b). Otherwise, the relationship between offspring size and parental reproductive success is obfuscated, and a quantitative comparison of observed and expected phenotypes may not be meaningful (Houle et al. 2011).

2.2.3 The Shape of the Offspring Fitness Curve

Smith and Fretwell (1974) suggest that parents should receive decreasing returns on offspring fitness as offspring size increases. This proposition has long been considered reasonable on biological grounds, given that the proportional unit contribution to investment declines as investment per offspring increases (Pianka 1976). Both Lloyd (1987) and Jørgensen et al. (2011) have since derived an asymptotic offspring fitness curve from first principles over a broad range of parameters. Smith and Fretwell also proposed that a minimum level of per-offspring investment is necessary for offspring to be viable (*x-min*). This minimum will not necessarily be governed by the physiological requirements of the offspring, such as the notion that offspring require x units of energy to complete embryonic development. Rather, various selection pressures will also cause *x-min* to vary among environments, such as competition for resources after embryonic development is complete (e.g., Allen et al. 2008).

Brockelman (1975: 678) remarked that "Smith and Fretwell's [offspring fitness] curve abruptly intersects the *x*-axis to the right of the origin, but a sigmoid curve which gradually approaches the *x*-axis may be more biologically realistic". In fact, both models are probably realistic, and whether offspring fitness approaches zero quickly (an "*r*-shaped" curve) or gradually (an "*s*-shaped" curve) could depend on whether selection favours fewer, larger offspring, or many small offspring (e.g., Fig. 1). If selection favours many small offspring, then offspring fitness may approach zero rapidly simply because selection on parental reproductive success has reduced offspring size to the physiological limit of viability. At the other extreme, when a strategy of fewer, larger offspring is favoured, far fewer parents will be producing offspring that are near the limit of physiological viability. Here, low but stochastic survival of smaller offspring might compel the curve towards the *x*-axis relatively slowly (Fig. 1). In this case, the concept of a clear value of *x-min* is obnubilated.

Empirical studies often seem most interested in the 'slope' or average gradient of the fitness curve. Some authors have even suggested that optimal size will be relatively large when the gradient of the fitness curve is relatively steep (e.g., Allen et al. 2008, Marshall et al. 2010). Others have implied the opposite (e.g., Hutchings 1997). Neither view is very useful. Optimal size is predicted by a non-linear function, such that a focus on the global properties of the relationship is warranted.

2.2.4 Fitting a Model to the Data

Estimating the shape of a univariate fitness curve is usually accomplished by fitting a cubic spline to the data (Schluter 1988). Splines can be invaluable in estimating the form of selection on a quantitative trait, especially because they require no prior information about the shape of the fitness relationship. However, a great deal of research has already focused on capturing the form of the offspring size-fitness relationship (Lloyd 1987; Jørgensen et al. 2011), so fitting a function whose form is restricted to a shape supported by first principles is perhaps a better approach. (Although the terms 'fitness curve' and 'fitness function' are generally used interchangeably, here we use the term 'function' to refer to the functions generated by statistical models to estimate the shape of the offspring fitness curve).

Many statistical models have been fit to experimental data (Table 2.1), but given the asymptotic shape of the offspring size-fitness relationship, our focus will be restricted to 2-parameter models that feature a specifiable asymptotic value of offspring survival (k). We focus on two asymptotic regression models (Figs. 2.3*A*, 2.3*B*), Hill Model (Fig. 2.3*C*), the logistic model (Fig. 2.3*D*), and the Weibull-1 model (Fig. 2.3*E*). Ritz (2010) provides an exceptional overview of the relationships among generalized linear models, the Hill model and Weibull models, including alternative parameterizations. Here, we review qualitative properties of these models, and we use simulations to assess how well each of these models can predict optimal offspring size (to within ± 5% of the true value) under a series of conditions likely to be encountered by size-number researchers.

2.3 Methods

Our simulations are based on the assumption that the offspring fitness curve will adopt some shape along the continuum between a very steep rise from minimum viable offspring size (x-min) to a maximum fitness (i.e., fecundity selection, Fig. 1 of main text) and a protracted s-shaped fitness curve (i.e., viability selection, Fig. 1 of main text). We created five artificial fitness curves that sample the continuum between fecundity selection and viability selection (Figs. 2.4, 2.5); these fitness curves were created by manually splicing together 3rd and 4th-order polynomials. Each curve maps a true value of offspring fitness, a proportion between 0 and 1, to a value of offspring size (x_i), an integer between 10 and 39. The x-variable is expressed in arbitrary units, and it simply reflects offspring size.

The simulations are designed to emulate a release-recapture experiment. Here, a researcher releases a number of offspring comprising discrete size classes (i.e., values of x), then as many offspring as possible are recaptured and assigned back to their original size class. Size-based differences in survival are then estimated based on the continuous survival probabilities (between 0 and 1) obtained for each size class. The shape of the offspring size-fitness curve can then be estimated by fitting a statistical model to the recapture data.

In this type of experiment, the accuracy of each size-specific survival estimate, and hence the estimate of the entire fitness curve, depends in part on the number of

offspring released at a given size *x*. Said differently, when offspring survival at a given value of *x* is, say, 10 % or 0.10, we are more confident that 0.10 is close to the true population value when the estimate is based on 1000 releases (i.e., 100/1000 = 0.10), compared to 100 releases (i.e., 10/100 = 0.10). We held the denominator constant at each value of *x* within a given simulation, but to examine the effect of sample size on the accuracy of our survival estimates, we varied this value among simulations. Specifically, in a given simulation, survival was estimated for a sample size of 50, 100 or 500 offspring of size *x* at each integer of *x*.

In a real experiment, the number of offspring released at each value of offspring size, *x*, is under experimenter control, but the overall number of offspring recaptured is not. Offspring survival (or recapture success) will also influence how accurately we can estimate the overall fitness curve because overall survival will affect the magnitude of the absolute differences among continuous survival probabilities. Hence, when average population survival is very low, it becomes difficult to estimate the shape of the offspring fitness curve because the actual size-based differences in survival are small and obscured by variation. A general expectation in release-recapture experiments, then, is that our ability to accurately estimate the offspring fitness curve is greatest with large sample sizes and high average offspring survival, and poorest with low sample sizes and low average survival. To examine the effect of population survival (or overall rate of recapture) on our ability to accurately estimate the shape of the offspring size-fitness curve, we varied the asymptotic value of offspring survival among simulations. Specifically, in a given simulation, the average asymptotic value of offspring survival was either 0.10, 0.25, 0.50, or 0.75.

The beta distribution is a family of continuous probability distributions defined between zero and one. The distribution is parameterized by α and β, where β defines the number of failures and α defines the number of successes in α + β Bernoulli trials. Hence, $\frac{\alpha}{\alpha+\beta}$ describes the proportion of successes out of all trials. Manipulating the sum of α and β is akin to manipulating the number of offspring released of a given size class, and raising the maximum value of α is akin to manipulating the asymptotic value of survival. We underline that it is the shape of the fitness curve that is of interest, and the actual

value of offspring survival denoted by our artificial fitness curves can be scaled to different asymptotic values of α. For example, in a population where offspring survival is high, we might expect an asymptotic value of offspring survival to be, say, 0.75, or 75%. Hence, if 500 offspring of each size class are released in this population, then the beta distribution that denotes asymptotic survival is parameterized by $\beta = 175$ and $\alpha = 325$. In a different population, the asymptotic survival value may only be, say, 0.10 or 10%, so the beta distribution that denotes asymptotic survival is parameterized by $\beta = 450$ and $\alpha = 50$. It is important to recognize that scaling can emulate differences in average offspring survival without altering the shape of the fitness curve. We summarize parameterizations of the beta distribution for each simulation in Appendix E.

All simulations were performed in R (R Development Core Team 2011). A simulation consisted of generating 50,000 unique datasets from one of the five artificial fitness curves, where the beta distribution was parameterized at each integer of x according to specific values of sample size and maximum offspring survival (Appendix E). In total we performed 60 simulations which generated 3 million unique datasets (i.e., 3 levels of sample size × 4 levels of asymptotic offspring survival × 5 artificial fitness curves = 60 simulations).

We generated each dataset by sampling randomly from an appropriately parameterized beta distribution at each integer of x on the fitness curve using the function *rbeta* (R Development Core Team 2011). The distribution of x-values (values of offspring size) were constant for all simulations on a given fitness curve, but varied among some fitness curves. In all cases, x-values were normally distributed, with the mean falling in the middle of the data range (Appendix E). We held constant the number of x-values in each simulation at 20, as studies estimating continuous survival probabilities often involve low replication of x values in exchange for greater replication of released individuals (e.g., Hutchings 1991, Einum and Fleming 2000, Houde et al. 2011). Note that our simulations are also applicable to studies that have collected binary survival data, as binary data can be binned into appropriate size-classes (values of x) to create continuous survival probabilities.

In every simulation, each of the five statistical models was fit to each of the 50,000 datasets. Models were fit in R (R Development Core Team 2011) using the

function *nls2* (Grothendieck 2010) for logistic regression, the Hill model and asymptotic regression models, and *drc* (Ritz and Strebig 2011) for the Weibull-1 model. Predictions from all five models – which were fit to identical datasets – were summarized and compared (Appendix E).

An accurate prediction of optimality occurs when there is quantitative agreement between a predicted optimal value and an observed trait value (Orzack and Sober 1994). Hence, one method of assessing how accurately a given model can predict optimal size would be to construct 95% confidence intervals for each of its 50,000 simulation runs, then one could compute how often the true optimal value (derived from the artificial fitness curve) falls within these confidence limits. Unfortunately, confidence intervals become larger as model uncertainty increases, so the former method could obviously overestimate the accuracy of a given statistical model. To obviate these issues, we assessed the accuracy of model predictions simply by summing the number of simulations (out of 50,000) in which a model predicted optimal offspring size to within ± 5% of the true value. We chose a value of ± 5% arbitrarily, but we venture that most ecologists would be comfortable if they could estimate optimal offspring size to within ± 5% of the true value. Runs Tests were performed on the residuals of every model, where a significant test value ($p < 0.05$) indicated that the model did not fit the data well. Runs Test were used to assess model fit because we were interested in how well each model is able to adopt the shape of the artificial fitness curve. Runs Tests were performed using the package *lawstat* (Noguchi et al. 2009). Maximum parental fitness (which corresponds to optimal size) was estimated for parents where reproductive effort (R) was 1000 units of energy, where investment per offspring (x) varied between 10 and 39 units of energy, and following Smith and Fretwell (1974) the number of offspring produced by parents (N) was $N = R/x$. Below, we assess how well the models fit the simulated data and how accurately each model predicted optimal size.

2.4 Results and Discussion

A summary of all simulations and model predictions, along with how well each model fit the data and the number of times each model accurately predicted optimal offspring size is found in (Appendix E). We found that the accuracy of model predictions

and model fit varied greatly among levels of offspring survival (Fig. 2.6*A*), levels of sample size (Fig. 2.6*B*) and among artificial fitness curves (Fig. 2.7). Notwithstanding, there were some outstanding patterns, which we describe below.

2.4.1 Asymptotic Regression

Asymptotic regression functions (Stevens 1951) typically increase from an *x*-intercept at a decreasing rate towards an asymptote. The unique property of these models is that they feature an estimable minimum viable offspring size, the *x*-intercept coefficient. The two asymptotic regression models that have been used in offspring size-number research are,

$$f(x) = k \cdot \left(1 - \frac{min}{x}\right)^{b} \quad \text{(Equation 2.1)}$$

$$f(x) = k \cdot \left(1 - e^{(-b \cdot (x - min))}\right) \quad \text{(Equation 2.2)}$$

where k is the known value of maximum fitness (e.g., maximum observed survival rate), min is the x-intercept to be estimated (i.e., minimum viable offspring size), b is the scaling exponent to be estimated, and e is the base of natural logarithms (Fig. 2.3*A*, 2.3*B*).

In our simulations, asymptotic regression did not provide adequate estimates of optimal size under the vast majority of conditions (Figs. 2.6, 2.7, 2.8, 2.9). Model (1) correctly estimated optimal size in only 19% of all simulations, and this is troubling because Model (1) is often fit to experimental data (e.g., animals: Einum and Fleming 2000; plants: Charpentier et al. 2012). Model (2) provided accurate estimates in only 31% of cases. Both models also produced inaccurate estimates of parental fitness, and the accuracy of Model (2) decreased as sample size and offspring survival increased (Figs. 2.8, 2.9). Runs Tests indicated that Models (1) and (2) did not fit the simulated data well in 30% of all cases. By comparison, a poor fit was observed in no more than 11% of all cases for other statistical models.

The problem with the asymptotic regression models evaluated herein is that they underestimated survival probabilities when offspring size was relatively large (e.g., Fig.

2.8). This can be deduced by the fact that these models grossly underestimated the correct value of parental fitness at optimality in almost all conditions (Figs. 2.8*B*, 2.9*B*). This indicates that their functions approach the upper fitness asymptote too slowly, regardless of the shape of the true fitness curve. So while asymptotic regression incidentally predicted optimal offspring size with accuracy under some conditions, these models were unable to provide a realistic representation of the true fitness curve.

This pattern is not a construct of the artificial fitness relationships we chose in our simulations, it is systematic: the same problem is also evident in published size-number studies that used asymptotic regression to model optimal offspring size with experimental data. Einum and Fleming (2000), for example, collected mark-recapture data for young Atlantic salmon (*Salmo salar*), then they estimated the relationship between offspring size and survival using Model (1). An inspection of their model predictions, however, suggests that offspring size must be approximately 8.6 standard deviations above their mean phenotypic value when offspring fitness is at 90 % of their maximum observed fitness value (assuming a mean offspring size of 0.105 g and standard deviation of 0.0251 g estimated from their Fig. 1*A*). Akin to the present study, their function approaches the asymptote very slowly, and this generates what appears to be an unreasonable prediction. Our findings suggest that Model (1) and Model (2) should not be used in experimental size-number research.

2.4.2 Logistic Regression

Most empirical studies that estimate the offspring fitness relationships use logistic regression (Table 1). Logistic regression is a form of generalized linear model that uses a logit link function, and this model generates *s*-shaped or sigmoidal functions bounded by zero and *k* (although *k* is usually set to 1.0; see Fig. 2.10 for a case study). The equation can be given by

$$f(x) = k \cdot \frac{e^{a+\beta \cdot x}}{1+e^{a+\beta \cdot x}}$$

(Equation 2.3)

where k is the known value of maximum fitness (e.g., maximum observed survival rate), a is the y-intercept, and β defines the steepness of the slope (Fig. 2.3*D*). The ubiquitous use of the logistic model to estimate offspring fitness relationships likely reflects the convenience of using a well-established linear model that happens to exhibit two non-linear regions when predicted values are back-transformed from logits into probabilities. Although a logistic function might accurately or adequately describe the relationship between offspring size and fitness in some cases, one must acknowledge that by equating a logistic curve with a fitness curve a particular *a priori* hypothesis has been accepted. Namely, one is assuming that offspring fitness is symmetric about a fitness of $0.5k$, and that offspring fitness approaches the x-axis slowly. Although the logistic model is in widespread use (Table 2.1), the assumption that offspring fitness approaches an upper asymptote from a value of $0.5k$ at the same rate as it approaches *x-min* from $0.5k$ is not based on theory. In fact, no theoretical model has ever used an offspring fitness curve that is necessarily symmetric about a fitness of $0.5k$ (Table 2.1), which suggests a different assumption prevails, at least among theorists.

Under simulation, the logistic model produced accurate estimates of optimal size in 43% of all cases, which makes it the least accurate of the sigmoidal models. While the logistic model often performed as well as, or better than, the Hill and Weibull-1 models when offspring survival and sample size were highest, it was typically less accurate under other conditions. The logistic model did not fit the simulated data in about 11% of all cases, which is similar to the rate of 8% generated by the Weibull-1 and Hill models.

2.4.3 Hill Model

The Hill equation was originally introduced in 1910 by Archibald V. Hill to describe the equilibrium relationship between the saturation of haemoglobin and oxygen tension (Hill 1910). Subsequently, it has been widely used to model dose-response relationships in pharmacokinetic models (reviewed by Goutelle et al. 2008), and more recently it has been adopted by Bonabeau et al. (1998) and Fischer et al. (2011) to describe the relationship between offspring size and fitness in theoretical simulations of offspring-size evolution. The Hill equation produces a flexible, s-shaped function given by the equation,

$$f(x) = k \cdot \frac{x^b}{x^b + a^b}$$

(Equation 2.4)

The function is bounded by zero and k, and b governs the steepness of the curve. The inflection point of the curve, a, is the estimated x-value which falls exactly in the middle of the function. Assuming $k = 1$, the inflection point will occur when $f(x) = 0.5$, and the function will always rise more quickly from 0 to 0.5 than from 0.5 to 1, producing a slight asymmetry about 0.5. Overall, the Hill model produced accurate estimates of optimal egg size in 46% of all simulation runs. In most cases, the Hill model predicted optimal size more accurately than the logistic equation, but less accurately than the Weibull-1 model (Figs. 2.6, 2.7, 2.8).

2.4.4 Weibull-1 Model

Weibull models (Weibull 1951) have been used extensively in eco-toxicological modelling (Ritz 2010), and while they have never been applied in offspring size-number research, it has long been recognized that they are useful for modelling survival in ecology and evolution (Pinder et al. 1978). Here we use a special case of the Weibull-1 model (Ritz 2010) where the lower and upper asymptotes are respectively fixed at 0 and k:

$$f(x) = k \cdot e^{-e^{b\{\ln(x) - \ln(a)\}}}$$

(Equation 2.5)

where k is the known value of maximum fitness (e.g., maximum observed survival rate), b defines the slope of the curve, and a is the x-value where the inflection point is located (Fig. 2.3*E*). In many respects, the Weibull-1 function is similar to that produced by the Hill equation: both are *s*-shaped, both increase relatively quickly from zero to the inflection point, a, and both approach the upper asymptote, k, slowly after surpassing the inflection point. The primary difference between the Hill and Weibull-1 curves is that the

slope of the Weibull-1 curve is much more pronounced between zero and *c*, such that the Weibull-1 function must approach the *x*-axis relatively abruptly.

The Weibull-1 model produced accurate estimates of optimal egg size in 49% of all simulation runs. On average, it correctly predicted optimal size more often than the Hill and logistic models when sample size and overall offspring survival were low, although its accuracy was similar to that of the Hill and logistic models when sample size and survival was high (Fig. 2.6). When prediction success is averaged across all simulations involving low offspring survival, the Weibull-1 model predicted optimal size between 8% and 10% more often than the logistic model (Fig. 2.6*A*). Prediction success was also between 7% and 8% greater than the logistic model when sample sizes were low to modest (Fig. 2.6*B*). Over all conditions, we found that values of parental fitness at optimality predicted by the Weibull-1 model were also closest to the true value (Figs. 2.8*B*, 2.9*B*). Therefore, the Weibull-1 model produced the most accurate estimates of optimal size and parental fitness on average, largely because it performed best when survival and sample size were low.

2.4.5 Which model should one fit?

An important lesson learned from our simulations is that it can be very difficult to accurately estimate optimal offspring size with experimental data (e.g., Figs. 2.6, 2.7) and both large sample sizes and elevated offspring survival will often be necessary to secure an accurate estimate. This is unfortunate because it is typically highly-fecund organisms with low offspring survival that are used in size-number studies (e.g, Atlantic salmon), and logistical constraints often have the effect of limiting sample size. With this lesson in mind, our simulations suggest that asymptotic regression should not be used to estimate the shape of an offspring-size fitness curve in experimental studies. Differences in the accuracy of the Hill, logistic and Weibull-1 models were usually unremarkable when sample sizes and offspring survival were high; however, the Weibull-1 model offered modest improvements over the Hill and logistic models when sample size and offspring survival were low. Given that low sample sizes and survival are often expected in size-number studies, the Weibull-1 model will, on average, provide the most accurate predictions. The Weibull-1 model can also be estimated easily in *R* (R Development Core

Team 2011) with the *drc* package (Ritz and Strebig 2011), which features the option of estimating *k*, or having *k* specified by the user. However, while we recommend the Weibull-1 model for experimental research, our simulations show that there is no silver bullet when it comes to modeling optimal offspring size (Appendix E) The choice of model should always be justified. Only after carefully designing an experiment, thoroughly exploring the data, then considering carefully which model should be applied can one potentially estimate the relationship between offspring size and fitness with defensible accuracy.

Table 2.1: Statistical models that have been used to map a positive relationship between offspring size and offspring fitness.

Common name	Model statement	Type	Reference
Asymptotic regression	$1 - (a/x)^b$	E & T	1–8
Asymptotic regression*	$1 - \exp(-b(x - a))$	T	9–16
Sigmoidal curve	$(1+p(\exp(-x/q)))/(1+m(\exp(-x/q)))$	T	17
Logistic regression	$\exp(a + \beta(x))/(1 + \exp(a + \beta(x)))$	E	18–29
Cubic spline	see Schluter (1988)	E	20–21, 30–34
Hill equation*	$x^b/(x^b + a^b)$	T	35–36
Power function	$a(x)^b$	E	37
Linear regression	$\beta(x) + a$	E	38–39
Logarithmic regression	$\beta(\ln(x)) + a$	E	40
Polynomial regression	$\beta_1(x) + \beta_2(x)^2 + a$	E	41

Note: 'Type' refers to the model's use in theoretical studies of offspring size-number strategies (T) or in experimental studies (E). All estimable parameters are in italics: 'x' is any measure of offspring size, 'exp' is the base of natural logarithms 'ln' is the natural logarithm, *b* is a scaling exponent that typically governs the asymptotic shape of the fitness curve, β is the linear slope of y on x, and *a* is a constant. See the original publications for a detailed description of these estimable parameters and their role in particular models. When slightly different parameterizations of the same model have been used (indicated by *), they are grouped under the most common form. Some studies have been omitted for brevity, but the list includes every function used to map a positive relationship between offspring size and offspring fitness. References: (1) (McGinley et al. 1987) (2) (McGinley 1989) (3) (Schultz 1991) (4) (Einum and Fleming 2000b) (5) (Hendry et al. 2001) (6) (Einum and Fleming 2004) (7) (Einum and Fleming 2007) (8) (Charpentier et al. 2012) (9) (Winkler and Wallin 1987) (10) (Parker et al. 1989) (11) (Lalonde 1991) (12) (Charnov et al. 1995) (13) (Lessells 2002) (14) (Mock et al. 2005) (15) (Guinnee et al. 2007) (16) (Marshall et al. 2010) (17) (Kindsvater et al. 2011) (18) (Hutchings 1997) (19) (Mojonnier 1998) (20) (Janzen et al. 2000b) (21) (Janzen et al. 2000a) (22) (Altwegg and Reyer 2003) (23) (Marshall and Keough 2006) (24) (Marshall et al. 2006) (25) (Marshall and Keough 2008) (26) (Marshall and Keough 2009) (27) (Bownds et al. 2010) (28) (Monro et al. 2010) (29) (Dias and Marshall 2010) (30) (Sinervo et al. 1992) (31) (Janzen 1993) (32) (Carrière and Roff 1995) (33) (Congdon J.D. et al. 1999) (34) (Rankin and Sponaugle 2011) (35) (Bonabeau et al. 1998) (36) (Fischer et al. 2011) (37) (Heath et al. 2003) (38) (Hutchings 1991) (39) (Sinervo and Doughty 1996) (40) (Dziminski et al. 2009) (41) (Janzen and Warner 2009)

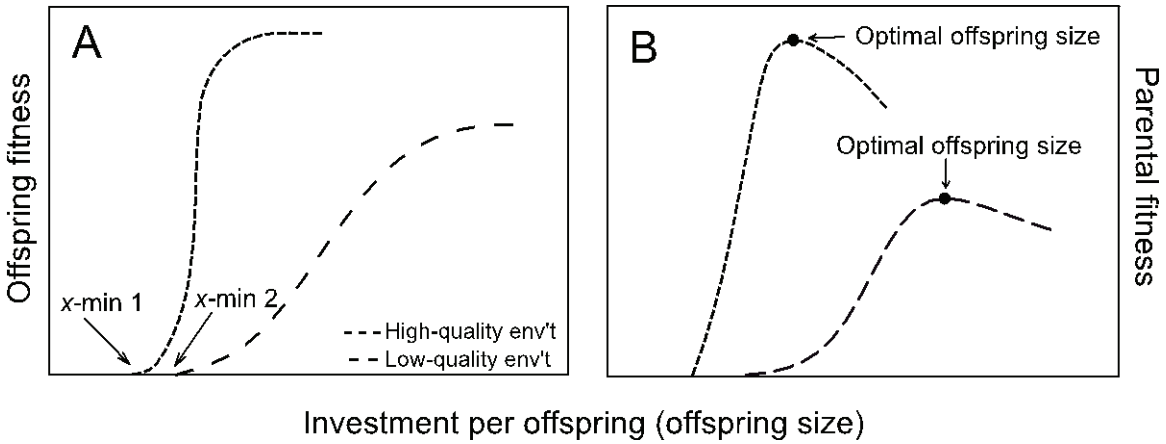

Figure 2.1: A possible set of relationships between investment per offspring and fitness. (*A*) In the high-quality environment, the minimum level of investment needed to produce a viable offspring (*x-min*) is relatively low and the fitness curve increases quickly from this minimum to an elevated asymptotic value of offspring fitness. The tail of the function approaches *x-min* quickly because optimal offspring size is small and near the limit of viability. In the low-quality environment, *x-min* is larger, and offspring fitness increases incrementally with offspring size up to a relatively low asymptotic value of fitness. The tail of the function is longer because low but stochastic survival of very small offspring (that are still well above the physiological minimum) compels the curve towards the *x*-axis more slowly. (*B*) The resultant parental fitness curves (the product of offspring fitness at size *x* and the number of offspring produced at size *x*) differ in shape by virtue of the shape of the offspring fitness curves. Optimal offspring size is the level of investment per offspring that maximizes parental fitness (see Fig. 2.2 for further development).

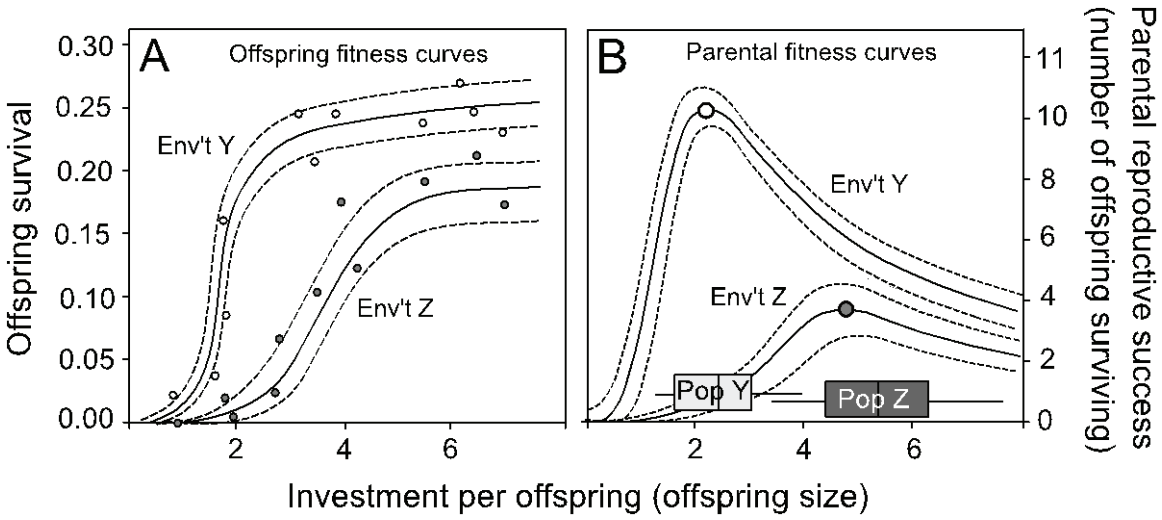

Figure 2.2: A hypothetical experiment in which offspring are released into two different environments, Y and Z, and survival (fitness) is subsequently assessed. (*A*) Offspring fitness relationships with confidence intervals are fit to the data. (*B*) Parental fitness is calculated as the product of offspring survival at size *x* and the number of offspring of size *x* that can be produced. Box-and-whisker plots show the distribution of natural offspring sizes from populations Y and Z that inhabit environments Y and Z, respectively. Comparing natural variation in offspring size to parental fitness curves constructed with experimental data reveals evidence of selection. Greater natural variation in population Z coincides with small fitness penalties for parents deviating from optimality, and vice versa for population Y. These fitness curves reveal that the strength of stabilizing selection on investment per offspring differs between populations, which explains why offspring size varies more in population Z and less in population Y. Finally, there would likely be quantitative agreement between the value predicted to maximize parental fitness (large circles) and population-averaged offspring size in both populations (e.g., if a one-sample t-test was performed). While this would indicate that selection has contributed to the evolution of offspring size, this general agreement could not be interpreted as evidence that offspring size is in an optimal state because optimality must be assessed at the level of the individual (for details see Orzack and Sober 1994).

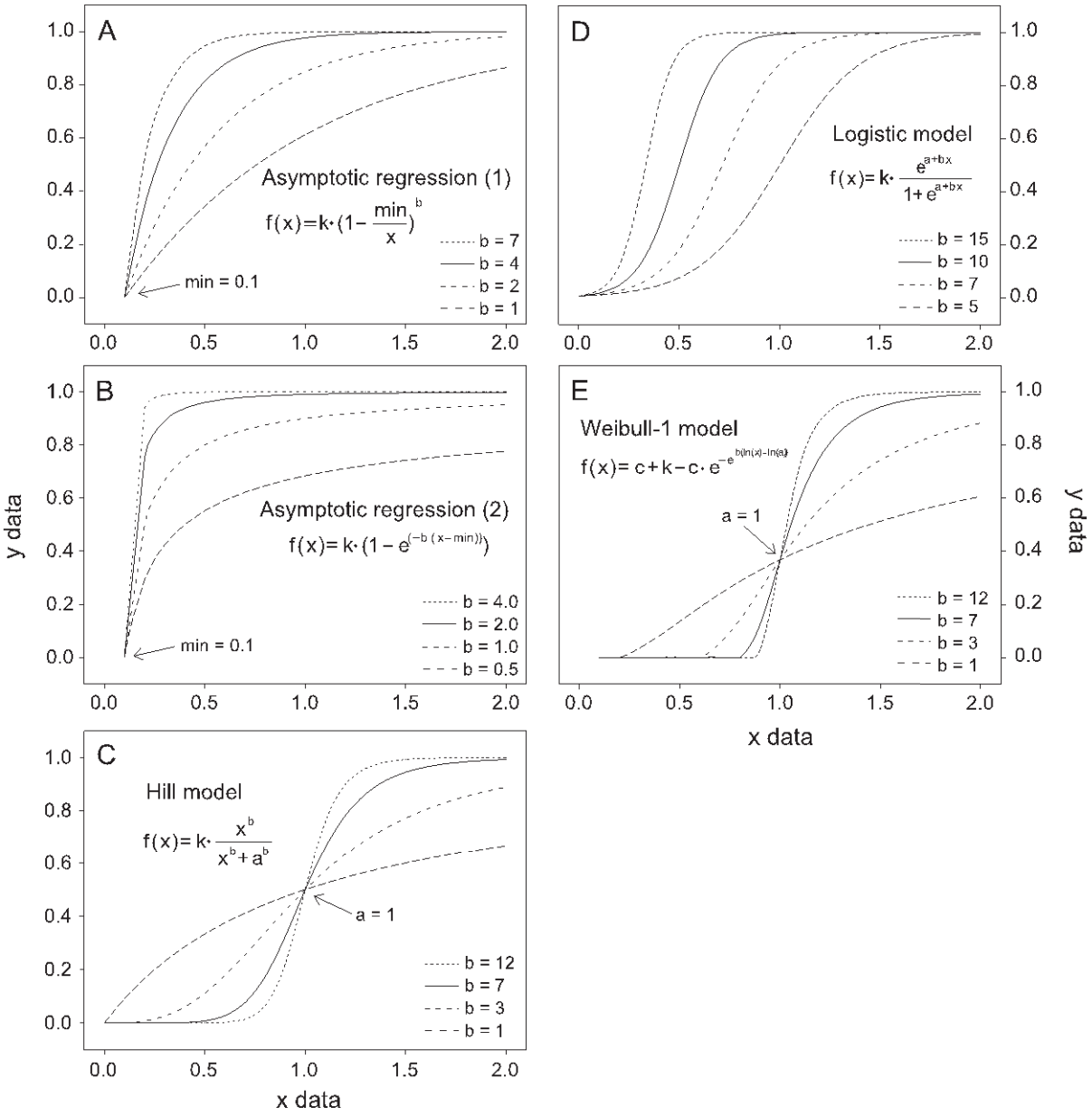

Figure 2.3: (*A*, *B*) Asymptotic regression models. (*C*) Hill model. (*D*) Logistic regression model. (*E*) Weibull-1 model. All models are fit with different slope values (*b*). Parameter values for the inflection point or *y*-intercept (*a*) or the *x*-intercept (*min*) are given in panels (*A*) through (*E*). Here, *ln* is the natural logarithm, and *e* is the base of natural logarithms. See text for further details.

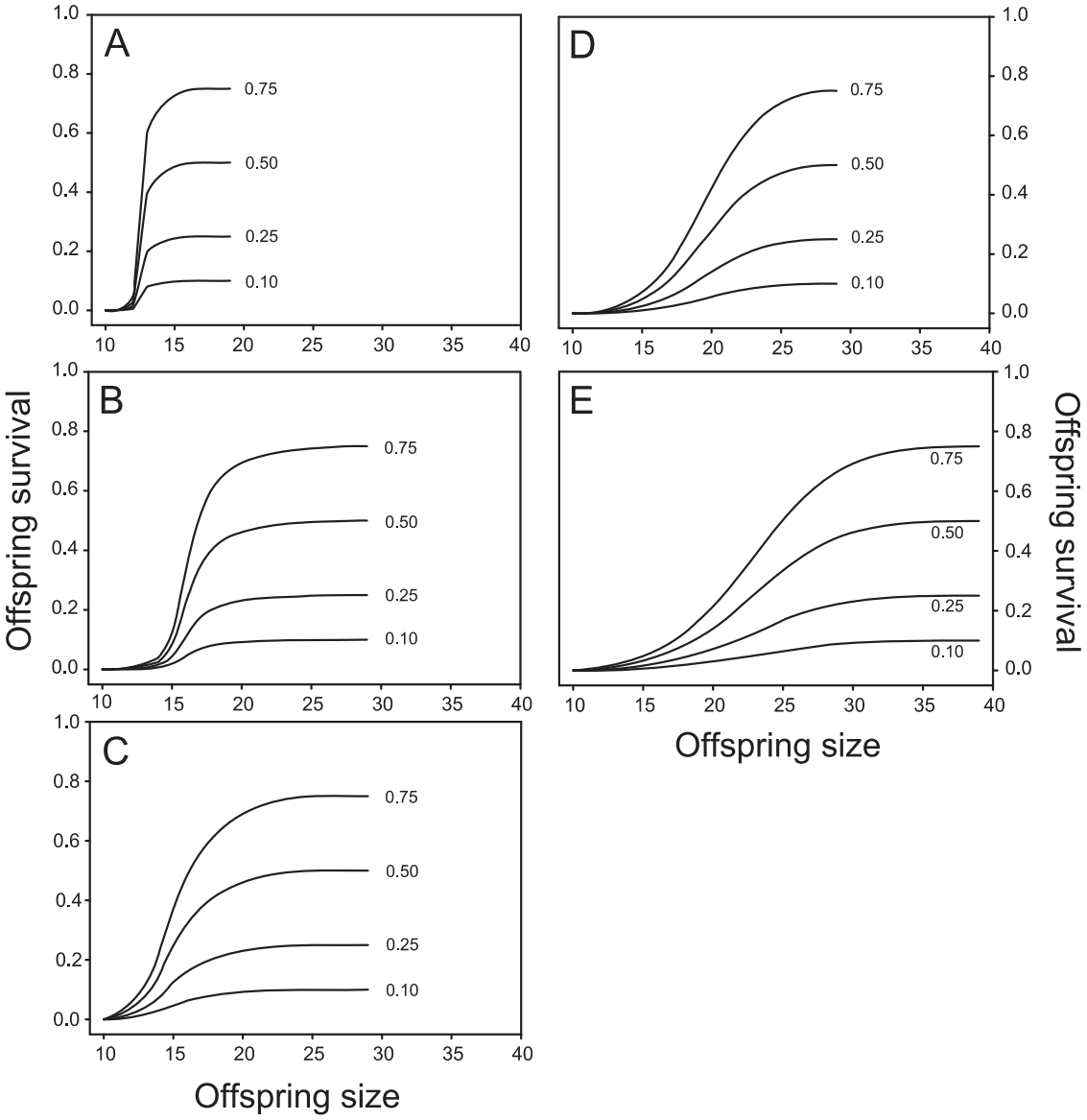

Figure 2.4: Artificial fitness curves scaled to different values of asymptotic offspring survival, 0.10 – 0.75. (*A*, *B*) Curves represent a form of fecundity selection. Offspring fitness increases rapidly with offspring size after the minimum viable offspring size (*x-min*) is surpassed, and the curve approaches the x-axis rapidly near *x-min*. (*C*) The curve exhibits qualities of fecundity selection and viability selection. (*D*) Fitness curve is perfectly symmetrical and viability selection is occurring. Offspring fitness increases slowly with size, and optimal size is large. (*E*) Strong viability selection. The curve is protracted, asymmetrical and s-shaped. Optimal size is very large, relative to *x*-min. The range of *x*-values spanned by a curve reflects the range values used in the simulations.

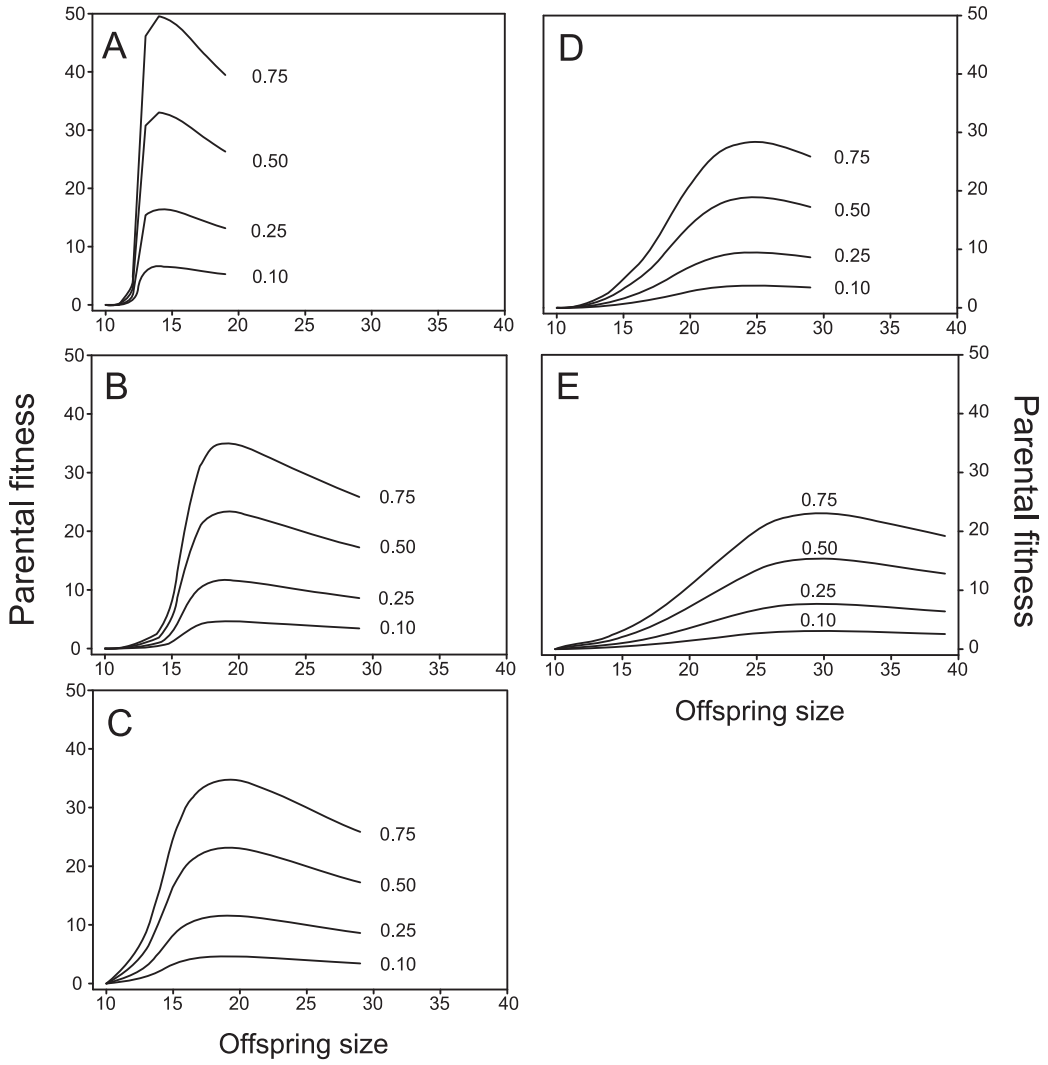

Figure 2.5: Artificial parental fitness curves in panels *A* – *E* correspond to artificial fitness functions in Fig. 2.4*A* – 2.4*E*, scaled to different values of asymptotic offspring survival, 0.10 – 0.75. Parental fitness was estimated for parents where reproductive effort (*R*) was 1000 units of energy and the number of offspring produced by parents (*N*) was $N = R/x$. Parental fitness is the product of expected offspring fitness at size *x* and the number of offspring that can be produced at size *x*. Parental fitness is expressed as the number of offspring surviving to time *t*, where *t* is the point at which offspring survival becomes random with respect to initial investment. The minimum cost of producing a viable offspring is 12 units (panels *A, B, D*), or 11 units (panels *C*, *E*).

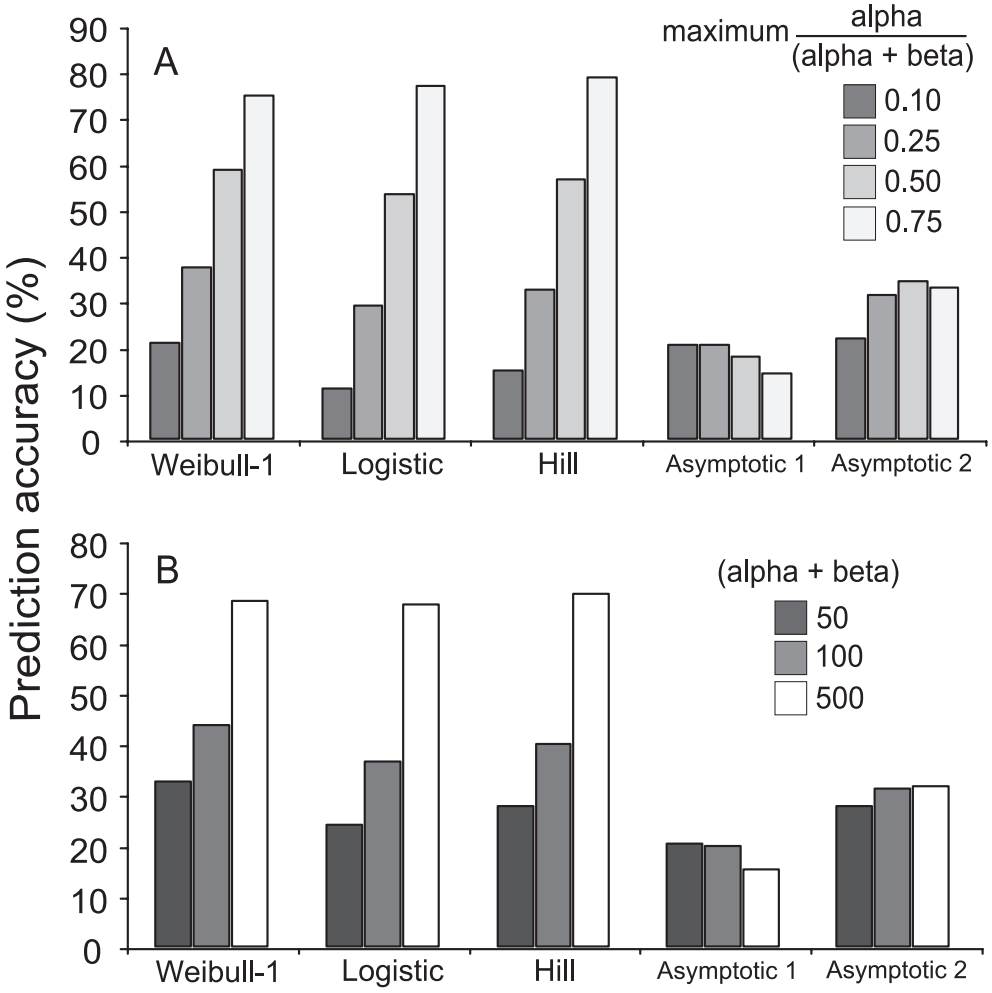

Figure 2.6: The results of all simulations are collapsed across all artificial fitness curves to demonstrate the overall effects of asymptotic offspring survival (*A*) and sample size (*B*) on each model's unique ability to estimate optimal size. Prediction accuracy (y-axis) is the percent of simulations in which models estimated optimal offspring size to within ± 5% of the true value. In panel *A*, "Maximum (Alpha/[Alpha + Beta])" is asymptotic survival; in panel *B*, "Alpha + Beta" is number of offspring initially released at a given level of offspring size. The Weibull-1 model better predicts optimal size at low values of survival and sample size, but this disparity disappears as survival and sample size increase.

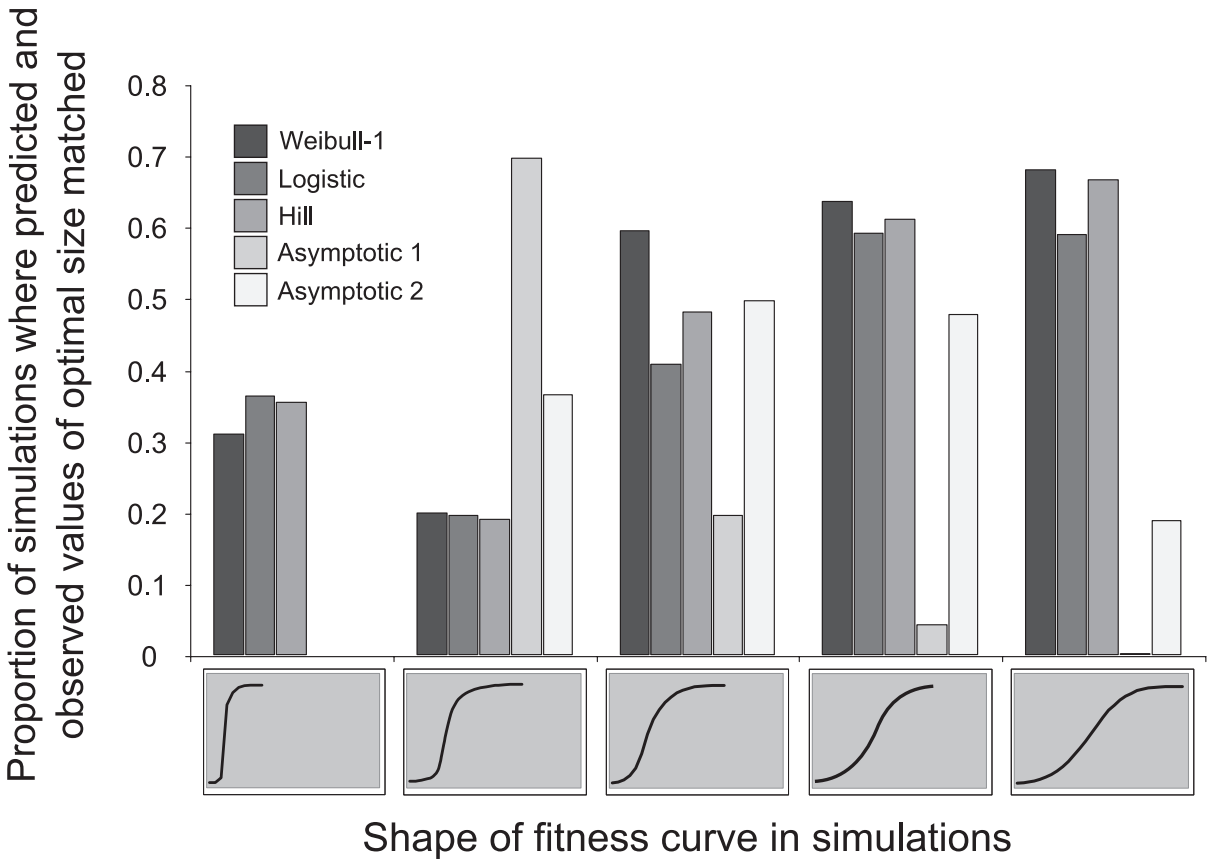

Figure 2.7: Prediction success of each statistical model for each of the five fitness curves. Here, the results are collapsed across all 12 simulation performed on a given curve (i.e., for each curve, simulations were performed under 4 levels of asymptotic offspring survival × 3 levels of sample size). A success is a simulation run in which a model predicts the correct value of optimal size to within ± 5% of the true value. The shape of the relevant fitness curve is sketched on the *x*-axis; see Fig. 2.4 for quantitatively accurate representations of these curves.

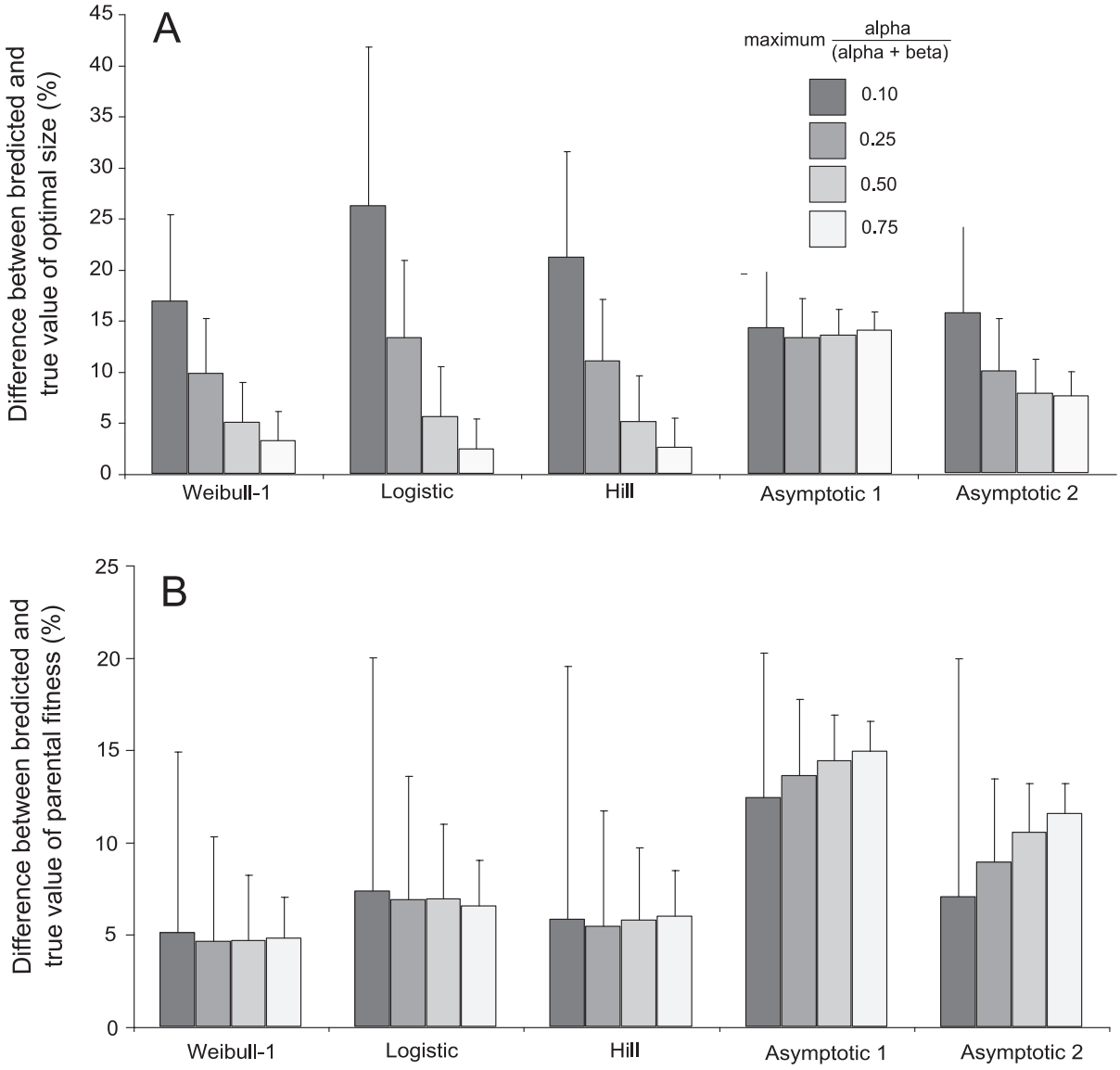

Figure 2.8: (*A*) For each of the 3 million times a given model was fit to a dataset, we calculated the deviation (in absolute terms) between a model's prediction of optimal size and the true value of optimal size. Here, deviations are displayed for each value of asymptotic offspring survival, 0.10 – 0.75, and we express these values as mean percent difference from the true value. For example, say the *x*-data for a given fitness curve ranged from 10 to 29, and that the true optimal size for this curve was 25. If the Weibull-1 model, for example, predicted an optimal value of 23 for a given simulation run, then its prediction differed from the true value by ((23 – 25)/20) × 100 = 10.0%. Error bars are the average deviation of the predicted value from the true value. These deviations were

calculated within simulation runs, then averaged across relevant simulations, and expressed in percent. (*B*) We also calculated the deviation (in absolute terms) between a model's prediction of parental fitness at optimality and the true value of parental fitness at optimality. Here, larger values represent poorer model predictions, as deviations are expressed in percent of maximum parental fitness (i.e., parental fitness at optimality). Error bars are the standard deviations calculated following the methods for panel *A*.

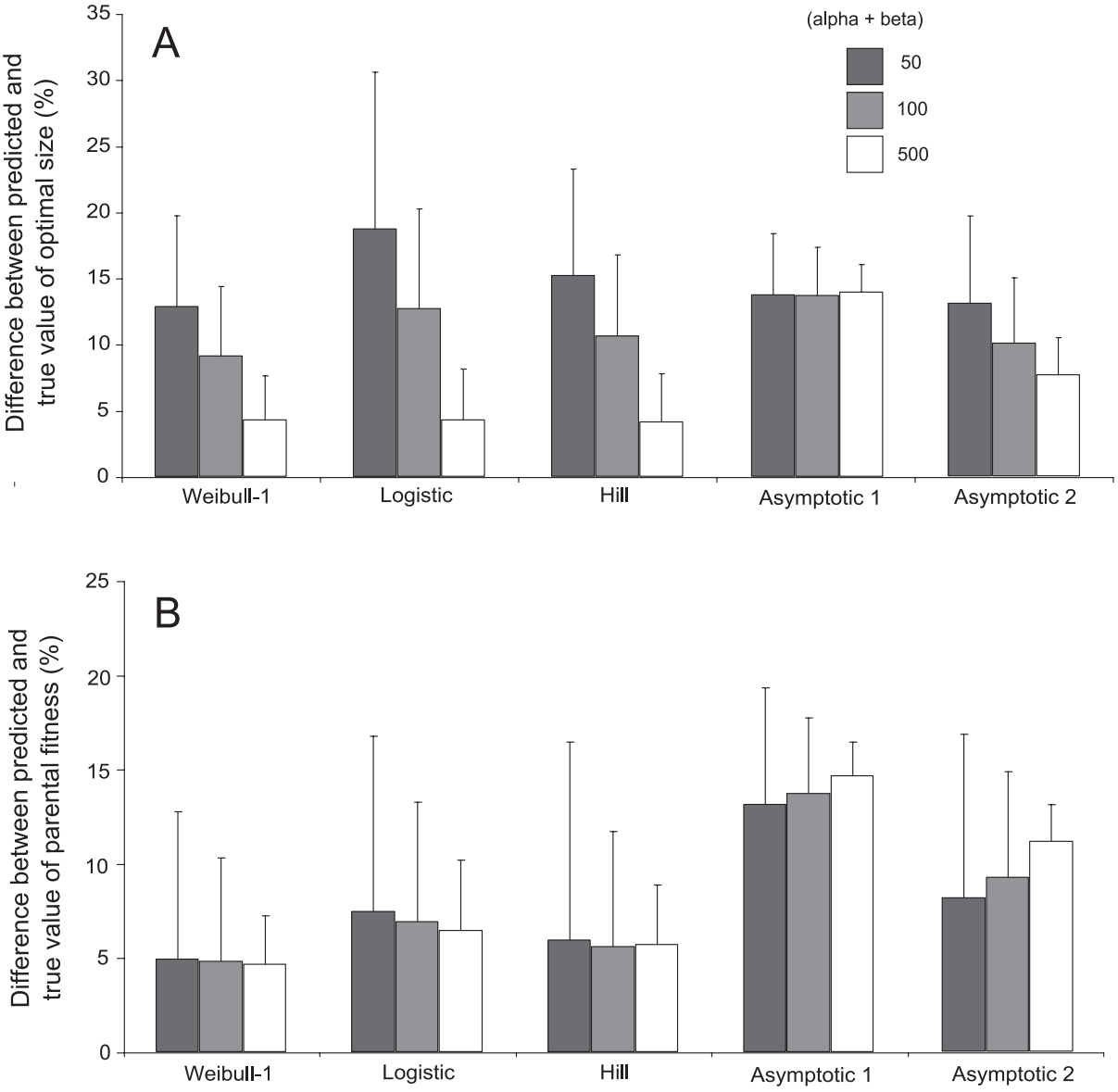

Figure 2.9: (*A*) For each of our 3 million simulation runs, we calculated the deviation (in absolute terms) between a model's prediction of optimal size and the true value of optimal size. Here, deviations are displayed for each level of sample size (50, 100, 500) and we express these deviations in mean percent difference from the true value. For example, say that the *x*-data for a given fitness curve ranged from 10 to 29, and that the true optimal size for this curve was 25. If the Weibull-1 model, for example, predicted an optimal value of 23 for a given simulation run, then its prediction differed from the true value by ((23 – 25)/20) × 100 = 10.0%. Error bars are the mean deviation of the predicted value from the true value. These deviations were calculated within simulation runs, then

averaged across relevant simulations, and expressed in percent. (*B*) For each of our 3 million simulation runs, we calculated the deviation (in absolute terms) between a model's prediction of parental fitness at optimality and the true value of parental fitness at optimality. Here, larger values represent poorer model predictions, as deviations are expressed in percent of maximum paretnal fitness (i.e., parental fitness at optimality). Error bars are the standard deviations calculated following the methods in panel *A*.

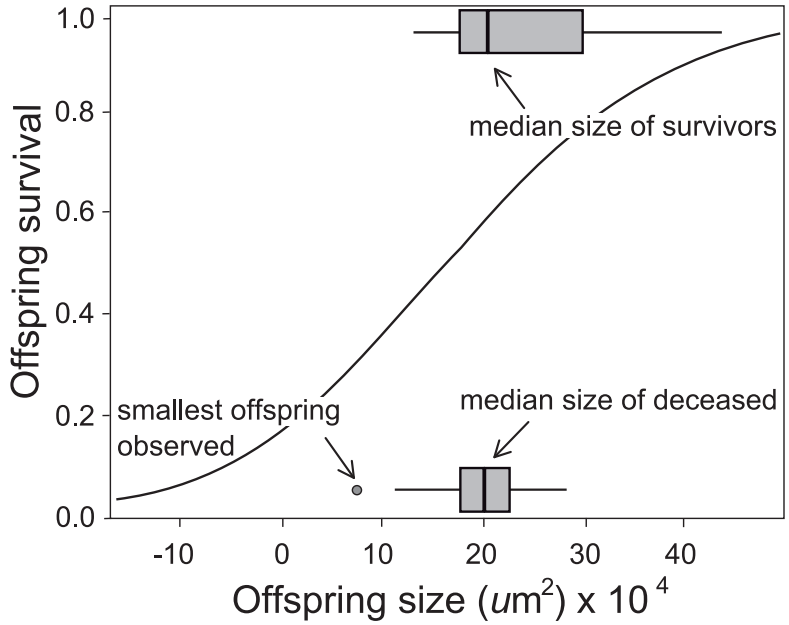

Figure 2.10: Binary logistic regression and offspring fitness relationships: a case study. The relationship between offspring size of the bryozoan *Watersipora subtorquata* and post-metamorphic survival was estimated using binary logistic regression (redrawn from Marshall and Keough (2009), their Fig. 1). Here, elevated values for offspring survival are predicted to occur below reasonable values of offspring size. Moreover, median values of offspring size are very similar among dead and live offspring, which suggests the offspring fitness curve should have approached maximum fitness more rapidly. While the authors acknowledge that survival predictions outside the phenotypic range may not be reliable (Marshall and Keough 2008:222), it might have been possible in the present case to achieve more realistic and perhaps more accurate predictions. For example, the offspring size data appear to exhibit a log-normal distribution, which is typical of body-size measures (Huxley 1932). Therefore, a log(x) transformation would have ensured that predicted values of offspring fitness never fell below an offspring size of zero (i.e., log(0) = $-\infty$), and the function would have approached maximum fitness more rapidly. Another general issue (which may or may not apply to the present case) is that ordinary binary logistic regression produces a function that is strictly bound to symmetry about a y-value of 0.5, and asymptotic offspring survival (k) is always modeled as $k = 1$. However, if asymptotic survival is lower than 1.0, then the function will be erroneously protracted, and both optimal offspring size and offspring survival in the lower range of x-values will

be overestimated. Where this particular problem is suspected, it can be obviated by binning binary data into appropriate size-classes (values of x), thereby creating continuous survival probabilities. Asymptotic offspring survival can then be specified in the model as the maximum observed survival probability.

Chapter 3: Environmental Quality Predicts Optimal Egg Size in the Wild

Published as: Rollinson, N. and J. A. Hutchings. in press. Environmental quality predicts optimal egg size in the wild. The American Naturalist.

3.1 Abstract

Parents can maximize their reproductive success by balancing the trade off between investment per offspring and fecundity. According to theory, environmental quality influences the relationship between investment per offspring and offspring fitness, such that well-provisioned offspring provide greater fitness returns for parents when environmental quality is lower. A major prediction of classic theory, then, is that optimal investment per offspring will increase as environmental quality decreases. To test this prediction, we release over 30 000 juvenile Atlantic salmon (*Salmo salar*) into eight wild stream environments, and we monitor subsequent growth and survival of juveniles. We estimate the shape of the relationship between investment per offspring (egg size) and offspring fitness in each stream. We find that optimal egg size is greater when the quality of the stream environment is lower (as estimated by a composite index of habitat quality). Across streams, the mean size of stream gravel and the mean amount of incident sunlight are the most important individual predictors of optimal egg size. Within streams, juveniles recaptured in stream subsections that featured larger gravels and greater levels of sunlight also grew relatively quickly, an association that complements our cross-stream analyses. The present study provides the first empirical verification that environmental quality alters the relationship between investment per offspring and offspring fitness, such that optimal investment per offspring increases as environmental quality decreases.

3.2 Introduction

Reproductive traits are strongly associated with fitness, and biologists have long endeavored to understand the causes and consequences of variation in these traits. Lack (1947) and Svärdson (1949) first recognized that reproductive effort is finite, such that parents must trade off investment per offspring (e.g., egg or seed size) and fecundity. Interestingly, while fecundity and investment per offspring can never be simultaneously maximized, the strength of the trade off between fecundity and offspring fitness is tied to environmental quality (e.g., Einum and Fleming 1999). This is because the shape of the relationship between investment per offspring and offspring fitness (i.e., the fitness

function) is expected to differ among environments. In low-quality environments, a relatively high level of initial offspring investment might be required just to ensure that offspring have a non-zero chance of survival (McGinley et al. 1987), or the investment per offspring–offspring fitness function might be relatively protracted, increasing incrementally to an asymptote (Hutchings 1997). In either case, well-provisioned offspring are predicted to fare better (Fox et al. 1997; Hutchings 1997; Einum and Fleming 1999), such that parents maximizing fecundity in low-quality environments do so at the expense of offspring fitness (Johnson et al. 2010). On the other hand, both well-provisioned and poorly-provisioned offspring fare well in high-quality environments, such that parents can maximize fecundity and offspring fitness simultaneously. Selection ultimately favours parents that balance the fitness gains accrued from increases in fecundity with the fitness losses resulting from investment-related decreases in offspring viability (Smith and Fretwell 1974), such that optimal investment per offspring is predicted to increase as environmental quality decreases.

Although a negative relationship between environmental quality and optimal investment per offspring is widely expected (Hendry et al. 2001; Bashey 2008; Rollinson et al. 2012), support for this preiction is surprisingly limited. While many empirical studies have shown that parents produce relatively well-provisioned offspring in low-quality environments (e.g., Johnston and Leggett 2002; Taborsky 2006; Bashey 2008), few studies have estimated the shape of the fitness function that relates investment per offspring to offspring fitness (for examples, see Hutchings 1991; Sinervo et al. 1992; Einum and Fleming 2000a; Dias and Marshall 2010). In the few instances where these fitness functions have been estimated, optimal investment per offspring has been found to be greater when offspring food rations are lower (e.g., Hutchings 1997) or when competition for resources is higher (e.g., Marshall and Keough 2008, 2009). But none of these studies constitutes a defensible test of the environmental quality hypothesis. A fair test requires that the unit of replication is the environment, and inferential statistics must be applied at this level (Hurlbert 1984).

Theoretical models and simulation studies are often used to predict how investment per offspring might evolve in different types of environment (e.g., Parker and Begon 1986; McGinley et al. 1987; Einum and Fleming 2004; Olofsson et al. 2009).

However, given that little empirical background exists, many of these models are based on unverified assumptions. For instance, it is not clear whether spatial or temporal differences in optimal investment per offspring at the intraspecific level are consistently associated with changes in minimum viable offspring size (the minimum investment per offspring required to ensure offspring have a non-zero chance of survival), yet this assumption underlies many theoretical models (e.g., McGinley et al. 1987; Einum and Fleming 2004; Olofsson et al. 2009). Therefore, an empirical focus on quantitative descriptions of investment per offspring – offspring fitness relationships, and their comparison across multiple environments, is necessary both to inform theory and to formally test the environmental quality hypothesis. Such a study is multifaceted because the selective agents that constitute 'environmental quality' and that ultimately drive the evolution of investment per offspring must be simultaneously identified.

Here, we use a novel method of estimating the relationship between investment per offspring and offspring fitness (Chapter 2) to test the prediction that optimal investment per offspring increases as the quality of the offspring environment decreases. We assess the strength of selection on investment per offspring, and whether minimum viable offspring size changes predictably with optimal investment per offspring. Finally, we use direct estimates of optimal values as a means of identifying components of the physical environment that comprise 'environmental quality' and potentially drive the evolution of offspring provisioning strategies in the wild.

3.2.1 Study Species and Predictions

Atlantic salmon (*Salmo salar*) and its sister species have a long history in the study of investment per offspring. One reason for this is because they provide little post-partum parental care, such that egg size (e.g., egg weight or egg diameter) is a good proxy for the amount of energy invested per offspring. Salmonid fish have been instrumental in providing tests of Smith and Fretwell's (1974) classic model (Hutchings 1991; Einum and Fleming 2000a), and in both developing and testing extensions of classic theory (Hendry et al. 2001; Einum and Fleming 2002; Einum et al. 2002; Rollinson and Hutchings 2010, 2011a).

Juvenile Atlantic salmon (individuals in the first year of life) emerge in spring from their nests in stream gravel. They quickly become territorial, establishing fixed food-based territories located in sections of stream that feature cover objects, such as pebbles and cobble stones, and that feature adequate water velocities and depths (Steingrimsson and Grant 2003). Growth and survival of juveniles has been linked to these three correlated habitat features (e.g., Nislow et al. 1999; Suttle et al. 2004; Finstad et al. 2007). Canopy closure is a fourth component of the physical environment which negatively impacts both survival and growth through its effects on local productivity (e.g., Murphy et al. 1986; Riley et al. 2009). While juvenile habitat preference within rivers is often predicted by concave preference functions where intermediate velocities, depths and gravel size (substrate composition) are favored (e.g., Guay et al. 2000; Hedger et al. 2005), habitat suitability at higher ecological scales decreases as gravel size and water velocity decrease, and as stream depth increases (Bjornn and Reiser 1991). Therefore, if optimal investment per offspring decreases as the quality of the juvenile environment increases, we predict that optimal egg size for Atlantic salmon will be larger in streams typified by small stream gravel, low water velocity, and greater depth. Finally, we predict that growth of wild juveniles will be positively related to the quality of the environment they occupied within streams. The latter prediction is intended to complement our evaluation of optimal egg size and environmental quality by examining sub-lethal effects of habitat on performance.

3.3 Methods

3.3.1 Study Area and Populations

Atlantic salmon populations in the inner Bay of Fundy collapsed in the 1990s, and a captive breeding program was initiated to recover these populations (Fraser et al. 2007). One aspect of this recovery effort involved studying the extent of local adaptation and weighing the relative conservation risks of inbreeding and outbreeding depression in salmon from the Economy River ("*Eco*"; 45°22'N , 63°54'W), the Great Village River ("*Grv*"; 45°22'N 63°36'W), and the Stewiacke River ("*Stw*"; 45°8'N 63°22'W) (Houde et al. 2011a; b). All three focal rivers are a part of Nova Scotia's Minas Basin, and the maximum pair-wise distance between river mouths is approximately 35 km (Fig. 3.1).

Genetic evidence from neutral markers suggests moderate gene-flow among rivers (F_{ST} values: *Grv*−*Stw* = 0.0353; *Eco*−*Grv* = 0.0673; *Eco*−*Stw* = 0.0953) (Tymchuk et al. 2010), and populations are characterized by similar levels of heterozygosity and gene diversity, although there is evidence that Economy River salmon underwent a recent population bottleneck (Table 3.1).

Data from a previous release experiment performed in 2008 (Houde et al. 2011a; b), as well as data from the present release experiment performed in 2009 (N. Rollinson, D.M. Keith, A.L.S. Houde, P.V. Debes, M.C. McBride, J.A. Hutchings, unpubl. data), provide little evidence of survival differences between inbred and outbred offspring. In the present study, we use data that was collected in 2009 as part of this larger conservation initiative, and we compare the effect of egg size on offspring performance across multiple environments. Importantly, we randomize the genetic contributions of parents with respect to egg size, and this allows for a robust assessment of the association between egg size *per se* and offspring fitness.

3.3.2 Breeding design

In 2001, the Department of Fisheries and Oceans (DFO) collected 56, 52, and 198 wild juvenile salmon (salmon in their 1st or 2nd year of life) from the Economy, Great Village, and Stewiacke Rivers, respectively. These wild individuals were reared to maturity in a common environment at the Coldbrook Biodiversity Facility, in Coldbrook, Nova Scotia. Near maturity, all individuals were genotyped at nine or more microsatellite loci (described in O'Reilly and Harvey 2009; Houde et al. 2011a) and were equipped with a passive integrated transponder ("PIT tag") so they could be readily identified. In 2003 and 2004, these mature wild-born fish were anesthetized and stripped of their gametes, and families from each parental river were generated (i.e., families arising from *Grv*×*Grv*, *Stw*×*Stw* and *Eco*×*Eco* cross-types). In addition to these three pure-bred cross-types, two outbred cross-types were created by crossing Stewiacke with Great Village gametes (i.e., *Grv*×*Stw* cross-types), and by crossing Stewiacke with Economy gametes (i.e., *Eco*×*Stw* cross-types) (Fig. 3.2). In both years, all families were kept in separate incubation trays and incubated through the eyed-ova stage at the Mersey Biodiversity Facility in Milton, Nova Scotia. All ova were then shaken vigorously at this stage

("shocked"), which helps identify ova that died during incubation. Dead ova were removed, and in both years all families were equilibrated by taking an even number of ova from each family (either 5 ova from each family, or 10 ova from each family), and pooling these eggs in common incubation chambers. Ova then hatched, and juveniles ("fry") were reared through first feeding (May) until mid-summer (July). These juveniles were then transferred back to Coldbrook and reared to maturity. At Coldbrook, all pooled juveniles generated in 2003 or 2004 were reared to maturity in captivity in a common environment, and they represent the parents used in the present study. All pooled individuals were genotyped, and based on known families (i.e., mating parents of known identity in 2003 and 2004), an exclusion-based family assignment program (FAP v. 3.6, Taggart 2007) was used to assign individuals back to their original family (see Houde et al. 2011a, 2011b). In total, 99.8% of these individuals, representing the parents used in the present study, were unambiguously assigned. Unassigned parents were not used in the present study.

All parents used in the present study were captive-born fish that had reached sexual maturity by 2008, with the exception of two sires that had been captured in the wild in 2001. Controlled breeding for the present study was performed on 31 October 2008, and 4 November 2008 at Coldbrook. Each dam (n = 45) was stripped of her eggs in sequence, and all eggs contained within each dam were divided by eye into four to eight groups of approximately equal number. Each group of eggs was fertilized with sperm from a different sire (n = 49 sires), such that four to eight half-sib families were produced for each dam; between one and seven of these half-sib families from a given dam were ultimately used in the present study. Each half-sib family comprised a particular cross-type, and these cross-types were defined based on the river of origin of the offspring's grandparents (i.e., wild salmon captured in 2001), and whether the offspring's immediate parents had arisen from breeding wild fish that had been collected from the same or different rivers (Fig. 3.2).

Fifteen cross-types were generated (Fig. 3.2). We produced a total of three 'pure' offspring cross-types, in which river-populations were not mixed (*Eco*×*Eco*, *Stw*×*Stw*, *Grv*×*Grv*), and three inbred offspring cross-types (*E inbred*, *S inbred*, *G inbred*). Inbred cross-types were simply a special case of 'pure' cross-types that were generated based on

known pedigrees, where first cousins or siblings were mated. We created three F_1 outbred cross-types (*Eco*×*Stw*, *Grv*×*Stw*, *Eco*×*Grv*), two F_2 outbred cross-types (*ES*×*ES*, *GS*×*GS*), and four backcrossed cross-types (*Eco*×*ES*, *Stw*×*ES*, *Grv*×*GS*, *Stw*×*GS*). Between 16 and 19 families were produced per cross-type (except for *S inbred*), and we used a reciprocal breeding design such that the same dams (n = 9 to 19 dams, depending on the cross-type) and sires (n = 9 to 19 sires, depending on the cross-type) from a parental cross-type (i.e., *Stw*, *Grv*, *Eco*, *ES* or *GS*) were represented in each offspring cross-type (Fig. 3.2*B*). Only 9 families were produced for *S inbred*, as there were fewer relatives that could be mated, owing to an avoidance of deliberate inbreeding in the captive-breeding program and the greater initial number of wild fish collected from the Stewiacke River. In total, 200 families were produced.

Between 14 and 25 green eggs (mean = 19.4 eggs) from each dam were retained at spawning, dried at 50°C in a drying oven, and weighed to the nearest 0.1 mg using an electronic balance. Egg size did not differ among dams of different cross-types (Fig. 3.3), and egg size varied little within dams (mean ± SD coefficient of variation in egg size within dams, 8.0 ± 2.9%, range = 2.8% - 16.7%). Embryos were incubated at ambient temperatures in incubation trays within a single incubation trough at Coldbrook until the eyed stage. Embryos were then transferred to the Aquatron Facility at Dalhousie University where they were kept until the beginning of the experiment in plastic containers (Lee's® "Kritter Keepers"). Each container measured 27.6×17.0×20.3 cm, but was modified to accommodate two families in 13.8×17.0×20.3 cm sections, and each container was perforated to allow water flow. Up to three containers (up to six families) occupied one 70 cm circular flow tank, and 60 flow tanks were used in total. Water levels were maintained so that embryos were submerged under 7 cm of water, and water volume was roughly 26.9 $L \cdot tank^{-1}$. Flow into each tank was $5 - 10$ $mls \cdot sec^{-1}$, and aeration originated from the middle of each tank, which promoted homogeneity in water quality. Ambient temperatures were maintained throughout incubation (mean ± SD: 3.1 ± 2.3 ºC at Coldbrook; 5.2 ± 1.6 ºC in the Aquatron). Percent development was estimated from the sum of daily development, where daily development is estimated by Kane (1988) as

(Equation 3.1) $$\ln(y) = 6.003e^{(-0.0307 \times T)}$$

Where e is the base of the natural logarithm, and T is the mean daily incubation temperature in degrees Celsius. Each daily value (y) was divided into 100 then summed across the incubation period to obtain percent development, where 100% development coincides with first-feeding.

3.3.2 Experimental Releases and Stream Selection

Embryo development was complete on May 11th, 2009, when offspring spawned on October 31st and November 4th had reached 100% and 97% development, respectively (Kane 1988). Approximately 3,000 unfed juveniles (also called 'fry') were then released into each of eight streams, with three release locations in the Stewiacke River, three locations in the Economy River, and three locations in the Great Village River (Fig 3.1; Table 3.2, Appendix E). Offspring cross-types were released into a given river only if a portion of their genome was derived from wild fish originally captured in the river, with the exception of 'pure' cross-types (i.e., *Eco×Eco, Stw×Stw, Grv×Grv*) which were released in all rivers (Table 3.2). All juveniles were released on the same day, and all juveniles were released at the same time and location with streams, with two exceptions: in the stream STW3, juveniles were divided evenly among two adjacent stream sections (the sections converged within 30 meters of the release points), and in STW2, half of the juveniles were released 65 m downstream from the uppermost release point.

Anecdotal evidence suggests that most streams historically supported naturally spawning Atlantic salmon; however, Minas Basin salmon stocks collapsed in the 1990s, and all streams are now apparently devoid of naturally spawning Atlantic salmon (Fraser et al. 2007). Our releases constituted the only salmon in their first year of life inhabiting each stream, although older salmon (parr aged 1 or 2 years, 6 – 12cm standard length) from previous release programs inhabited most streams, as did naturally-spawned brook trout (*Salvelinus fontinalis*). Streams were selected for experimental releases based on the average gradient of the land estimated from topographical maps, and their close proximity to roads. Site visits were performed in order to ensure that streams were not too wide and not too fast. Based on these subjective criteria, streams were assumed to

represent hospitable environments for juvenile Atlantic salmon prior to release, although no direct habitat measurements had been taken prior to the onset of our study.

3.3.3 Habitat Measurements and Analysis

In July 2009, habitat stations were established at five-meter intervals in each stream, beginning ten meters upstream from the release point and ending 500 meters downstream from the release point. At every station, we established a transect that bisected the stream. We recorded water depth using a measuring stick at three equidistant locations along the transect, then mean water depth was calculated for the transect (Depth, cm). Stream width (Width, m) was the linear bank-to-bank distance across the transect, measured using a measuring tape. Water velocity (Velocity, $m \times s^{-1}$) was estimated by calculating the time it took an orange to travel two meters in the fastest part of the current (following Purchase and Hutchings 2008), beginning at the transect bisection. At the centre of each transect, we measured the proportion of a given area in which sunlight was directly obstructed by physical objects (e.g., leaves, trunks, branches) using a convex densitometer; this measure is expressed as the proportion of closed canopy area. Two measures of canopy closure were taken. Low Canopy Closure is an estimate of the amount of underbrush that inhibited incident sunlight from reaching the stream, such that only obstructions below a height of 3 m contributed towards the proportion of closed area. High Canopy Closure is an estimate of the extent to which forest canopy obstructed sunlight, such that only obstructions above a height of 3 m contributed to the proportion of closed area. Both types of canopy closure were estimated facing each cardinal direction, then these four estimates were averaged to produce one estimate of High Canopy Closure per station, and one estimate of Low Canopy Closure per station. Substrate type was estimated by eye for a 5 m area between each transect, and each transect bisection fell in the middle of this 5 m survey area. Proportional composition of the substratum was estimated to the nearest 5% following Boudreault's (1984) size classes: class 1 is sand (< 5 mm); class 2 are gravels (5–40 mm); class 3 are pebbles (40–80 mm); class 4 are cobbles (80–250 mm); class 5 are boulders (250–500 mm); class 6 is bedrock (> 500 mm). The granulometric index (GI) was then calculated as $GI = \Sigma(Gc \times Gu)$, where GI is the granulometric index of a station, Gc is the

granulometric class and Gu is the proportion of the substrate composed of that class (Hedger et al. 2005).

Velocity, Depth and GI of our individual 5-m stream sections were correlated, so we reduced these data by using principle components analysis. We estimated scores for each individual stream section by including log(Velocity), log(Depth) and GI from all 886 habitat stations comprising all 8 streams into a single principle components analysis (Levin et al. 2002; Mäki-Petays et al. 2002). The first of the three principle components accounted for 57.3% of the variation in the data, and this component was positively associated with log(Velocity) and GI, and negatively associated with log(Depth) (eigenvalue = 1.72; loadings: GI = 0.493, log(Velocity) = 0.621, log(Depth) = −0.610). Importantly, this index of habitat quality reflects a continuum between shallow, fast-flowing stream sections comprised of larger, heavier gravel (positive principle component scores) representing favorable juvenile habitat (Bjornn and Reiser 1991), and deeper, slow-moving stream sections where smaller gravel accumulated (negative principle components) representing unfavorable juvenile habitat (also see Nislow et al. 1999; Guay et al. 2000; Suttle et al. 2004; Armstrong and Nislow 2006; Finstad et al. 2007). Principal component scores for each of our 8 streams were obtained by dividing the surface area of each 5-m section of stream (5-m × Width) by the total area of the stream surveyed (Table 3.3), then multiplying this value by the principle component score of the stream section, and summing all section values within a stream. The mean score of each stream is therefore weighted, such that stream sections contribute in proportion to their area to the overall mean. We note that sites STW1 and STW2 are two independent release sites that are located within the same stream (Little Branch Brook, Stewiacke: upper and lower release sites, see Fig. 3.1), such that these release sites probably do not constitute independent environments. Similarities inhabitat measurements corroborate this assumption (Table 3.4). We therefore pooled data from these two sites when using inferential statistics to relate stream-specific estimates of optimal egg size (below) to environmental measures, such that only eight ‘environments’ were compared.

3.3.4 Recapture of Juveniles

Streams were electrofished by a team of three individuals using one backpack electrofisher and a lip-seine net between 25 August and 3 October, 2009. Two streams featured beaver dams and exhibited low recapture rates, so to increase sample size these streams were electrofished twice, about one month apart (Table 3.3). Electrofishing was initiated at the habitat station furthest from the release point and progressed systematically upstream through all areas (except near beaver dams) until we had reached the last station, which was always located ten meters above the release point. Our maximum survey distances (300 – 510 m downstream) extended beyond average downstream dispersal distances of juveniles (Webb et al. 2001; Einum and Nislow 2005). When a juvenile was captured in the seine net, it was placed in a perforated 50 ml vial, then the vial was labeled with the nearest habitat station. After fishing, juveniles were removed from the vials and anesthetized, using food safe clove oil (Hilltech Canada, Vankleek Hill, ON, Canada). Wet mass was measured to the nearest 0.01g, and a portion of the tail fin was clipped and placed in an individual 1 ml vial filled with 95% ethanol. Juveniles were re-released.

Multiple-pass electrofishing is necessary to reliably estimate survival rates (Bohlin et al. 1989). We performed only single-pass electrofishing, so overall recapture rates at our sites are very likely underestimates of true survival. This does not necessarily pose a problem when estimating optimal egg size: a fitness function can be scaled to any maximum value without fundamentally altering its shape, and it is both the shape of the function and minimum viable egg size that determines optimal egg size (Smith and Fretwell 1974). In the present study, we assume that offspring arising from small eggs were as easy to catch as offspring arising from large eggs, such that the shape of the estimated function is accurate, even if true survival is underestimated (see also Achord et al. 2003; Bailey and Kinnison 2010).

3.3.5 Parentage Assignments

The parents and grandparents of offspring generated for the present study had been previously genotyped at five or more tetranucleotide microsatellite loci (O'Reilly and Harvie 2009; Houde et al. 2011a). Fin clips of juveniles recaptured in our study streams were also genotyped at five to seven tetranucleotide microsatellite loci (for

details see Houde et al. 2011a, 2011b). Based on known genotypes of all dams and sires used in the present study, as well as records of mated pairs logged at Coldbrook in autumn of 2008, an exclusion-based macro for Microsoft Excel® (C. Harvie, Department of Fisheries and Oceans Canada) was used to assign juveniles back to their original family, such that we could identify each juvenile's mother and father. In some cases, juveniles were assigned to more than one family when 5 loci were used, so these juveniles were genotyped at up to two more loci, then the assignment was rerun. Familial assignment was over 90% successful for most streams (Table 3.3).

3.3.6 Estimates of Optimal Egg Size

We released offspring from a total of 32, 36 and 42 dams into streams in the Great Village, Economy and Stewiacke Rivers, respectively. The median number of juveniles released per dam ranged from 59 to 81 for the three different rivers. The number of half-sib families released per dam ranged between one and seven (median = 4), although more than one family was released per dam in 87.4% of cases (Appendix E). After parentage assignments, we summed the number of juveniles from a given dam (and hence, a given mean egg size) that was released into a stream. Next, we divided this value into the number of offspring recaptured in the stream from that dam. It is therefore difficult to attribute variation in recapture rate to genetic effects, because offspring cross-types varied within dam and hence within levels of egg size. Logistic regression was used to estimate the relationship between direct estimates of Egg Size (mg, dry mass) and offspring recapture probability (fitness) for each stream; linear selection differentials (β) were also estimated (Lande and Arnold 1983). The egg size–offspring fitness function was estimated for each stream using the Weibull-1 model (Rollinson and Hutchings, in press).

(Equation 3.2)
$$f(x) = k \cdot \mathrm{e}^{-\mathrm{e}^{b\{\ln(x)-\ln(a)\}}}$$

where k is maximum fitness observed in a given stream, e is the base of natural logarithms, ln is the natural logarithm, b is a shape parameter and a is the inflection point.

Weibull-1 models were fit in *R* (The R Development Core Team 2012) with the *drc* package (Ritz and Strebig 2011). Each level of egg size in these analyses was weighted by the number of individuals originally released from the relevant level of egg size (Appendix E). Importantly, the Weibull-1 model produces a sigmoidal function, and this reflects a biologically-realistic situation in which low but stochastic offspring survival near minimum viable egg size compels the fitness function slowly towards the x-axis (Chapter 2). No model estimate of minimum viable egg size (i.e., an x-intercept) is therefore possible. For each stream, we estimated minimum viable egg size as the predicted value of egg size where survival was 5 % of *k*.

Optimal egg size was derived from the Weibull-1 function following Smith and Fretwell (1974). However, confidence intervals provided by the *drc* package are only valid when all observations have a common variance, and this assumption could not be satisfied for our data. We therefore used simulation to approximate confidence intervals for optimal egg size estimates. Our simulations first accounted for the uncertainty in the 'true' value of offspring survival (T) for a given level of egg size (i) in each stream. While T_i was always unknown, our simulation assumes T_i falls somewhere within the 95% confidence limits of the observed recapture rate for every observed level of egg size. We used the binomial distribution to construct 95% confidence intervals on each observed recapture probability within each stream (Zar 1984, p. 378). One random sample $\hat{p}i$ was obtained from each binomial distribution for each level of egg size within each stream. For the purposes of simulation, $\hat{p}i$ was assumed to accurately represent T_i for a given level of egg size. Having obtained one possible value of T_i (namely, $\hat{p}i$), we next accounted for the uncertainty estimating T_i. We parameterized a beta distribution based on the number of recaptures (α) and the number of non-recaptures (β) comprising $\hat{p}i$ (e.g., if $\hat{p}i = 0.13$ and 100 fish were released at that level of egg size, then $\alpha = 13$ and $\beta = 87$). We drew one random sample ($S\hat{p}i$) from the beta distribution of $\hat{p}i$. Finally, all values of $S\hat{p}i$ for a given stream were regressed against dry egg mass using Equation (3.2) to estimate optimal egg size. We repeated this procedure 10,000 times to generate a distribution of optimal egg sizes for each stream. Confidence limits were obtained from the upper and lower 2.5% of these distributions.

3.3.7 Optimal Egg Size and Environmental Quality

We used linear regression to test whether optimal egg size was predicted by stream-averaged principle component scores. Values of optimal egg size for streams STW1 and STW2 were estimated from pooled data (as was the principle component score), such that each regression featured 8 data points. We further used linear regression to test the relationship between optimal egg size and direct measures of the physical environment: GI, Low Canopy Closure, High Canopy Closure, Velocity, and Depth. Stream-averaged estimates of GI, Velocity and Depth were calculated within streams as the weighted average of stream sections in which the measurement was taken (weights were determined by the two-dimensional area of each section). Stream-averaged estimates of Low Canopy Closure and High Canopy Closure were simple averages of closure across stations in a stream. All linear regressions were weighted by the total number of juvenile recaptures in a given stream.

3.3.8 Juvenile Growth Models

Juveniles often occupy the same territory for at least two months in Atlantic Canada (Steingrimsson and Grant 2003), so we tested relationships between the physical environment of 5-meter stream sections and growth of juveniles that occupied these sections. This analysis was performed within streams and at the individual level, rather than across streams at the group level, because juveniles that find suitable habitat within environments that are otherwise adverse might grow at the same rate as juveniles finding suitable habitat within high-quality environments (Nislow et al. 2004). Between 2 May and 3 May 2009 (i.e., just prior to juvenile release), we estimated mean juvenile mass-at-release for each dam in our study. We collected 4 to 22 juveniles (mean = 14.2 juveniles) from each dam, and each juvenile was weighed to the nearest 0.001g. After recapturing juveniles between August and September 2009, we modeled specific growth in the wild (Ω, % × day^{-1}) following Ostrovsky (1995),

$$\Omega = \frac{M_t^b - M_0^b}{b \cdot t} \cdot 100$$

(Equation 3.3)

where M_0 is the mean weight of larvae from dam_m estimated prior to release, M_t is the weight at recapture of a juvenile whose mother is dam_m, b is the allometric weight exponent for the relationship between specific growth rate and body weight (0.31 for Atlantic salmon juveniles, Elliott and Hurley 1997) and t is the number of days between release and recapture. In this model, we consider the releases sites STW1 and STW2 to be the same stream, and juveniles captured on the second visit to GRV1 and STW3 were not included in the analysis. To obtain our final growth model, we followed the information-theoretic approach of Burnham and Anderson (2002) using a mixed analysis with maximum likelihood parameter estimation. In our base model (in which subsequent models were nested), we assumed that dam and sire identity (n = 41 dams and 46 sires) contribute to variation in juvenile growth, and that average growth is different among the 8 streams in our study. We hypothesized that initial egg size (dry mass, mg) increases the ability of juveniles to acquire food and territories (Einum and Fleming 2000a), but that the relationship between growth and egg size would differ among streams,

(Equation 3.4) $$Y_{i(j)mp} = \beta_0 + \tau_j + u_{0j} + (\beta_1 + u_{1j}) \times x_{i(j)} + \gamma_m + \delta_p + \varepsilon_{i(j)mp}$$

Where i's are individual juveniles nested in stream j, β_0 is a fixed intercept, x is dry egg mass (mg), β_1 is the mean slope of growth as a function of dry egg mass across all streams, τ is the random intercept for stream j, u_0 is the random intercept modifier and u_1 is the random slope modifier for the relationship between dry egg mass and growth in stream j, γ is the random intercept for dam m, δ is the random intercept for sire p, and ε is error. This base model was then parameterized with up to five predictors (fixed effects) that might have additive effects on of juvenile growth: GI, Low Canopy Closure, High Canopy Closure, Velocity, and Depth. These predictors reflect the physical environment within the 5-m stream section where individuals were recaptured, and every model parameterized by these predictors reflects a biologically-plausible hypothesis (Burnham and Anderson 2002). Specifically, we hypothesized that low Velocity decreases the ability of juveniles to acquire food items drifting in the current (Nislow et al. 1999), so we tested for a negative relationship between Velocity and growth. Juveniles tend to

avoid deep streams and deep sections of stream (e.g., Guay et al. 2000; Hedger et al. 2005), so we expected a negative relationship between Depth and growth. Substrate size is positively correlated with the number and quality of available territories (Suttle et al. 2004; Finstad et al. 2007, 2009), so we tested for a positive relationship between individual growth and GI. A decrease in incident sunlight can decrease local productivity at small spatial scales (Murphy et al. 1986; O'Grady 1993; Riley et al. 2009), so we tested for a negative relationship between canopy closure and growth. In total, we compared 16 models. Unfortunately, due to a loss of some temperature data-loggers, stream temperature could not be incorporated into our growth models, although the term τ (Equation 3.4) accounts for differences in mean growth among streams.

3.4 Results

A total of 30,516 salmon were released, 1210 juveniles were captured, and 1084 could be assigned to their original mother. Average recapture rate per mother ranged from 0.0238 (UCI = 0.0305, LCI = 0.0185) at STW1 to 0.0478 (UCI = 0.0648, LCI = 0.0351) at GRV3. Within each stream, recapture rate was positively and significantly related to egg size, and coefficients of determination (r^2) were typically large (Table 3.5). Linear selection differentials were also positive and significant in each stream, with $\beta \pm$ SE values ranging from 0.382 ± 0.122 at GRV3 to 1.26 ± 0.138 at ECO3 (Table 3.5) and averaging ($\pm$ SD) 0.690 ± 0.299 across all streams.

3.4.1 Optimal Egg Size

Estimates of optimal egg size averaged ($\pm$SD) 48.1 ± 3.78 mg across all eight streams, and estimates ranged from 41.8 mg to 54.0 mg (Fig. 3.4). These values approach the maximum value of egg size produced by the hatchery-reared dams (mean observed egg size: 31.5 ± 6.35 mg, range: 21.9 – 48.8 mg, n = 45 dams), and our simulations suggest that confidence limits are very wide in all cases (Fig. 3.5). We found no relationship between optimal egg size and the maximum distance surveyed from the release point (Optimal size = $37.6 + 0.0218 \times$(Distance), $r^2 = 0.186$, n = 8, p = 0.29), suggesting that potential size-biased dispersal (Einum et al. 2011) did not influence our estimates of optimal egg size. The relationship between optimal egg size and

environmental quality (Principle Component 1) was significant and negative (Fig. 3.6*A*), as was the relationship between optimal egg size and GI (Fig. 3.6*B*). Optimal size did not correlate with Low Canopy Closure (Optimal size = 43.8 + 13.0×(Low Canopy Closure), r^2 = 0.185, n = 8, p = 0.29) or High Canopy Closure (Optimal size = 46.3 + 2.02×(High Canopy Closure), r^2 < 0.01, n = 8, p = 0.92). However, when these two metrics were averaged to create a single index of closure (Average Canopy Closure), this derived metric correlated positively and nearly significantly with optimal egg size (Fig. 3.6*C*). Neither average stream Depth (Optimal size = 38.4 + 0.593×(Depth), r^2 = 0.291, n = 8, p = 0.18) nor average Velocity was related to optimal egg size (Optimal size = 49.8 – 4.66×(Velocity), r^2 < 0.01, n = 8, p = 0.92).

Minimum viable egg size averaged (±SD) 21.9 ± 3.10 mg and ranged from 18.4 – 26.5 mg (Fig. 3.4). The mean and range was centered on the smallest value of egg size observed in the present study, which was 21.9 mg, suggesting that our estimates of minimum viable egg size were reasonable for their respective environments. Indeed, values of minimum survival used to estimate minimum viable egg size were low (average survival at minimum viable egg size = 0.6 ± 0.2 %), such that very few offspring survived below minimum viable size. Minimum viable egg size was not related to k (r^2 = 0.064, n = 8, p = 0.55), where k the maximum observed survival in a given stream. However, optimal egg size and minimum viable egg size were positively linearly related (Optimal size = 27.8 + 0.926× (Minimum size), r^2 = 0.580, n = 8, p = 0.028). Interestingly, environmental quality did not predict minimum viable egg size (Minimum size = 21.8 – 3.29×(PC1), r^2 = 0.348, n = 8, p = 0.12), although the relationship was in the predicted direction. The strength of linear selection on offspring size (β) was strongly related to minimum viable egg size (Minimum size = 14.7 + 10.5×(β), r^2 = 0.926, n = 8, p < 0.001), and there was also a positive but non-significant association between the strength of linear selection and optimal egg size (Optimal size = 42.4 + 8.16×(β), r^2 = 0.382, n = 8, p = 0.10).

3.4.2 Juvenile Growth

Across all streams, mean mass-at-recapture (± SD) was 1.92 ± 0.663g (n = 996). Mean specific growth varied from 0.942 ± 0.125 % body weight×day^{-1} in ECO3 (n = 94)

to 1.32 ± 0.167 % body weight×day^{-1} in GRV2 (n = 144). The model that best predicted individual growth of juveniles featured GI, Low Canopy Closure and High Canopy Closure (Table 3.6), as well as the predictors expressed in the base model (Equation 3.4). Relationships between growth, GI and canopy closure were in the direction predicted by our *a priori* hypotheses (Table 3.7). While the Akaike weight (w_i) for this best model was low (0.392, Table 3.6), several lines of evidence suggest that it is a far superior model given the data. First, the two competitive models in our confidence set each differed from this best model by one additional parameter, but both these models had essentially the same values of log-likelihood as the best model (Table 3.6). Therefore, the more complex models are not competitive (Burnham and Anderson 2002, p. 131) but are within a few AIC_c units of the best model because they feature one additional parameter without improving model fit (as measured by the log-likelihood). Second, we estimated the relative importance of GI, Canopy Closure (as a single predictor), Velocity and Depth as predictors of growth by summing w_i across models where the appropriate predictor appears (Burnham and Anderson 2002, p. 167). We found that GI and Canopy Closure had a relative importance of 0.998 and 0.999, respectively, whereas that of Depth was 0.364, and that of Velocity was 0.357. Finally, effect sizes for GI and both metrics of canopy closure were always relatively large (e.g., $|t| > 1.96$ in all cases), whereas those for Velocity and Depth were always relatively small (e.g., $|t| < 1.96$ in all cases).

3.5 Discussion

The evolution of offspring size has been well explored in a theoretical context (e.g., Smith and Fretwell 1974; Parker and Begon 1986; Bonabeau et al. 1998; Rees and Venable 2007), yet a sound theoretical development as well as a broad understanding of this trait is ultimately founded in the empirical study of offspring size – number tradeoffs. The present study is the first to provide direct, empirical, quantitative support for the prediction that optimal egg size increases as environmental quality decreases. Furthermore, we find that intraspecific variation in optimal egg size is positively associated with variation in minimum viable egg size, and this provides empirical verification for theoretical studies that assume a correlation between minimum viable egg size and optimal values (McGinley et al. 1987; Einum and Fleming 2004; Olofsson et al.

2009; Charpentier et al. 2012). Our data suggest that the extent of offspring survival at low values of egg size shifts the entire fitness function towards greater or lesser values of egg size, and owing to variation in the sigmoidal shape of the fitness function, this creates a positive but unsettled association between minimum viable egg size and the optimal value (Fig. 3.4). We also observed strong linear selection on egg size, and interestingly, the strength of selection exhibited a positive association with minimum viable egg size. This is likely because a consistent range of phenotypes was examined across all 8 streams, such that low survival when egg size is small increased minimum viable egg size while concomitantly elevating the slope of the linear selection regression.

We used estimates of optimal size as a means of identifying aspects of the physical environment that comprise 'environmental quality' and potentially drive the evolution of offspring provisioning strategies in Atlantic salmon. We found that the same components of the physical environment that influenced optimal egg size at a broad spatial scale also influenced individual growth of juveniles at a very small spatial scale. Juveniles that held territories in stream sections featuring relatively large gravel and a relatively open forest canopy grew relatively quickly. Similarly, across streams, the most important predictor of optimal egg size was the mean size of stream gravels (GI); mean incident sunlight (canopy closure) also correlated weakly and nearly significantly with optimal size. Water depth and velocity within stream sections did not appear to influence juvenile growth, and while stream depth and velocity were indirectly correlated with optimal egg size as principle components of environmental quality, we found that neither variable was important as an individual predictor of optimal egg size.

Understanding the evolution of egg size in any species requires identifying the life stage at which investment per offspring influences offspring performance, as well as the mechanisms through which investment-related biases in performance occur. In species with little post-partum care, effects of egg size on the performance of embryos (e.g., hatching success) are often very weak (Pepin et al. 1997; Rombough 2007; Riddick and Wu 2012; for exceptions see Einum et al. 2002; Marshall and Bolton 2007). However, immediately following embryonic development (e.g., just after hatching), there is typically a critical period in which performance is strongly related to offspring size (Fox et al. 1997; Nislow et al. 2004; Marshall and Keough 2008). Indeed, size-biased survival

of offspring is usually associated with differences in the competitive ability of juveniles (Svensson and Sinervo 2000; Bashey 2008), or with differences in the amount of time juveniles are able to persist without securing food (reviewed by Kamler 2006). Many empirical studies have verified that competition among juveniles affects the evolution of investment per offspring, primarily through its effects on juvenile resource acquisition (Hutchings 1991; Svensson and Sinervo 2000; Bashey 2008).

In the present study, we focused on how the physical environment of a stream predicts environmental quality for juvenile salmon. Differences in the strength of size-biased mortality were observed among streams, likely as a result of how the physical environment affected juvenile resource acquisition immediately following release (Einum and Fleming 2000a). The size of stream substrates is a recognized driver of population regulation in Atlantic salmon, where carrying capacity and population growth rate of one-year old fish has been linked to the presence and spatial distribution of gravel-shelter objects (Finstad et al. 2007, 2009). In part, this may occur because offspring production in salmon streams eclipses the amount of juvenile habitat that is available, such that the acquisition of a fixed, food-based territory centered within river gravels is paramount to juvenile survival (Einum and Fleming 2000b; Nislow et al. 2004; Kvingedal and Einum 2011). Failure to secure a territory usually means that juveniles become 'drifters' (Bujold et al. 2004) that engage in risky foraging behavior (Vehanen 2003) and face increased mortality during their dispersal downstream (Finstad et al. 2007; Kvingedal and Einum 2011; Einum et al. 2011).

The size of stream gravel reflects the availability and quality of juvenile territories for simple geometric reasons, given that an amalgam of large gravel will have relatively more and larger inter-gravel spaces, and that juveniles use these gravel interstices for shelter (Heggenes 1988). Sheltering behavior is extremely important for juvenile salmon: it decreases the proportion of time spent swimming against the current, the number of aggressive encounters with conspecifics (Suttle et al. 2004), and presumably the risk of depredation. Juveniles in the vicinity of shelter objects even display lower resting metabolic rates than those not in the presence of shelters (Millidine et al. 2006). Individual growth increases with the presence and size of cover objects within juvenile territories (Suttle et al. 2004; Finstad et al. 2007), probably by virtue of lower metabolic

costs, lower aggression among neighboring territory holders (Jaeger 1981; Suttle et al. 2004), and an increase in vulnerable prey items (invertebrates) associated with larger, less embedded gravel (Mebane 2001; Suttle et al. 2004). In the present study, predicted values of optimal egg size suggest that parental reproductive success will be greater if parents decrease fecundity and increase investment per offspring when river gravel is relatively small, and this finding is highly concordant with the early life history of Atlantic salmon.

In addition, we found that the degree of canopy closure was negatively related to juvenile growth within stream sections. Negative relationships between juvenile growth, survival, and canopy closure have been previously documented at both large and small spatial scales (Murphy et al. 1986; O'Grady 1993; Riley et al. 2009). Increases in incident sunlight can stimulate the growth of periphyton, and this has the direct result of increasing local abundance of aquatic invertebrates (Zimmermann and Death 2002; Fuller et al. 2007). Therefore, juvenile salmon occupying territories in sunlit areas have greater access to food. Influxes of terrestrial invertebrates into streams are also substantial and can comprise more than 50% of the annual energy budget of stream-dwelling salmonids (Nakano and Murakami 2001; Erős et al. 2012). Incident sunlight in riparian zones may attract forest-dwelling insects to the stream and further increase food availability in sunlit areas. Interestingly, we found that optimal egg size correlated positively, but not significantly, with average stream canopy cover. This pattern warrants further investigation, as it is consistent with theoretical expectations (e.g., McGinley et al. 1987), and with broad patterns of egg-size variation in relation to ecosystem productivity (Johnston and Leggett 2002).

On a cautionary note, overall survival was low and sample sizes were modest in the present study. In these circumstances, it is difficult to estimate optimal egg size with accuracy (Chapter 2). Indeed, the confidence intervals estimated for predicted values of optimal egg size were also very large. We cannot discount the possibility that the correlation between optimal egg size and environmental quality is not repeatable. But we also emphasize the corroboratory nature of our data: we found that juveniles occupying areas of stream featuring more sunlight and larger gravel grew more quickly, and this complements our cross-stream analysis which identifies gravel size and incident sunlight as potential drivers of environmental quality. Estimates of optimal egg size were also

similar for STW1 and STW2, where independent releases of 3545 juveniles occurred at different sites in the same stream. This provides limited evidence that our estimates of optimal egg size are repeatable within the environments surveyed.

Table 3.1: Mean (SD) genetic estimates for Atlantic salmon river-populations

No. Loci	River	N	Gene diversity	Allelic richness	He	d^2	F	F_{IS}
8	Stw	99	0.837 (0.059)	11.31 (4.18)	0.804 (0.149)	491.4 (383.0)	0.069 (0.327)	0.030 (0.047)
	Grv	37	0.844 (0.059)	10.97 (3.35)	0.878 (0.133)	709.1 (471.6)	0.013 (0.093)	-0.045 (0.084)
	Eco	30	0.738 (0.062)	6.85 (1.45)	0.921 (0.084)	573.8 (252.6)	-0.018 (0.044)	-0.261 (0.148)
16	Grv	37	0.804 (0.014)	10.89 (20.22)	0.816 (0.008)	579.5 (70626.6)	0.023 (0.006)	-0.017 (0.011)
	Eco	30	0.702 (0.011)	6.68 (4.22)	0.840 (0.007)	488.6 (46295.1)	-0.001 (0.003)	-0.205 (0.017)

SOURCE: All values from Houde et al. 2011b; table modified from Houde et al. 2011b.

NOTE: The eight microsatellite loci were SSsp1605, SSsp2201, SSsp2210, SSsp2215, SSsp2216, SSspG7 Ssa197, and Ssa202. The sixteen microsatellite loci were the eight loci plus Ssa171, Ssa85, Ssa486, Ssa144, SsaD71, Ssa58, U3, Ssosl417. Heterozygosity is He, and is estimated as the proportion of heterozygous loci for each individual. The estimated genomic diversity for each individual is d^2. For each individual, the inbreeding coefficient (F_{IS}) was estimated using the method-of-moments estimator.

Table 3.2: Number of Atlantic salmon released by cross-type and stream.

Name of cross-type	Cross-type	GRV Streams	ECO Streams	STW Streams
Economy Pure	*Eco×Eco*	175	175	175
Economy Inbred	*E inbred*	0	175	0
Great Village Pure	*Grv×Grv*	175	175	175
Great Village Inbred	*G inbred*	175	0	0
Stewiacke Pure	*Stw×Stw*	175	175	175
Stewiacke Inbred	*S inbred*	0	0	175
Economy-Great Village F_1 Hybrid	*Eco×Grv*	328	350	0
Economy-Stewiacke F_1 Hybrid	*Eco×Stw*	0	353	353
Great Village – Stewiacke F_1 Hybrid	*Grv×Stw*	380	0	354
Great Village – GS Backcross	*Grv×GS*	700	0	353
Economy – ES Backcross	*Eco×ES*	0	700	350
Stewiacke – GS Backcross	*Stw×GS*	715	0	365
Stewiacke – ES Backcross	*Stw×ES*	0	442	442
Great Village – Stewiacke F_2 Hybrid	*GS×GS*	700	0	350
Economy – Stewiacke F_2 Hybrid	*ES×ES*	0	558	279
Release total per stream		3523	3103	3546

NOTE: Values represent release total per stream, and there were multiple release streams per river (see Fig. 3.1).

Table 3.3: Details of releases, electrofishing and recaptures for all streams.

Site	Releases (juveniles/families)	Recaptured/assigned	Survey date	Effort (s)	Surveyed (m/m^2)
ECO 1	3103/132	123/117	06-Sep	2388	510/1984
ECO 2	3103/132	127/117	07-Sep	3011	655/1782
ECO 3	3103/132	120/94	23-Sep	3893	520/1511
STW 1	3546/161	95/87	09-Sep	3354	500/1656
STW 2	3546/161	110/103	20-Sep	3920	520/1697
STW 3	3546/161	78/74	29-Aug	1773	265/574
STW 3		48/42	03-Oct	2135	260/563
GRV 1	3523/118	65/56	26-Aug	2294	480/1933
GRV 1		55/42	25-Sep	3006	480/1933
GRV 2	3523/118	158/145	25-Aug	2005	405/1430
GRV 3	3523/118	231/207	16-Sep	5381	510/1548

NOTE: Under Releases, we note the number of juveniles released and the number of full-sib families these juveniles comprised. We also note the number of juveniles recaptured and successfully assigned to their original family. Two sites, GRV1 and STW3 were sampled on two independent days (date surveyed) because of low captures on the first survey. The time that the electrofisher was active is 'Effort' and is measured in seconds. We recorded the total river surface area that was electrofished ('Surveyed', m^2) as well as the total linear stream reach electrofished ('Surveyed', m). The low assignment rate at ECO3 reflects deliberate capture and analysis of a small sample of larger juveniles. This was done in order to ensure that larger juveniles were in fact not released as part of the present study (i.e., fast-growing juveniles). None of these larger juveniles could be assigned.

Table 3.4: Summary of habitat measurements for each stream.

	Max distance	Area	$\bar{x}$ low canopy	$\bar{x}$ high canopy	$\bar{x}$ GI	$\bar{x}$ velocity	$\bar{x}$ depth	$\bar{x}$ PC1
ECO1	505	2057	0.241	0.735	2.62	0.476	20.3	-0.480
			(0.302)	(0.280)	(0.634)	(0.184)	(12.0)	
ECO2	500	1856	0.495	0.709	2.22	0.420	16.5	-0.640
			(0.379)	(0.290)	(0.579)	(0.159)	(8.12)	
ECO3	510	1555	0.135	0.946	2.90	0.433	14.9	0.0085
			(0.266)	(0.0764)	(0.481)	(0.148)	(7.57)	
STW1	500	1705	0.259	0.664	3.29	0.473	18.8	-0.089
			(0.268)	(0.260)	(0.627)	(0.185)	(9.01)	
STW2	510	1714	0.146	0.859	3.41	0.450	18.9	-0.216
			(0.202)	(0.129)	(0.473)	(0.227)	(9.53)	
STW3	300	552	0.396	0.655	2.88	0.399	14.3	-0.189
			(0.355)	(0.305)	(0.473)	(0.181)	(6.04)	
GRV1	495	1970	0.412	0.644	3.07	0.501	17.9	-0.090
			(0.334)	(0.266)	(0.527)	(0.233)	(7.54)	
GRV2	390	1378	0.200	0.745	4.23	0.460	12.3	1.038
			(0.295)	(0.253)	(1.20)	(0.180)	(3.99)	
GRV3	500	1563	0.375	0.696	3.57	0.388	12.2	0.490
			(0.330)	(0.265)	(0.757)	(0.149)	(4.28)	

NOTE: Means (SD) are across all 5-m stream sections within a stream. The distance between the release point and the most distal habitat station is Max distance (m). The total surface area of stream sampled for habitat measurements is Area (m^2). The weighted mean principal component score for each stream is PC1. Habitat measurements in some streams could not be completed up to the full 500 meters downstream from the release

point when streams joined with the river mainstem or other major water bodies. Streams GRV1 and STW3 were blocked with beaver dams (*Castor canadensis*) downstream from the release point. Habitat measurements were avoided near dams so that dams did not bias site-averaged habitat estimates. Juveniles were often found downstream from dams.

Table 3.5: Mean estimates of optimal egg size (Opt, mg dry mass) and minimum viable egg size (xMin, mg dry mass), as well as logistic regression coefficients (intercept = a, slope = b) and selection differentials (β) for the relationship between egg dry mass and recapture probability.

Stream	Opt	xMin	b (SE)	a (SE)	r^2	β (SE)	n	♀
ECO 1			0.0412	-4.16	0.427**	0.515	117	
	45.3	20.1	(0.00820)	(0.280)		(0.0928)		
ECO 2			0.0675	-5.12	0.625**	0.947	117	36
	54.0	25.9	(0.00896)	(0.306)		(0.137)		
ECO 3			0.0719	-5.40	0.719**	1.26	94	
	49.4	26.5	(0.00770)	(0.264)		(0.138)		
STW 1			0.0522	-4.76	0.472**	0.859	87	
	52.5	24.1	(0.00872)	(0.291)		(0.140)		
STW 2			0.0637	-5.06	0.616**	0.810	103	42
	50.5	24.7	(0.00795)	(0.265)		(0.104)		
STW 1&2			0.0576	-4.88	0.678**	0.830	190	
	49.9	24.0	(0.00628)	(0.209)		(0.0933)		
STW 3			0.0520	-4.59	0.482**	0.621	116	
	47.1	20.4	(0.00853)	(0.284)		(0.110)		
GRV 1			0.0358	-4.11	0.255*	0.488	102	
	50.9	20.8	(0.0112)	(0.365)		(0.146))		
GRV 2			0.0450	-4.17	0.353**	0.479	145	32
	41.8	19.3	(0.0111)	(0.363)		(0.106)		
GRV 3			0.0476	-4.03	0.255*	0.382	207	
	46.1	18.4	(0.0148)	(0.486)		(0.122)		

NOTE: Sample size (n) is the number of individuals in each stream that were recaptured and assigned to their original mother. The number of levels of egg size (the number of

dams) for each river is ♀. STW1 and STW2 are different release sites within the same stream, and STW 1&2 is the analysis on pooled data. R-square values are for logistic regressions, and all continuous survival probabilities were logit-transformed after adding a value of 0.025 for logistic regressions. *p < 0.01, **p < 0.001.

Table 3.6: Model selection for growth of juvenile salmon in 8 streams.

Hypothesis	K	LogLik	AIC_c	$\Delta AICc$	w_i	Confidence Set
(B)	7	454.8	-895.4	53.99	0	No
(B) + G	8	460.7	-905.2	44.18	0	No
(B) + CL + CH	9	477.3	-936.3	13.04	0.001	No
(B) + V	8	456.0	-895.9	53.52	0	No
(B) + D	8	455.0	-893.8	55.55	0	No
(B) + G + CL + CH	10	484.8	-949.4	0.00	0.392	Yes
(B) + G + V	9	460.9	-903.7	45.72	0	No
(B) + G + D	9	460.7	-903.2	46.14	0	No
(B) + CL + CH + D	10	478.4	-936.7	12.72	0.001	No
(B) + CL + CH + V	8	456.0	-895.9	53.52	0	No
(B) + V + D	9	456.0	-893.8	55.55	0	No
(B) + G + CL + CH + V	11	485.4	-948.4	0.95	0.244	Yes
(B) + G + CL + CH + D	11	485.4	-948.5	0.90	0.250	Yes
(B) + G + V + D	10	460.9	-901.6	47.76	0	No
(B) + CL + CH + V + D	11	479.6	-936.9	12.44	0.001	No
(B) + G + CL + CH + V + D	12	485.6	-946.9	2.49	0.113	Yes

NOTE: The 'Hypothesis' column is model parameterization, and all models are nested in the base model (Equation 3.4), which is summarized as (B). Tandem predictors are low canopy closure (CL) and high canopy closure (CH), and individual predictors are the granulometric index (G), water velocity (V) and water depth (D), all of which are modeled as fixed effects. The number of model parameters is *K*, the value of the maximized log likelihood is '*LogLik*', Akaike weight is w_i. Models in our confidence set have a relative likelihood of ≥ 0.05, which corresponds to a ΔAIC_c of 6.0.

Table 3.7: Summary of the model that best predicts variation in mass-specific growth (% body weight×day^{-1}) for 996 juveniles from 8 different streams in Nova Scotia.

Predictor	Effect	Variance (SD)	Estimate (SE)	t
Sire	RI	0.00195 (0.0441)	–	–
Dam	RI	0.00287 (0.0535)	–	–
Site	RI	0.0233 (0.153)	–	–
Egg Size by Site	RS	0.00000649 (0.00255)	–	–
Residual	Error	0.0194 (0.139)	–	–
Intercept	FE	–	1.23 (0.0835)	14.7
Egg Size	FE	–	−0.00244 (0.00195)	−1.25
Granulometric index	FE	–	0.0270 (0.00695)	3.88
Low Canopy Closure	FE	–	−0.0524 (0.0171)	−3.07
High Canopy Closure	FE	–	−0.126 (0.0196)	−6.42

NOTE: Parameter estimates are reported after fitting models using restricted maximum likelihood parameter estimation, but maximum likelihood was used during the model-selection process. Random effects (intercepts = RI, slopes = RS) and residual variance are reported first, followed by estimates for fixed effects (FE).

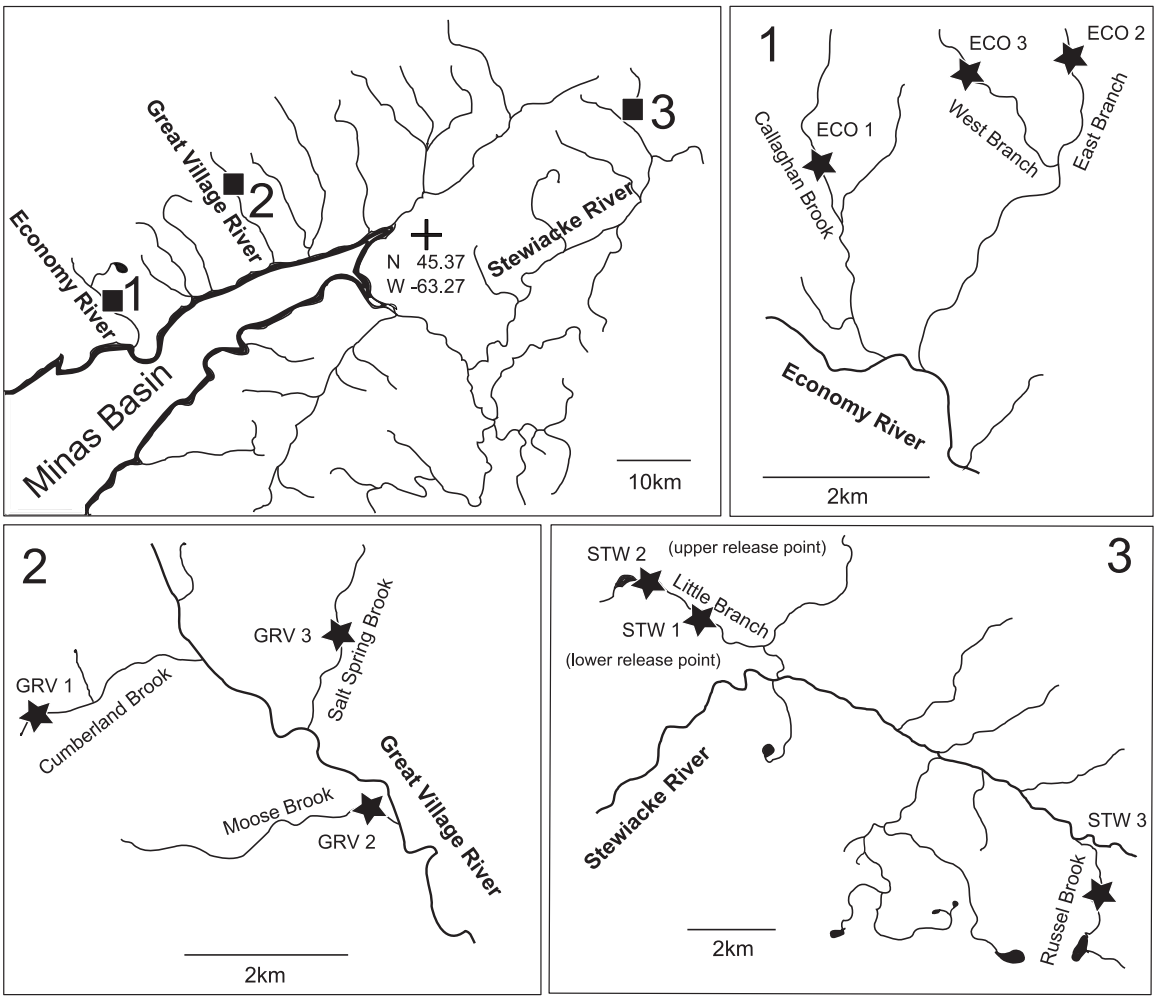

Figure 3.1: Map of experimental release areas. Dark squares in upper left panel are enlarged in subsequent panels to show location of experimental releases within streams (stars).

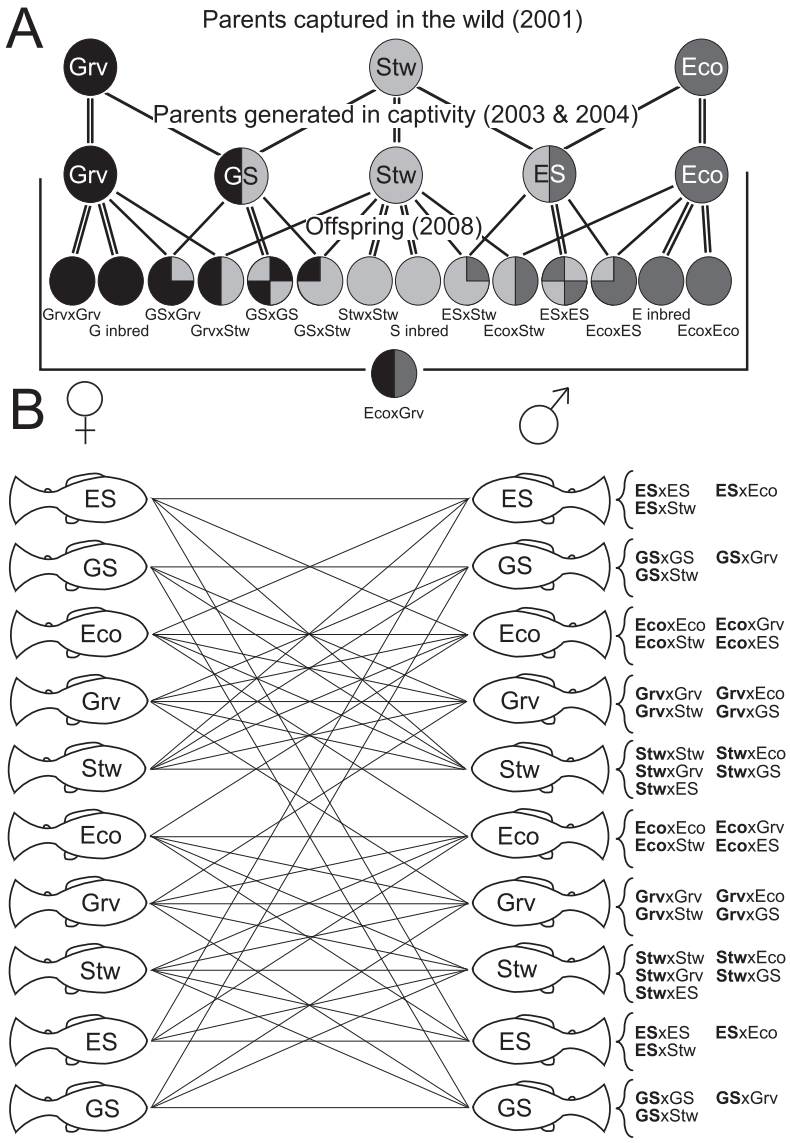

Figure 3.2: (*A*) Each circle represents a cross-type, and shading indicates the proportion of genome derived from wild types captured in Great Village (black), Stewiacke (grey) and Economy (dark grey). Grey lines leading to each circle indicate how a given cross-type was created. (*B*) The reciprocal breeding design of the present study. Parents of a given cross-type were mated with many individuals belonging to the same and different cross-types. All possible offspring cross-types that can be generated from a parental cross-type are depicted on the right of the panel.

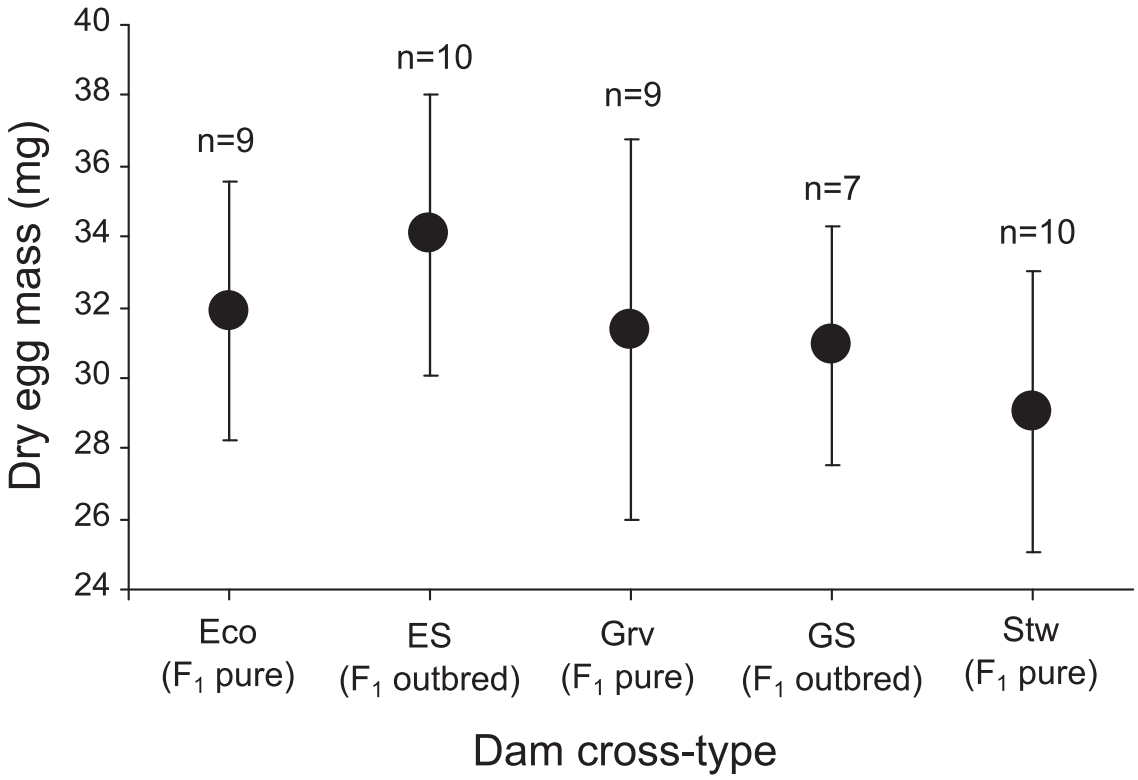

Figure 3.3: Mean ± 95% confidence intervals of mean egg mass for dams of different cross-types. All dams were spawned in 2003 or 2004 by mating two parents that were originally captured in the wild in 2001. An '*Eco*' cross-type are those dams arising from mating two parents originally captured in the Economy River. An '*ES*' cross-type is a dam arising from mating one parent originally captured in the Economy River with one parent captured in the Stewiacke River. Mean egg size did not differ among cross-types (ANOVA: $F_{4,44} = 0.79$, $p = 0.54$).

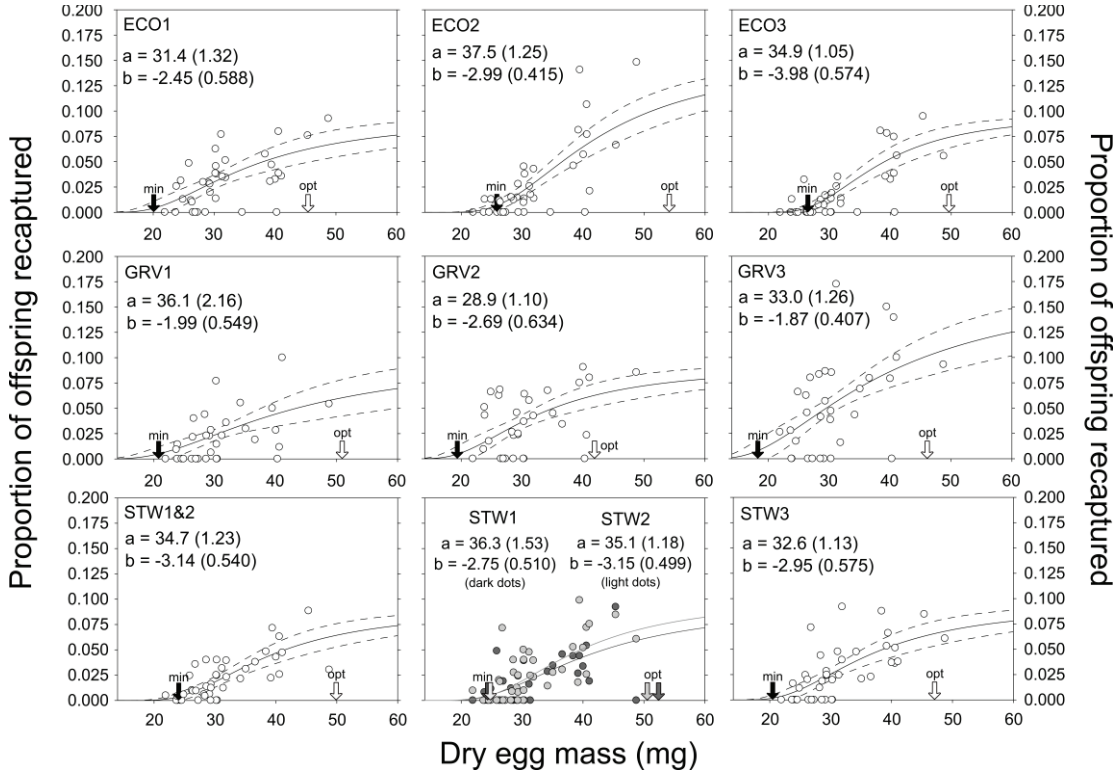

Figure 3.4: Fitness functions ± 95% Weibull-1 confidence intervals for egg size versus proportion of offspring recaptured. Black arrows are minimum viable egg size, white arrows are optimal egg size. For clarity, confidence intervals are not shown on fitness functions for STW2 or STW1, where independent offspring releases occurred at separate locations within the same stream. The same families and number of individuals were released in STW1 and STW2, such that the pooled sample ("STW1 & STW2") is the average offspring recapture rate in STW1 and STW2. Parameter estimates are from Weibull-1 models (Equation 3.2).

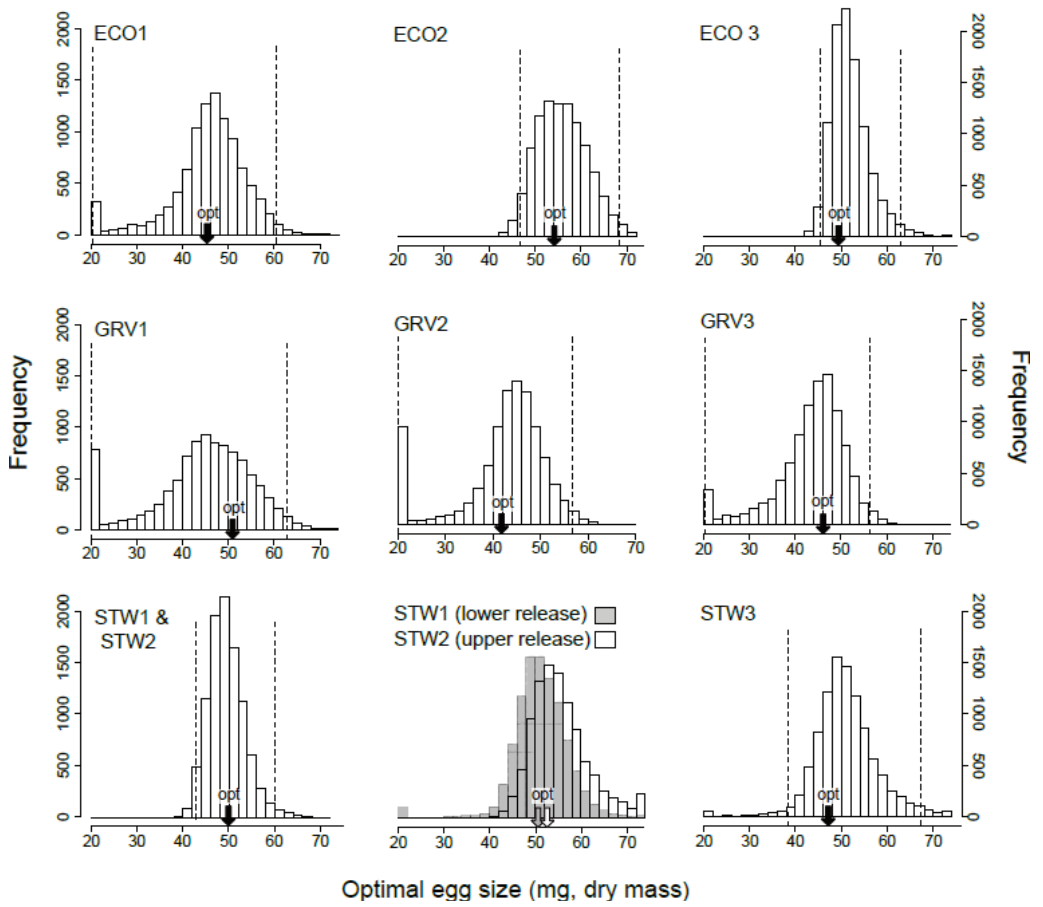

Figure 3.5: Optimal egg size distributions arose from 10,000 simulations incorporating various sources of sampling error. Black arrows are optimal egg size estimates from Weibull-1 functions. Dashed lines are 95% confidence intervals for optimal egg size based on simulations. For simplicity, minimum viable egg size was set at 20.0 mg, just lower than the smallest value of egg size observed in the present study. Bimodal distributions in streams GRV1–3 arose because the original egg-size–recapture relationship was weak, resulting in several simulation runs in which optimal egg size was minimal due to a lack of correlation between egg size and recapture probability.

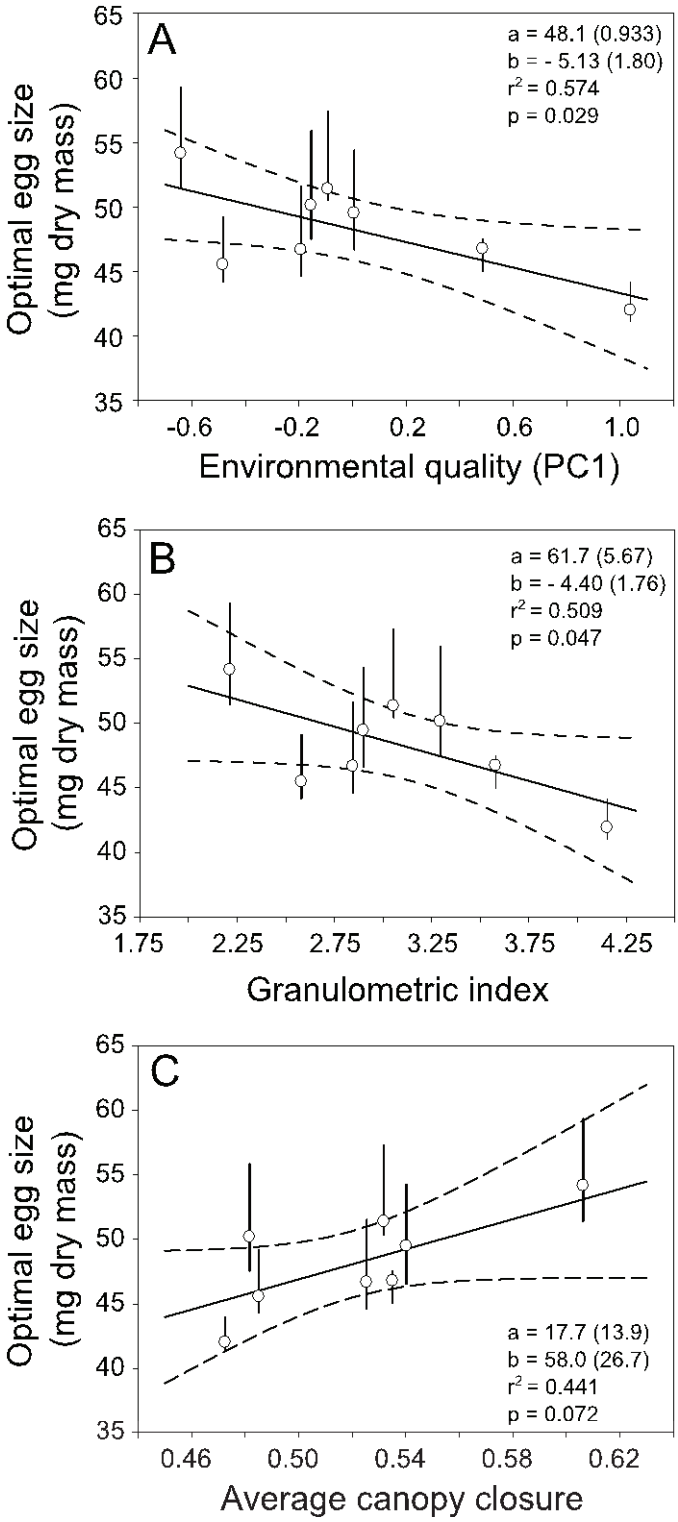

Figure 3.6: Relationships between optimal egg size and environmental measures of 8 streams. Note that 95% confidence intervals placed on optimal egg size estimates are based on Weibull-1 models and are conservative.

Chapter 4: Why Does Egg Size Increase with Maternal Size? Testing an Extension of Smith and Fretwell's Classic Model

Published as "Rollinson, N., and J. A. Hutchings. 2010. Why does egg size increase with maternal size? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (*Salmo salar*). Evolutionary Ecology Research 12:949-960."

4.1 Abstract

Some extensions of Smith and Fretwell's classic model predict that larger mothers can increase reproductive success by increasing investment per offspring, but only if increases in maternal size have a predictable and negative influence on the offspring environment. These extensions of Smith and Fretwell's model are based on the premise that fecundity also increases with maternal body size, such that if egg, larval or juvenile dispersal is limited, offspring developing at higher densities (e.g., in larger clutches of eggs) experience more stressful conditions during development. A common observation is indeed that investment per offspring increases with maternal size, so this pattern may be a form of maternal compensation for the negative effect of fecundity on environmental quality. We tested this hypothesis using Atlantic salmon eggs and larvae. We expected that offspring developing in larger clutches would be relatively prone to mutual physical disturbance, and that these larvae would expend more energy while seeking or creating oxygenated areas within the nest. Therefore, offspring in larger clutches would use more energy during embryonic or larval development, resulting in a smaller subsequent juvenile size. At the eyed-egg stage, we buried 12 full-sib families of Atlantic salmon (*Salmo salar*) in 24 gravel egg pockets at high density (200 eggs per egg pocket, or 1.26 eggs/cm^3, n = 12) and low density (25 eggs per egg pocket, or 0.318 eggs/cm^3, n = 12) and monitored subsequent juvenile size, survival, time of emergence, and developmental stage at emergence. Using this paired design, we find no evidence that density *per se* affects offspring phenotypes. However, we find that offspring from larger eggs emerge later and at an earlier developmental stage than offspring from smaller eggs. Later emergence, as well as emergence at an earlier developmental stage, may decrease survival prospects of juveniles, and given that this is relatively prone to occur when investment per offspring is greater, the present study suggests that there can be unexpected disadvantages associated with increasing investment per offspring. In sum, we do not provide evidence that positive intraspecific correlations between egg size and maternal size represents a form of maternal compensation for sibling competition, but we provide new evidence that bigger might not always be better.

4.2 Introduction

In a given environment, there is a single level of per-offspring investment that will maximise maternal fitness (Smith and Fretwell 1974). Pronounced within-population variation in egg size is observed in many taxa, however, and much of this variation correlates positively with maternal body size (Roff 1992; Hendry et al. 2001). Although this pattern may reflect physiological or morphological constraints on egg size imposed by maternal size (Congdon and Gibbons 1987; Sakai and Harada 2001), it may also arise because the mother's phenotype predicts the rearing environment of her offspring (Hendry et al. 2001; Hendry and Day 2003). For example, fecundity increases with maternal size (Roff 1992), so relatively intense competition for resources might be expected among offspring from larger mothers, at least when offspring dispersal is limited (Kivelä and Välimäki 2008). In highly competitive environments, both maternal and offspring fitness increase with per-offspring investment (Hutchings 1991; Einum and Fleming 1999), so larger eggs may be favoured when juvenile density is high. Many theoretical studies have extended Smith and Fretwell's classic model to incorporate an influence of sibling competition on the offspring size – fitness curve, and these models generally predict that investment per offspring should increase with maternal resource level or maternal size (insects: Parker and Begon 1986; reptiles: McGinley 1989; plants: Venable 1992; salmonid fish: Hendry et al. 2001; Einum et al. 2002; Hendry and Day 2003).

The model developed by Hendry and Day (2003; also see Hendry et al. 2001) was developed with a special emphasis on salmonids, and it predicts that larger females should produce larger eggs only if larger females decrease the quality of the offspring incubation environment. Indeed, both egg size and fecundity of salmonid fishes correlate positively with maternal size, so this group provides a good opportunity to explore the general idea that underlies the aforementioned extensions of Smith and Fretwell's model. Prior to spawning, salmonids migrate to their natal river, and mothers bury their eggs underneath the gravel in a series of egg pockets (collectively called a "redd"). Both the number of egg pockets and the number of eggs per pocket increases with maternal fecundity, such that large eggs tend to occur in higher densities than small eggs (Fleming 1998; Einum et al. 2002). After the eggs hatch, the larvae (or "alevins") remain beneath

the gravel in the vicinity of the egg pocket to absorb their yolk sac, which takes approximately 40-90 days (Quinn 2005). The larvae then navigate up through the small pore spaces and emerge as juveniles (or "fry").

If the egg size – maternal size correlation represents a form of maternal compensation for sibling competition, then the effect of sibling competition on juvenile performance is predicted to occur in the egg pocket (Hendry et al. 2001; Einum et al. 2002; Hendry and Day 2003) or during the juvenile stage after offspring have emerged from the egg pocket (McGinley 1989; Einum and Fleming 2002; Einum et al. 2008). In the present study, we restrict our focus to the larval stage in Atlantic salmon, which occurs between hatching and emergence from the egg pocket. Recent evidence suggests a negative correlation between larval rearing density and yolk conversion efficiency on artificial substrates (Houde et al. 2011a), such that larvae developing in the presence of relatively more siblings lose relatively more energy during development. This likely reflects mutual physical disturbance, whereby larvae stocked at high densities interact more frequently with their siblings and accrue higher metabolic costs during development. In semi-natural environments, sub-gravel movement of larvae also increases with increasing larval density, both as a direct consequence of mutual physical disturbance and as a behavioural response to high CO_2 levels ("ventilation swimming") which increases interstitial flow in high-density environments (Bams 1969). Hence, part of the reason large salmonids lay large eggs may reflect compensation for the negative effects of increased sub-gravel larval density on energy use during development.

Here we manipulate larval size and larval density of Atlantic salmon (*Salmo salar*) to test this primary hypothesis. If density affects larval development by increasing larval movement and metabolic demand, then juveniles emerging from low-density treatments will weigh more (dry weight) than juveniles emerging from high densities. We also test an auxiliary hypothesis: if larger eggs are less sensitive to differences in environmental quality (Hutchings 1991; Einum and Fleming 1999) then body size of juveniles hatching from smaller eggs will be negatively affected by rearing density, but body size of juveniles hatching from large eggs will not. We note that dry body mass of larvae/juveniles is highly correlated with total energy content of juveniles ($r^2 > 0.99$, n = 48; see Chapter 5), such that the use of juvenile dry mass as a proxy for juvenile energy

content is justified. Finally, in the present study, we also explore relationships between emergence time, emergence success, rearing density and egg size.

4.3 Methods

Atlantic salmon were reared to maturity in the Aquatron facility at Dalhousie University, Nova Scotia, Canada. Twelve females and 11 males were stripped of their eggs or milt between December 7th and 9th, 2009, and female fork length was measured to the nearest cm. A sample of twenty-five unfertilized eggs was collected from each female. These eggs were frozen at -4°C and subsequently weighed to the nearest mg. Each clutch was then fertilized with milt from one male (1♂:1♀ mating), but due to a shortage of males, one particular male was used to fertilize the eggs of two different females. After fertilization, eggs were divided equally among two perforated plastic containers (10 × 12 cm or 10 × 22cm) which were placed in one of eight 70 cm circular flow tanks, with the exception of one female (see below). Up to eight families were kept in a given flow tank, and water in all tanks was maintained at ambient temperature and was continuously aerated, using air stones. Dead eggs were removed from their containers every 3 to 4 days until the eyed stage. Eggs trays were then briefly shaken ("shocked"), which kills the majority of eggs that failed to develop properly, and all dead eggs were removed. For one female, eggs were divided equally among four plastic containers and incubated separately in four flow tanks, two of which were not occupied by other eggs used in this study. Water in these extra two tanks was (Mean ± SD) 0.34 ± 0.035ºC higher than in the eight other tanks, and this temperature difference persisted until March 10th, which resulted in an a 7.5% difference in degree days (Mean ± SD, Hot: 458.9 ± 0.64 ºD, Cold: 424.15 ± 3.18 ºD). Mean temperature for all eggs during the entire incubation period was (Mean ± SD) 6.57 ± 0.018ºC.

We constructed water-tight containers designed to simulate natural egg pockets within salmon redds (Fig. 4.1). Containers were constructed from 15.2 cm diameter PVC piping cut into 30 cm pieces. The base of each container was fixed with a plastic, water-tight cap, but the top was left open. Dechlorinated water was fed into the container through 0.25 cm tubing fixed in a small hole that was drilled 2cm above base of each container. Water flowed upward through the container, then out the top of the container

through a 0.75 cm tube fixed to the lip. Emergence traps were created by cutting 1cm-diameter tubing into 0.75 cm sections then fixing those sections into similar-sized perforations in transparent, circular, 15 cm × 5 cm plastic trays. The trays were then secured in the top of each container. Gravel was purchased from Conrad Bros Ltd. (Dartmouth, Nova Scotia) and sieved into three size classes (Mean ± SD): 1.54 ± 0.19 cm, 2.12 ± 0.27 cm and 2.82 ± 0.34 cm. These classes were then mixed in a 3:3:2 ratio resulting in a mean gravel diameter of approximately 2.01 cm, which is a spawning gravel size typical for Atlantic salmon (Louhi et al. 2008).

Eyed eggs were transferred to the experimental replicates on March 9th and 10th, 2010. For each of the 12 females, eggs were taken from all relevant incubation trays and pooled. To reduce the subsequent within-replicate variation in juvenile size, the very largest and very smallest eggs were removed (by eye) and discarded. A subsample of 28-32 eggs from each female was then weighed to the nearest mg to obtain an estimate of egg wet mass, and unless otherwise noted, these values of egg wet mass were used in subsequent analyses. For each mother, twenty-five eggs were placed in one replicate (low density) and 200 were placed in the next replicate (high density). For the female with four incubation trays, 11 of the 25 eggs allocated to the low-density treatment were from the warmer two tanks, as were 87 of the 200 eggs allocated to the high-density treatment, such that an equal ratio of warm to cold eggs occurred in both treatments. A 1-cm layer of fine gravel (4 – 6 mm diameter) was placed on the bottom of each replicate, followed by a 1-cm layer of gravel mixture. Eggs were then carefully placed in the centre of each replicate on the gravel mixture. Eggs were then covered with 23 cm of gravel, and the replicates of each female were subsequently place side-by-side on steel racks to complete development. Flow rate was maintained at 3 mls sec^{-1}. Based on the estimated volume of gravel placed in each replicate, as well as the known volume of experimental replicates, this flow rate corresponds to an interstitial velocity of ~0.04 cm sec sec^{-1}, which is typical of salmon redds (Lapointe et al. 2004; Zimmermann and Lapointe 2005). Flow was monitored and adjusted every two days, temperature was recorded daily, and a natural photoperiod was initiated after eggs were buried in gravel.

Based on our observations of egg dispersion after placement, we estimate that actual volume of our artificial egg pockets (the area in which eggs settled in each

replicate) was 157 cm^3 for high-density treatments, such that egg density in high-density treatments was roughly 1.27 eggs/cm^3. Horizontal dispersion of eggs was similar between high and low densities, but there was no vertical stratification of eggs at low densities. Hence, we estimate that the volume of low-density egg pockets was roughly 78.5 cm^3, or 0.318 eggs/cm^3. After hatching, when larvae were capable of distributing themselves evenly throughout the entire column of gravel, the minimum density of larvae before the beginning of emergence was 0.15 larvae/cm^3 and 0.018 larvae/cm^3 for high- and low-density treatments, respectively. These values are likely to be underestimates, given that we would expect a more clumped larval distribution after hatching. (Note that values are corrected for the volume of gravel, whereby only 1370 ml of water was available to the larvae in each replicate.)

Juveniles began emerging from the gravel on April 22nd, 2010. When juveniles were detected in an emergence trap, they were captured with a turkey baster, over-anaesthetized in 0.1mg/L Tricaine methanesulfonate, rinsed in fresh water, blotted dry, placed in labelled clear plastic bags, and frozen at -4ºC. Between May 23rd and June 10th, 2010, juveniles were dried at 55 ºC in a drying oven and weighed to the nearest 0.001g. When a visible yolk sac was observed on juveniles, it was removed (while the juveniles remained frozen), dried, and weighed separately. All samples were dried on parchment paper.

Paired samples t-tests were used to test for density-related effects, where samples were paired by female of origin. Paired differences were obtained by subtracting values of low-density treatments from values of high-density treatments. We tested the shape of the relationship between density-related effects and egg wet mass by regressing paired differences against egg wet mass, using least squares regression. Proportional data were logit-transformed before analysis, and all reported values are mean ± SE, unless otherwise noted. Finally, when a paired t-test revealed no difference between treatments for a given juvenile trait, we took the average trait value to obtain one data point per replicate pair (i.e., one data point per female). However, given that the means generated in our high density treatments were a more reliable estimate of the true mean (because *n* was much higher), we calculated weighted means across high and low density treatment according to the formula,

(Equation 4.1) $$W\bar{x} = \frac{((HD\bar{x} \times HDn) + (LD\bar{x} \times LDn))}{(HDn + LDn)}$$

Where $W\bar{x}$ is the weighted mean, HD$\bar{x}$ and LD$\bar{x}$ are the means of the high density and low density treatment, respectively, and HD*n* and LD*n* are the number of juveniles contributing to the HD$\bar{x}$ and LD$\bar{x}$ means, respectively. Weighted means were then used in regression analyses.

4.4 Results

Mean EWM of 25 unfertilized eggs, which were collected immediately after spawning (i.e., well before the eyed-egg stage), ranged from 111.7 mg – 163.7 mg. As expected, we observed a positive correlation between the length of females spawned in the present study and the mean size of eggs they produced (Y = 1.78×(fork length, cm) + 44.3, r^2 = 0.348, n = 12, p = 0.044, linear regression).

When eggs were allocated to experimental replicates (i.e., at the eyed-egg stage of development), mean EWM of the 12 females ranged from 114.2 ± 1.6 to 184.2 ± 1.5 mg. Emergence occurred between April 22nd and June 8th, 2010. Median day of emergence (expressed as the number of days after the first juvenile in the experiment emerged from the gravel) did not differ between high density treatments (HD: 14.29 ± 0.88 days) and low density treatments (LD: 14.14 ± 0.78 days) (t = 0.33, n = 12, p = 0.75, paired t-test). Weighted median day of emergence increased with initial egg size (Y = 0.100×(EWM) – 1.77, r^2 = 0.393, n = 11, p = 0.039, linear regression, Fig. 2*A*), but only when the female whose eggs were incubated at slightly warmer temperatures (see Methods) was not included in this analysis (Fig. 2*A*). We note that the regression is not significant when this data point is included (Y = 2.99 + 0.0719×(EWM), r^2 = 0.306, n = 12, p = 0.062, linear regression). Mean dry mass of juveniles at emergence ranged from 22.8 – 44.0 mg and did not differ between high-density treatments (HD: 36.82 ± 1.93 mg) and low-density treatments (LD: 36.70 ± 1.88 mg) (t = 0.65, n = 12, p = 0.53, paired t-test). Paired differences between HD and LD treatments in mean dry mass of juveniles did not

correlate with original egg size (r^2 = 0.070, p = 0.41, linear regression), indicating that offspring of all sizes were equally unaffected by density. No difference in survival was detected between high-density treatments (HD: 90.96 ± 2.05 %) and low-density treatments (LD: 92.67 ± 2.02 %) (t = 0.34, n = 12, p = 0.74, paired t-test).

Dry yolk weight expressed as a percentage of dry juvenile body mass did not differ between high-density treatments (HD: 1.91 ± 0.54 %) and low-density treatments (LD: 1.82 ± 0.49 %) (t = 0.26, n = 12, p = 0.80, paired t-test). The average dry yolk weight across all treatments was low (< 2% dry body mass), but this is only because a substantial proportion of juveniles did not emerge with any residual yolk. In fact, mean (± SD) dry yolk weight expressed as a percentage of juvenile body weight was 17.9 ± 0.0886 % when only considering individuals that actually emerged with residual yolk.

Dry yolk weight increased with egg mass (Logit(Y) = 0.0346×(EWM) – 9.86, r^2 = 0.512, n = 12, p = 0.009, linear regression). The percentage of individuals emerging with a visible yolk did not differ among high-density treatments (HD: 10.12 ± 2.72 %) and low-density treatments (LD: 9.57 ± 2.11 %) (t = -0.13, n = 12, p = 0.90, paired t-test), but weighted mean proportion of individuals emerging from replicates with visible yolk correlated positively with egg size (Logit(Y) = 0.0409×(EWM) – 9.07, r^2 = 0.588, n = 12, p = 0.004, linear regression, Fig. 2*B*).

4.5 Discussion

In the present study, we tested an extension of Smith and Fretwell's classic model by examining the effect of egg density on offspring survival and weight in Atlantic salmon (Hendry and Day 2003). We did not observed any significant effect of density on juvenile traits, and eggs of all sizes appeared to be equally unaffected by density. Our data do not support the hypothesis that an increase in egg or larval density in the egg pocket results in an increase in juvenile mortality or energy use, such that the positive correlation between egg size and female body size in Atlantic salmon is likely unrelated to sub-gravel egg or larval density *per se*. However, the maternal phenotype might influence the offspring environment in many different ways, such that the present study should only be interpreted as evidence that larval density probably does not influence the quality of the offspring environment in Atlantic salmon.

We observed that juveniles from small eggs tended to emerge earlier than did juveniles from large eggs, and that offspring from large eggs were more likely to emerge with a residual yolk sac (which indicates that development was not complete). Our data therefore provide two potential mechanisms by which larger offspring might exhibit disadvantages that are correlated with their size in some environments. These potential mechanisms warrant further discussion, given that the observed pattern may affect how offspring size scales with offspring fitness in some environments, and the specific mechanisms identified herein might be important in shaping offspring provisioning strategies in salmon.

A positive correlation between egg size and development time has been documented in diverse taxa (Morgulis 1909; Gillooly et al. 2002; Teletchea et al. 2009) and this pattern has been ascribed to the allometric effect of size on metabolic energy allocation at the cellular level (West et al. 1997, 2001; Gillooly et al. 2002). Therefore, in many species there exists an inherent trade off between investment per offspring and development time. This is extremely interesting, given that there tends to be positive directional selection on body size and negative directional selection on phenological timing in many taxa (Kingsolver and Diamond 2011). Of course, the ecological relevance of these opposing selection pressures clearly depends on the magnitude of the effect of egg size on development time, and the relative benefits of a larger body size and earlier emergence.

Intraspecific differences in incubation time as a function of egg size are not trivial in some instances: for example, Rombough (1985) predicted that eggs of Chinook salmon (*Oncorhynchus tshawytscha*) weighing 200 mg can complete development 14 days sooner than eggs weighing 500 mg at 10°C, and 31 days sooner at 5°C. In the present study, the predicted difference in emergence time between the largest and smallest eggs was about 7.0 days (Fig. 2*A*). This value loosely corresponds to the value of 4.9 days predicted by substituting our egg size data into the equation for embryonic development of freshwater fish ($\log_{10}$ development time (days) = 3.002 + 0.599($\log_{10}$ Egg Diameter (mm)) – 1.91($\log_{10}$ Incubation Temperature °C + 2), r^2 = 0.92) (Teletchea et al. 2009). The maximum predicted difference in emergence time observed herein (roughly 5 – 7 days), which is based solely on egg size, might be large enough to be relevant in terms of

offspring survival. For example, Einum and Fleming (2000a) measured selection gradients on body size at emergence and on emergence date for juvenile Atlantic salmon, and they found that the strength of selection on both traits was similar but in opposing directions: a 1 SD delay in emergence timing (about 10 days in their study, their Fig. 5) resulted in a 39% (± 0.138 %) decrease in survival, whereas a 1 SD increase in body length at emergence resulted in a 24.9% (± 0.131%) increase in survival.

There are also reasons to expect that emergence time may affect survival of juvenile Atlantic salmon. Earlier emergence may increase the time available for growth (Cutts et al. 1999; Einum and Fleming 2000a), and it may result in a better selection of territories (Fausch 1984; Hughes 1992; but see Cutts et al. 1999). Moreover, prior access to feeding territories which confers an advantage in territorial disputes (Mason and Chapman 1965; Cutts et al. 1999). On the other hand, the potential advantage of earlier emergence may be offset by increased predation (Brännäs, 1995) or lower environmental quality (Crecco and Savoy 1985; Bailey and Kinnison 2010). There would appear to be enough evidence to warrant further investigation into the fitness trade-off between investment per offspring and development time in Atlantic salmon; indeed, Kingsolver and Diamond (2011) point out that there is no study which jointly and explicitly evaluates the fitness trade off between body size and development time.

Larvae hatching from larger eggs are bigger (Einum and Fleming 1999), they have more energy (Kamler 2006), and they take longer to completely absorb their yolk (Killeen et al. 1999). The latter point may help explain why juveniles from small eggs emerge sooner than those from larger eggs, though in the present study, juveniles from larger eggs emerged relatively late and yet were still more likely to emerge with a visible yolk sac, compared to individuals from smaller eggs. In fact, 21 % of juveniles emerging from the nest stocked with the largest eggs had a visible yolk, compared to less than 1% for the nest stocked with the smallest eggs (Fig. 2*B*). Larger juveniles often fare better than small juveniles post-emergence (Hutchings 1991; Einum and Fleming 1999, 2000a; b) but, to our knowledge, only two studies have evaluated the effect of residual yolk on juvenile survival in the wild. Letcher and Terrick (2001) found no evidence that yolk-sac juveniles fared worse in the wild than fully-developed juveniles, though the type and abundance of predators was not studied. On the other hand, Fresh and Schroder (1987)

found that juvenile survival 1 day after release was consistently inversely related to estimates of percent yolk, whereas the effect of mass and length on survival was inconsistent and variable in direction. Electrofishing surveys coupled with an analysis of stomach contents suggested that large rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) were abundant and were preferentially capturing yolk-sac juveniles.

Laboratory studies suggest that the "ontogenetic stage" (*sensu* Hale 1999) does not have an effect on larval swimming performance of many salmonid species, at least when yolk-sac absorbtion is nearly complete (Hale 1999). However, in these laboratory studies, the definition of "ontogenetic stage" is based on ratios of individual larval lengths to population-mean length at yolk-sac absorbtion, such that "ontogenetic stage" is probably does not accurately define the size of a larval yolk sacs (see Hale 1996, 1999). Notwithstanding, "ontogenetic stage" of salmon (*sensu* Hale 1999) correlates positively with juvenile length (Killeen et al. 1999), and maximum swim velocity and distance travelled after a simulated predation attempt increases linearly or asymptotically with juvenile length (Hale 1996, 1999). Thus, these laboratory studies suggest that juveniles emerging before their yolk sac is fully absorbed might not be performing to their full potential in the short term. But this does not necessarily mean that large juveniles with remaining yolk sacs will perform worse than smaller, well-developed juveniles. In sum, there is mixed field-based evidence that the presence of a yolk sac increases predation risk (Fresh and Schroder 1987; but see Letcher and Terrick 2001), and laboratory-based studies provide limited evidence that the presence of a yolk sac *per se* does not affect larval swimming performance. The relative importance of size at emergence and developmental stage on predator avoidance is clearly not understood, but at best, the evidence seems to suggests that yolk sac juveniles might incur a modest increase in predation risk.

Finally, we note that range of clutch biomasses for the HD treatments (22.9g – 36.9g) that were used in the present study was at the lower end of what would be expected in the field. Biomass per egg pocket increases in terms of female size according to the function 13.17 + 0.01(Female Body Mass, g) (I.A. Fleming, unpublished data, cited in Einum et al. (2002)). Given that the range of female sizes used in the present

study was 1240 – 3310g (pre-oviposition), a clutch biomass of roughly 25.6 – 46.3g per egg pocket may have more closely emulated wild conditions for HD treatments.

The present study does not provide evidence that sub-gravel egg or larval density affects the phenotypes of emergent juveniles. Accordingly, we do not provide any evidence that the positive correlation between egg size and female body size is related to sub-gravel density *per se*. Our data do, however, reveal potential reasons why large eggs may be disadvantageous in some environments. Future studies should investigate further the relationships between percent yolk at emergence, emergence time, and fitness of juveniles to evaluate the relative benefits associated with different maternal provisioning strategies.

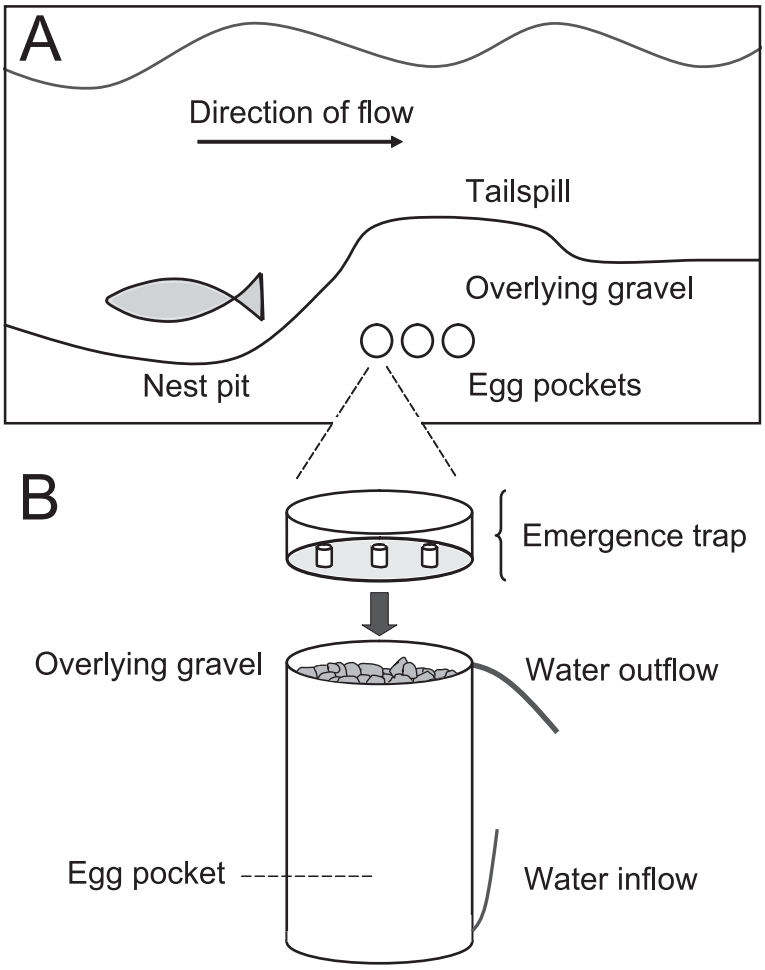

Figure 4.1: (*A*) Salmon deposit their eggs in a series of egg pockets within their "redd" (nest) in riverine gravels. Facing upstream, the mother digs a depression (nest pit) into which she spawns a batch of eggs. While using her tail to cover this batch of eggs with gravel, she creates a new depression immediately upstream from the former depression, into which she spawns another batch of eggs (the nest pit becomes an egg pocket, covered with the tailspill). This process is repeated until all her eggs are spawned. She then covers her last batch of eggs, which leaves the nest as depicted. (*B*) A representation of an artificial egg pocket (nest replicate) used in the present study

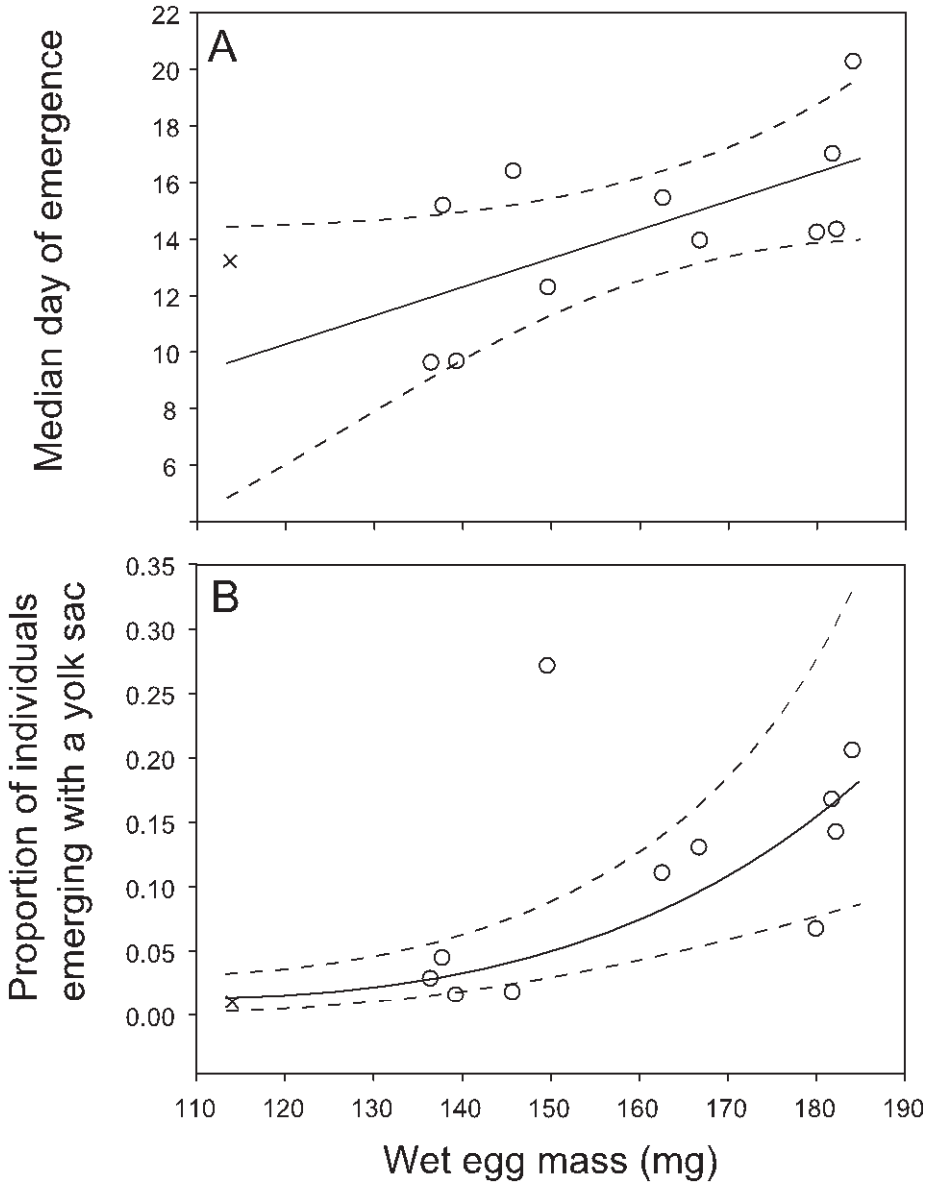

Figure 4.2: (*A*) Weighted median day of emergence plotted against egg wet mass (Y = 0.100×(EWM) – 1.77, r^2 = 0.393, n = 11, p = 0.039) with 95% confidence intervals. "X" is a data point omitted in this regression (see text). (*B*) Egg wet mass plotted against weighted mean proportion of juveniles emerging from the nest environment with a visible yolk sac (Logit(Y) = 0.0409×(EWM) – 9.07, r^2 = 0.588, n = 12, p = 0.004); the regression is presented using all available data, and this regression remains significant while omitting the "X" data point (r^2 = 0.391, n = 11, p = 0.024).

Chapter 5: Body Size-Specific Maternal Effects on the Offspring Environment Shape Juvenile Phenotypes in Atlantic Salmon

Published as: "Rollinson, N. and Hutchings, J. A. 2011. Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon. Oecologia. 166: 889-898."

5.1 Abstract

Positive associations between maternal investment per offspring and maternal body size have been explained as adaptive responses by females to predictable, body size-specific maternal influences on the offspring's environment. As a larger per-offspring investment increases maternal fitness when the quality of the offspring environment is low, optimal egg size may increase with maternal body size if larger mothers create relatively poor environments for their eggs or offspring. Here, we manipulate egg size and rearing environments (gravel size, nest depth) of Atlantic salmon (*Salmo salar*) in a 2 × 2 × 2 factorial experiment. We find that the incubation environment typical of large and small mothers can exert predictable effects on offspring phenotypes, but the nature of these effects provides little support to the prediction that smaller eggs are better-suited to nest environments created by smaller females (and vice versa). Our data indicate that the magnitude and direction of phenotypic differences between small and large offspring vary among maternal nest environments, underscoring the point that removal of offspring from the environmental context in which they are provisioned in the wild can bias experimentally derived associations between offspring size and metrics of offspring fitness. The present study also contributes to a growing literature which suggests that the fitness consequences of egg size variation are often more pronounced during the early juvenile stage, as opposed to the egg or larval stage.

5.2 Introduction

Classic egg size theory predicts that, in a given environment, females should divide the energy available for reproduction into offspring of an optimal size (Smith and Fretwell 1974). Population mean egg size may indeed evolve according to the value predicted under the Smith-Fretwell model (reptiles: Sinervo et al. 1992; insects: Carrière and Roff 1995; fish: Einum and Fleming 2000b); however, pronounced within-population variation in egg size is common, and it is difficult to explain under the classic model (McGinley et al. 1987; Bernardo 1996). Although this variation can take many forms, a common observation is that egg size increases with maternal body size (reptiles:

Congdon and Gibbons 1987; plants: Sakai and Harada 2001; fish: Hendry and Day 2003; wasps: Lalonde 2005; crustaceans: Sato and Suzuki 2010).

Concomitantly, larger offspring generally fare better than smaller offspring, especially when environmental quality is poor (Hutchings 1991; Fox et al. 1997; Einum and Fleming 1999). This has led to the supposition that larger mothers are generally more fit than smaller mothers because the former produce eggs of "higher quality" (Marshall et al. 2010). This supposition can sometimes be attributed to an inappropriate focus on offspring fitness, rather than maternal fitness (Marshall and Uller 2007). More importantly, however, this supposition is largely based on studies that measure offspring traits in a common environment after removing them from the environmental context in which they were provisioned (Marshall et al. 2010). It is now recognized that maternal effects on the offspring environment, such as nest site choice or fecundity, can affect offspring phenotypes (Resetarits 1996; Beckerman et al. 2006; Plaistow et al. 2007; Brown and Shine 2009). For example, size-related increases in reproductive effort and fecundity may indeed increase the reproductive success of relatively large mothers, but these larger more fecund mothers may concomitantly reduce the quality of their offspring's environment by increasing sib competition. Hence, when egg or juvenile dispersal is limited, larger mothers may increase per-offspring investment to offset the negative effects of sib competition, whereas no such compensation would be necessary for smaller, less fecund mothers (Parker and Begon 1986; Einum et al. 2002; Hendry and Day 2003). Models of egg-size evolution that incorporate predictable effects of the maternal phenotype (especially body size) on the offspring environment have existed for a quarter century (Parker and Begon 1986; Hendry et al. 2001; Hendry and Day 2003; Kindsvater et al. 2010) and they provide one possible framework in which the egg size – maternal size correlation can be understood.

Atlantic salmon (*Salmo salar*) provide a unique opportunity to test these models, as this species exhibits a positive egg size – maternal size correlation (Hendry et al. 2001), and they display a suite of characteristics that might promote a correspondence of female body size and optimal egg size: they are highly fecund, they provide no post-oviposition parental care, maternal size varies widely within populations, and they and bury their eggs in individual nesting locations within riverine gravels (Fleming 1996).

Moreover, the physical characteristics of the egg and larval environment should vary predictably with maternal size. For example, a positive correspondence is observed between maternal size and the size of gravels in which she spawns her eggs (Kondolf and Wolman 1993; Kondolf 2000). This may reflect a constraint on nest site selection imposed by maternal body size, whereby only larger, stronger females are able to move larger, heavier gravels with relative ease. Interestingly, small eggs and larvae may actually be better suited to smaller gravels. This is because larger offspring may have difficulty emerging through the small pore spaces typical of smaller gravels (Quinn et al. 1995). Therefore, part of the reason small females produce small eggs may relate to constraints on nest-site selection, whereby the only nesting substrates available to small females are those in which large eggs and large larvae are less likely to survive.

The physical characteristics of the egg and larval environment also vary with the maternal phenotype by virtue of a positive correlation between maternal size and egg burial depth ("nest depth"), which is observed both within populations and across salmonid species (Crisp and Carling 1989; Steen and Quinn 1999). Whereas smaller females may simply lack the size and strength to dig deep nests, larger females may dig relatively deep nests to reduce the chance of subsequent nest destruction by mothers digging at the same site ("nest superimposition"), or by shifting surface gravels ("streambed scour") during the prolonged egg incubation period (Hayes 1987; Van Den Berghe and Gross 1989; Montgomery et al. 1996; Fukushima et al. 1998; Rennie and Millar 2000; Mogensen and Hutchings 2012). However, an increase in nest depth may give rise to further, predictable differences in the physical environment, such as warmer incubation temperatures (Hannah et al. 2004; Anibas et al. 2009) and lower dissolved oxygen levels (Ingendahl 2001; Malcolm et al. 2004; but see Peterson and Quinn 1996), both of which may favour increased per-offspring investment (Beacham and Murray 1985, 1990; Fleming and Gross 1990; Einum et al. 2002). In other words, larger females may increase nest depth to decrease the chance of total reproductive failure, but in so doing, these larger females may simultaneously decrease the quality of the offspring's physical environment.

In the present study, we test whether predictable, body size-specific maternal influences on the offspring's physical environment cause variation in offspring

phenotypes. Specifically, we test whether an increase in nest depth *per se* increases the amount of energy offspring require to successfully emerge from the egg pocket. We also test whether smaller eggs are better suited to smaller incubation gravels by virtue of a size-specific ability of small juveniles to navigate the relatively small pore spaces between small gravels (Quinn et al. 1995). The experiment is a 2 × 2 × 2 factorial design with two levels of gravel size, nest depth and egg size. Broadly, we expect a positive relationship between the energy offspring use for emergence and nest depth, and a negative relationship between the energy offspring use for emergence and gravel size. Moreover, if large eggs fare worse in small gravel, then we expect an egg size × gravel size interaction, whereby more energy is expended by large offspring emerging from small gravel, and we expect this effect to be exacerbated by nest depth (egg size × gravel size × nest depth).

5.3 Methods

Eggs for the present study were obtained from a subsample of captive-bred females that were reared to maturity at the Coldbrook Biodiversity facility. The breeding design and spawning methods are described in Chapter 3.

Initial mean egg weight for each female was estimated from a sample of 14 – 25 eggs collected in Coldbrook, prior to egg fertilization. Eggs from three females were chosen for this experiment based on similarity in mean egg weight and above-average coefficient of variation (CV) in egg weight (CV for selected females: 13.4 % – 15.2 %). On March 6th and 7th, 2009, "large" and "small" eggs from each of the three female were separated by eye in all relevant crosses (Einum and Fleming 1999), and 8 – 15 eggs per size class were weighed and then frozen at –4 ºC. Large eggs weighed (mean ± SD) 100.0 ± 5.3 mg, 91.9 ± 4.6 mg and 97.6 ± 4.7 mg for females A, B and C, respectively. Small eggs weighed 71.0 ± 5.5 mg, 67.7 ± 6.2 mg and 71.9 ± 7.2 mg for females A, B and C, respectively. Hence, among females, egg weight was similar for "large" and "small" eggs. Within females, egg weight varied by 27.2 ± 1.5 %, but eggs were genetically similar (siblings or half-sibs from the same mother).

To select appropriate levels of gravel size, we regressed fork length (cm) of all females spawned against mean egg weight (mg) and obtained the equation Length =

0.204×(EWM, mg) + 26.5. We then substituted our values of mean small egg weight (70 mg) and mean large egg weight (97 mg) into this equation to predict the length of a female that typically spawns eggs of these sizes (40.8 cm and 46.3 cm fork length, respectively). Finally, we substituted predicted fork length into the equation which relates median spawning gravel size to female fork length, i.e., Median Gravel Diameter (mm) = 0.4(Fish Length, cm), given by Kondolf and Wolman (1993). This equation is based on a meta-analysis and includes data from 8 species of salmonids. Based on these equations, we expect eggs of 70 mg and 97 mg to be spawned in median gravel sizes of 1.6 cm and 1.9 cm, respectively. Gravel was purchased from Conrad Bros Ltd. (Dartmouth, Nova Scotia), and sieved into two size classes to match the above values. Mean ± SD diameter of 75 randomly selected pebbles was 1.54 ± 0.19 cm (small gravel) and 2.12 ± 0.27 cm (large gravel). An additional size class measuring 2.82 ± 0.34 cm (control gravel) was used for control replicates (see below for rationale). For each size class, mean, median and geometric mean gravel diameter was very similar. Values of nest depth (15 cm and 30 cm) were selected based on the only published account of egg burial depth for salmon in Nova Scotia (White 1942), which falls within the range of burial depths typical of Atlantic salmon (DeVries 1997).

We designed water-tight containers to simulate natural egg pockets within salmon nests (Fig. 4.1). Containers were constructed from 15.2 cm-diameter PVC piping cut into 23 cm pieces (for 15 cm nest depth), 37 cm pieces (30 cm nest depth), or 30 cm pieces (23 cm nest depth, controls). The base of each container was fixed with a plastic, water-tight cap, but the top was left open. Dechlorinated water was fed into the container through 0.25 cm tubing fixed in a small hole that was drilled 2 cm above base of each container. Water flowed upward through the container, then out the top of the container through a 0.75 cm tube fixed to the lip. Flow rate was maintained at 10.3 ± 1.70 ml s^{-1} throughout the course of the experiment, and mean ± SD temperature from the date of allocation to the first day of emergence (7 March 2009 to 12 May 2009) was 5.5 ± 1.8 ºC. Emergence traps were created by cutting 1cm-diameter tubing into 0.75 cm sections then fixing those sections into similar-sized perforations in transparent, circular, 15 cm × 5 cm plastic trays; these removable trays were then placed in the top of each container (Fig. 4.2).

The experiment was a 2 × 2 × 2 factorial design, with two levels of egg size, two levels of gravel size and two levels of nest depth. The dependent variable was always a juvenile trait (e.g., day of emergence, weight at emergence) averaged within each replicate, such that the number of data points and replicates was equivalent. Specifically, we placed small or large eggs from each female (egg size) in small or large gravel (gravel size) and buried the eggs 15 cm or 30 cm below the gravel-water interface (nest depth). Each treatment was replicated twice, resulting in 48 experimental egg pockets in total (3 females × 2 egg sizes × 2 nest depths × 2 gravel sizes × 2 replicates per treatment = 48 replicates). As replicates containing eggs from the same mother were not independent, sib-grouping (or equivalently, female of origin) was a random effect (see below).

Control replicates were intended to represent a benign environment where we could observe any differences between juvenile traits as a result of egg size. A very large gravel size (2.82 ± 0.34 cm) was used so that gravel was indeed present in the offspring environment but did not impede offspring emergence. An intermediate burial depth was chosen (23 cm) under the assumption that burial depth (nest depth) affects the energy offspring use for emergence only when gravel size impedes offspring migration. Hence, controls replicates consisted of small or large eggs buried in control gravel, 23 cm below the gravel-water interface, and each control was replicated twice resulting in 12 controls (3 females × 2 egg sizes × 2 replicates per egg size = 12 replicates). Controls were always analyzed separately from experimental replicates, as control replicates could not be incorporated into the factorial design.

Juveniles began emerging from the gravel on 12 May 2009. When juveniles were detected in an emergence trap, they were captured with a turkey baster, over-anesthetised in 0.1mg L^{-1} tricaine methanesulfonate, rinsed in freshwater, blotted dry and weighed to the nearest 0.1 mg. They were then placed in individually-labelled Epindorph tubes and frozen at –4 °C. All juveniles were subsequently vacuum-dried for 18 hours, using a Labconco® 4.5 L freeze-dryer, then dry weight was measured to the nearest 0.1 mg. To estimate their energy content, a subsample of 4 juveniles from each egg pocket replicate was homogenized and then combusted in a Parr® 1266 semi-micro oxygen bomb calorimeter. Each sample of 4 juveniles was compressed to a 3 mm-diameter pellet, and pellets were combusted using 1 of 2 Parr® 1107 micro-bombs as described by Michaud

and Taggart (2007). Within each replicate, the juveniles that were combusted differed in emergence date by no more than 5 days (mean ± SD: 1.88 ± 1.87 days), and the homogenized sample always consisted of the 2nd, 3rd, 4th and 5th juveniles to emerge. Eight eyed eggs from each female (4 large eggs and 4 small eggs) were also dried and individually combusted to estimate initial energy of large and small eggs (3 females × 2 egg sizes × 4 eggs = 24 samples).

Data were analyzed using the Proc Mixed function in SAS v. 9.1.3. Female of origin was a random effect (with a compound symmetry covariance structure), and all models were fully factorial. Weighted fits were used when cell means were computed from unequal sample sizes, and proportional data were arcsine square-root transformed before analysis. Reported differences are least square mean differences ± SE, unless otherwise noted.

5.4 Results

Juvenile salmon emerged from the gravel between 12 May 2009 and 5 June 2009; temperature during this period was (mean ± SD) 11.1 ± 1.3 ºC. We observed significant main effects of all factors on median day of emergence (Table 5.1). Small gravel delayed emergence by 3.12 ± 0.61 days, juveniles emerged 1.71 ± 0.62 days sooner from shallow nests, and juveniles from small eggs emerged 1.98 ± 0.61 days before those from large eggs ($p \leq 0.014$ in all cases, Table 5.1). Juveniles from small eggs also emerged significantly sooner (2.64 ± 0.67 days) in controls (Table 5.1).

Mean (± SE) mass-specific energy averaged over all eggs allocated to the experiment was 24.52 ± 0.21 kJ g^{-1}. There was no difference in the specific energy of small eggs (mean ± SE, 24.45 ± 0.29 kJ g^{-1}, n = 12) and large eggs (mean ± SE, 24.59 ± 0.33 kJ g^{-1}, n = 12) before they were allocated to the experiment ($F_{1,20} = 0.12$, n = 24 eggs, p = 0.73). Mean (± SE) specific energy of all juveniles was 21.49 ± 0.060 kJ g^{-1} (n = 48 replicates). We observed significant main effects of all factors on specific energy (Table 1). Specific energy decreased by 0.29 ± 0.11 kJ g^{-1} in small gravel, by 0.23 ± 0.11 kJ g^{-1} in deep nests, and by 0.31 ± 0.11 kJ g^{-1} for juveniles from small eggs ($p \leq 0.049$ in all cases, Table 5.1). Mean (± SE) specific energy of juveniles emerging from controls

was 21.03 ± 0.22 kJ g^{-1} (n = 12 replicates), but the difference between small and large juveniles (0.064 ± 0.47 kJ g^{-1}) was not significant (Table 5.1).

We estimated mean total energy content of juveniles emerging from each replicate ("total energy"). For each treatment, the average effects of egg size, gravel size and nest depth were added to the mean global specific energy (21.49 kJ g^{-1}, see above) to obtain predicted specific energy, which is treatment-specific. Predicted specific energy was then multiplied by mean dry weight of juveniles for each replicate to obtain total energy. We found that total energy decreased by 0.0289 ± 0.0039 kJ in small gravel ($F_{1,38}$ = 55.1, p < 0.0001). We also observed effects of egg size ($F_{1,38}$ = 1177.5, p <0.0001) and depth ($F_{1,38}$ = 16.8, p = 0.0002), and a depth × egg size interaction ($F_{1,38}$ = 7.1, p = 0.011), whereby there was no consistent effect of depth on small juveniles, though a negative effect of depth was apparent for large juveniles (Fig. 5.1). In controls, total energy differed by 32.2 % between small and large juveniles (small eggs = 0.307 ± 0.007 kJ; large eggs = 0.425 ± 0.007 kJ; $F_{1,8}$ = 323.5, p < 0.0001).

We corrected total energy of juveniles for the estimated energy contained in the eggs prior to allocation to the experiment. First, mean dry weight of each female's eggs (which differs slightly among females, see Methods) was multiplied by a common value of 24.51 kJ g^{-1} to obtain an estimate of initial egg energy content (mean egg energy ± SD: small eggs = 0.557 ± 0.021 kJ, n = 3 females; large eggs = 0.808 ± 0.040 kJ, n = 3 females). Then for each female, total energy of juveniles in each replicate was subtracted from initial egg energy, and this value was divided by initial egg energy. The result is estimated proportion of energy used by offspring from allocation to emergence ("energy used"). In controls, mean ± SE energy used was 44.2 ± 0.8 %, and large juveniles used slightly, but not significantly more energy (2.9 ± 0.1 %) from allocation to emergence ($F_{1,8}$ = 5.1, p = 0.053). Mean ± SE energy used in experimental replicates was 46.7 ± 0.5%. Energy used increased by 4.38 ± 0.62% in small gravel ($F_{1,38}$ = 49.7, p < 0.0001) and in deep nests by 2.32 ± 0.62% ($F_{1,38}$ = 14.0, p = 0.0006, Fig. 5.2). The depth × egg size interaction was not significant ($F_{1,38}$ = 3.5, p = 0.069), but the direction of this relationship indicated that small juveniles fared worse in shallow nests and small gravel (i.e., their 'home' environment).

Finally, the percentage difference (% difference = $[x_1 - x_2]/[\{x_1 + x_2\}/2]$) in energy between large eggs and small eggs allocated to the experiment was 36.8 % (small eggs = 0.557 ± 0.021 kJ, n = 3 females; large eggs = 0.808 ± 0.040 kJ, n = 3 females, see above). For each nest environment, we calculated how this initial size-related difference in egg energy compared with size-related differences in juvenile energy at the end of the experiment. First, we eliminated multiple observations from the same females by averaging total juvenile energy within females for each treatment, such that we obtained three values of total energy for each treatment. Pairwise differences in total energy between juveniles from large eggs and juveniles from small eggs were then calculated for each environment, where values for large and small juveniles were paired by female of origin. These independent estimates (n = 3 per environment) were then averaged across all three females to obtain one pairwise difference per environment. Differences in total energy between juveniles from large eggs and juveniles from small eggs were then expressed as percentages, such that these percentages could be directly compared to the initial difference of 36.8 % (Table 5.2). We observed that the initial difference of 36.8% was reduced to a minimum difference of (mean ± SE) 25.1 ± 2.4% and increased to a maximum difference of 49.8 ± 2.6%, depending on the environments compared. In other words, environmental effects reduced the initial difference in offspring total energy by a maximum of 28.6% (from 37.7% to 25.1%), and increased the difference by a maximum of 30.0% (from 36.8% to 49.8%, Table 5.2).

5.5 Discussion

In the present study, we manipulate offspring rearing environments and egg size simultaneously while controlling for genetic factors so that causative relationships between egg size, juvenile energy use and timing of emergence can be established for multiple environments. Broadly, we observe that the incubation environments typical of large and small salmonid mothers result in phenotypic differences among emergent juveniles. The magnitude of the phenotypic differences between juveniles from large and small eggs depends both on environment and on the phenotypic trait examined.

Significantly more energy is used by juveniles emerging from deep nests, regardless of initial egg size (Fig. 5.2). Although our assessment of environmental quality

is incomplete, insofar as it is based on nest depth *per se*, this result is consistent with the hypothesis that larger mothers provide environments of poor quality for their offspring (Einum et al. 2002; Hendry and Day 2003). However, we caution that the overall effect of nest depth on juvenile energy use is very small (Fig. 5.2), and that uncorrected energy values even intimate that juveniles from large eggs fare relatively poorly in deep nests. Our study should not, therefore, be considered the first experimental evidence of reductions in the quality of the offspring environment with increases in maternal size (Einum et al. 2002; Hendry and Day 2003). Gravel size, on the other hand, has a greater effect on juvenile energy use than nest depth (Fig. 5.2), but we find no evidence the size of incubation gravels exert different effects on offspring of different sizes (i.e., an egg size × gravel size interaction), at least insofar as incubation gravels affect energy use of juveniles. This indicates that small females probably do not 'match' small eggs with the anticipated selective environment (Hendry et al. 2001). We cannot, however, discount the possibility that in the wild, gravel size influences aspects of the physical environment that we do not measure in our study, such as oxygen delivery (e.g., Quinn et al. 1995), which then have disparate effects on eggs or offspring of different sizes (Einum et al. 2002). Notwithstanding, our data suggest that small mothers would fare better if they spawned eggs in the larger gravel sizes that are typical of larger mothers (Kondolf and Wolman 1993), such that the use of small spawning gravels by small mothers is likely related to interference competition for spawning sites with larger gravels (van den Berghe and Gross 1989), or an inability of small mothers to efficiently move larger gravels. More broadly, few experimental studies have addressed whether an increase in egg size with maternal size can be explained as a maternal response to predictable differences in the quality of the offspring environment, and none have lent support to this hypothesis (Wiklund et al. 1987; Lalonde 2005; Chapter 4). Similarly, the present study suggests that that this hypothesis cannot help explain egg-size variation within populations of Atlantic salmon, at least with respect to the factors we tested.

While we provide little evidence in support of the focal hypothesis, our study nonetheless provides valuable insight into the potential effects of nest-site selection and nest construction on offspring phenotypes. Specifically, we find that initial energetic differences between large and small eggs can be reduced or increased, depending on the

environments that are compared (Table 5.2). In other words, relative placement of nests by mothers can increase or reduce initial disparities in total energy contained in the egg. For the sake of comparison, the magnitude of the differences in the present study (up to ± 30% of the original difference, Table 5.2) are comparable to differences in egg size observed among environments when adaptive egg size plasticity has been inferred (range: 13.5 – 30%; reviewed in Fischer et al. 2011), such that the effects observed in the present study might be ecologically important. Similarly, the presence of simple effects in our study (e.g., Fig. 5.2) demonstrates that the maternal nest environment will affect offspring phenotypes in absolute terms. Hence, if one is interested in how per-offspring investment affects offspring performance, then the present study shows that removing offspring from the context in which offspring were provisioned overestimates the predictability of the relationship between offspring size and performance.

Comparing experimental data to control data underscores this point. For example, emergence time is an important fitness metric in salmonids (Einum and Fleming 2000a), and because small embryos complete development faster than large embryos (Gillooly et al. 2002), it is thought that small offspring might gain an advantage over large offspring by virtue of earlier access to feeding territories (see Chapter 4). Indeed, we observe in our controls (presumably a common and relatively benign environment) that larger juveniles emerge 2.64 ± 0.67 days later than smaller juveniles ($F_{1,8}$ = 15.5, p = 0.004, see Results). However, if we compare emergence timing of large juveniles from their 'home' environments (large gravel, deep nests) to that of small juveniles from their 'home' environments (small gravel, shallow nests), we observe a difference of only 0.87 ± 0.84 days. This is a 67 % reduction in the mean difference that is observed between controls, and this difference in emergence time is not significant ($F_{1,8}$ = 1.09, p = 0.33, ANOVA). A similar pattern is observed for specific energy, which varies between egg sizes among environments, but not between egg sizes within controls (Table 1). Hence, future studies should endeavour to estimate ecologically relevant relationships among offspring size and performance by examining the size-performance relationship in the environmental context in which offspring are provisioned. Environmental biases, such as those documented in the present study, can presumably alter the extent to which investment per

offspring predicts offspring performance, such that large environmental effects might reduce the strength of direct selection on investment per offspring.

In sum, our data reveal that the physical environment created by mothers of different sizes can play a role in shaping offspring phenotypes, but we do not provide evidence that eggs of different sizes are better-suited to different types of incubation environments. Therefore, the present study underlines the possibility that the egg size – maternal size correlation is a spandrel (*sensu* Gould and Lewontin 1979) while contributing to a growing literature which suggests that the fitness consequences of egg size variation on offspring performance are more pronounced during the early juvenile stage, as opposed to the egg or larval stage (Einum and Fleming 2002; Beckerman et al. 2006; Rombough 2006; Plaistow et al. 2007).

Table 5.1: Fixed effects ANOVA table for analysis of experimental and control data.

	Median Day of Emergence				Caloric Density			
	Experiment		Controls		Experiment		Controls	
	$F_{1,38}$	p	$F_{1,8}$	p	$F_{1,38}$	P	$F_{1,8}$	p
Egg Size	10.6	0.002	15.5	0.004	7.3	0.010	0.2	0.90
Gravel Size	27.3	<0.001	–	–	6.5	0.015	–	–
Nest Depth	6.7	0.014	–	–	4.1	0.049	–	–
Egg × Gravel	0.3	0.60	–	–	0.03	0.87	–	–
Egg × Depth	0.03	0.86	–	–	1.5	0.23	–	–
Gravel × Depth	0.1	0.74	–	–	0.1	0.75	–	–
Egg × Gravel × Depth	0.2	0.65	–	–	0.2	0.64	–	–

Table 5.2: Pairwise differences (percent ± SE) in total energy of large and small juveniles for each environment.

		Large Eggs			
		15LG	15SG	30LG	30SG
Small Eggs	15LG	40.6 ± 1.4	32.9 ± 2.4	33.5 ± 1.4	25.1 ± 2.4
		(9.9)	(-11.3)	(-9.4)	(-37.7)
	15SG	49.7 ± 0.3	42.0 ± 1.1	42.6 ± 0.2	34.4 ± 3.1
		(29.7)	(13.2)	(14.7)	(-6.7)
	30LG	43.1 ± 0.3	35.3 ± 1.4	35.9 ± 0.5	27.6 ± 3.8
		(15.7)	(-4.2)	(-2.4)	(-28.6)
	30SG	49.8 ± 2.6	42.1 ± 1.9	42.8 ± 2.6	34.5 ± 4.2
		(30.0)	(13.5)	(15.0)	(-6.4)

NOTE: Values in the header of the table indicate nest burial depth (15cm or 30cm) and capital letters denote large gravel (LG) and small gravel (SG); treatments are separated by egg size. Cells represents the percent difference in total energy of large juveniles compared to small juveniles for a given pair of environments (% difference = $[x_1 - x_2]/[\{x_1 + x_2\}/2]$). In other words, 40.6 ± 1.4 indicates that the difference between large and small juveniles in energy at the end of the experiment was 40.6 %, when those two environments are compared. Values in parentheses are the percent change from the original difference of 36.8%. For example, the 40.6% difference in juvenile energy content represents a 9.9% change from the original difference of 36.8%. The pairwise difference between large and small juveniles from controls was 31.9 ± 1.6%, which is a 14.6% reduction of the initial difference.

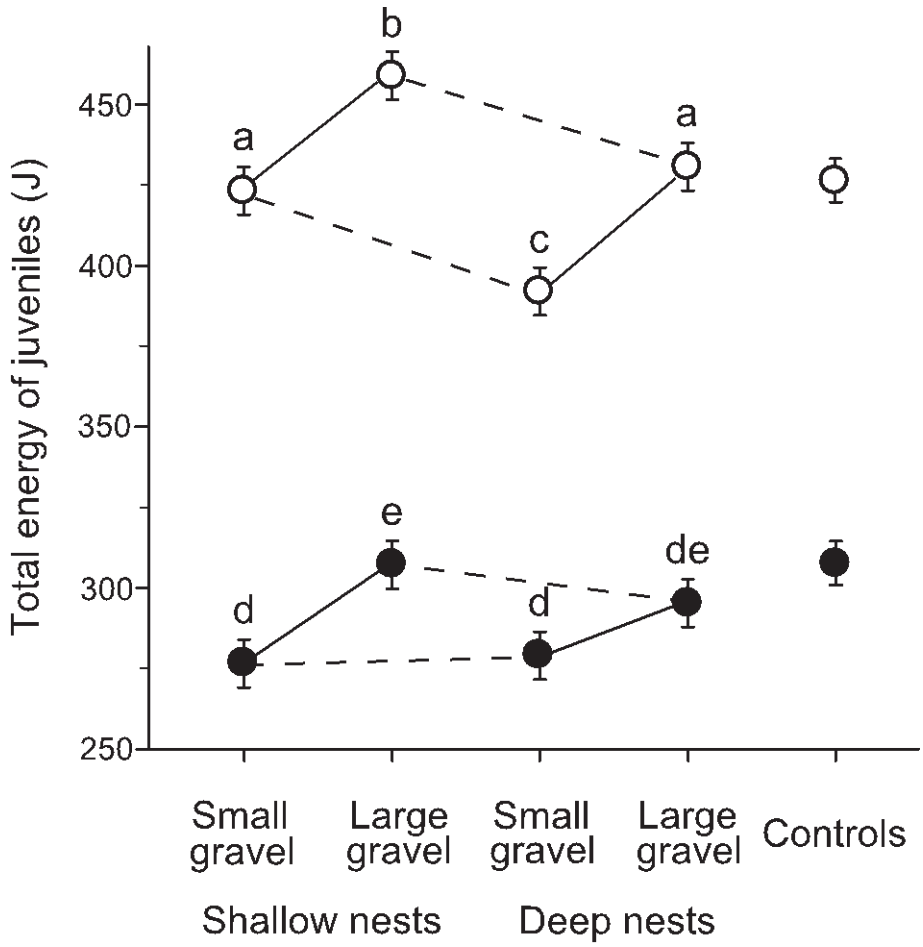

Figure 5.1: Total energy of juvenile salmon (least square mean ± SE) at emergence from shallow or deep nests covered by small or large gravel. Juveniles emerged from large egg (open circles) or small egg treatments (closed circles). Solid lines represent the linear effect of gravel size within each level of depth. Dashed lines are the linear effect of nest depth within each level of gravel size. The asymmetry in the linear effects of depth between small and large eggs is indicative of the egg size × nest depth interaction (see text). Letters denote significant differences ($\alpha = 0.05$), where adjusted p-values for multiple comparisons were computed from the simulated distribution of the maximum absolute value of a multivariate *t* random vector (200,000 simulations).

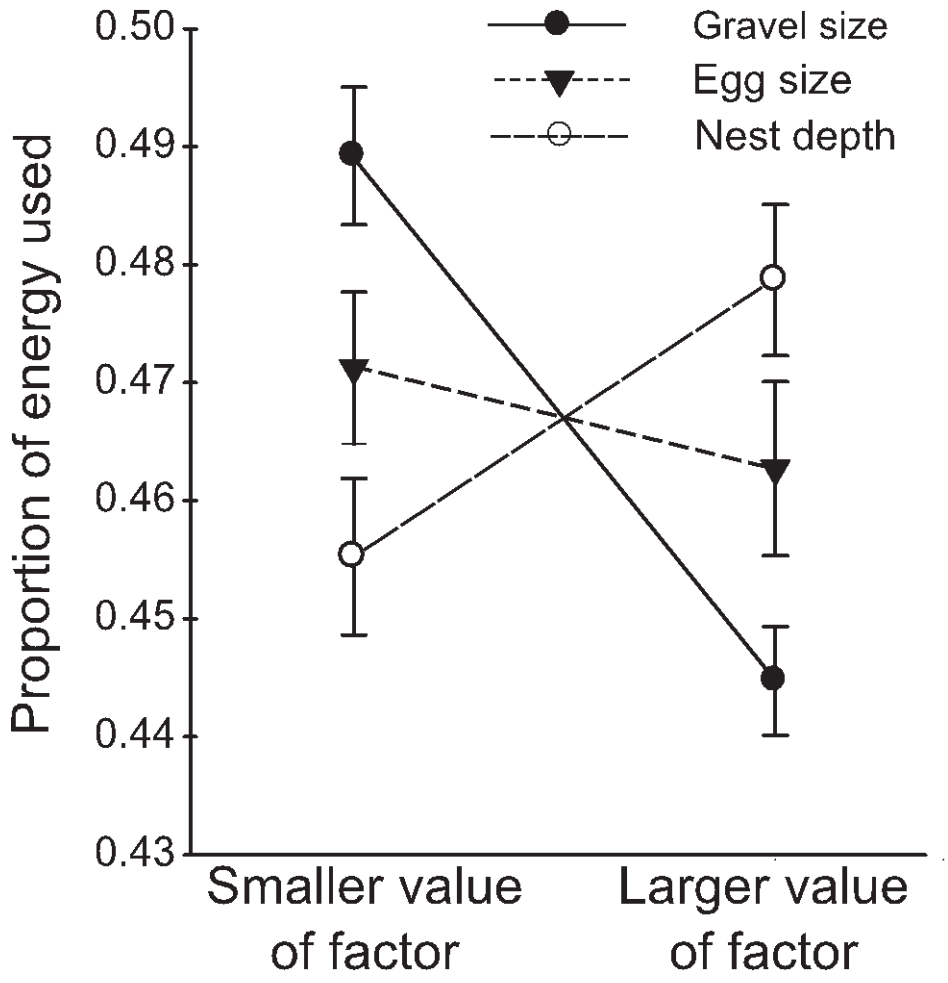

Figure 5.2: Values on the y-axis represent the proportion of total energy that offspring used to develop from the eyed-egg stage to the fry stage and emerge from the egg pocket. To calculate this, the energetic content of juveniles upon emergence was subtracted from the amount of energy contained in the original eggs, then this value was divided by the amount of energy in the original eggs. Drawn are the main effects (mean ± SE) of gravel size ($F_{1,38} = 49.7$, $p < 0.0001$, closed circles), egg size (triangles, NS), and nest depth ($F_{1,38} = 14.0$, $p = 0.0006$, open circles).

Chapter 6: Why Does Egg Size of Salmonids Increase with the Mean Size of Population Spawning Gravels?

Published as: "Rollinson, N. and Hutchings, J. A. 2011. Why does egg size of salmonids increase with the mean size of population spawning gravels? Canadian Journal of Fisheries and Aquatic Sciences 68:1307-1315"

6.1 Abstract

Population mean egg size of salmonids increases with the mean size of gravels in which a population spawns. One popular explanation for this pattern is that large larvae cannot navigate small gravel interstices, so mothers must decrease per-offspring investment when spawning gravels are small. We manipulated the size of incubation gravels and egg size of Atlantic salmon (*Salmo salar*) to test whether size-related entombment of larvae occurs. We find little evidence of size-related entombment, but we find evidence that gravel size does not affect all larval sizes equally. Larger larvae emerge from small gravels before development is complete and with a visible yolk sac, possibly due to oxygen limitation in small gravels. Smaller larvae always complete development in the gravel and emerge without a yolk sac. Although growth and survival may increase with juvenile size following yolk-sac absorption, juveniles with yolk sacs may fare worse when depredation rates are high. The egg size–gravel size correlation may therefore reflect increased post-emergence mortality among larger offspring in small-gravel environments. Alternatively, compaction stress in fine gravels coupled with size asymmetries in larval strength may have caused the patterns we observed, in which case our data might not help explain the egg size–gravel size correlation.

6.2 Introduction

Mothers are expected to allocate the energy available for reproduction into offspring of an optimal size (Smith and Fretwell 1974). Given that investment per offspring is a heritable trait, variation in offspring size among populations is often assumed to be the result of natural selection (e.g., Gregersen et al. 2008). Conceptually, there are at least two ways in which direct selection can result in a divergence of mean egg size among populations. First, selection might favour a single level of investment per offspring across all offspring life stages, such that a consistent difference among populations in the strength or direction of selection on offspring size leads to population divergence in per-offspring investment (Smith and Fretwell 1974; Johnson et al. 2010). Second, divergence might also be the result of opposing patterns of selection at different stages of offspring development (Hendry et al. 2001). For example, depredation rate of

seeds might increase with seed size, but seedling vigor and subsequent seedling growth rate might also be positiely related to seed size (Parciak 2002). Therefore, the level of investment per offspring that maximizes parental fitness becomes function of the relative strength of selection on seed size during different life stages (Hendry et al. 2001), and the strength or direction of selection during these stages might vary among populations. Although this type of mechanism can likely account for population divergence in investment per offspring (e.g., Parciak 2002), few studies have explicitly examined its role in maintaining intraspecific egg-size variation.

Salmonids are largely autumnal spawners that deposit their eggs into riverine or lacustrine gravels. Embryos develop over winter and hatch into yolk-sac larvae (or "alevins") in the following spring. Once yolk-sac absorption is complete, individuals emerge from the gravel as juveniles (or "fry"), and in some species, they establish feeding territories near their natal area. Size asymmetries at the juvenile stage (Hutchings 1991; Einum and Fleming 2000b) and at the embryo or larval stage (Einum et al. 2002) can affect maternal and offspring fitness in salmonids, such that this group has stimulated a wealth of research on offspring size-number strategies (Einum et al. 2004).

Famously, a clear, positive correlation between population-specific spawning gravel size and population mean egg size has been observed among 21 Alaskan populations of sockeye salmon (*Oncorhynchus nerka*) (Quinn et al. 1995; Quinn 2005) (Fig. 6.1). It has been hypothesized that this egg size – gravel size correlation results from a decrease in offspring fitness with increasing egg size in small gravels. Only small larvae might be able to navigate the small pore spaces in finer gravels, whereas larger larvae might become entombed in the nest cavity. However, as the size of incubation gravels increases, there may be no size-related advantage during the embryo or larval stage, and given that larger juveniles generally exhibit greater fitness in the wild (Einum and Fleming 2000a), a larger egg size might maximize maternal fitness when gravel sizes are large. Although this widely discussed hypothesis has never been explicitly tested (Witzel and MacCrimmon 1983; Quinn et al. 1995; Quinn 2005), it suggests that opposing patterns of selection at different life stages led to adaptive divergence in investment per offspring among environments (Hendry et al. 2001).

The maximum difference between the smallest and largest population-mean egg size in the original sockeye salmon populations was 25.6 % (Quinn et al. 1995). The magnitude of this difference is indeed sufficient to warrant an investigation of adaptive hypotheses, as limited data suggest that a difference in egg size of 14 – 30 % is typical when reasonable evidence of adaptive variation in egg size exists (summarized by Fischer et al. 2011). On the other hand, although variation in egg size can be related to proximate factors, such as variation in the offspring selective environment (e.g., Fox et al. 1997), it can also be traced to other factors, such as the rate at which a mother can provision primary oocytes (Sakai and Harada 2001). The point is that a correlation between environmental variables and population-mean phenotypes *per se* is not reasonable evidence of adaptive divergence among populations. Manipulative experiments that are based on clear, *a priori* hypotheses are necessary to test whether inter-population variation in egg size reflects an adaptive response to local environments.

Here, we manipulate incubation environments of larval Atlantic salmon to test whether large embryos and larvae exhibit poor survival in small gravels because of size-related larval entombment. Assuming that size-related entombment is a function of larval size *per se*, we expect that larvae hatching from relatively large embryos will experience lower survival than larvae hatching from smaller embryos in fine gravels, but not in larger gravels. If large larvae have difficulty navigating the small interstices of fine gravels, then we also expect that they will use more energy during emergence from these gravels. Thus, we predict further that, in smaller gravels, large juveniles surviving to emergence will have a lower specific energy content (in Joules per gram of tissue) compared to smaller juveniles, but no difference in specific energy will be observed between large and small individuals that have emerged from a large gravel substrate.

6.3 Methods

Eggs for the present study were obtained from a subsample of captive-bred females that were reared to maturity at the Coldbrook Biodiversity Facility in Coldbrook, Nova Scotia. The breeding design and spawning methods are described in Chapter 3. The containers that were used to simulate natural egg pockets within salmon redds in the present study are described in Chapter 4 (Fig. 4.1).

The experiment was a 3 × 3 factorial design with three levels of egg size and three levels of gravel size, with three replicates per treatment (3 egg sizes × 3 gravel sizes × 3 replicates = 27 replicates in total). Gravel was purchased from Conrad Bros Ltd. (Dartmouth, Nova Scotia), and sieved into three size classes. Mean ± SD diameter of 75 randomly selected pebbles (measured along the median or "B" axis) was 28.2 ± 3.4 mm (large gravel), 11.7 ± 1.6 mm (medium gravel) and 8.9 ± 1.6 mm (small gravel). The mean, median and geometric mean gravel size was very similar for each size class. The range of geometric mean gravel sizes identified by Quinn et al. (1995) was 1.6 – 77.8 mm (Pebble Count method) and 0.90 – 32.1 mm (Bulk Samples).

Initial mean egg mass for each of the females spawned in the present study was estimated from a sample of 14 – 25 unfertilized eggs collected in Coldbrook. Based on these estimates, we identified six females that produced "small" eggs, six that produced "medium" eggs, and five that produce "large" eggs; the females that contributed eggs to each egg-size class came from a mix of three different populations, and hybrids thereof (Table 6.1). One female in each of these size classes was spawned on 4 November 2008, and the rest were spawned on 31 October 2008. On 10 and 11 March 2009, embryos were selected from relevant families (Table 6.1) and allocated evenly to the appropriate egg size treatment. Allocation proceeded as in the following example: 19 large embryos might have been collected from each of four half-sib families (76 embryos in total) that share a common mother. In this case, two embryos per family (eight embryos in total) would then have been allocated to each of the nine "large eggs" replicates, with four of the 76 embryos remaining unused. One or two of the unused embryos from each family were retained and weighed to the nearest 0.1 mg to obtain embryo mass (Table 6.1), the others were discarded. Estimated mean embryo mass (± SE) allocated to each small, medium, and large egg-size replicate was 69.5 ± 1.1 mg, 103.6 ± 1.5 mg, and 138.0 ± 2.6 mg, respectively, thus exceeding the range of population mean egg sizes identified by Quinn et al. (1995), which were between 84.5 ± 2.7 mg and 113.5 ± 1.2 mg. The "small" and "large" egg-size replicates each contained 44 embryos, "medium" egg-size replicates contained 45 embryos, and all embryos were buried under 23 cm of gravel.

A natural photoperiod was initiated on 31 March 2009. Juvenile emergence occurred between 13 May and 18 June 2009. When juveniles were detected in an

emergence trap, they were captured with a turkey baster, over-anaesthetized in 0.1 $mg \cdot L^{-1}$ tricaine methanesulphonate, rinsed in freshwater, blotted dry, weighed to the nearest 0.1 mg, and measured to the nearest mm (standard length) using calipers. We also noted the presence or absence of a yolk sac, as the mean percent yolk content of juveniles emerging from a replicate is highly correlated with the percent of individuals emerging with a visible yolk sac ($r^2 = 0.971$, $n = 12$ replicates, $p < 0.001$; data from Chapter 4). Juveniles were then frozen in individual 3 ml plastic tubes at −4 °C. All replicates were excavated on 24 June 2009, which is six days after the last juvenile had emerged, and the remaining live and dead alevins were counted.

Between 12 and 15 July 2010, we combusted a subsample of juveniles from each replicate to estimate their energetic content. First, the median day of emergence was calculated separately for each replicate. Then three ($n = 4$ replicates) or four juveniles ($n = 23$ replicates) that emerged on the median day of emergence, or that emerged as close as possible to the median day of emergence, were vacuum dried for 18 hours, using a Labconco® 4.5 L freeze-dryer ($n = 104$ juveniles dried in total). Dry mass was then measured to the nearest 0.1 mg. To estimate energetic content of tissue, the subsample was homogenized and then combusted in a Parr® 1266 semimicro oxygen bomb calorimeter. Each subsample was compressed to a 3 mm-diameter pellet, and pellets were combusted using 1 of 2 Parr® 1107 micro-bombs, as described by Michaud and Taggart (2007).

Factorial ANOVAs and Tukey Post Hoc Tests were used to assess treatment effects, and weighted fits were used when cell means were computed from unequal sample sizes. Proportional data were ($\text{logit}(x + 0.025)$) transformed before analysis.

6.4 Results

We found a tight relationship between wet and dry mass of the 104 dried juveniles (dry mass, mg = 0.188×(wet mass, mg) - 3.23, $r^2 = 0.983$, $p < 0.001$), so all values of juvenile mass reported below are dry mass values predicted from wet mass. Over the course of the experiment, ten juveniles escaped from their replicates and were found in the overflow sink. They could not be assigned back to their original container, but they

varied in size (range 11.5 – 52.6 mg, mean ± SD, 21.3 ± 4.2 mg), which indicates that they did not come from a single replicate.

Emergence began on 13 May 2009 at 102.8 % development and finished on 18 June 2009 at 164.8 % development. We detected significant effects of egg size ($F_{[2,18]}$ = 5.8, p = 0.011), gravel size ($F_{[2,18]}$ = 142.9. p < 0.001) and their interaction ($F_{[4,18]}$ = 3.3, p = 0.032) on percent development at emergence. Similarly, we detected significant effects of egg size ($F_{[2,18]}$ = 6.4, p = 0.008) and gravel size ($F_{[2,18]}$ = 185.5, p < 0.001) on median day of emergence, as well as a gravel size × egg size interaction ($F_{[4,18]}$ = 4.3, p = 0.013). Both estimates of emergence timing indicated that individuals from small embryos emerged relatively late in finer gravels, but there was no difference among egg size treatments in emergence time in large gravel (Fig. 6.2*A*).

Survival increased with increasing gravel size ($F_{[2,18]}$ = 66.5, p < 0.001), but survival was not related to egg size ($F_{[2,18]}$ = 0.52, p = 0.60) and the egg size × gravel size interaction was not significant ($F_{[4,18]}$ = 2.6, p = 0.070, Fig. 6.2*B*). Dry mass at emergence was significantly affected by egg size ($F_{[2,18]}$ = 3696.6, p < 0.001) and gravel size ($F_{[2,18]}$ = 487.6, p < 0.001), and the egg size × gravel size interaction was significant ($F_{[4,18]}$ = 19.5, p < 0.001). For large-egg treatments, juvenile mass did not differ between medium-gravel and small-gravel treatments, apparently because juveniles were smaller than expected in the large egg–medium gravel treatment. Differences in mass were apparent for medium and small egg treatments at each level of gravel size (Fig. 6.2*C*).

Specific energy of juveniles averaged 21.86 ± 0.12 $kJ \cdot g^{-1}$ across all treatments. We detected a significant effect of egg size ($F_{[2,18]}$ = 4.8, p = 0.021) and an egg size × gravel interaction ($F_{[4,18]}$ = 3.4, p = 0.031). Specific energy was significantly greater in the large egg–small gravel treatment, compared to small egg–small gravel and small egg–medium gravel treatments, but there was no difference among treatments in large gravel (Fig. 6.3*B*). To obtain total energy of juveniles (in Joules), we multiplied the specific energy value obtained from each replicate by the mean dry mass of juveniles emerging from that replicate. In the subsequent ANOVA, we observed significant effects of egg size ($F_{[2,18]}$ = 1166.5, p < 0.001), gravel size ($F_{[2,18]}$ =145.4, p < 0.001) and the egg size × gravel size term was significant ($F_{[4,18]}$ = 4.1, p = 0.015). Total energy increased with increasing gravel size for the medium-egg treatments, but total energy was not different

among the two smaller gravel sizes for both small and large eggs. Within egg-size treatments, total energy was always greatest in large gravel (Fig. 6.3*A*).

Individuals from small embryos never emerged with a yolk sac at any level of gravel size, whereas many individuals from medium and large embryos emerged from small gravels with a yolk sac (Fig. 6.3*C*). Accordingly, the proportion of fry emerging with a yolk sac ('proportion with yolk') was zero-heavy and not normally distributed (Kolmogorov-Smirnov Test Statistic = 0.352, $p < 0.001$). These data pose a statistical problem, as they indicate an invariance of 'proportion with yolk' in the 'small egg' level of the 'egg size' factor. Most statistical tests assume that data are drawn from populations with the same distribution of data, even if the distribution is non-normal. In our case, the proportion of individuals emerging with a yolk sac from small-egg treatments was always zero, and hence distribution-free, so a more appropriate approach is to calculate the probability of drawing a zero from a population with a mean and standard deviation equal to those observed in the other treatments. To evaluate whether treatments with a non-zero mean were significantly greater than zero, we used one-tailed *z* tests,

(Equation 6.1) $$z = (x_i - \bar{x}) \times \sigma^{-1}$$

where x_i is 0, $\bar{x}$ is the weighted mean of a given treatment, and σ is the weighted standard deviation (Zar 1984, p. 84). We also applied a Bonferonni correction to account for multiple paired comparisons (five tests were performed, so $\alpha = 0.01$). Weighted mean proportion of individuals emerging with a yolk sac was greater than zero for the large egg–small gravel treatment ($\text{logit}(\bar{x} + 0.025) = 1.20$, $(\text{logit}(\sigma + 0.025) = 1.56$, $\text{logit}(x_i + 0.025) = -3.66$, $z = -3.11$, $\alpha = 0.01$, $p = 0.0009$) and for the medium egg-small gravel treatment ($\bar{x} = -0.933$, $\sigma = 1.00$, $x_i = -3.66$, $z = -2.72$, $\alpha = 0.01$, $p = 0.003$), whereas other treatments were not greater than zero (Fig. 6.3*C*). This suggests an egg size × gravel size interaction, as there is no difference among levels of egg size in larger gravels, but proportion with yolk increases in juveniles from medium and large eggs when gravel size is small (Fig. 6.3*C*). Finally, proportion with yolk correlated positively with specific energy (Spearman's rho = 0.516, $n = 27$, $p = 0.006$), indicating that juveniles emerging with yolk sacs had a higher specific energy.

Six days after the last juvenile emerged from the gravel (24 June 2009), we assumed that all larvae surviving within the replicates were unable to escape ('entombed'), and we excavated all replicates. We analyzed these data to provide insight into the mechanism(s) of mortality within the gravel (e.g., oxygen limitation versus entombment). All live individuals detected were emaciated, suggesting entombment. Only four live individuals were found in the nine medium-gravel replicates, all four of which were in two large-egg replicates. No live individuals were found in any of the large-gravel replicates. Hence, we analyzed entombment data only within small gravels using a one-way ANOVA, where we tested the effect of egg size. The percentage of live individuals entombed in small-, medium-, and large-egg treatments with small gravels was 11.1%, 12.3%, and 23.7%, respectively, and these values are inversely related to survival to emergence in small gravels (Fig. 6.2*B*). The one-way ANOVA was not significant ($F_{[2,6]}$ = 2.5, P = 0.16), however, which indicates that survival after entombment was not related to egg size in small gravel.

Dead individuals were too decomposed to determine stage-at-death (i.e., yolk-sac *vs* non-yolk-sac larvae). The percent of dead individuals found after excavation was, not surprisingly, higher in small gravel ($F_{[1,12]}$ = 53.4, p < 0.001) but unaffected by egg size ($F_{[2,12]}$ = 2.2, p = 0.15) or their interaction ($F_{[2,12]}$ = 1.4, p = 0.29). Note that no dead individuals were detected in the large gravel replicates, so the 'large gravel' level of the gravel size factor was not included in the preceding analysis.

6.5 Discussion

In the present study, we test whether inter-population variation in egg size could be maintained by size-specific survival of embryos or larvae in particular incubation substrates. We observed that the effect of egg size on offspring phenotypes differed markedly among incubation substrates, whereby the egg size × gravel size interaction term was significant in four of five ANOVAs. Although our survival data suggest that individuals from small embryos fared similarly in both small and medium gravel (which cannot be said of individuals from medium and large embryos) the relevant interaction term for this effect was not significant (p = 0.07). Our data do not, therefore, support our focal hypothesis. Below, we re-evaluate traditional hypotheses for the egg size–gravel

size correlation, and examine whether our data can help explain the egg size–gravel size correlation.

First, we observed interactions among egg size and specific energy, day of emergence, and percent of juveniles emerging with a yolk sac. These interactions are not independent, as specific energy increases with percent yolk, and earlier emergence may also correlate with percent yolk (developmental stage) within gravel-size fractions. These interactions broadly reflect a positive correlation between initial egg size and the proportion of juveniles emerging with yolk in smaller gravels, and a lack of this correlation in larger gravels. Moreover, small larvae emerged relatively late in small gravel, but not in larger gravels, and this is reflected in the lower specific energy (or percent yolk) of small larvae in small gravels. Overall, these patterns suggest that relatively large larvae emerge prematurely in small incubation gravels, whereas small larvae delay emergence until yolk absorption is complete. A common observation is indeed that premature emergence occurs when incubation gravels are small (Phillips et al. 1975; Witzel and MacCrimmon 1981; Olsson and Persson 1986), and that larger larvae take longer to absorb their larger yolk sacs (Killeen et al. 1999). Our findings are novel, however, in that premature emergence in stressful environments is much more common among larger larvae.

The patterns observed in the present study might have nothing to do with the egg size–gravel size correlation observed by Quinn et al. (1995). It is possible that compaction stress in fine gravels encouraged larvae of all sizes to emerge prematurely (Witzel and MacCrimmon 1983). Compaction may have concomitantly result in delayed emergence in small larvae, assuming small larvae are weaker and have more difficulty pushing their way through the small interstitial spaces. This can explain every pattern we observed in the present study, and it is perhaps the parsimonious explanation available for the data.

Notwithstanding, it is worth examining other explanations for the patterns observed in the present study. Flow in artificial incubators can be largely confined to the smooth incubator walls when gravel size is small (Rose and Rizk 1949; Witzel and MacCrimmon 1983), and given that larval mobility is restricted in smaller gravels (Dill and Northcote 1970), oxygen stress may have occurred in the present study. Assuming

zero mortality in our replicates, estimated total oxygen consumption for small-, medium- and large- egg replicates was 0.0853 $mg \cdot h^{-1}$, 0.141 $mg \cdot h^{-1}$ and 0.191 $mg \cdot h^{-1}$, respectively, at the eyed embryo stage, and 0.0985 $mg \cdot h^{-1}$, 0.261 $mg \cdot h^{-1}$ and 0.479 $mg \cdot h^{-1}$, respectively, just after hatching (i.e., the beginning of the larval stage) (calculations following Rombough 2006). For large-egg replicates, this suggests an increase of 60.1 % in oxygen consumption from the embryo stage to the larva stage, compared to a 13.4 % increase for small-egg replicates. Of course, we did not detect a size-related difference in survival-to-emergence, or in the number of larvae that were surviving while entombed in small gravels at the end of the experiment, so if a size-related oxygen effect occurred, it was likely sub-lethal.

Yet it is unlikely that size-related patterns of emergence reflect different hypoxic tolerances of small and large embryos. Small embryos have been long-expected to fare better in low-oxygen environments because of larger surface area-to-volume ratios, which was presumed to translate into more favourable rates of gas exchange (Krogh 1959); however, this supposition has been discredited. Although geometric constraints dictate that egg and embryo surface area increases at a rate of $(\text{embryo mass})^{0.67}$, the rate of metabolic expansion is much slower ($b = 0.44$, Einum et al. 2002; $b = 0.30$, Rombough 2006), such that larger embryos actually have a greater surface area for gas exchange relative to metabolic oxygen demand. Despite this difference, embryo size has no effect on hypoxic tolerance during the embryo stage, so it is unlikely that embryo surface area is a major factor limiting oxygen uptake (Rombough 2006; but see Einum et al. 2002). Size-related differences in hypoxic tolerance during the larval stage are also unlikely to result in adaptive, inter-population egg-size variation. Although hypoxic tolerance is indeed lower for larvae from larger embryos, this effect is confined to the first half of the larval period when gills are underdeveloped and oxygen acquisition must be primarily cutaneous. Moreover, this effect of initial egg size on larval hypoxic tolerance is very small (Rombough 2006), so it is unlikely that hypoxic tolerance *per se* is responsible for the patterns observed in the present study.

On the other hand, metabolic rate increases relatively quickly with size in larvae, so a small larva requires less oxygen per unit of time than a larger larva, all else being equal (Rombough 2006). Assuming larval oxygen consumption scales at the rate (embryo

mass)$^{0.62}$ (Rombough 2006), we estimate that the per capita oxygen consumption is roughly 16.7 % higher in the sockeye population with the largest eggs, compared to that with the smallest (Quinn et al. 1995). This difference may be important in small incubation gravels where flow rates are relatively low, such that smaller larvae could persist in a given interstitial space for a longer period before running low on oxygen. We observed that total energy of juveniles increased with gravel size, which is indeed consistent with lower yolk-conversion efficiency in small-gravel environments (e.g., Olsson and Persson 1986) or at lower levels of dissolved oxygen *per se* (e.g., Mason 1969). We suggest that oxygen depletion rates by larvae, rather than hypoxic tolerance *per se*, may explain why large juveniles emerge prematurely from small gravels. In this scenario, however, larval biomass per unit area is likely an important factor that influences oxygen availability, but unfortunately Quinn et al. (1995) do not report whether larval density (or fecundity) varies with gravel size among their focal populations.

If size-specific oxygen use by larvae is responsible for the patterns of emergence observed in the present study, then a novel hypothesis for the egg size–gravel size correlation arises. Although the relative importance of residual yolk and size-at-emergence on the probability of depredation is not understood, there is credible evidence to suggest that yolk-sac juveniles fare worse when depredation is high. For example, in a large-scale release programme spanning two years, juvenile survival was consistently and inversely related to a metric of residual yolk content, whereas selection on juvenile size was inconsistent and variable in direction (Fresh and Schroder 1987). An analysis of predator stomach contents in this study suggested that individuals with yolk sacs were being preferentially depredated (Fresh and Schroder 1987). If yolk-sac larvae are indeed more prone to depredation, then small individuals may have a greater fitness when incubated in small gravels because they emerge as juveniles, not as yolk-sac larvae. Although it is clear that yolk-sac juveniles also emerge under natural conditions (Garcia de Leaniz et al. 2000), our hypothesis depends critically on the benefits of increased size-at-emergence versus the propensity of these larger individuals with yolk-sacs to be depredated, so further testing is certainly warranted. Overall, more research is needed to

understand both the mechanism responsible for the pattern of emergence observed in the present study, and to understand the egg size–gravel size correlation.

Another long-standing hypothesis for the egg size–gravel size correlation is that egg and embryo predators, such as sculpins (*Cottus* sp.) and gobies (*Neogobius* sp.), are be better-able to penetrate larger gravels, such that large eggs might be favoured in large gravels if predators are gape-limited (Quinn et al. 1995; Einum et al. 2002). We think this hypothesis is unlikely. Although egg and embryo predators may be an important source of mortality (Foote and Brown 1998), there is little evidence that gobies and sculpins can differentially penetrate the gravels sizes described in the 21 populations of sockeye salmon (Biga et al. 1998; Chotkowski and Marsden 1999; Palm et al. 2009). Moreover, assuming egg diameter (d) in mm scales to egg wet mass according to the equation given by Einum and Fleming (2002),

(Equation 6.2) $$d = (6 \times v \times (1/\pi))^{1/3}$$

Where v is egg volume, and given that the range of egg masses described by Quinn et al. (1995) was 84.5 mg – 113.5 mg, this hypothesis requires that the predators be gape-limited to the extent that an estimated 0.56 mm difference in egg or embryo diameter decreases the likelihood of depredation in larger gravels.

It is altogether possible that the egg size – gravel size correlation is spurious, and that the present study fails to support the focal hypothesis for this reason. However, the present study has three important drawbacks, any of which may explain why we observed no pattern of size-related survival. First, we cannot exclude the possibility that the original sockeye salmon populations (Quinn et al. 1995) exhibit traits at the embryo, larval or maternal stage that act in concert with variation in egg size to effect the positive egg size–gravel size correlation. For example, the depth at which mothers bury their embryos may vary with spawning substrate (e.g., Kondolf and Wolman 1993), but we buried embryos at a depth of 23 cm at all levels of gravel size, which is the average burial depth of Atlantic salmon embryos (Devries 1997). Burial depth may correlate negatively with dissolved oxygen levels (e.g., Ingendahl 2001; Malcolm et al. 2004; but see Peterson and Quinn 1996), and given that large larvae have a higher per-capita oxygen

consumption (Rombough 2006), mothers may do well to spawn small eggs in shallow nests when gravel size is small and flow is limited. We note that survival in our smallest gravel was very low at all levels of egg size, which underlines the possibility that the present study did not effectively emulate conditions in the wild. Second, the gravel sizes used in the present study were homogenous, and this is unlikely to occur in nature. A silt, sand and cobble mixture, for example, may have the same geometric mean gravel size a homogeneous pebble mixture, but the two mixtures may not have the same effects on offspring survival or phenotypes (Tappel and Bjornn 1983). Our results may have differed if we had more closely emulated gravel mixtures found in the wild, rather than using homogenous gravel mixtures. Third, and with the other caveats in mind, it is diffcult to overlook the possibility that we committed a Type II error with respect to size-related survival in different gravel size fractions. The relevant interaction term appraoched significance ($p = 0.07$), and small embryos appeared to fare similarly in both small and medium gravels, which would provide at least some support to the focal hypothesis.

In sum, our data do not support the hypothesis that size-related entombment of salmonid larvae occurs (Quinn et al. 1995; Quinn 2005), though caveats particular to the present experiment may have precluded a proper test of the focal hypothesis. We identify the possibility that inter-population variation in egg size is maintained by size-related oxygen demand of larvae, coupled with a propensity for larger larvae to deplete oxygen reserves more quickly in fine gravels, which ultimately results in premature emergence of large larvae from small gravels. It is also possible that large larvae emerge sooner from small gravel because they are simply stronger than larvae from small embryos, in which case it seems less likely that the pattern we observed in the present study can explain the egg size–gravel size correlation. The present study shows that the mechanism(s) involved in effecting the positive correlation between egg size and gravel size, if any, may be more complex than originally anticipated.

Table 6.1: Summary of embryo allocation to the experiment.

♀ ID	Egg Size	♀ Crosstype	Embryo Mass (SE)	No. embryos	No. families
1	Small	*Stw*×*Stw*	66.7 (2.8)	12	4
2	Small	*Stw*×*Stw*	73.9 (1.5)	12	4
3	Small	*Stw*×*Stw*	70.1 (2.6)	4	4
4	Small	*Grv*×*Grv*	70.3 (1.6)	5	5
5	Small	*Grv*×*Grv*	67.6 (2.9)	6	4
6	Small	*Stw*×*Stw*	61.5 (1.0)	5	5
7	Medium	*Stw*×*Stw*	107.7 (0.9)	9	4
8	Medium	*Eco*×*Eco*	100.1 (4.3)	8	5
9	Medium	*Grv*×*Grv*	103.5 (1.2)	6	5
10	Medium	*Eco*×*Eco*	103.6 (3.9)	7	3
11	Medium	*Eco*×*Stw*	108.2 (1.9)	8	2
12	Medium	*Grv*×*Stw*	98.5 (3.4)	7	2
13	Large	*Grv*×*Grv*	138.5 (2.3)	4	4
14	Large	*Eco*×*Stw*	137.4 (1.0)	8	2
15	Large	*Eco*×*Stw*	150.9 (0.7)	10	2
16	Large	*Grv*×*Grv*	149.4 (1.2)	11	6
17	Large	*Stw*×*Stw*	130.0 (1.9)	11	6

NOTE: "♀ Cross-type" reflects the genetic background of the female (Stewiacke River; Great Village River; Economy River; See Chapter 3). "No. embryos" is the number allocated to each relevant replicate from a given female. "No. families" is the number of half-sib families (of embryos) allocated to the experiment from a given female. Embryo mass is in mg.

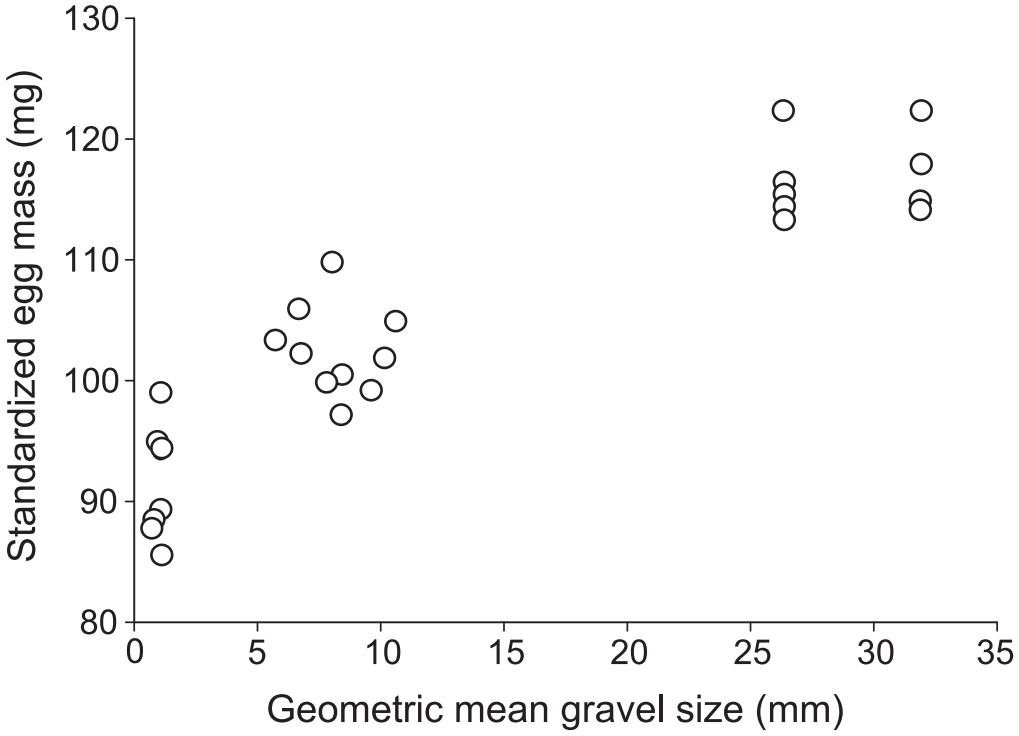

Figure 6.1: The relationship between population mean egg size of sockeye salmon (*Oncorhynchus nerka*) and geometric mean size of spawning gravels. Multiple points for the same gravel size are samples from the same population in different years. Egg mass is adjusted to a common maternal body size of 450 mm fork length. Modified from Quinn et al. (1995).

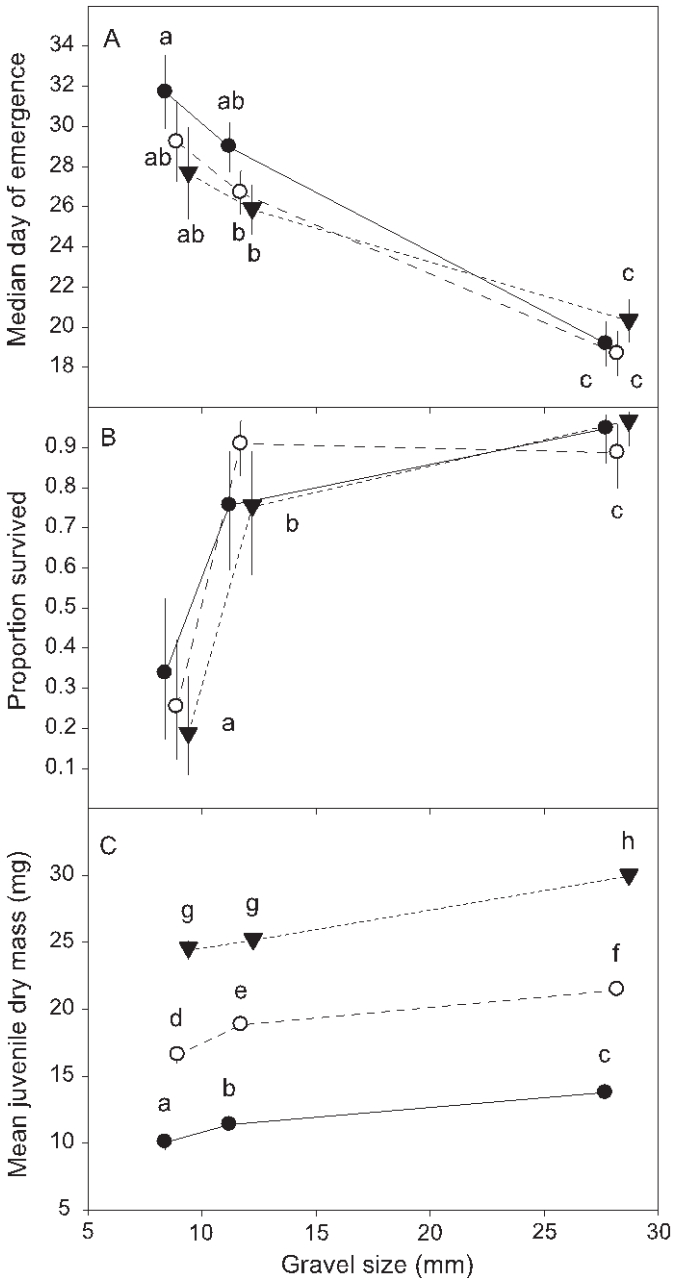

Figure 6.2: Letters denote significant differences after a Tukey's HSD test; means are least-square means and error bars are 95 % confidence intervals (*A*) Median day of juvenile emergence for each level of egg size and gravel. Note that post-hoc tests show an identical pattern of significance when emergence time is expressed as percent development (*B*) Embryo-to-juvenile survival as a function of egg size and gravel size. Proportions are back-transformed from logits. Note that only the main effect of gravel was significant, and that the single letter at each level of gravel size denotes differences for the main effect of gravel size. (*C*) Mean dry mass of juveniles.

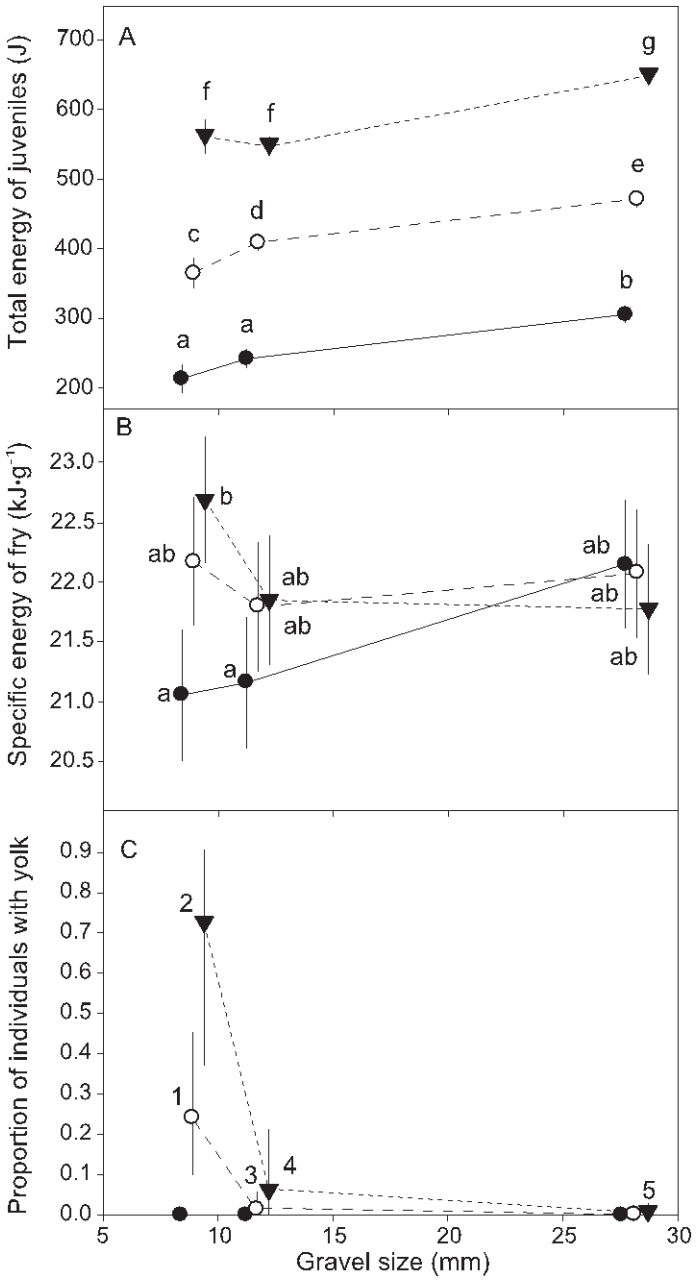

Figure 6.3: Letters denote significant differences after a Tukey's HSD test; error bars are 95 % confidence intervals. (*A*) Least square mean specific energy of juveniles. (*B*) Least square mean total energy of juveniles. (*C*) Weighted mean proportion of individuals that emerged from the gravel with a visible yolk sac as a function of gravel size and egg size. Data are back-transformed from logits. Values identify one-tailed *z*-tests (α = 0.01) performed on logit-transformed data, which evaluated whether non-zero means were significantly greater than zero (1. z = -2.72, p = 0.003; 2. z = -3.11, p = 0.0009; 3. z = -0.57, p = 0.28; 4. z = -1.09, p = 0.14; 5. z = -0.56, p = 0.28).

Chapter 7: Discussion and Conclusion

7.1 Introduction

Investment per offspring has been traditionally been viewed through the lens of optimality. The rationale for focussing on investment per offspring in an optimality context is, perhaps, because this trait is clearly and directly related to reproductive success. In other words, natural selection might be expected to overcome local constraints (e.g., genetic constraints) and produce an optimal investment per offspring (Orzack and Sober 1994). In reality, however, investment per offspring is extremely variable within populations (Bernardo 1996). This is difficult to explain under the classic model because optimality implies that an individual or a group of individuals exhibit the phenotype which confers maximum individual fitness, and that variation in the phenotype within or among individuals will be minimal. Perhaps it is this disparity between theory and observation, coupled with the long-standing optimality paradigm, that explains why so many theoretical and empirical studies have attempted to explain egg-size variation within populations in adaptive terms (e.g., Kaplan and Cooper 1984; Parker and Begon 1986; Einum and Fleming 2002; Hendry and Day 2003; Marshall et al. 2008; Olofsson et al. 2009; Morrongiello et al. 2012).

Most of this thesis is focussed on tests of adaptive hypotheses for investment per offspring, primarily because so many adaptive hypotheses exist. To conclude, this final chapter explores non-adaptive explanations for variation in investment per offspring, drawing both from the literature and from research presented herein. The focus of this discussion will be primarily on mechanisms that can result in variation that occurs among individuals within populations, as opposed to processes such as genetic drift which might cause variation in mean phenotypes among populations. To ensure the discussion is clear, I underline that the general motivation behind extensions of the Smith-Fretwell model is not, at least in my view, to explain *all* of the variation observed at the appropriate scale in adaptive terms. Rather, these models are generally developed to provide insight into the conditions and mechanisms that might influence the evolution of investment per offspring. The goal of this section is first to briefly examine different models and

hypotheses that explain egg-size variation in adaptive terms, and to examine the quantity and quality of the evidence in support of these hypotheses. The second objective is to explore known evolutionary constraints on investment per offspring, and to explore whether any specific constraints are likely to provide general explanations for the mismatch between Smith and Fretwell's classic model and empirical observations.

7.2 Diversified Bet-Hedging

There are models that explain egg-size variation within environments and populations as examples of diversified bet hedging. In these models, variation in egg size within individuals (i.e., within a clutch) or variation in mean egg size among individuals in a given environment occurs because geometric mean fitness is greater for genotypes that randomly vary the size of their offspring in a given reproductive episode (Kaplan and Cooper 1984; Marshall et al. 2008; Olofsson et al. 2009). The problem with diversified bet-hedging is that the environmental and ecological conditions that favour this strategy are very specific. The environment must be temporally variable, the relationship between offspring size and fitness must be convex or 'dome shaped', and overlapping generations reduce the fitness benefits for genotypes that vary investment per offspring (McGinley et al. 1987; Schultz 1991; Einum and Fleming 2004; Marshall et al. 2008). While reasonable empirical evidence of diversified bet-hedging exists for reproductive traits in some plants (e.g., the timing of seed germination, Simons 2009), empirical evidence of diversified bet-hedging in animals is always correlative and generally unconvincing (Crump 1981; Koops et al. 2003; Allen et al. 2008; Dziminski et al. 2009; Morrongiello et al. 2012). In fact, no study has even demonstrated a dome-shaped relationship between offspring size and offspring fitness in animals (for one possible example, see Kaplan 1992). Admittedly, empirical tests of diversified bet hedging are logistically difficult and must span many generations, and this might explain a general lack of good evidence for this phenomenon. On the other hand, it is difficult to believe that diversified bet hedging for offspring size is widespread in animals, given that it is favoured only under very specific ecological and environmental conditions. In the overwhelming majority of cases, variation in investment per offspring within individuals, among individuals, and over time, probably does not reflect diversified bet hedging.

7.3 Adaptive Egg-Size Plasticity

Some extensions of the Smith-Fretwell model assume that multiple environments effectively exist within a population (either spatially or temporally), and that it is a matching of maternal provisioning strategies to these environments that, in part, creates variation in mean egg size either among individuals (i.e., if environments vary spatially) or variation in mean egg size across time (McGinley et al. 1987; Schultz 1991). Adaptive egg-size plasticity generally requires that allocation to reproductive tissues *and* oviposition or parturition occur in the same environment, so this mechanism is not realistic for many species (see e.g., Tyler and Sumpter 1996; Kinnison et al. 2001). For other species, there is unequivocal evidence that adaptive phenotypic plasticity in egg size occurs. For example, the seed beetle, *Stator limbatus*, adjusts the size and number of eggs in response to the quality of the host plant upon which eggs are laid, and this facultative response increases reproductive success across host plants (Fox et al. 1997). For some species, then, this phenomenon might contribute substantially to variation in investment per offspring that is observed among individuals or across time. In fact, convincing examples of adaptive egg-size plasticity are not uncommon (e.g., water fleas: Perrin 1989; beetles: Fox et al. 1997; bony fishes: Bashey 2006; Taborsky 2006; bryozonas: Allen et al. 2008; flies: Vijendravarma et al. 2010), but there are also many species that apparently do not manipulate egg size even though allocation to reproductive tissues and oviposition occur in the same environment (e.g., soil mites: Plaistow et al. 2007). Notwithstanding a publication bias, it would appear as though many (but not all) species that provision their offspring and oviposit in the same environment do exhibit adaptive egg-size plasticity. Therefore, egg-size plasticity can partly account for differences in mean egg size among individuals in a population (i.e., if environments vary spatially, Fox et al. 1997), or changes in a population mean from time *A* to time *B* (i.e, if the environment varies temporally, Perrin 1989). Yet, even though mean investment per offspring changes among environments, unexplained variation in egg size in many of these studies is still very high (e.g., Kawecki 1995; Creighton 2005). This suggests that constraints on investment per offspring still comprise an important contribution to egg-size variation in these species and populations.

7.4 Optimal Trait Combinations

Adaptive egg-size plasticity stipulates that a matching of egg size to a local environment is a facultative response, such that environmental conditions can usually be assessed on an ongoing basis and reproductive patterns can be adjusted to maximize reproductive success in the anticipated environment. Yet, a different mechanism might also lead to a matching of investment per offspring and local environments. According to Parker and Begon (1986), Hendry and Day (2003) and Hendry et al. (2001), reproductive effort and/or maternal body size might influence the offspring environment in a predictable manner, creating spatially heterogeneous selective environments for offspring. Under these conditions, genetic correlations between investment per offspring and reproductive effort (or maternal body size) might be favoured by selection (Sinervo and Svensson 2002). To be clear, this phenomenon is distinct from adaptive phenotypic plasticity, primarily because the bivariate relationship between investment per offspring and body size (or reproductive effort) is the result of an underlying genetic correlation, i.e., an obligate rather than a facultative change in investment with increasing body size or reproductive effort (Schwarzkopf et al. 1999; Karl et al. 2007; Bauerfiend and Fischer 2008).

Positive phenotypic correlations between maternal size in investment per offspring occur in many taxa (Hendriks and Mulder 2008), and these correlations could in theory be an example of this phenomenon (Parker and Begon 1986; Hendry and Day 2003). While there is reasonable evidence of a positive genetic correlation between investment per offspring and reproductive effort (or female body size) in many different taxa (Schwarzkopf et al. 1999; Caley et al. 2001; Czesak and Fox 2003; Beck and Beck 2005; Schroderus et al. 2012), there is a decided lack of evidence that increases in fecundity or female body size affect offspring fitness (through sibling competition, for example) within reproductive episodes (salmon: Chapter 4, Chapter 5; butterflies: Wiklund et al. 1987; wasps: Lalonde 2005). In fact, positive phenotypic correlations between maternal size or reproductive effort and investment per offspring are observed even when investment per offspring has no discernable effect on offspring fitness (Wiklund et al. 1987; Lalonde 2005). The correlation is also observed in species that provide a great deal of post-partum parental care (reviewed by Hendriks and Mulder

2008). The latter pattern would be surprising if, in general, the correlation served the purpose of offsetting negative effects of sibling competition, given that ongoing parental care obscures the fitness consequences of variation in initial offspring investment (Monteith et al. 2012). Therefore, the positive phenotypic correlation between investment per offspring and maternal size contributes to variation in investment per offspring observed within populations, but there is currently no reason to suspect that it has evolved as a form of maternal compensation for size-specific maternal effects on the offspring environment.

7.5 Constraints and the Classic Model

Smith and Fretwell's classic model can help explain both the mean phenotype and the distribution of phenotypes in a given population (see Chapter 2). One needs only to assume that a given population effectively inhabits one particular environment with respect to selection on offspring size. In this interpretation, it is understood that variation among individuals in a population arises because of constraints on investment per offspring (e.g., genetic constraints) that result in individual deviations from optimal investment per offspring. While many extensions of Smith and Fretwell's model propose mechanisms of egg-size evolution that are worth investigating, and while there is evidence of adaptive plasticity in egg size in some species, there is unequivocal evidence of physiological and genetic constraints on investment per offspring in many taxa. In many of these cases, these non-adaptive explanations can provide a parsimonious explanation for most of the among-individual variation in egg size that occurs within populations.

First, there is a sound basis to suspect that reproductive traits in general should be relatively variable compared to morphological and physiological traits, such that a relatively large amount of variation in investment per offspring within populations might be expected. Given that resources are limited, as is the ability of an organism to acquire them, some structures and functions are likely given priority for resource allocation. For good reason, allocation to maintenance is generally prioritized over allocation to growth and reproduction (Schubert et al. 2009; Marshall and Sinclair 2010), such that reproductive traits might be more likely to be affected by individual variation in energy

budgets than many other traits (Glazier 2002). This predisposition of reproductive traits (and more generally, life-history traits) to environmental influences can perhaps explain why these traits have a relatively low heritability (Houle 1992), and why investment per offspring is generally a variable trait. Therefore, we have a general, *a priori* reason to expect that investment per offspring is relatively sensitive to variation in individual energy budgets, and that it will vary among individuals for this reason.

The Smith-Fretwell model also assumes hierarchical allocation to reproduction, such that organisms will devote some optimal fraction of energy to reproduction, then this fraction will be divided between size and number of offspring (also see Van Noordwijk and De Jong 1986). However, the assumption of independent allocation to traits that compete for resources is probably not realistic in general (e.g., King et al. 2011), or for investment per offspring (Schwarzkopf et al. 1999). There is reasonable evidence that investment per offspring and reproductive effort are often positively genetically correlated, such that neither trait can evolve independently (Schwarzkopf et al. 1999; Caley et al. 2001; Czesak and Fox 2003; Beck and Beck 2005; Schroderus et al. 2012). The genetic correlation has profound implications on the evolution of this trait, and a new theoretical literature has begun to explore how investment per offspring might evolve under multivariate selection on a suite of correlated life-history traits, such as the reproductive costs of variation in reproductive effort, coupled with variation in female condition and age-specific mortality schedules (Kindsvater et al. 2010, 2011). The point, though, is that investment per offspring will vary within populations if reproductive effort also varies, and there is currently no empirical basis to view this genetic correlation as anything other than a constraint that limits adaptive evolution of investment per offspring.

In Chapter 5, we also demonstrate that maternal effects on the offspring environment might obscure the relationship between investment per offspring and fitness (also see, e.g., Lloyd 2004; Mitchell et al. 2013, and references therein). Of course, the present body of research used 'simulated' maternal effects, insofar as we estimated from the literature how females might construct the nest environment, but the underlying point is clear: maternally-induced variation in the offspring environment might make investment per offspring less visible to selection. This might decrease the strength of

stabilizing selection on parental investment per offspring (Figs. 2.1; 2.2), thereby resulting in more variation among individuals in a population (McGinley et al. 1987).

There are many other mechanisms that cause mean egg size to differ among individuals in a population or environment. Morphological constraints, such as the size of the pelvic opening in reptiles, can increase the variance observed among individuals in some populations (Congdon and Gibbons 1987; Sinervo and Licht 1991). Variation in maternal androgen levels can also result in variation in offspring size (Bowden et al. 2004), given that androgens inhibit follicular development during certain follicular stages (Staub and De Beer 1997). The overarching point is that investment per offspring is a complex, environmentally-sensitive trait, and that variation in this trait affected by morphology and (apparently) unrelated physiological functions.

Investment per offspring has hitherto been viewed through the lens of optimality, but a realistic expectation is that offspring size will vary extensively within populations, due to a rich diversity of constraints on the evolution of investment per offspring. To come full circle, this means that optimality *sensu* Smith and Fretwell (1974) should not be expected, but it does not mean that the classic model and other models of egg-size evolution are not useful. As we explain in Chapter 2, comparing observed values of investment per offspring to the expected (optimal) values from the classic model can be an important first step towards understanding whether individuals in a population are near an adaptive peak. This procedure may also help one understand the extent to which constraints (and perhaps which particular constraints) influence the evolution of investment per offspring in a given population (Orzack and Sober 1994). Extensions of the classic model are also useful because they describe potential mechanisms that influence egg-size evolution. While these extensions may be less broadly applicable and more difficult to interpret than the classic model, they can nonetheless be applied and tested with specific species or populations in which the model assumptions are likely to be satisfied.

Appendix A: Copyright Permission Letters

A.1 Copyright Permission for Chapter 2

License number	3122040786684
License date	Apr 04, 2013
Licensed content publisher	Ecological Society of America
Licensed content title	Ecology
Licensed content date	Jan 1, 1920
Type of use	Thesis/Dissertation
Requestor type	Academic institution
Format	Electronic
Portion	chapter/article
Title or numeric reference of the portion(s)	Chapter 2
Author of portion(s)	Njal Rollinson
Issue, if republishing an article from a serial	N/A
Publication date of portion	August 2013
Rights for	Main product
Duration of use	Life of current edition
Creation of copies for the disabled	no
With minor editing privileges	yes
For distribution to	Canada
In the following language(s)	Original language of publication
With incidental promotional use	no
Lifetime unit quantity of new product	0 to 499
Made available in the following markets	professional
The requesting person/organization	Njal Rollinson
Author/Editor	Njal Rollinson
The standard identifier	PhD Thesis
Title	The adaptive significance of egg-size variation within and among populations of Atlantic salmon
Publisher	Dalhousie University
Expected publication date	Aug 2013
Estimated size (pages)	250
Total (may include CCC user fee)	0.00 USD

A.2 Copyright Permission for Chapter 5

SPRINGER LICENSE
TERMS AND CONDITIONS

Feb 22, 2013

This is a License Agreement between Njal Rollinson ("You") and Springer ("Springer") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Springer, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3090410773481
License date	Feb 15, 2013
Licensed content publisher	Springer
Licensed content publication	Oecologia
Licensed content title	Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon
Licensed content author	Njal Rollinson
Licensed content date	Jan 1, 2011
Volume number	166
Issue number	4
Type of Use	Thesis/Dissertation
Portion	Full text
Number of copies	6
Author of this Springer article	Yes and you are a contributor of the new work
Order reference number	
Title of your thesis / dissertation	The adaptive significance of egg-size variation within and among populations of Atlantic salmon
Expected completion date	Aug 2013
Estimated size(pages)	250
Total	0.00 CAD
Terms and Conditions	

A.3 Copyright Permission for Chapter 6

NRC RESEARCH PRESS LICENSE
TERMS AND CONDITIONS

Feb 22, 2013

This is a License Agreement between Njal Rollinson ("You") and NRC Research Press ("NRC Research Press") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by NRC Research Press, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3090421223144
License date	Feb 15, 2013
Licensed content publisher	NRC Research Press
Licensed content publication	Canadian Journal of Fisheries and Aquatic Sciences
Licensed content title	Why does egg size of salmonids increase with the mean size of population spawning gravels?
Licensed content author	Njal Rollinson, Jeffrey A. Hutchings
Licensed content date	Aug 1, 2011
Volume number	68
Issue number	8
Type of Use	Thesis/Dissertation
Requestor type	Author (original work)
Format	Print and electronic
Portion	Full article
Order reference number	
Title of your thesis / dissertation	The adaptive significance of egg-size variation within and among populations of Atlantic salmon
Expected completion date	Aug 2013
Estimated size(pages)	250
Total	0.00 USD
Terms and Conditions	

Appendix B: Beta Distribution Parameterizations used in Chapter 2

B.1 Parameterizations of the Beta Distribution for the Artificial Fitness Curve in Fig. 2.4*A*.

Maximum α/(α+β) =		0.10			0.25			0.50			0.75	
(α+β) =	50	100	500	50	100	500	50	100	500	50	100	500
$x = 10$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 11$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 12$	0.25	0.50	2.50	0.63	1.25	6.25	1.25	2.50	12.50	1.88	3.75	18.75
$x = 13$	4.00	8.00	40.00	10.00	20.00	100.00	20.00	40.00	200.00	30.00	60.00	300.00
$x = 14$	4.63	9.25	46.25	11.56	23.13	115.63	23.13	46.25	231.25	34.69	69.38	346.88
$x = 15$	4.88	9.75	48.75	12.19	24.38	121.88	24.38	48.75	243.75	36.56	73.13	365.63
$x = 16$	4.98	9.95	49.75	12.44	24.88	124.38	24.88	49.75	248.75	37.31	74.63	373.13
$x = 17$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 18$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 19$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00

NOTE: Values of alpha (α), where α is the number of successes in α + β Bernoulli trials, are given for each integer of offspring size (x) for sample sizes of 50, 100 and 500 (i.e., the value of α + β), and for asymptotic values of offspring fitness of 0.10, 0.25, 0.50 and 0.75 (i.e., the average maximum value of α/(α + β)). The corresponding values of β, the number of failures α + β Bernoulli trails, are not given at each value of x, but they can be deduced by subtracting α from the value of α + β.

B.2 Parameterizations of the Beta Distribution for the Artificial Fitness Curve in Fig. 2.4*B*.

Maximum α/(α+β) =		0.10			0.25			0.50			0.75	
Maximum (α+β) =	50	100	500	50	100	500	50	100	500	50	100	500
$x = 10$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 11$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 12$	0.05	0.10	0.50	0.13	0.25	1.25	0.25	0.50	2.50	0.38	0.75	3.75
$x = 13$	0.13	0.25	1.25	0.31	0.63	3.13	0.63	1.25	6.25	0.94	1.88	9.38
$x = 14$	0.25	0.50	2.50	0.63	1.25	6.25	1.25	2.50	12.50	1.88	3.75	18.75
$x = 15$	0.75	1.50	7.50	1.88	3.75	18.75	3.75	7.50	37.50	5.63	11.25	56.25
$x = 16$	2.25	4.50	22.50	5.63	11.25	56.25	11.25	22.50	112.50	16.88	33.75	168.75
$x = 17$	3.50	7.00	35.00	8.75	17.50	87.50	17.50	35.00	175.00	26.25	52.50	262.50
$x = 18$	4.13	8.25	41.25	10.31	20.63	103.13	20.63	41.25	206.25	30.94	61.88	309.38
$x = 19$	4.45	8.90	44.50	11.13	22.25	111.25	22.25	44.50	222.50	33.38	66.75	333.75
$x = 20$	4.63	9.25	46.25	11.56	23.13	115.63	23.13	46.25	231.25	34.69	69.38	346.88
$x = 21$	4.75	9.50	47.50	11.88	23.75	118.75	23.75	47.50	237.50	35.63	71.25	356.25
$x = 22$	4.83	9.65	48.25	12.06	24.13	120.63	24.13	48.25	241.25	36.19	72.38	361.88
$x = 23$	4.88	9.75	48.75	12.19	24.38	121.88	24.38	48.75	243.75	36.56	73.13	365.63
$x = 24$	4.93	9.85	49.25	12.31	24.63	123.13	24.63	49.25	246.25	36.94	73.88	369.38
$x = 25$	4.95	9.90	49.50	12.38	24.75	123.75	24.75	49.50	247.50	37.13	74.25	371.25
$x = 26$	4.98	9.95	49.75	12.44	24.88	124.38	24.88	49.75	248.75	37.31	74.63	373.13
$x = 27$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 28$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 29$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00

NOTE: Values of alpha (α), where α is the number of successes in α + β Bernoulli trials, are given for each integer of offspring size (x) for sample sizes of 50, 100 and 500 (i.e., the value of α + β), and for asymptotic values of offspring fitness of 0.10, 0.25, 0.50 and 0.75 (i.e., the average maximum value of α/(α + β)). The corresponding values of β, the number of failures α + β Bernoulli trails, are not given at each value of x, but they can be deduced by subtracting α from the value of α + β.

B.3 Parameterizations of the Beta Distribution for the Artificial Fitness Curve in Fig. 2.4*C*.

Maximum α/(α+β) =		0.10			0.25			0.50			0.75	
Maximum (α+β) =	50	100	500	50	100	500	50	100	500	50	100	500
$x = 10$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 11$	0.15	0.30	1.50	0.38	0.75	3.75	0.75	1.50	7.50	1.13	2.25	11.25
$x = 12$	0.38	0.75	3.75	0.94	1.88	9.38	1.88	3.75	18.75	2.81	5.63	28.13
$x = 13$	0.75	1.50	7.50	1.88	3.75	18.75	3.75	7.50	37.50	5.63	11.25	56.25
$x = 14$	1.50	3.00	15.00	3.75	7.50	37.50	7.50	15.00	75.00	11.25	22.50	112.50
$x = 15$	2.50	5.00	25.00	6.25	12.50	62.50	12.50	25.00	125.00	18.75	37.50	187.50
$x = 16$	3.25	6.50	32.50	8.13	16.25	81.25	16.25	32.50	162.50	24.38	48.75	243.75
$x = 17$	3.75	7.50	37.50	9.38	18.75	93.75	18.75	37.50	187.50	28.13	56.25	281.25
$x = 18$	4.13	8.25	41.25	10.31	20.63	103.13	20.63	41.25	206.25	30.94	61.88	309.38
$x = 19$	4.40	8.80	44.00	11.00	22.00	110.00	22.00	44.00	220.00	33.00	66.00	330.00
$x = 20$	4.63	9.25	46.25	11.56	23.13	115.63	23.13	46.25	231.25	34.69	69.38	346.88
$x = 21$	4.75	9.50	47.50	11.88	23.75	118.75	23.75	47.50	237.50	35.63	71.25	356.25
$x = 22$	4.85	9.70	48.50	12.13	24.25	121.25	24.25	48.50	242.50	36.38	72.75	363.75
$x = 23$	4.93	9.85	49.25	12.31	24.63	123.13	24.63	49.25	246.25	36.94	73.88	369.38
$x = 24$	4.98	9.95	49.75	12.44	24.88	124.38	24.88	49.75	248.75	37.31	74.63	373.13
$x = 25$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 26$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 27$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 28$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 29$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00

NOTE: Values of alpha (α), where α is the number of successes in α + β Bernoulli trials, are given for each integer of offspring size (x) for sample sizes of 50, 100 and 500 (i.e., the value of α + β), and for asymptotic values of offspring fitness of 0.10, 0.25, 0.50 and 0.75 (i.e., the average maximum value of α/(α + β)). The corresponding values of β, the number of failures α + β Bernoulli trails, are not given at each value of x, but they can be deduced by subtracting α from the value of α + β.

B.4 Parameterizations of the Beta Distribution for the Artificial Fitness Curve in Fig. 2.4*D*.

Maximum α/(α+β) =		0.10			0.25			0.50			0.75	
Maximum (α+β) =	50	100	500	50	100	500	50	100	500	50	100	500
$x = 10$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 11$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 12$	0.05	0.10	0.50	0.13	0.25	1.25	0.25	0.50	2.50	0.38	0.75	3.75
$x = 13$	0.13	0.25	1.25	0.31	0.63	3.13	0.63	1.25	6.25	0.94	1.88	9.38
$x = 14$	0.25	0.50	2.50	0.63	1.25	6.25	1.25	2.50	12.50	1.88	3.75	18.75
$x = 15$	0.50	1.00	5.00	1.25	2.50	12.50	2.50	5.00	25.00	3.75	7.50	37.50
$x = 16$	0.75	1.50	7.50	1.88	3.75	18.75	3.75	7.50	37.50	5.63	11.25	56.25
$x = 17$	1.13	2.25	11.25	2.81	5.63	28.13	5.63	11.25	56.25	8.44	16.88	84.38
$x = 18$	1.63	3.25	16.25	4.06	8.13	40.63	8.13	16.25	81.25	12.19	24.38	121.88
$x = 19$	2.25	4.50	22.50	5.63	11.25	56.25	11.25	22.50	112.50	16.88	33.75	168.75
$x = 20$	2.75	5.50	27.50	6.88	13.75	68.75	13.75	27.50	137.50	20.63	41.25	206.25
$x = 21$	3.38	6.75	33.75	8.44	16.88	84.38	16.88	33.75	168.75	25.31	50.63	253.13
$x = 22$	3.88	7.75	38.75	9.69	19.38	96.88	19.38	38.75	193.75	29.06	58.13	290.63
$x = 23$	4.25	8.50	42.50	10.63	21.25	106.25	21.25	42.50	212.50	31.88	63.75	318.75
$x = 24$	4.50	9.00	45.00	11.25	22.50	112.50	22.50	45.00	225.00	33.75	67.50	337.50
$x = 25$	4.75	9.50	47.50	11.88	23.75	118.75	23.75	47.50	237.50	35.63	71.25	356.25
$x = 26$	4.88	9.75	48.75	12.19	24.38	121.88	24.38	48.75	243.75	36.56	73.13	365.63
$x = 27$	4.95	9.90	49.50	12.38	24.75	123.75	24.75	49.50	247.50	37.13	74.25	371.25
$x = 28$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 29$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00

NOTE: Values of alpha (α), where α is the number of successes in α + β Bernoulli trials, are given for each integer of offspring size (x) for sample sizes of 50, 100 and 500 (i.e., the value of α + β), and for asymptotic values of offspring fitness of 0.10, 0.25, 0.50 and 0.75 (i.e., the average maximum value of α/(α + β)). The corresponding values of β, the number of failures α + β Bernoulli trails, are not given at each value of x, but they can be deduced by subtracting α from the value of α + β.

B.5 Parameterizations of the Beta Distribution for the Artificial Fitness Curve in Fig. Fig. 2.4*E*.

Maximum α/(α+β) =		0.10			0.25			0.50			0.75	
Maximum (α+β) =	50	100	500	50	100	500	50	100	500	50	100	500
$x = 10$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$x = 11$	0.05	0.10	0.50	0.13	0.25	1.25	0.25	0.50	2.50	0.38	0.75	3.75
$x = 12$	0.09	0.17	0.85	0.21	0.43	2.13	0.43	0.85	4.25	0.64	1.28	6.38
$x = 13$	0.12	0.23	1.15	0.29	0.58	2.88	0.58	1.15	5.75	0.86	1.73	8.63
$x = 14$	0.21	0.42	2.10	0.53	1.05	5.25	1.05	2.10	10.50	1.58	3.15	15.75
$x = 15$	0.31	0.62	3.10	0.78	1.55	7.75	1.55	3.10	15.50	2.33	4.65	23.25
$x = 16$	0.45	0.90	4.50	1.13	2.25	11.25	2.25	4.50	22.50	3.38	6.75	33.75
$x = 17$	0.63	1.26	6.30	1.58	3.15	15.75	3.15	6.30	31.50	4.73	9.45	47.25
$x = 18$	0.85	1.70	8.50	2.13	4.25	21.25	4.25	8.50	42.50	6.38	12.75	63.75
$x = 19$	1.13	2.25	11.25	2.81	5.63	28.13	5.63	11.25	56.25	8.44	16.88	84.38
$x = 20$	1.45	2.90	14.50	3.63	7.25	36.25	7.25	14.50	72.50	10.88	21.75	108.75
$x = 21$	1.78	3.55	17.75	4.44	8.88	44.38	8.88	17.75	88.75	13.31	26.63	133.13
$x = 22$	2.15	4.30	21.50	5.38	10.75	53.75	10.75	21.50	107.50	16.13	32.25	161.25
$x = 23$	2.55	5.10	25.50	6.38	12.75	63.75	12.75	25.50	127.50	19.13	38.25	191.25
$x = 24$	2.95	5.90	29.50	7.38	14.75	73.75	14.75	29.50	147.50	22.13	44.25	221.25
$x = 25$	3.38	6.75	33.75	8.44	16.88	84.38	16.88	33.75	168.75	25.31	50.63	253.13
$x = 26$	3.75	7.50	37.50	9.38	18.75	93.75	18.75	37.50	187.50	28.13	56.25	281.25
$x = 27$	4.00	8.00	40.00	10.00	20.00	100.00	20.00	40.00	200.00	30.00	60.00	300.00
$x = 28$	4.25	8.50	42.50	10.63	21.25	106.25	21.25	42.50	212.50	31.88	63.75	318.75
$x = 29$	4.45	8.90	44.50	11.13	22.25	111.25	22.25	44.50	222.50	33.38	66.75	333.75
$x = 30$	4.63	9.25	46.25	11.56	23.13	115.63	23.13	46.25	231.25	34.69	69.38	346.88
$x = 31$	4.75	9.50	47.50	11.88	23.75	118.75	23.75	47.50	237.50	35.63	71.25	356.25
$x = 32$	4.83	9.65	48.25	12.06	24.13	120.63	24.13	48.25	241.25	36.19	72.38	361.88
$x = 33$	4.88	9.75	48.75	12.19	24.38	121.88	24.38	48.75	243.75	36.56	73.13	365.63
$x = 34$	4.93	9.85	49.25	12.31	24.63	123.13	24.63	49.25	246.25	36.94	73.88	369.38
$x = 35$	4.95	9.90	49.50	12.38	24.75	123.75	24.75	49.50	247.50	37.13	74.25	371.25
$x = 36$	4.98	9.95	49.75	12.44	24.88	124.38	24.88	49.75	248.75	37.31	74.63	373.13
$x = 37$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 38$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00
$x = 39$	5.00	10.00	50.00	12.50	25.00	125.00	25.00	50.00	250.00	37.50	75.00	375.00

NOTE: Values of alpha (α), where α is the number of successes in α + β Bernoulli trials, are given for each integer of offspring size (x) for sample sizes of 50, 100 and 500 (i.e., the value of α + β), and for asymptotic values of offspring fitness of 0.10, 0.25, 0.50 and 0.75 (i.e., the average maximum value of α/(α + β)). The corresponding values of β, the number of failures α + β Bernoulli trails, are not given, but they can be deduced by subtracting α from the value of α + β.

Appendix C: Values of Offspring Size (x) used in Chapter 2 Simulations

x-value	freq(*x*) A	μA	freq(*x*) B-D	μB	μC	μD	freq(*x*) E	μE
10	1	0.000	1	0.000	0.000	0.000	1	0.000
11	2	0.000	0	0.000	0.010	0.000	0	0.010
12	2	0.050	1	0.010	0.030	0.010	0	0.017
13	2	0.800	0	0.025	0.075	0.025	1	0.023
14	3	0.925	1	0.050	0.150	0.050	0	0.042
15	3	0.975	1	0.150	0.300	0.100	0	0.062
16	2	0.995	1	0.450	0.500	0.150	1	0.090
17	2	1.000	1	0.700	0.650	0.225	0	0.126
18	2	1.000	2	0.820	0.750	0.325	1	0.170
19	1	1.000	2	0.880	0.825	0.450	1	0.225
20			2	0.925	0.880	0.550	0	0.290
21			2	0.95	0.925	0.675	0	0.355
22			1	0.965	0.950	0.775	2	0.430
23			1	0.975	0.970	0.850	0	0.510
24			1	0.985	0.985	0.900	2	0.590
25			1	0.990	0.990	0.950	2	0.675
26			0	0.995	0.995	0.975	2	0.750
27			1	1.000	1.000	0.990	0	0.800
28			0	1.000	1.000	1.000	2	0.850
29			1	1.000	1.000	1.000	0	0.890
30							1	0.925
31							1	0.950
32							0	0.965
33							1	0.975
34							0	0.980
35							0	0.985
36							1	0.990
37							0	0.995
38							0	1.000
39							1	1.000

NOTE: "Freq(*x*)" is the frequency of the "*x*-value" in each dataset simulated for fitness curves A – E. True offspring fitness at a given integer of *x* is "μ" for curves A – E. Offspring fitness μA – μE correspond to values on curves in Fig. 2.4*A–E*, although herein k = 1.

Appendix D: Results Summary of Chapter 2 Simulations

D.1 Results Summary of Simulations Performed on the Fitness Curve Depicted in Fig. 2.4*A*.

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	0	0	0	0	0
		OES (SD)	19.3 (1.27)	21.2 (2.18)	20.2 (1.59)	18.1 (0.89)	19.3 (1.33)
0.10	50	P fitness (SD)	6.68 (0.88)	7.08 (1.04)	6.82 (1.48)	6.28 (0.73)	6.74 (0.82)
		Sig. Runs Tests	8.8	14.5	12.0	5.8	7.2
		a (SE)	-3.37 (1.13)	-4.90 (1.77)	4.71 (1.71)	10.2 (0.78)	9.95 (1.11)
		b (SE)	13.6 (0.74)	0.32 (0.12)	15.57 (1.08)	1.86 (0.48)	0.14 (0.04)
		Successes	0.1	0.3	0.1	0	0
		OES (SD)	18.2 (1.03)	19.2 (1.61)	18.7 (1.26)	17.3 (0.72)	18.1 (0.99)
0.10	100	P fitness (SD)	6.62 (0.59)	6.90 (0.68)	6.71 (0.74)	6.16 (0.50)	6.55 (0.55)
		Sig. Runs Tests	12.8	23.4	19.2	7.7	10
		a (SE)	-4.55 (1.30)	-6.27 (1.96)	6.16 (1.82)	10.4 (0.51)	10.3 (0.65)
		b (SE)	13.0 (0.51)	0.44 (0.14)	14.4 (0.61)	2.43 (0.49)	0.190 (0.04)
		Successes	9.1	15.4	12.8	0	0
		OES (SD)	15.8 (0.85)	15.6 (1.14)	15.7 (1.06)	16.1 (0.43)	16.3 (0.50)
0.10	500	P fitness (SD)	6.94 (0.36)	7.37 (1.45)	7.17 (0.79)	5.93 (0.22)	6.22 (0.24)
		Sig. Runs Tests	20.1	25.9	24.3	16	23
		a (SE)	-11.6 (3.56)	-19.7 (6.78)	18.1 (5.56)	10.5 (0.28)	10.6 (0.31)
		b (SE)	12.6 (0.22)	1.52 (0.52)	13.1 (0.25)	3.7 (0.58)	0.30 (0.05)
		Successes	0.4	1.2	0.8	0	0
		OES (SD)	17.4 (0.95)	17.9 (1.47)	17.7 (1.19)	16.9 (0.63)	17.4 (0.8)
0.25	50	P fitness (SD)	16.8 (1.22)	17.6 (1.57)	17.1 (1.41)	15.3 (0.99)	16.2 (1.07)
		Sig. Runs Tests	14.9	27.4	22.9	8.7	11.9
		a (SE)	-5.96 (1.71)	-8.73 (2.87)	8.28 (2.44)	10.5 (0.39)	10.5 (0.47)
		b (SE)	12.8 (0.4)	0.64 (0.21)	13.9 (0.47)	2.87 (0.51)	0.23 (0.04)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	2.7	6.3	4.7	0	0
		OES (SD)	16.4 (0.88)	16.4 (1.28)	16.4 (1.11)	16.4 (0.49)	16.7 (0.58)
0.25	100	P fitness (SD)	17.1 (1.02)	18.1 (1.42)	17.5 (1.25)	15.0 (0.70)	15.8 (0.75)
		Sig. Runs Tests	18.7	27.8	24.9	11.9	16.9
		a (SE)	-8.72 (2.68)	-14.2 (4.68)	13.0 (3.83)	10.6 (0.3)	10.6 (0.34)
		b (SE)	12.6 (0.28)	1.08 (0.35)	13.32 (0.33)	3.42 (0.54)	0.28 (0.04)
		Successes	36.3	56.3	51.1	0	0
		OES (SD)	14.8 (0.54)	14.4 (0.65)	14.6 (0.66)	15.5 (0.35)	15.7 (0.39)
0.25	500	P fitness (SD)	17.7 (0.55)	18.6 (0.93)	18.3 (0.90)	14.5 (0.32)	15.2 (0.35)
		Sig. Runs Tests	14.8	18.7	16.3	24.8	36
		a (SE)	-17.9 (4.02)	-30.7 (8.16)	28.6 (6.96)	10.6 (0.23)	10.6 (0.24)
		b (SE)	12.5 (0.12)	2.41 (0.63)	12.8 (0.14)	4.35 (0.66)	0.36 (0.05)
		Successes	7.5	14.1	11.4	0	0
		OES (SD)	15.8 (0.84)	15.6 (1.13)	15.7 (1.04)	16.1 (0.41)	16.3 (0.48)
0.50	50	P fitness (SD)	34.8 (1.97)	36.9 (2.65)	36.0 (2.49)	29.7 (1.13)	31.2 (1.22)
		Sig. Runs Tests	17.0	21.9	20.5	13.3	18.9
		a (SE)	-11.5 (3.65)	-19.9 (8.77)	18.1 (6.08)	10.6 (0.27)	10.6 (0.30)
		b (SE)	12.6 (0.22)	1.53 (0.64)	13.1 (0.26)	3.73 (0.58)	0.31 (0.05)
		Successes	21.2	36.0	31.6	0	0
		OES (SD)	15.1 (0.69)	14.8 (0.84)	14.9 (0.84)	15.8 (0.38)	16.0 (0.38)
0.50	100	P fitness (SD)	35.4 (1.48)	37.4 (1.89)	36.7 (1.95)	29.3 (0.83)	30.7 (0.90)
		Sig. Runs Tests	16.3	20.1	18.4	19.0	27.1
		a (SE)	-15.6 (4.26)	-27.6 (9.07)	25.4 (7.48)	10.6 (0.24)	10.6 (0.26)
		b (SE)	12.5 (0.16)	2.15 (0.70)	12.9 (0.18)	4.12 (0.62)	0.34 (0.05)
		Successes	86.9	89.0	91.4	0	0
		OES (SD)	14.4 (0.34)	13.9 (0.27)	13.9 (0.34)	15.3 (0.10)	15.3 (0.23)
0.50	500	P fitness (SD)	35.5 (0.59)	37.3 (0.74)	37.0 (0.78)	28.6 (0.39)	29.8 (0.43)
		Sig. Runs Tests	16.2	34.2	27.0	52.6	68.6
		a (SE)	-23.7 (3.78)	-43.7 (10.1)	41.2 (8.74)	10.6 (0.22)	10.6 (0.23)
		b (SE)	12.5 (0.07)	3.44 (0.78)	12.7 (0.09)	4.72 (0.75)	0.40 (0.06)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	38.3	54.8	51.0	0	0
		OES (SD)	14.8 (0.53)	14.3 (0.64)	14.5 (0.64)	15.6 (0.37)	15.8 (0.36)
0.75	50	P fitness (SD)	53.4 (1.93)	56.4 (2.57)	55.6 (2.49)	43.6 (0.97)	45.5 (1.04)
		Sig. Runs Tests	13.2	18.6	15.9	22.1	31.2
		a (SE)	-18.8 (4.59)	-34.1 (11.8)	31.6 (9.18)	10.6 (0.23)	10.6 (0.24)
		b (SE)	12.5 (0.12)	2.67 (0.89)	12.8 (0.14)	4.36 (0.67)	0.36 (0.06)
		Successes	71.8	79.0	79.3	0	0
		OES (SD)	14.4 (0.39)	14.0 (0.40)	14.1 (0.44)	15.3 (0.2)	15.4 (0.33)
0.75	100	P fitness (SD)	53.5 (1.27)	56.3 (1.57)	55.7 (1.61)	43.1 (0.71)	44.9 (0.77)
		Sig. Runs Tests	13.0	25.0	19.7	37.6	52.3
		a (SE)	-22.5 (4.29)	-41.5 (11.2)	38.8 (9.58)	10.6 (0.23)	10.6 (0.23)
		b (SE)	12.5 (0.09)	3.26 (0.87)	12.7 (0.11)	4.61 (0.73)	0.39 (0.06)
		Successes	99.8	86.4	94.4	0	0
		OES (SD)	14.1 (0.37)	13.8 (0.03)	13.8 (0.06)	15.3 (0.01)	15.3 (0.02)
0.75	500	P fitness (SD)	52.7 (0.58)	55.3 (0.66)	55.0 (0.64)	42.5 (0.35)	44.2 (0.38)
		Sig. Runs Tests	21.0	40.5	33.4	86.5	94.1
		a (SE)	-27.0 (2.94)	-48.8 (8.66)	46.8 (7.75)	10.6 (0.22)	10.6 (0.22)
		b (SE)	12.4 (0.05)	3.84 (0.67)	12.7 (0.06)	4.95 (0.82)	0.42 (0.07)

NOTE: Alpha (α) is the number of successes and beta (β) is the number of failures in α+β Bernoulli trials. Levels of asymptotic offspring survival (max α/(α+β)) and sample size (α+β) are indicated for each simulation (which comprises one combination of (max α/(α+β)) and (α+β)). For each simulation, we generated 50,000 datasets, then we fit each model to each of the 50,000 datasets to assess how well each model was able to estimate optimal offspring size. "Successes" is the percent of runs (out of 50,000) where the model estimated optimal size to within ± 5% of the true value. "OES (SD)" is the mean (± SD) value of optimal size estimated by the model; the true value of optimal size is 14.1, which can be derived from the fitness curve. "P fitness" is the mean (± SD) maximum value of parental fitness estimated by the model; the true maximum values are 6.61 (max α/(α+β) = 0.10), 16.5 ((max α/(α+β) = 0.25), 33.0 ((max α/(α+β) = 0.50), and 49.6 ((max α/(α+β) = 0.75)). "Sig. Runs Tests" is the percent of runs tests (out of 50,000) that were significant, with the null expectation that about 5 % will be significant due to chance alone. "a (SE)" and "b (SE)" are the mean parameter estimates and mean standard error given by each model (see main text for model statements).

D.2 Results Summary of Simulations Performed on the Fitness Curve Depicted in Fig. 2.4*B*.

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	2.0	0	1.7	15.8	1.0
		OES (SD)	24.4 (2.35)	27.5 (6.29)	25.6 (3.20)	21.1 (1.36)	24.4 (2.29)
0.10	50	P fitness (SD)	4.44 (0.58)	4.66 (0.68)	4.48 (1.07)	4.18 (0.47)	4.44 (0.53)
		Sig. Runs Tests	4.7	8.1	6.3	4.0	4.7
		a (SE)	-2.33 (0.84)	-3.46 (1.27)	3.27 (1.22)	10.6 (1.52)	10.0 (2.49)
		b (SE)	17.6 (1.43)	0.16 (0.06)	21.3 (2.09)	1.07 (0.29)	0.07 (0.02)
		Successes	0.2	0.1	0.2	33.1	0.7
		OES (SD)	23.5 (1.41)	26.1 (2.81)	24.5 (1.74)	20.4 (0.96)	23.0 (1.48)
0.10	100	P fitness (SD)	4.47 (0.40)	4.62 (0.47)	4.5 (0.43)	4.16 (0.33)	4.39 (0.36)
		Sig. Runs Tests	6.0	12.4	8.5	4.6	5.8
		a (SE)	-3.09 (0.90)	-4.24 (1.33)	4.17 (1.21)	10.8 (1.01)	10.6 (1.44)
		b (SE)	16.5 (0.97)	0.22 (0.07)	19.0 (1.07)	1.37 (0.28)	0.09 (0.02)
		Successes	3.0	3.6	3.0	93.3	17.6
		OES (SD)	21.4 (0.86)	22.0 (1.26)	21.8 (1.04)	19.0 (0.60)	20.7 (0.79)
0.10	500	P fitness (SD)	4.64 (0.20)	4.85 (0.22)	4.72 (0.22)	4.12 (0.14)	4.28 (0.15)
		Sig. Runs Tests	10.0	18.6	13.7	8.10	11.6
		a (SE)	-6.05 (1.17)	-8.21 (1.70)	8.05 (1.51)	10.9 (0.60)	11.0 (0.70)
		b (SE)	15.9 (0.42)	0.48 (0.10)	17.0 (0.43)	1.96 (0.29)	0.14 (0.02)
		Successes	0.1	0.3	0.2	40.5	0.7
		OES (SD)	23.0 (1.15)	24.7 (1.99)	23.8 (1.40)	20.2 (0.82)	22.4 (1.18)
0.25	50	P fitness (SD)	11.4 (0.84)	11.9 (0.99)	11.5 (0.91)	10.4 (0.67)	11.0 (0.71)
		Sig. Runs Tests	7.6	16.2	11.2	5.6	7.4
		a (SE)	-3.94 (1.05)	-5.41 (2.25)	5.27 (1.40)	11.0 (0.82)	11.1 (1.07)
		b (SE)	16.3 (0.76)	0.30 (0.13)	18.2 (0.81)	1.57 (0.28)	0.11 (0.02)
		Successes	0.9	1.4	1.0	75.3	4.1
		OES (SD)	22.1 (0.99)	22.9 (1.55)	22.5 (1.21)	19.5 (0.69)	21.4 (0.95)
0.25	100	P fitness (SD)	11.6 (0.62)	12.1 (0.72)	11.8 (0.68)	10.3 (0.47)	10.8 (0.50)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	9.0	18.6	13.5	6.8	9.3
		a (SE)	-5.19 (1.14)	-7.19 (1.73)	6.96 (1.52)	11.0 (0.65)	11.2 (0.78)
		b (SE)	16.0 (0.52)	0.41 (0.10)	17.4 (0.55)	1.82 (0.28)	0.13 (0.02)
		Successes	19.4	19.7	18.4	92.6	54
		OES (SD)	20.5 (0.63)	20.6 (0.77)	20.6 (0.72)	18.5 (0.51)	20.0 (0.53)
0.25	500	P fitness (SD)	11.8 (0.30)	12.3 (0.33)	12.1 (0.34)	10.2 (0.21)	10.6 (0.23)
		Sig. Runs Tests	9.7	14.2	12.3	11.6	17.9
		a (SE)	-8.36 (1.08)	-11.4 (1.77)	11.3 (1.57)	11.0 (0.52)	11.2 (0.56)
		b (SE)	15.8 (0.22)	0.68 (0.10)	16.7 (0.25)	2.26 (0.33)	0.16 (0.02)
		Successes	2.6	3.8	3.0	91.8	9.4
		OES (SD)	21.5 (0.85)	22.0 (1.22)	21.8 (1.02)	19.2 (0.58)	20.9 (0.76)
0.50	50	P fitness (SD)	23.3 (1.08)	24.5 (1.26)	23.8 (1.21)	20.5 (0.79)	21.4 (0.82)
		Sig. Runs Tests	8.8	16.6	12.6	7.5	10.2
		a (SE)	-6.14 (1.24)	-8.56 (1.92)	8.28 (1.67)	11.04 (0.59)	11.2 (0.68)
		b (SE)	15.9 (0.42)	0.5 (0.11)	17.1 (0.45)	1.98 (0.30)	0.14 (0.02)
		Successes	9.1	10.4	9.2	97.0	28.4
		OES (SD)	20.8 (0.71)	21.0 (0.92)	21.0 (0.84)	18.8 (0.50)	20.3 (0.61)
0.50	100	P fitness (SD)	23.6 (0.80)	24.7 (0.90)	24.1 (0.89)	20.47 (0.57)	21.3 (0.59)
		Sig. Runs Tests	8.6	13.9	11.4	9.6	13.7
		a (SE)	-7.53 (1.19)	-10.41 (1.91)	10.2 (1.68)	11.02 (0.54)	11.3 (0.59)
		b (SE)	15.9 (0.29)	0.62 (0.11)	16.8 (0.32)	2.16 (0.32)	0.15 (0.02)
		Successes	55.5	52.6	51.8	74.3	92.7
		OES (SD)	20.0 (0.41)	20.0 (0.49)	20.0 (0.47)	18.1 (0.32)	19.5 (0.50)
0.50	500	P fitness (SD)	23.7 (0.36)	24.6 (0.40)	24.2 (0.39)	20.4 (0.26)	21.0 (0.27)
		Sig. Runs Tests	9.4	10.4	9.9	20.1	35.1
		a (SE)	-9.83 (0.85)	-13.4 (1.53)	13.3 (1.34)	11.0 (0.50)	11.2 (0.52)
		b (SE)	15.8 (0.13)	0.81 (0.09)	16.5 (0.16)	2.43 (0.36)	0.17 (0.02)
		Successes	18.0	19.0	17.4	94.4	52.2
		OES (SD)	20.4 (0.59)	20.6 (0.72)	20.5 (0.67)	18.6 (0.50)	20.0 (0.48)
0.75	50	P fitness (SD)	35.5 (1.10)	37.0 (1.21)	36.3 (1.21)	30.6 (0.75)	31.7 (0.75)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	6.8	9.0	7.9	10.3	15.2
		a (SE)	-8.38 (1.22)	-11.6 (1.95)	11.4 (1.73)	11.0 (0.53)	11.2 (0.57)
		b (SE)	15.8 (0.24)	0.69 (0.11)	16.7 (0.27)	2.27 (0.34)	0.16 (0.02)
		Successes	38.2	37.0	35.6	86.9	80.7
		OES (SD)	20.1 (0.47)	20.2 (0.57)	20.2 (0.54)	18.3 (0.44)	19.7 (0.47)
0.75	100	P fitness (SD)	35.5 (0.76)	36.9 (0.83)	36.3 (0.83)	30.6 (0.53)	31.5 (0.54)
		Sig. Runs Tests	6.9	9.0	8.0	14.6	22.8
		a (SE)	-9.33 (1.02)	-12.8 (1.74)	12.7 (1.54)	11.0 (0.51)	11.2 (0.53)
		b (SE)	15.8 (0.17)	0.77 (0.10)	16.6 (0.20)	2.38 (0.35)	0.17 (0.02)
		Successes	91.8	89.0	89.0	42.2	99.9
		OES (SD)	19.8 (0.41)	19.8 (0.43)	19.8 (0.42)	18 (0.05)	19.1 (0.33)
0.75	500	P fitness (SD)	35.5 (0.34)	36.7 (0.38)	36.3 (0.37)	30.5 (0.24)	31.3 (0.25)
		Sig. Runs Tests	9.9	10.5	8.9	37.2	61.2
		a (SE)	-10.7 (0.64)	-14.5 (1.30)	14.5 (1.12)	11.0 (0.49)	11.2 (0.51)
		b (SE)	15.7 (0.09)	0.88 (0.08)	16.4 (0.11)	2.53 (0.39)	0.18 (0.02)

NOTE: Alpha (α) is the number of successes and beta (β) is the number of failures in α+β Bernoulli trials. Levels of asymptotic offspring survival (max α/(α+β)) and sample size (α+β) are indicated for each simulation (which comprises one combination of (max α/(α+β)) and (α+β)). For each simulation, we generated 50,000 datasets, then we fit each model to each of the 50,000 datasets to assess how well each model was able to estimate optimal offspring size. "Successes" is the percent of runs (out of 50,000) where the model estimated optimal size to within ± 5% of the true value. "OES (SD)" is the mean (± SD) value of optimal size estimated by the model; the true value of optimal size is 19.0, which can be derived from the fitness curve. "P fitness" is the mean (± SD) maximum value of parental fitness estimated by the model; the true maximum values are 4.68 (max α/(α+β) = 0.10), 11.7 ((max α/(α+β) = 0.25), 23.4 ((max α/(α+β) = 0.50), and 35.1 ((max α/(α+β) = 0.75)). "Sig. Runs Tests" is the percent of runs tests (out of 50,000) that were significant, with the null expectation that about 5% will be significant due to chance alone. "a (SE)" and "b (SE)" are the mean parameter estimates and mean standard error given by each model (see main text for model statements).

D.3 Results Summary of Simulations Performed on the Fitness Curve Depicted in Fig. 2.4*C*.

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	9.2	0.3	4.9	53.4	7.8
		OES (SD)	24.0 (2.70)	26.9 (6.94)	25.2 (3.67)	20.8 (1.49)	24.0 (2.62)
0.10	50	P fitness (SD)	4.28 (0.56)	4.52 (0.67)	4.35 (1.66)	4.08 (0.46)	4.31 (0.52)
		Sig. Runs Tests	4.1	5.3	4.5	3.7	4.1
		a (SE)	-2.17 (0.79)	-3.30 (1.21)	3.10 (1.17)	10.42 (1.58)	9.61 (2.76)
		b (SE)	17.5 (1.53)	0.16 (0.06)	21.4 (2.22)	1.04 (0.28)	0.07 (0.02)
		Successes	10.8	0.6	3.9	62.9	19.6
		OES (SD)	23.3 (1.52)	26.0 (2.99)	24.3 (1.82)	20.2 (0.99)	22.8 (1.51)
0.10	100	P fitness (SD)	4.31 (0.38)	4.47 (0.45)	4.34 (0.41)	4.08 (0.32)	4.28 (0.35)
		Sig. Runs Tests	4.3	6.5	5.2	3.9	4.4
		a (SE)	-2.89 (0.81)	-4.02 (1.20)	3.96 (1.12)	10.7 (1.02)	10.4 (1.49)
		b (SE)	16.3 (1.00)	0.21 (0.06)	18.9 (1.07)	1.35 (0.27)	0.09 (0.02)
		Successes	48.1	17.4	26.7	10.8	77.1
		OES (SD)	21.6 (0.85)	22.7 (1.24)	22.2 (0.99)	19.0 (0.63)	20.7 (0.86)
0.10	500	P fitness (SD)	4.39 (0.18)	4.57 (0.2)	4.46 (0.19)	4.06 (0.15)	4.21 (0.16)
		Sig. Runs Tests	3.9	10.3	5.9	6.7	6.0
		a (SE)	-4.78 (0.77)	-6.36 (1.14)	6.36 (1.02)	10.8 (0.55)	10.8 (0.64)
		b (SE)	15.6 (0.44)	0.37 (0.06)	17.0 (0.43)	1.89 (0.25)	0.13 (0.02)
		Successes	11.8	1.8	4.2	62.1	28.4
		OES (SD)	22.9 (1.21)	25.0 (1.94)	22.3 (0.30)	20.0 (0.86)	22.3 (1.25)
0.25	50	P fitness (SD)	10.9 (0.79)	11.4 (0.91)	10.9 (0.83)	10.2 (0.67)	10.7 (0.71)
		Sig. Runs Tests	4.5	7.7	5.8	4.3	4.6
		a (SE)	-3.46 (0.84)	-4.78 (1.27)	4.71 (1.16)	10.9 (0.80)	10.8 (1.08)
		b (SE)	16.1 (0.79)	0.26 (0.07)	18.2 (0.81)	1.53 (0.26)	0.10 (0.02)
		Successes	29.3	8.8	14	32.7	60.7
		OES (SD)	22.1 (0.96)	23.4 (1.44)	22.7 (1.13)	19.4 (0.71)	21.3 (0.98)
0.25	100	P fitness (SD)	10.9 (0.57)	11.4 (0.64)	11.1 (0.60)	10.1 (0.47)	10.6 (0.50)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	4.3	9.2	6.2	5.3	5.1
		a (SE)	-4.31 (0.82)	-5.89 (1.26)	5.82 (1.13)	10.9 (0.60)	11.0 (0.74)
		b (SE)	15.7 (0.55)	0.34 (0.07)	17.4 (0.55)	1.78 (0.25)	0.12 (0.02)
		Successes	91	61.6	74.5	0.1	71.4
		OES (SD)	20.8 (0.60)	21.4 (0.75)	21.2 (0.66)	18.5 (0.51)	20.0 (0.57)
0.25	500	P fitness (SD)	11.1 (0.26)	11.5 (0.28)	11.2 (0.28)	10.1 (0.22)	10.4 (0.22)
		Sig. Runs Tests	3.0	9.0	4.9	13.4	9.8
		a (SE)	-6.08 (0.59)	-8.05 (0.99)	8.11 (0.87)	10.9 (0.44)	11.0 (0.46)
		b (SE)	15.4 (0.23)	0.48 (0.06)	16.6 (0.24)	2.19 (0.26)	0.15 (0.02)
		Successes	49.6	18.8	28.0	78.3	13.4
		OES (SD)	21.6 (0.82)	22.6 (1.15)	22.1 (0.95)	20.8 (0.82)	19.1 (0.62)
0.50	50	P fitness (SD)	22.0 (0.97)	23.0 (1.07)	22.4 (1.03)	21.1 (0.82)	20.3 (0.80)
		Sig. Runs Tests	4.2	7.3	5.2	6.1	6.6
		a (SE)	-4.84 (0.81)	-6.59 (1.27)	6.53 (1.12)	0.13 (0.02)	1.93 (0.26)
		b (SE)	15.6 (0.46)	0.39 (0.07)	17.1 (0.46)	11.0 (0.63)	10.9 (0.53)
		Successes	77.2	41.3	54.9	82.7	1.8
		OES (SD)	21.1 (0.67)	21.8 (0.88)	21.5 (0.76)	20.3 (0.65)	20.2 (0.57)
0.50	100	P fitness (SD)	22.1 (0.7)	23.0 (0.76)	22.5 (0.74)	20.9 (0.59)	18.7 (0.53)
		Sig. Runs Tests	3.4	8.2	5.1	7.2	9.5
		a (SE)	-5.61 (0.71)	-7.52 (1.14)	7.52 (1.00)	0.15 (0.02)	2.10 (0.26)
		b (SE)	15.5 (0.32)	0.45 (0.06)	16.7 (0.32)	11.1 (0.51)	10.9 (0.47)
		Successes	99.1	95.8	98.8	30.7	0
		OES (SD)	20.3 (0.47)	20.8 (0.50)	20.7 (0.51)	19.5 (0.50)	18.1 (0.28)
0.50	500	P fitness (SD)	22.1 (0.31)	22.8 (0.33)	22.5 (0.33)	20.7 (0.27)	20.1 (0.26)
		Sig. Runs Tests	2.4	8.7	3.8	20.6	32.6
		a (SE)	-6.88 (0.41)	-8.97 (0.76)	9.12 (0.63)	0.17 (0.02)	2.36 (0.28)
		b (SE)	15.4(0.13)	0.54 (0.04)	16.4 (0.15)	11.1 (0.42)	10.9 (0.41)
		Successes	92.2	61.4	75.8	84.2	0.1
		OES (SD)	20.8 (0.57)	21.4 (0.71)	21.2 (0.64)	20.0 (0.54)	18.5 (0.51)
0.75	50	P fitness (SD)	33.2 (0.96)	34.4 (1.02)	33.7 (1.01)	31.2 (0.78)	30.3 (0.79)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	3.3	5.8	3.9	7.8	10.5
		a (SE)	-6.08 (0.71)	-8.09 (1.13)	8.13 (1.01)	0.15 (0.02)	2.19 (0.27)
		b (SE)	15.4 (0.28)	0.48 (0.06)	16.6 (0.28)	11.1 (0.49)	10.9 (0.45)
		Successes	98.4	84.7	93.6	52.5	0
		OES (SD)	20.6 (0.52)	21.1 (0.57)	20.9 (0.53)	19.7 (0.49)	18.3 (0.43)
0.75	100	P fitness (SD)	33.2 (0.69)	34.3 (0.72)	33.7 (0.71)	31.1 (0.56)	30.3 (0.56)
		Sig. Runs Tests	2.7	6.7	3.7	12.4	19.1
		a (SE)	-6.54 (0.55)	-8.59 (0.92)	8.69 (0.80)	0.16 (0.02)	2.3 (0.28)
		b (SE)	15.4 (0.19)	0.52 (0.05)	16.47 (0.20)	11.1 (0.44)	10.9 (0.42)
		Successes	99.4	100	100	0	4.3
		OES (SD)	20.1 (0.22)	20.6 (0.49)	20.3 (0.47)	18.0 (0.03)	19.2 (0.38)
0.75	500	P fitness (SD)	33.1 (0.32)	34.1 (0.33)	33.6 (0.33)	30.2 (0.25)	30.9 (0.26)
		Sig. Runs Tests	2.2	10.7	3.2	57.5	40.3
		a (SE)	-7.29 (0.29)	-9.43 (0.61)	9.62 (0.48)	10.9 (0.41)	11.1 (0.40)
		b (SE)	15.3 (0.09)	0.58 (0.04)	16.3 (0.10)	2.44 (0.30)	0.17 (0.02)

NOTE: Alpha (α) is the number of successes and beta (β) is the number of failures in α+β Bernoulli trials. Levels of asymptotic offspring survival (max α/(α+β)) and sample size (α+β) are indicated for each simulation (which comprises one combination of (max α/(α+β)) and (α+β)). For each simulation, we generated 50,000 datasets, then we fit each model to each of the 50,000 datasets to assess how well each model was able to estimate optimal offspring size. "Successes" is the percent of runs (out of 50,000) where the model estimated optimal size to within ± 5% of the true value. "OES (SD)" is the mean (± SD) value of optimal size estimated by the model; the true value of optimal size is 20.6, which can be derived from the fitness curve. "P fitness" is the mean (± SD) maximum value of parental fitness estimated by the model; the true maximum values are 4.85 (max α/(α+β) = 0.10), 12.1 ((max α/(α+β) = 0.25), 24.3 ((max α/(α+β) = 0.50), and 36.4 ((max α/(α+β) = 0.75)). "Sig. Runs Tests" is the percent of runs tests (out of 50,000) that were significant, with the null expectation that about 5 % will be significant due to chance alone. "a (SE)" and "b (SE)" are the mean parameter estimates and mean standard error given by each model (see main text for model statements).

D.4 Results Summary of Simulations Performed on the Fitness Curve Depicted in Fig. 2.4*D*.

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	10.9	2.9	5.1	27.9	18.0
		OES (SD)	28.3 (2.19)	30.5 (3.08)	29.5 (2.49)	23.4 (1.58)	27.7 (2.46)
0.10	50	P fitness (SD)	3.86 (0.72)	4.25 (0.85)	4.03 (0.79)	3.06 (0.57)	3.47 (2.22)
		Sig. Runs Tests	4.1	3.9	3.6	7.0	5.2
		a (SE)	-3.38 (1.10)	-5.46 (1.78)	5.09 (1.52)	11.5 (1.49)	11.6 (1.89)
		b (SE)	20.0 (1.03)	0.24 (0.09)	22.7 (1.37)	0.93 (0.26)	0.06 (0.02)
		Successes	24.9	12.1	16.0	13.5	34
		OES (SD)	27.0 (1.67)	28.3 (2.18)	27.8 (1.91)	22.8 (1.20)	26.4 (1.84)
0.10	100	P fitness (SD)	3.79 (0.49)	4.10 (0.56)	3.94 (0.53)	3.01 (0.29)	3.33 (0.93)
		Sig. Runs Tests	4.7	4.0	4.0	14.1	9.0
		a (SE)	-4.14 (1.08)	-6.34 (1.43)	6.07 (1.42)	11.6 (1.13)	11.8 (1.32)
		b (SE)	19.2 (0.68)	0.30 (0.07)	21.3 (0.76)	1.10 (0.24)	0.07 (0.01)
		Successes	73.7	59.3	65.0	0	64.1
		OES (SD)	25.2 (0.90)	25.7 (1.09)	25.5 (1.01)	21.9 (0.68)	24.6 (0.94)
0.10	500	P fitness (SD)	3.68 (0.20)	3.90 (0.22)	3.80 (0.21)	2.92 (0.13)	3.16 (0.15)
		Sig. Runs Tests	7.0	3.0	2.9	65.7	48.6
		a (SE)	-5.71 (0.80)	-8.20 (1.01)	8.13 (1.00)	11.6 (0.81)	12.0 (0.84)
		b (SE)	18.5 (0.30)	0.41 (0.05)	19.98 (0.27)	1.36 (0.24)	0.09 (0.01)
		Successes	34.4	21.7	25.2	11.0	41.6
		OES (SD)	26.5 (1.44)	27.3 (1.83)	27.0 (1.64)	22.8 (1.06)	26.0 (1.55)
0.25	50	P fitness (SD)	9.50 (0.99)	10.3 (1.10)	9.88 (1.06)	7.46 (0.61)	8.24 (0.72)
		Sig. Runs Tests	3.9	4.0	3.7	22.9	13.8
		a (SE)	-4.70 (1.04)	-7.15 (1.44)	6.88 (1.40)	11.8 (0.97)	12.1 (1.07)
		b (SE)	19.0 (0.53)	0.34 (0.07)	20.9 (0.56)	1.20 (0.24)	0.08 (0.01)
		Successes	58.7	44.2	49.1	1.7	60.7
		OES (SD)	25.6 (1.11)	26.1 (1.36)	25.9 (1.25)	22.3 (0.82)	25.2 (1.16)
0.25	100	P fitness (SD)	9.30 (0.67)	9.94 (0.74)	9.65 (0.71)	7.32 (0.42)	8.01 (0.50)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	4.1	3.8	3.7	44.0	28.9
		a (SE)	-5.44 (0.92)	-8.05 (1.26)	7.86 (1.22)	11.8 (0.83)	12.2 (0.87)
		b (SE)	18.7 (0.37)	0.40 (0.06)	20.3 (0.36)	1.33 (0.24)	0.09 (0.01)
		Successes	83.4	83.6	84.5	0	59.8
		OES (SD)	24.7 (0.64)	24.9 (0.74)	24.8 (0.70)	21.8 (0.51)	24.3 (0.64)
0.25	500	P fitness (SD)	9.08 (0.30)	9.61 (0.33)	9.39 (0.32)	7.22 (0.19)	7.81 (0.23)
		Sig. Runs Tests	7.0	3.7	3.3	91.6	80.9
		a (SE)	-6.29 (0.58)	-9.08 (0.71)	9.00 (0.70)	11.8 (0.70)	12.24 (0.68)
		b (SE)	18.4 (0.19)	0.46 (0.04)	19.7 (0.15)	1.50 (0.24)	0.10 (0.01)
		Successes	69.1	55.0	60.0	0.3	66.9
		OES (SD)	25.3 (0.95)	25.7 (1.16)	25.5 (1.07)	22.2 (0.73)	24.9 (0.99)
0.50	50	P fitness (SD)	18.5 (1.10)	19.7 (1.20)	19.1 (1.16)	14.6 (0.72)	15.9 (0.83)
		Sig. Runs Tests	3.4	3.7	3.5	51.5	34.8
		a (SE)	-5.70 (0.87)	-8.38 (1.20)	8.21 (1.17)	11.8 (0.79)	12.2 (0.81)
		b (SE)	18.6 (0.33)	0.42 (0.06)	20.0 (0.31)	1.38 (0.24)	0.09 (0.01)
		Successes	81.0	74.5	77.8	0	66.1
		OES (SD)	24.8 (0.75)	25.2 (0.90)	25.1 (0.84)	21.9 (0.59)	24.4 (0.78)
0.50	100	P fitness (SD)	18.3 (0.79)	19.4 (0.87)	18.9 (0.83)	14.5 (0.52)	15.7 (0.60)
		Sig. Runs Tests	4.1	3.5	3.3	74.6	57.8
		a (SE)	-6.07 (0.71)	-8.81 (0.95)	8.71 (0.92)	11.8 (0.74)	12.2 (0.73)
		b (SE)	18.4 (0.25)	0.44 (0.05)	19.8 (0.22)	1.45 (0.24)	0.10 (0.01)
		Successes	82.3	92.6	89.8	0	37.1
		OES (SD)	24.4 (0.49)	24.6 (0.53)	24.5 (0.52)	21.6 (0.49)	24.0 (0.42)
0.50	500	P fitness (SD)	18.1 (0.35)	19.0 (0.39)	18.6 (0.37)	14.4 (0.23)	15.5 (0.28)
		Sig. Runs Tests	27.8	3.3	3.4	98.9	93.8
		a (SE)	-6.59 (0.49)	-9.37 (0.48)	9.35 (0.49)	11.8 (0.70)	12.2 (0.68)
		b (SE)	18.3 (0.15)	0.48 (0.02)	19.5 (0.10)	1.53 (0.25)	0.10 (0.01)
		Successes	83.7	82.6	84.5	0	62.7
		OES (SD)	24.7 (0.64)	25.0 (0.75)	24.9 (0.71)	21.8 (0.52)	24.3 (0.67)
0.75	50	P fitness (SD)	27.3 (1.02)	28.9 (1.07)	28.3 (1.05)	21.7 (0.70)	23.4 (0.76)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	4.1	3.5	3.4	76.1	62.1
		a (SE)	-6.32 (0.75)	-9.10 (0.96)	9.02 (0.95)	11.8 (0.74)	12.2 (0.73)
		b (SE)	18.4 (0.25)	0.46 (0.05)	19.7 (0.21)	1.48 (0.25)	0.10 (0.01)
		Successes	81.9	89.2	87.6	0	47.3
		OES (SD)	24.5 (0.54)	24.7 (0.60)	24.6 (0.58)	21.7 (0.48)	24.0 (0.53)
0.75	100	P fitness (SD)	27.1 (0.73)	28.6 (0.77)	28.0 (0.75)	21.6 (0.50)	23.3 (0.55)
		Sig. Runs Tests	8.5	3.4	3.4	91.1	81.8
		a (SE)	-6.49 (0.61)	-9.27 (0.71)	9.24 (0.71)	11.8 (0.71)	12.2 (0.70)
		b (SE)	18.3 (0.19)	0.47 (0.04)	19.6 (0.15)	1.52 (0.25)	0.10 (0.01)
		Successes	80.6	95.2	91.4	0	16.7
		OES (SD)	24.2 (0.36)	24.5 (0.50)	24.4 (0.48)	21.5 (0.50)	23.9 (0.28)
0.75	500	P fitness (SD)	26.9 (0.33)	28.4 (0.37)	27.8 (0.35)	21.5 (0.23)	23.1 (0.26)
		Sig. Runs Tests	57.1	3.4	5.6	100	98.4
		a (SE)	-6.72 (0.45)	-9.52 (0.34)	9.52 (0.38)	11.8 (0.69)	12.2 (0.66)
		b (SE)	18.3 (0.14)	0.49 (0.02)	19.5 (0.07)	1.56 (0.25)	0.11 (0.01)

NOTE: Alpha (α) is the number of successes and beta (β) is the number of failures in α+β Bernoulli trials. Levels of asymptotic offspring survival (max α/(α+β)) and sample size (α+β) are indicated for each simulation (which comprises one combination of (max α/(α+β) and (α+β)). For each simulation, we generated 50,000 datasets, then we fit each model to each of the 50,000 datasets to assess how well each model was able to estimate optimal offspring size. "Successes" is the percent of runs (out of 50,000) where the model estimated optimal size to within ± 5% of the true value. "OES (SD)" is the mean (± SD) value of optimal size estimated by the model; the true value of optimal size is 25.0, which can be derived from the fitness curve. "P fitness" is the mean (± SD) maximum value of parental fitness estimated by the model; the true maximum values are 3.52 (max α/(α+β) = 0.10), 8.81 ((max α/(α+β) = 0.25), 17.6 ((max α/(α+β) = 0.50), and 26.4 ((max α/(α+β) = 0.75)). "Sig. Runs Tests" is the percent of runs tests (out of 50,000) that were significant, with the null expectation that about 5 % will be significant due to chance alone. "a (SE)" and "b (SE)" are the mean parameter estimates and mean standard error given by each model (see main text for model statements).

D.5 Results Summary of Simulations Performed on the Fitness Curve Depicted in Fig. 2.4*E*.

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Successes	16.6	1.3	6.8	3.8	30.8
		OES (SD)	33.8 (3.27)	37.7 (7.87)	35.9 (3.80)	25.3 (1.94)	32.3 (3.24)
0.10	50	P fitness (SD)	3.06 (0.47)	3.32 (0.64)	3.13 (0.51)	2.69 (0.51)	2.89 (0.37)
		Sig. Runs Tests	3.9	5.9	4.1	5.2	4.3
		a (SE)	-2.54 (0.88)	-3.48 (732.7)	3.75 (1.26)	12.1 (1.99)	12.2 (2.87)
		b (SE)	24.1 (1.71)	0.09 (73.19)	28.7 (2.37)	0.77 (0.20)	0.04 (0.01)
		Successes	35.2	6.4	17.8	0.1	48.2
		OES (SD)	32.2 (2.39)	35.8 (3.58)	33.7 (2.67)	24.0 (1.43)	29.9 (2.39)
0.10	100	P fitness (SD)	3.01 (0.33)	3.21 (0.39)	3.07 (0.35)	2.70 (0.24)	2.84 (0.50)
		Sig. Runs Tests	4.3	4.9	4.3	8.2	5.6
		a (SE)	-3.00 (0.84)	-4.52 (1.18)	4.3 (1.12)	11.7 (1.54)	12.0 (2.07)
		b (SE)	22.5 (1.24)	0.17 (0.05)	26.0 (1.29)	0.90 (0.19)	0.05 (0.01)
		Successes	80.8	52.7	68.4	0	17.6
		OES (SD)	30.1 (1.24)	31.5 (1.64)	30.9 (1.41)	22.7 (0.88)	27.4 (1.31)
0.10	500	P fitness (SD)	2.99 (0.14)	3.16 (0.16)	3.07 (0.15)	2.69 (0.11)	2.76 (0.11)
		Sig. Runs Tests	5.3	3.7	3.5	37.1	22.6
		a (SE)	-4.37 (0.66)	-6.23 (0.92)	6.17 (0.87)	11.7 (1.03)	12.2 (1.15)
		b (SE)	21.4 (0.58)	0.26 (0.04)	23.7 (0.48)	1.17 (0.19)	0.07 (0.01)
		Successes	45.7	14.7	25.7	0	55.1
		OES (SD)	31.6 (1.86)	34.1 (2.68)	32.89 (2.13)	24.2 (1.22)	29.5 (1.94)
0.25	50	P fitness (SD)	7.57 (0.65)	8.10 (0.74)	7.78 (0.70)	6.70 (0.49)	7.05 (0.52)
		Sig. Runs Tests	3.9	4.4	4.0	12.3	7.2
		a (SE)	-3.51 (0.85)	-5.27 (14.9)	5.04 (1.16)	12.1 (1.27)	12.6 (1.56)
		b (SE)	22.2 (0.98)	0.21 (1.42)	25.1 (0.96)	1.02 (0.19)	0.06 (0.01)
		Successes	69.5	34.3	48.8	0	41.9
		OES (SD)	30.64 (1.44)	32.37 (1.94)	31.6 (1.64)	23.5 (0.99)	28.3 (1.51)
0.25	100	P fitness (SD)	7.5 (0.45)	7.99 (0.51)	7.72 (0.48)	6.68 (0.35)	6.95 (0.37)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	3.7	4.2	3.8	22.4	13.1
		a (SE)	-4.04 (0.73)	-5.97 (1.10)	5.77 (1.01)	12.0 (1.05)	12.6 (1.19)
		b (SE)	21.7 (0.71)	0.25 (0.04)	24.2 (0.63)	1.13 (0.18)	0.07 (0.01)
		Successes	86.1	88.8	94.2	0	1.4
		OES (SD)	29.3 (0.83)	30.3 (1.00)	29.9 (0.89)	22.6 (0.73)	26.7 (0.84)
0.25	500	P fitness (SD)	7.39 (0.20)	7.8 (0.22)	7.6 (0.21)	6.66 (0.16)	6.83 (0.16)
		Sig. Runs Tests	4.8	3.5	3.5	68.5	52.1
		a (SE)	-4.90 (0.49)	-7.03 (0.66)	6.97 (0.62)	11.9 (0.87)	12.6 (0.89)
		b (SE)	21.1 (0.38)	0.3 (0.03)	23.11 (0.28)	1.31 (0.19)	0.08 (0.01)
		Successes	81.6	49.7	65.8	0	25.3
		OES (SD)	30.2 (1.21)	31.6 (1.56)	31.0 (1.35)	23.1 (0.87)	27.7 (1.26)
0.50	50	P fitness (SD)	15.0 (0.76)	15.9 (0.83)	154 (0.81)	13.4 (0.61)	13.8 (0.61)
		Sig. Runs Tests	3.5	4.0	3.7	29.5	17.2
		a (SE)	-4.35 (0.73)	-6.32 (1.05)	6.18 (0.98)	11.9 (1.01)	12.5 (1.13)
		b (SE)	21.5(0.64)	0.26 (0.04)	23.8 (0.54)	1.18 (0.19)	0.07 (0.01)
		Successes	87.4	75.9	86.5	0	7.6
		OES (SD)	29.6 (0.98)	30.7 (1.22)	30.3 (1.08)	22.8 (0.78)	27.1 (1.02)
0.50	100	P fitness (SD)	14.9 (0.53)	15.8 (0.58)	15.3 (0.56)	13.4 (0.42)	13.7 (0.43)
		Sig. Runs Tests	3.5	3.6	3.6	50.4	34.5
		a (SE)	-4.75 (0.60)	-6.85 (0.84)	6.76 (0.79)	11.9 (0.91)	12.6 (0.96)
		b (SE)	21.3 (0.47)	0.29 (0.03)	23.3 (0.37)	1.27 (0.19)	0.08 (0.01)
		Successes	78.4	99.5	97.9	0	0
		OES (SD)	28.9 (0.75)	29.8 (0.63)	29.5 (0.54)	22.1 (0.36)	26.3 (0.57)
0.50	500	P fitness (SD)	14.7 (0.23)	15.5 (0.26)	15.1 (0.25)	13.3 (0.19)	13.6 (0.20)
		Sig. Runs Tests	16.7	3.5	4.3	87.7	80.1
		a (SE)	-5.23 (0.41)	-7.39 (0.43)	7.4 (0.43)	11.8 (0.86)	12.5 (0.87)
		b (SE)	21.0 (0.29)	0.32 (0.02)	22.9 (0.17)	1.36 (0.20)	0.08 (0.01)
		Successes	85.5	88.9	94.0	0	1.3
		OES (SD)	29.3 (0.85)	30.4 (1.00)	29.9 (0.91)	22.5 (0.73)	26.7 (0.87)
0.75	50	P fitness (SD)	22.3 (0.71)	23.4 (0.76)	22.9 (0.75)	20.0 (0.62)	20.5 (0.59)

Max α/(α+β)	α+β		Weibull-1	Logistic	Hill	Asymptotic 1	Asymptotic 2
		Sig. Runs Tests	3.6	3.3	3.5	51.3	36.5
		a (SE)	-4.92 (0.62)	-7.02 (0.83)	6.98 (0.79)	11.87 (0.91)	12.5 (0.97)
		b (SE)	21.2 (0.47)	0.30 (0.03)	23.1 (0.35)	1.30 (0.20)	0.08 (0.01)
		Successes	83.0	97.5	97.1	0	0
		OES (SD)	29.0 (0.74)	30.0 (0.80)	29.6 (0.69)	22.3 (0.57)	26.5 (0.67)
0.75	100	P fitness (SD)	22.2(0.51)	23.3 (0.55)	22.8 (0.53)	20.0 (0.45)	20.5 (0.43)
		Sig. Runs Tests	5.7	3.2	3.4	72.1	58.5
		a (SE)	-5.12 (0.50)	-7.25 (0.62)	7.26 (0.61)	11.9 (0.88)	12.5 (0.90)
		b (SE)	21.1 (0.36)	0.31 (0.03)	23.0 (0.25)	1.34 (0.20)	0.08 (0.01)
		Successes	68.6	99.3	99.1	0	0
		OES (SD)	28.5 (0.70)	29.5 (0.25)	29.4 (0.41)	22.0 (0.05)	26.2 (0.59)
0.75	500	P fitness (SD)	22.1 (0.23)	23.1 (0.25)	22.6 (0.24)	20.0 (0.20)	20.3 (0.19)
		Sig. Runs Tests	37.2	3.0	5.8	93.9	90.9
		a (SE)	-5.39 (0.38)	-7.57 (0.31)	7.63 (0.34)	11.8 (0.85)	12.5 (0.85)
		b (SE)	20.9 (0.27)	0.33 (0.01)	22.8 (0.13)	1.40 (0.20)	0.08 (0.01)

NOTE: Alpha (α) is the number of successes and beta (β) is the number of failures in α+β Bernoulli trials. Levels of asymptotic offspring survival (max α/(α+β)) and sample size (α+β) are indicated for each simulation (which comprises one combination of (max α/(α+β) and (α+β)). For each simulation, we generated 50,000 datasets, then we fit each model to each of the 50,000 datasets to assess how well each model was able to estimate optimal offspring size. "Successes" is the percent of runs (out of 50,000) where the model estimated optimal size to within ± 5% of the true value. "OES (SD)" is the mean (± SD) value of optimal size estimated by the model; the true value of optimal size is 30.0, which can be derived from the fitness curve. "P fitness" is the mean (± SD) maximum value of parental fitness estimated by the model; the true maximum values are 3.08 (max α/(α+β) = 0.10), 7.71 ((max α/(α+β) = 0.25), 15.4 ((max α/(α+β) = 0.50), and 23.1 ((max α/(α+β) = 0.75)). "Sig. Runs Tests" is the percent of runs tests (out of 50,000) that were significant, with the null expectation that about 5 % will be significant due to chance alone. "a (SE)" and "b (SE)" are the mean parameter estimates and mean standard error given by each model (see main text for model statements).

Appendix E: Summary of Releases by Dam, Offspring Crosstype, and Stream (Chapter 3).

♀	♀ cross-type	Egg Mass (mg)	River	Eco×Eco	E inbred	Grv×Grv	G inbred	Stw×Stw	S inbred	Eco×Grv	Eco×Stw	Grv×Stw	Grv×GS	GS×GS	Stw×GS	Eco×ES	Stw×ES	ES×ES	No. Families	Total
1			E	-	-	-	-	-	-	-	-	-	-	-	-	68	49	94a	4	211
1	ES	45.4	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
1			S	-	-	-	-	-	-	-	-	-	-	-	-	34	49	47[a]	4	130
2			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
2	GS	35.1	G	-	-	-	-	-	-	-	-	-	86	146[a]	58	-	-	-	4	290
2			S	-	-	-	-	-	-	-	-	-	43	73[a]	29	-	-	-	4	145
3			E	-	-	14[a]	-	-	-	40	-	-	-	-	-	-	-	-	3	54
3	G	48.8	G	-	-	14[a]	55[b]	-	-	40	-	18	2	-	-	-	-	-	8	129
3			S	-	-	14[a]	-	-	-	-	-	18	1	-	-	-	-	-	4	33
4			E	23[a]	20[a]	-	-	-	-	21	40	-	-	-	-	34	-	-	7	138
4	E	29.2	G	23[a]	-	-	-	-	-	21	-	-	-	-	-	-	-	-	3	44
4			S	23[a]	-	-	-	-	-	-	40	-	-	-	-	17	-	-	4	80
5			E	37[a]	-	-	-	-	-	22	4	-	-	-	-	18	-	-	5	81
5	E	28.6	G	37[a]	-	-	-	-	-	22	-	-	-	-	-	-	-	-	3	59
5			S	37[a]	-	-	-	-	-	-	4	-	-	-	-	9	-	-	4	50
6			E	33[a]	38[a]	-	-	-	-	14	3	-	-	-	-	18	-	-	7	106
6	E	30.4	G	33[a]	-	-	-	-	-	14	-	-	-	-	-	-	-	-	3	47
6			S	33[a]	-	-	-	-	-	-	3	-	-	-	-	9	-	-	4	45
7			E	-	-	-	-	4[a]	-	-	32	-	-	-	-	-	28	-	4	64
7	S	39.5	G	-	-	-	-	4[a]	-	-	-	10	-	-	26	-	-	-	4	40
7			S	-	-	-	-	4[a]	4	-	32	10	-	-	13	-	28	-	7	91
8			E	-	-	-	-	-	-	-	-	-	-	-	-	64	34	90[a]	4	188
8	ES	40.6	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
8			S	-	-	-	-	-	-	-	-	-	-	-	-	32	34	45[a]	4	111
9			E	-	-	-	-	34[a]	-	-	53	-	-	-	-	-	36	-	4	123

♀	♀ cross-type	Egg Mass (mg)	River	Eco×Eco	E inbred	Grv×Grv	G inbred	Stw×Stw	S inbred	Eco×Grv	Eco×Stw	Grv×Stw	Grv×GS	GS×GS	Stw×GS	Eco×ES	Stw×ES	ES×ES	No. Families	Total
9	S	40	G	-	-	-	-	34[a]	-	-	-	55	-	-	88	-	-	-	4	177
9			S	-	-	-	-	34[a]	105	-	53	55	-	-	44	-	36	-	7	327
10			E	-	-	-	-	7[a]	-	-	4	-	-	-	-	-	11	-	4	22
10	S	30.3	G	-	-	-	-	7[a]	-	-	-	-	-	-	6	-	-	-	3	13
10			S	-	-	-	-	7[a]	-	-	4	-	-	-	3	-	11	-	5	25
11			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
11	S	26.8	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
11			S	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	1	14
12			E	-	-	-	-	-	-	22	-	-	-	-	-	-	-	-	1	22
12	E	34.6	G	-	-	-	-	-	-	22	-	-	-	-	-	-	-	-	1	22
12			S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
13			E	-	-	-	-	-	-	-	-	-	-	-	-	76	29	92[a]	4	197
13	ES	39.2	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
13			S	-	-	-	-	-	-	-	-	-	-	-	-	38	29	46[a]	4	113
14			E	17[a]	38[a]	-	-	-	-	33	17	-	-	-	-	38	-	-	7	143
14	E	41.1	G	17[a]	-	-	-	-	-	33	-	-	-	-	-	-	-	-	3	50
14			S	17	-	-	-	-	-	-	17	-	-	-	-	19	-	-	4	53
15			E	-	-	-	-	-	-	-	-	-	-	-	-	10	20	32[a]	4	62
15	ES	25.9	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
15			S	-	-	-	-	-	-	-	-	-	-	-	-	5	20	16[a]	4	41
16			E	23[a]	16	-	-	-	-	9	17	-	-	-	-	20	-	-	6	85
16	E	26.2	G	23[a]	-	-	-	-	-	9	-	-	-	-	-	-	-	-	3	32
16			S	23[a]	-	-	-	-	-	-	17	-	-	-	-	10	-	-	4	50
17			E	-	-	-	-	-	-	-	-	-	-	-	-	48	-	-	1	48
17	ES	30.3	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
17			S	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	1	24
18			E	-	-	-	-	-	-	-	-	-	-	-	-	80	28	38[a]	4	146
18	ES	31.9	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
18			S	-	-	-	-	-	-	-	-	-	-	-	-	40	28	19[a]	4	87
19			E	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	1	25
19	G	26.5	G	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	1	25
19			S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0

♀	♀ cross-type	Egg Mass (mg)	River	Eco×Eco	E inbred	Grv×Grv	G inbred	Stw×Stw	S inbred	Eco×Grv	Eco×Stw	Grv×Stw	Grv×GS	GS×GS	Stw×GS	Eco×ES	Stw×ES	ES×ES	No. Families	Total
20			E	-	-	-	-	57[a]	-	-	55	-	-	-	-	-	5	-	4	117
20	S	31.9	G	-	-	-	-	57[a]	-	-	-	40	-	-	92	-	-	-	4	189
20			S	-	-	-	-	57[a]	-	-	55	40	-	-	46	-	5	-	6	203
21			E	-	-	31[a]	-	-	-	20	-	-	-	-	-	-	-	-	3	51
21	G	29.4	G	-	-	31[a]	36[a]	-	-	20	-	64	60	-	-	-	-	-	7	211
21			S	-	-	31[a]	-	-	-	-	-	64	30	-	-	-	-	-	4	125
22			E	-	-	-	-	-	-	-	-	-	-	-	-	34	33	68[a]	4	135
22	ES	28.2	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
22			S	-	-	-	-	-	-	-	-	-	-	-	-	17	33	34[a]	4	84
23			E	-	-	39[a]	-	-	-	35	-	-	-	-	-	-	-	-	3	74
23	G	30.3	G	-	-	39[a]	36[a]	-	-	35	-	45	36	-	-	-	-	-	7	191
23			S	-	-	39[a]	-	-	-	-	-	45	18	-	-	-	-	-	3	102
24			E	-	-	13	-	-	-	14	-	-	-	-	-	-	-	-	2	27
24	G	27.3	G	-	-	13	-	-	-	14	-	-	32	-	-	-	-	-	3	59
24			S	-	-	13	-	-	-	-	-	-	16	-	-	-	-	-	2	29
25			E	30[a]	24[a]	-	-	-	-	14	16	-	-	-	-	16	-	-	7	100
25	E	26.4	G	30[a]	-	-	-	-	-	14	-	-	-	-	-	-	-	-	3	44
25			S	30[a]	-	-	-	-	-	-	16	-	-	-	-	8	-	-	4	54
26			E	-	-	39[a]	-	-	-	-	-	-	-	-	-	-	-	-	2	39
26	G	31.2	G	-	-	39[a]	-	-	-	-	-	-	48	-	-	-	-	-	3	87
26			S	-	-	39[a]	-	-	-	-	-	-	24	-	-	-	-	-	3	63
27			E	12[a]	39[a]	-	-	-	-	14	17	-	-	-	-	22	-	-	7	104
27	E	30.2	G	12[a]	-	-	-	-	-	14	-	-	-	-	-	-	-	-	3	26
27			S	12[a]	-	-	-	-	-	-	17	-	-	-	-	11	-	-	4	40
28			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
28	GS	23.9	G	-	-	-	-	-	-	-	-	-	22	34[a]	14	-	-	-	4	70
28			S	-	-	-	-	-	-	-	-	-	11	17	7	-	-	-	4	35
29			E	-	-	-	-	-	-	-	-	-	-	-	-	56	33	90[a]	4	179
29	ES	29.3	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
29			S	-	-	-	-	-	-	-	-	-	-	-	-	28	33	45	4	106
30			E	-	-	-	-	25[a]	-	-	-	-	-	-	-	-	16	-	3	41
30	S	21.9	G	-	-	-	-	25[a]	-	-	-	41	-	-	10	-	-	-	4	76

♀	♀ cross-type	Egg Mass (mg)	River	Eco×Eco	E inbred	Grv×Grv	G inbred	Stw×Stw	S inbred	Eco×Grv	Eco×Stw	Grv×Stw	Grv×GS	GS×GS	Stw×GS	Eco×ES	Stw×ES	ES×ES	No. Families	Total
30			S	-	-	-	-	25[a]	16	-	-	41	-	-	5	-	16	-	6	103
31			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
31	S	24.4	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
31			S	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	1	7
32			E	-	-	-	-	33[a]	-	-	25	-	-	-	-	-	20	-	4	78
32	S	23.8	G	-	-	-	-	33[a]	-	-	-	11	-	-	15	-	-	-	4	59
32			S	-	-	-	-	33[a]	20	-	25	11	-	-	15	-	20	-	7	124
33			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
33	GS	36.6	G	-	-	-	-	-	-	-	-	-	86	152[a]	26	-	-	-	4	264
33			S	-	-	-	-	-	-	-	-	-	43	76	13	-	-	-	4	132
34			E	-	-	8[a]	-	-	-	18	-	-	-	-	-	-	-	-	3	26
34	G	40.6	G	-	-	8[a]	16	-	-	18	-	18	26	-	-	-	-	-	6	86
34			S	-	-	8[a]	-	-	-	-	-	18	13	-	-	-	-	-	4	39
35			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
35	GS	28.6	G	-	-	-	-	-	-	-	-	-	44	110[a]	64	-	-	-	4	218
35			S	-	-	-	-	-	-	-	-	-	22	55[a]	32	-	-	-	4	109
36			E	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	1	25
36	E	40.3	G	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	1	25
36			S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
37			E	-	-	12[a]	-	-	-	11	-	-	-	-	-	-	-	-	3	23
37	G	23.7	G	-	-	12[a]	32[a]	-	-	11	-	27	26	-	-	-	-	-	7	108
37			S	-	-	12[a]	-	-	-	-	-	27	13	-	-	-	-	-	4	52
38			E	-	-	-	-	-	-	-	-	-	-	-	-	32	25	-	2	57
38	ES	31.4	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
38			S	-	-	-	-	-	-	-	-	-	-	-	-	16	25	-	2	41
39			E	-	-	19	-	-	-	13	-	-	-	-	-	-	-	-	2	32
39	G	24.6	G	-	-	19	-	-	-	13	-	26	-	-	-	-	-	-	3	58
39			S	-	-	19	-	-	-	-	-	-	-	-	-	-	-	-	1	19
40			E	-	-	-	-	15	-	-	42	-	-	-	-	-	21	-	3	78
40	S	24.9	G	-	-	-	-	15	-	-	-	-	-	-	-	-	-	-	1	15
40			S	-	-	-	-	15	9	-	42	-	-	-	-	-	21	-	4	87
41			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0

♀	♀ cross-type	Egg Mass (mg)	River	Eco×Eco	E inbred	Grv×Grv	G inbred	Stw×Stw	S inbred	Eco×Grv	Eco×Stw	Grv×Stw	Grv×GS	GS×GS	Stw×GS	Eco×ES	Stw×ES	ES×ES	No. Families	Total
41	GS	28.4	G	-	-	-	-	-	-	-	-	-	64	116[a]	48	-	-	-	4	228
41			S	-	-	-	-	-	-	-	-	-	32	58[a]	24	-	-	-	4	114
42			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
42	GS	29.4	G	-	-	-	-	-	-	-	-	-	50	78	34	-	-	-	3	162
42			S	-	-	-	-	-	-	-	-	-	25	39	17	-	-	-	3	81
43			E	-	-	-	-	-	-	-	28	-	-	-	-	-	-	-	1	28
43	S	26.9	G	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	1	25
43			S	-	-	-	-	-	-	-	28	25	-	-	-	-	-	-	2	53
44			E	-	-	-	-	-	-	-	-	-	-	-	-	66	54	54[a]	4	174
44	ES	38.4	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
44			S	-	-	-	-	-	-	-	-	-	-	-	-	33	54	27	4	114
45			E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
45	GS	34.2	G	-	-	-	-	-	-	-	-	-	118	64	234	-	-	-	3	416
45			S	-	-	-	-	-	-	-	-	-	62	32	117	-	-	-	3	211

a - Total number of offspring from 2 different full-sib families of the same cross-type, arising from matings with 2 different sires

b - Total number of offspring from 3 different full-sib families of the same cross-type, arising from matings with 3 different sires

NOTE: 'River' is the Economy (E), Great Village (G) or Stewiacke (S) River. All values given in the table are values for each stream within river. The number of full-sib families released from a given dam in each stream within river is 'No. Families'. For example, 49 offspring from dam 1 (♀ 1) of the genotype *Stw×ES* were released into each of the three streams in the Economy and Stewiacke Rivers, but no offspring were released into any streams in the Great Village River. The number of offspring released from each dam in each stream within river is 'Total'. Note that 'Total' represents the denominator of the division that was used to determine survival for a given level of egg size in a given stream. For example, survival for an egg size of 45.5 mg (produced by dam 1) in a given Economy stream was determined by the number of offspring recaptured in the relevant stream divided by 211.

Literature Cited

Abrams P (2001) Adaptationism, optimality models, and tests of adaptive scenarios. In: Orzack SH, Sober E (eds) Adaptationism and optimality. Cambridge University Press, New York, pp 273–302

Achord S, Levin PS, Zabel RW (2003) Density-dependent mortality in Pacific salmon: the ghost of impacts past? Ecology Letters 6:335–342

Allen RM, Buckley YM, Marshall DJ (2008) Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. American Naturalist 171:225 – 337

Altwegg R, Reyer HU (2003) Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882

Anibas C, Fleckenstein JH, Volze N, Buis K, Verhoeven R, Meire P, Batelaan O (2009) Transient or steady-state? Using vertical temperature profiles to quantify groundwater-surface water exchange. Hydrological Processes 23:2165–2177

Armstrong JD, Nislow KH (2006) Special section critical habitat during the transition from maternal provisioning in freshwater fish , with emphasis on Atlantic salmon (Salmo salar) and brown trout (Salmo trutta). Journal of Zoology 269:403–413

Arnold S, Wade M (1984a) On the measurement of natural and sexual selection: theory. Evolution 39:709–719

Arnold S, Wade M (1984b) On the measurement of natural and sexual selection: applications. Evolution 38:720–734

Bailey MM, Kinnison MT (2010) Habitat-mediated size selection in endangered Atlantic salmon fry: selectional restoration assessment. Evolutionary Applications 3:352–362

Bams R. (1969) Symposium on Salmon and Trout in Streams. In: Northcote T. (ed) Symposium on Salmon and Trout in Streams. Institute of Fisheries, University of British Columbia, Vancouver, pp 71–87

Bashey F (2006) Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy Poecilia reticulata. Evolution 60:348–361

Bashey F (2008) Competition as a selective mechanism for larger offspring size in guppies. Oikos 117:104–113

Bauerfiend SS, Fischer K (2008) Maternal body size as a morphological constraint on egg size and fecundity in butterflies. Basic and Applied Ecology 9:443–451

Beacham T, Murray C (1985) Effect of female size, egg size, and water temperature on developmental biology of chum salmon (Oncorhynchus keta) from the Ninat River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 42:1755–1765

Beacham T, Murray C (1990) Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. Transactions of the American Fisheries Society 119:927–941

Beck CW, Beck RE (2005) The effect of packing constraints on optimal investment in offspring. Evolutionary Ecology Research 7:1077–1088

Beckerman AP, Benton TG, Lapsley CT, Koesters N (2006) How effective are maternal effects at having effects? Proceedings of the Royal Society B: Biological Sciences 273:485–493

Van Den Berghe EP, Gross MR (1989) Natural selection resulting from female breeding competition in a Pacific salmon (coho: Oncorhynchus kisutch). Evolution 43:125–140

Bernardo J (1996) The particular maternal effects of propagule size, especially egg size: patterns, models, quality of evidence and interpretation. American Zoologist 36:216–236

Biga H, Janssen J, Marsden JE (1998) Effect of substrate size on lake trout egg predation by mottled sculpin. Journal of Great Lakes Research 24:464–473

Bjornn TC, Reiser DW (1991) Habitat requirements of salmonids in streams. American Fisheries Society Special Publication 19:83–138

Bohlin T, Hamrin S, Heggberget T, Rasmussen G, Saltveit S (1989) Electrofishing—theory and practice with special emphasis on salmonids. Hydrobiologia 173:9–43

Bonabeau E, Deneubourg J-L, Theraulaz G (1998) Within-brood competition and the optimal partitioning of parental investment. American Naturalist 153:419–427

Boudreault A (1984) Methodologie utilisee pour la photointerpretation des rivieres a saumon de la Cote-Nord. Loretteville, Quebec

Bowden R, Harms H, Paitz R, Janzen F (2004) Does optimal egg size vary with demographic stage because of physiological constraint? Functional Ecology 18:522–529

Bownds C, Wilson R, Marshall DJ (2010) Why do colder mothers produce larger eggs? An optimality approach. The Journal of Experimental Biology 213:3796–3801

Brockelman WY (1975) Competition, the fitness of offspring, and optimal clutch size. American Naturalist 109:677–699

Brown GP, Shine R (2009) Beyond size-number trade-offs: clutch size as a maternal effect. Philosophical transactions of the Royal Society of London Series B, Biological sciences 364:1097–106

Bujold V, Cunjak RA, Dietrich JP, Courtemanche DA (2004) Drifters versus residents : assessing size and age differences in Atlantic salmon (Salmo salar) fry. Canadian Journal of Fisheries and Aquatic Sciences 282:273–282

Burnham KP, Anderson DR (2002)Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach, 2nd edn. Springer, New York

Caley MJ, Schwarzkopf L, Shine R (2001) Does total reproductive effort evolve independently of offspring size? Evolution 55:1245–1248

Capinera JL (1979) Qualitative variation in plants and insects: effect of propagule size on ecological plasticity. American Naturalist 114:350–361

Carrière Y, Roff DA (1995) The evolution of offspring size and number: a test of the Smith–Fretwell model in three species of crickets. Oecologia 102:389–396

Charnov EL, Downhower JF, Brown LP (1995) Optimal offspring size in small litters. Evolutionary Ecology 9:57–63

Charpentier A, Anand M, Bauch CT (2012) Variable offspring size as an adaptation to environmental heterogeneity in a clonal plant species: integrating experimental and modelling approaches. Journal of Ecology 100:184–195

Chotkowski MA, Marsden JE (1999) Round goby and mottled sculpin predation on lake trout eggs and fry: field predictions from laboratory experiments. Journal of Great Lakes Research 25:26–35

Congdon J, Gibbons J (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

Congdon J.D., Nagle RD, Dunham AE, Beck CW, Kinney OM, Yeomans SR (1999) The relationship of body size to survivorship of hatchling snapping turtles (Chelydra serpentina): an evaluation of the “bigger is better” hypothesis. Oecologia 121:224–235

Crecco VA, Savoy TF (1985) Effects of biotic and abiotic factors on growth and relative survival of young American shad, Alosa sapidissima, in the Connecticut River. Canadian Journal of Fisheries and Aquatic Sciences:1640–1648

Creighton JC (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. Behavioral Ecology 16:1031–1036

Crisp DT, Carling PA (1989) Observations on siting, dimensions and structure of salmonid redds. Journal of Fish Biology 34:119–134

Crump ML (1981) Variation in propagule size as a function of environmental uncertainty for tree frogs. American Naturalist 177:724–737

Cutts C., Metcalfe NB, Taylor AC (1999) Competitive asymmetries in territorial juvenile Atlantic salmon. Oikos 86:479–486

Czesak ME, Fox CW (2003) Evolutionary ecology of egg size and number in a seed beetle : genetic trade-off differs between environments. Evolution 57:1121–1132

DeVries P (1997) Riverine salmonid egg burial depths: review of published data and implications for scour studies. Canadian Journal of Fisheries and Aquatic Sciences 54:1685–1698

Dias GM, Marshall DJ (2010) Does the relationship between offspring size and performance change across the life-history? Oikos 119:154–162

Dill LM, Northcote TG (1970) Effects of gravel size, egg depth and egg density on intragravel movement and emergence of coho salmon (Oncorhynchus kisutch) alevins. Journal of the Fisheries Research Board of Canada 27:1191–1199

Dziminski M a., Vercoe PE, Roberts JD (2009) Variable offspring provisioning and fitness: a direct test in the field. Functional Ecology 23:164–171

Einum S, Finstad AG, Robertsen G, Nislow KH, McKelvey S, Armstrong JD (2011) Natal movement in juvenile Atlantic salmon: a body size-dependent strategy? Population Ecology 54:285–294

Einum S, Fleming IA (1999) Maternal effects of egg size in brown trout (Salmo trutta): norms of reaction to environmental quality. Proceedings of the Royal Society B: Biological Sciences:2095–2100

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

Einum S, Fleming IA (2000b) Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565–567

Einum S, Fleming IA (2002) Does within-population variation in fish egg size reflect maternal influences on optimal values? The American Naturalist 160:756–765

Einum S, Fleming IA (2004) Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. Evolutionary Ecology Research 6:443–455

Einum S, Fleming IA (2007) Of chickens and eggs: diverging propagule size of iteroparous and semelparous organisms. Evolution 61:232–238

Einum S, Hendry AP, Fleming IA (2002) Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B: Biological Sciences 269:2325–2330

Einum S, Kinnison MT, Hendry AP (2004) Evolution of egg size and number. In: Hendry AP, Stearns SC (eds) Evolution Illuminated: Salmon and their Relatives. Oxford University Press, Oxford, pp 128–153

Einum S, Nislow KH (2005) Local-scale density-dependent survival of mobile organisms in continuous habitats: an experimental test using Atlantic salmon. Oecologia 143:203–210

Einum S, Nislow KH, Mckelvey S, Armstrong JD (2008) Nest distribution shaping within-stream variation in Atlantic salmon juvenile abundance and competition over small spatial scales. The Journal of Animal Ecology 77:167–172

Elliott J, Hurley MA (1997) A functional model for maximum growth of Atlantic salmon parr, Salmo salar, from two populations in northwest England. Functional Ecology 11:592–603

Erős T, Gustafsson P, Greenberg LA, Bergman E (2012) Forest-stream linkages: effects of terrestrial invertebrate input and light on diet and growth of brown trout (Salmo trutta) in a boreal forest stream. PloS one 7:e36462

Fausch KD (1984) Profitable stream positions for salmonids: relating specific growth rate to net energy gain. Canadian Journal of Fisheries and Aquatic Sciences 62:441–451

Finstad AG, Einum S, Forseth T, Ugedal O (2007) Shelter availability affects behaviour, size-dependent and mean growth of juvenile Atlantic salmon. Freshwater Biology 52:1710–1718

Finstad AG, Einum S, Ugedal O, Forseth T (2009) Spatial distribution of limited resources and local density regulation in juvenile Atlantic salmon. The Journal of Animal Ecology 78:226–35

Fischer B, Taborsky B, Kokko H (2011) How to balance the offspring quality–quantity trade off when environmental cues are unreliable. Oikos 120:258–270

Fleming IA (1996) Reproductive strategies of Atlantic salmon: ecology and evolution. Reviews in Fish Biology and Fisheries 6:379–416

Fleming IA (1998) Pattern and variability in the breeding system of Atlantic salmon (Salmo salar), with comparisons to other salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55:59–76

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

Foote CJ, Brown GS (1998) Ecological relationship between freshwater sculpins (genus Cottus) and beach-spawning sockeye salmon (Oncorhynchus nerka) in Iliamna Lake, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 55:1524–1533

Fox C, Thakar M, Mousseau T (1997) Egg size plasticity in a seed beetle: an adaptive maternal effect. American Naturalist 149:149–193

Fraser DJ, Jones MW, McParland TL, Hutchings JA (2007) Loss of historical immigration and the unsuccessful rehabilitation of extirpated salmon populations. Conservation Genetics 8:527–546

Fresh KL, Schroder SL (1987) The influence of the abundance, size , and yolk reserves of juvenile chum salmon (Oncorhynchus keta) on predation by freshwater fishes in a small coastal stream. Canadian Journal of Fisheries and Aquatic Sciences 44:236–243

Fukushima M, Quinn T, Smoker W (1998) Estimation of eggs lost from superimposed pink salmon (Oncorhynchus gorbuscha) redds. Canadian Journal of Fisheries and Aquatic Sciences 55:618–625

Fuller RL, LaFave C, Anastasi M, Molina J, Salcedo H, Ward S (2007) The role of canopy cover on the recovery of periphyton and macroinvertebrate communities after a month-long flood. Hydrobiologia 598:47–57

Garcia de Leaniz C, Fraser N, Huntingford F a. (2000) Variability in performance in wild Atlantic salmon, Salmo salar L., fry from a single redd. Fisheries Management and Ecology 7:489–502

Gibson RJ (1993) The Atlantic salmon in fresh water: spawning, rearing and production. Reviews in Fish Biology and Fisheries 3:39–73

Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH (2002) Effects of size and temperature on developmental time. Nature 417:70–73

Glazier DS (2002) Resource-allocation rules and the heritability of traits. Evolution 56:1696–1700

Goutelle S, Maurin M, Rougier F, Barbaut X, Bourguignon L, Ducher M, Maire P (2008) The Hill equation: a review of its capabilities in pharmacological modelling. Fundamental & Clinical Pharmacology 22:633–648

Gregersen F, Haugen TO, Vøllestad LA (2008) Contemporary egg size divergence among sympatric grayling demes with common ancestors. Ecology of Freshwater Fish 17:110–118

Grothendieck G (2010) nls2: Non-linear regression with brute force.

Guay JC, Boisclair D, Rioux D, Leclerc M, Lapointe M, Legendre P (2000) Development and validation of numerical habitat models for juveniles of Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 57:2065–2075

Guinnee MA, Gardner A, Howard AE, West SA, Little TJ (2007) The causes and consequences of variation in offspring size: a case study using Daphnia. Journal of Evolutionary Biology 20:577–587

Hale ME (1996) The development of fast-start performance in fishes: escape kinematics of the Chinook salmon (Oncorhynchus tshawytscha). American Zoologist 36:695–709

Hale ME (1999) Locomotor mechanics during early life history: effects of size and ontogeny on fast-start performance of salmonid fishes. Journal of Experimental Biology 202

Hannah DM, Malcolm I a., Soulsby C, Youngson AF (2004) Heat exchanges and temperatures within a salmon spawning stream in the Cairngorms, Scotland: seasonal and sub-seasonal dynamics. River Research and Applications 20:635–652

Hayes JW (1987) Competition for spawning space between brown (Salmo trutta) and rainbow trout (S. gairdneri) in a lake inlet tributary, New Zealand. Canadian Journal of Fisheries and Aquatic Sciences 44:40–47

Heath DD, Heath JW, Bryden CA, Johnson RM, Fox CW (2003) Rapid evolution of egg size in captive salmon. Science 299:1738–1740

Hedger DR, Dodson JJ, Bergeron NE, Caron F (2005) Habitat selection by juvenile Atlantic salmon : the interaction between physical habitat and abundance. Journal of Fish Biology 67:1054–1071

Heggenes J (1988) Substrate preferences of brown trout fry (Salmo trutta) in artificial stream channels. Canadian Journal of Fisheries and Aquatic Sciences1 45:1801–1806

Hendriks A, Mulder C (2008) Scaling of offspring number and mass to plant and animal size: model and meta-analysis. Oecologia 155:705–716

Hendry AP, Day T (2003) Revisiting the positive correlation between female size and egg size. Evolutionary Ecology Research 5:421–429

Hendry AP, Day T, Cooper AB (2001) Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387–407

Hill A V. (1910) The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. Journal of Physiology 40:iv–vii

Houde ALS, Fraser DJ, O'Reilly P, Hutchings JA (2011a) Maternal and paternal effects on fitness correlates in outbred and inbred Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 68:534–549

Houde ALS, Fraser DJ, O'Reilly P, Hutchings JA (2011b) Relative risks of inbreeding and outbreeding depression in the wild in endangered salmon. Evolutionary Applications 4:634–647

Houle (1992) Comparing evolvability and variability of quantitative traits. Genetics 130:195–204

Houle D, Pelabon C, Wagner GP, Hansen TF (2011) Measurement and meaning in biology. Quarterly Review of Biology 86:3–34

Hughes NF (1992) Ranking of feeding positions by drift-feeding Arctic grayling (Thymallus articus) in dominance hierarchies. Canadian Journal of Fisheries and Aquatic Sciences 49:1994–1998

Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211

Hutchings JA (1991) Fitness Consequences of Variation in Egg Size and Food Abundance in Brook Trout Salvelinus fontinalis. Evolution 45:1162–1168

Hutchings JA (1997) Life history responses to environmental variability in early life. In: Chambers RC, Trippel EA (eds) Early Life History and Recruitment in Fish Populations. Chapman & Hall, London, pp 139–168

Huxley J (1932) Problems of relative growth. Dial Press, New York

Ingendahl D (2001) Dissolved oxygen concentration and emergence of sea trout fry from natural redds in tributaries of the River Rhine. Journal of Fish Biology 58:325–341

Jaeger RG (1981) Dear enemy recognition and the costs of aggression between salamanders. American Naturalist 117:962–974

Janzen FJ (1993) An experimental analysis of natural selection on body size of hatchling turtles. Ecology 74:332–341

Janzen FJ, Tucker JK, Paukstis GL (2000a) 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

Janzen FJ, Tucker JK, Paukstis GL (2000b) Experimental analysis of an early life-history stage: selection on size of hatchling turtles. Ecology 81:2290–2304

Janzen FJ, Warner DA (2009) Parent-offspring conflict and selection on egg size in turtles. Journal of Evolutionary Biology 22:2222–2230

Johnson DW, Christie MR, Moye J (2010) Quantifying evolutionary potential of marine fish larvae: heritability, selection, and evolutionary constraints. Evolution 64:2614–2628

Johnston TA, Leggett WC (2002) Maternal and environmental gradients in the egg size of an iteroparous fish. Ecology 83:1777–1791

Jørgensen C, Auer SK, Reznick DN (2011) A model for optimal offspring size in fish, including live-bearing and parental effects. American Naturalist 177:E119–35

Kamler E (2006) Parent – egg – progeny relationships in teleost fishes : an energetics perspective. Reviews in Fish Biology and Fisheries:399–421

Kane TR (1988) Relationship of Temperature and Time of Initial Feeding of Atlantic Salmon. The Progressive Fish-Culturist 50:93–97

Kaplan RH (1992) Greater maternal investment can decrease offspring survival in the frog Bombina orientalis. Ecology 73:280–288

Kaplan RH, Cooper WS (1984) The evolution of developmental plasticity in reproductive characteristics: an application of the “adaptive coin-flipping” principle. American Naturalist 123:393–410

Karl I, Lorenz MW, Fischer K (2007) Energetics of reproduction: consequences of divergent selection on egg size, food limitation, and female age for egg composition and reproductive effort in a butterfly. Biological Journal of the Linnean Society 91:403–418

Kawecki T (1995) Adaptive plasticity of egg size in response to competition in the cowpea weevil, Callosobruchus maculatus (Coleoptera: Bruchidae). Oecologia 102:81–85

Killeen J, McLay HA, Johnston I. (1999) Development in Salmo trutta at different temperatures, with a quantitative scoring method for intraspecific comparisons. Journal of Fish Biology 55:382–404

Kindsvater HK, Alonzo S. H, Mangel M, Bonsall MB (2010) Effects of age- and state-dependent allocation on offspring size and number. Evolutionary Ecology Research 12:327–346

Kindsvater HK, Bonsall MB, Alonzo SH (2011) Survival costs of reproduction predict age-dependent variation in maternal investment. Journal of Evolutionary Biology 24:2230–2240

King EG, Roff DA, Fairbairn DJ (2011) The evolutionary genetics of acquisition and allocation in the wing dimorphic cricket, Gryllus firmus. Evolution 65:2273–2285

Kingsolver JG, Diamond SE (2011) Phenotypic selection in natural populations: what limits directional selection? The American Naturalist 177:346–357

Kinnison MT, Unwin MJ, Hendry AP, Quinn TP (2001) Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55:1656–1667

Kivelä SM, Välimäki P (2008) Competition between larvae in a butterfly Pieris napi and maintenance of different life-history strategies. The Journal of Animal Ecology 77:529–539

Kondolf GM (2000) Assessing salmonid spawning gravel quality. Transactions of the American Fisheries Society 129:262–281

Kondolf GM, Wolman MG (1993) The sizes of salmonid spawning gravels. Water Reseource Research 29:2275–2285

Koops MA, Hutchings JA, Adams BK (2003) Environmental predictability and the cost of imperfect information : influences on offspring size variability. Evolutionary Ecology Research 5:29–42

Krist M (2011) Egg size and offspring quality: a meta-analysis in birds. Biological Reviews of the Cambridge Philosophical Society 86:692–716

Krogh A (1959) The comparative physiology of respiratory mechanisms. University of Pennsylvania Press, Philadelphia

Kvingedal E, Einum S (2011) Prior residency advantage for Atlantic salmon in the wild: effects of habitat quality. Behavioral Ecology and Sociobiology 65:1295–1303

Lack D (1947) The Significance of clutch-size. Ibis 89:302–352

Lalonde R (1991) Optimal offspring provisioning when resources are not predictable. American Naturalist 138:680–686

Lalonde R (2005) Egg size variation does not increase offspring performance under interspecific competition in Nasonia vitripennis, a gregarious parasitoid. Journal of Animal Ecology 74:630–635

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Lapointe MF, Bergeron NE, Bérubé F, Pouliot M-A, Johnston P (2004) Interactive effects of substrate sand and silt contents, redd-scale hydraulic gradients, and interstitial velocities on egg-to-emergence survival of Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 61:2271–2277

Latta RG (2010) Natural selection, variation, adaptation, and evolution: a primer of interrelated concepts (RG Latta, Ed.). International Journal of Plant Sciences 171:930–944

Lessells CM (2002) Parentally biased favouritism: why should parents specialize in caring for different offspring? Philosophical Transactions of the Royal Society of London Series B, Biological sciences 357:381–403

Letcher BH, Terrick TD (2001) Effects of developmental stage at stocking on growth and survival of Atlantic salmon fry. North American Journal of Fisheries Management 21:102–110

Levin PS, Achrod S, Feist BE, Zabel RW (2002) Non-indigenous brook trout and the demise of Pacific salmon: a forgotten threat? Proceedings of the Royal Society B: Biological Sciences 269:1663–1670

Lloyd DG (1987) Selection of offspring size at independence and other size-versus-number strategies. American Naturalist 129:800–817

Lloyd JD (2004) Nest-site preference and maternal effects on offspring growth. Behavioral Ecology 15:816–823

Louhi P, Mäki-Petäys A, Erkinaro J (2008) Spawning habitat of Atlantic salmon and brown trout: general criteria and intragravel factors. River Research and Applications 24:330–339

Mäki-Petays A, Huusko A, Erkinaro J, Muotka T (2002) Transferability of habitat suitability criteria of juvenile Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 59:218–228

Malcolm IA, Soulsby C, Youngson AF, Hannah DM, Mclaren IS, Thorne A (2004) Hydrological influences on hyporheic water quality : implications for salmon egg survival. Hydrological Processes 18:1543–1560

Mangel M, Ludwig D (1992) Definition and evaluation of the fitness of behavioural and developmental programs. Annual Review of Ecology and Systematics 23:507–536

Marshall DJ, Bolton TF (2007) Effects of egg size on the development time of non-feeding larvae. Biological Bulletin 212:6–11

Marshall DJ, Bonduriansky R, Bussière LF (2008) Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. Ecology 89:2506–2517

Marshall DJ, Cook CN, Emlet RB (2006) Offspring size effects mediate competitive interactions in a colonial marine invertebrate. Ecology 87:214–225

Marshall DJ, Heppell SS, Munch SB, Warner RR (2010) The relationship between maternal phenotype and offspring quality: Do older mothers really produce the best offspring? Ecology 91:2862–2873

Marshall DJ, Keough MJ (2006) Complex life cycles and offspring provisioning in marine invertebrates. Integrative and Comparative Biology 46:643–651

Marshall DJ, Keough MJ (2008) The relationship between offspring size and performance in the sea. American Naturalist 171:214–224

Marshall DJ, Keough MJ (2009) Does interspecific competition affect offspring provisioning? Ecology 90:487–495

Marshall KE, Sinclair BJ (2010) Repeated stress exposure results in a survival-reproduction trade-off in Drosophila melanogaster. Proceedings Biological sciences / The Royal Society 277:963–969

Marshall DJ, Uller T (2007) When is a maternal effect adaptive? Oikos 116:1957–1963

Mason JC, Chapman DW (1965) Significance of early emergence, environmental rearing capacity, and behavioural ecology of juvenile coho salmon in stream channels. Journal of the Fisheries Research Board of Canada 22:173–190

McGinley MA (1989) The influence of a positive correlation between clutch size and offspring fitness on the optimal offspring size. Evolutionary Ecology 3:150–156

McGinley MA, Temme DH, Geber MA (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. American Naturalist 130:370–398

Mebane CA (2001) Testing bioassessment metrics: macroinvertebrate, sculpin, and salmonid responses to stream habitat, sediment, and metals. Environmental Monitoring and Assessment 67:293–322

Michaud J, Taggart C (2007) Lipid and gross energy content of North Atlantic right whale food,Calanus finmarchicus, in the Bay of Fundy. Endangered Species Research 3:77–94

Millidine KJ, Armstrong JD, Metcalfe NB (2006) Presence of shelter reduces maintenance metabolism of juvenile salmon. Functional Ecology 20:839–845

Mitchell TS, Warner DA, Janzen FJ (2013) Phenotypic and fitness consequences of maternal nest-site choice across multiple early life stages. Ecology 94:336–345

Mock DW, Schwagmeyer PL, Parker GA (2005) Male house sparrows deliver more food to experimentally subsidized offspring. Animal Behaviour 70:225–236

Mogensen S, Hutchings JA (2012) Maternal fitness consequences of interactions among agents of mortality in early life of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 69:1539–1555

Mojonnier L (1998) Natural selection on two seed-size traits in the common morning glory Ipomoea purpurea (Convolvulaceae): patterns and evolutionary consequences. American Naturalist 152:188–203

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

Monteith KM, Andrews C, Smiseth PT (2012) Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. Journal of Evolutionary Biology 25:1815–1822

Montgomery D, Buffington J, Peterson N, Schuett-Hames D, Quinn TP (1996) Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival. Canadian Journal of Fisheries and Aquatic Sciences 53:1061–1070

Morgulis S (1909) The influence of the size of the egg and temperature on the growth of the frog. American Naturalist 43:57–58

Morrongiello JR, Bond NR, Crook DA, Wong BBM (2012) Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. The Journal of Animal Ecology 81:806–817

Murphy ML, Heifetz J, Johnson SW, Koski K V., Thedinga JF (1986) Effects of clear-cut logging with and without buffer strips on juvenile salmonids in Alaskan streams. Canadian Journal of Fisheries and Aquatic Sciences 43:1521–1533

Nakano S, Murakami M (2001) Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America 98:166–170

Nislow KH, Einum S, Folt CL (2004) Testing predictions of the critical period for survival concept using experiments with stocked Atlantic salmon. Journal of Fish Biology 65(Supplem:188–200

Nislow KH, Folt CL, Parrish DL (1999) Favorable foraging locations for young Atlantic salmon: application to habitat and population restoration. Ecological Applications 9:1085–1099

Noguchi K, Hui WLW, Gel YR, Gastwirth JL, Miao W (2009) lawstat: an R package for biostatistics, public policy, and law.

Van Noordwijk AJ, De Jong G (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist 128:137–142

O'Grady MF (1993) Initial observations on the effects of varying levels of deciduous bankside vegetation on salmonid stocks in Irish waters. Aquaculture and Fisheries Management 24:563–573

O'Reilly PT, Harvie CJ (2009) Conservation of genetic variation in the inner Bay of Fundy Atlantic salmon captive breeding and rearing program. Darmouth, NS

Olofsson H, Ripa J, Jonzén N (2009) Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proceedings Biological sciences / The Royal Society 276:2963–2969

Olsson TI, Persson BG (1986) Effects of gravel size and peat material concentrations on embryo survival and alevin emergence of brown trout, Salmo trutta L. Hydrobiologia 135:9–14

Orzack SH, Sober E (1994) Optimality models and the test of adaptationism. American Naturalist 143:361–380

Ostrovsky I (1995) The parabolic pattern of animal growth: determination of equation parameters and their temperature dependencies. Freshwater Biology 33:357–371

Palm D, Lindberg M, Brannas E, Lundqvist H, Ostergren J, Carlsson U (2009) Influence of European sculpin, Cottus gobio , on Atlantic salmon Salmo salar, recruitment and the effect of gravel size on egg predation - implications for spawning habitat restoration. Fisheries Management and Ecology 16:501–507

Parciak W (2002) Seed size, number and habitat of a fleshy-fruited plant: consequences for seedling establishment. Ecology 83:794–808

Parker GA, Begon M (1986) Optimal Egg Size and Clutch Size: Effects of Environment and Maternal Phenotype. American Naturalist 128:573–592

Parker GA, Maynard Smith J (1990) Optimality theory in evolution biology. Nature 348:27–33

Parker GA, Mock DW, Lamey TC (1989) How selfish should stronger sibs be? American Naturalist 133:846–868

Pepin P, Orr DC, Anderson JT (1997) Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences 54(Supplem:2–10

Perrin N (1989) Population density and offspring size in the cladoceran Simocephalus vetulus (Muller). Functional Ecology 3:29–36

Peterson N, Quinn T (1996) Spatial and temporal variation in dissolved oxygen in natural egg pockets of chum salmon, in Kennedy Creek, Washington. Journal of Fish Biology 48:131–143

Phillips RW, Lantz RL, Claire EW, Moring JR (1975) Some effects of gravel mixtures on emergence of coho salmon and steelhead trout fry. Transactions of the American Fisheries Society 104:461–466

Pianka ER (1976) Natural selection of optimal reproductive tactics. American Zoologist 16:775–784

Pinder JEI, Wiener JG, Smith MH (1978) The Weibull distribution: a new method of summarizing survivorship data. Ecology 59:175–179

Plaistow SJ, St. Clair JJH, Grant J, Benton TG (2007) How to put all your eggs in one basket: empirical patterns of offspring provisioning throughout a mother's lifetime. American Naturalist 170:520–529

Purchase C, Hutchings J (2008) A temporally stable spatial pattern in the spawner density of a freshwater fish: evidence for an ideal despotic distribution. Canadian Journal of Fisheries and Aquatic Sciences 65:382–388

Quinn T (2005) The Behaviour and Ecology of Pacific Salmon and Trout. University of British Columbia Press, Toronto

Quinn TP, Hendry AP, Wetzel LA (1995) The influence of life history trade-offs and the size of incubation gravels on egg size variation in sockeye salmon (Oncorhynchus nerka). Oikos 74:425–438

R Development Core Team (2011) R: A language and environment for statistical computing.

Rankin TL, Sponaugle S (2011) Temperature influences selective mortality during the early life stages of a coral reef fish. PloS one 6:e16814

Rees M, Venable LD (2007) Why do big plants make big seeds ? Journal of Ecology 95:926–936

Rennie CD, Millar RG (2000) Spatial variability of stream bed scour and fill: a comparison of scour depth in chum salmon (Oncorhynchus keta) redds and adjacent bed. Canadian Journal of Fisheries and Aquatic Sciences 57:928–938

Resetarits WJ (1996) Oviposition site choice and life history evolution. Integrative and Comparative Biology 36:205–215

Riddick EW, Wu Z (2012) Mother-Offspring Relations: Prey Quality and Maternal Size Affect Egg Size of an Acariphagous Lady Beetle in Culture. Psyche: A Journal of Entomology 2012:1–7

Riley WD, Pawson MG, Quayle V, Ives MJ (2009) The effects of stream canopy management on macroinvertebrate communities and juvenile salmonid production in a chalk stream. Fisheries Management and Ecology 16:100–111

Ritz C (2010) Toward a unified approach to dose-response modeling in ecotoxicology. Environmental Toxicology and Chemistry 29:220–229

Ritz C, Strebig J (2011) drc: analysis of dose-response curves.

Roff (1992) The Evolution of Life Histories. Chapman and Hall, New York

Rollinson N, Hutchings JA (2010) Why does egg size increase with maternal size ? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (Salmo salar). Evolutionary Ecology Research 12:949–960

Rollinson N, Hutchings JA (2011a) Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon. Oecologia 166:889–898

Rollinson N, Hutchings JA (2011b) Why does egg size of salmonids increase with the mean size of population spawning gravels? Canadian Journal of Fisheries and Aquatic Sciences 68:1307–1315

Rollinson N, Hutchings JA (2013a) Environmental quality predicts optimal egg size in the wild. American Naturalist. in press

Rollinson N, Hutchings JA (2013b) The relationship between offspring size and fitness: integrating theory and empiricism. Ecology 94:315–324

Rombough PJ (2007) Oxygen as a constraining factor in egg size evolution in salmonids. Canadian Journal of Fisheries and Aquatic Sciences 64:692–699

Rose HE, Rizk AM (1949) Further researches in fluid flow through beds of granular material. Proceedings of the Institution of Mechanical Engineers 160:493–503

Sakai S, Harada Y (2001) Why do large mothers produce large offspring ? Theory and a test. The American Naturalist 157:348–359

Sato T, Suzuki N (2010) Female size as a determinant of larval size, weight, and survival period in the coconut crab, Birgus latro. Journal of Crustacean Biology 30:624–628

Schluter D (1988) Estimating the form of selection on a quantitative trait. Evolution 42:849–861

Schroderus E, Koivula M, Koskela E, Mappes T, Oksanen TA, Poikonen T (2012) Can number and size of offspring increase simultaneously?-a central life-history trade-off reconsidered. BMC Evolutionary Biology 12:44

Schubert KA, De Vries G, Vaanholt LM, Meijer HAJ, Daan S, Verhulst S (2009) Maternal energy allocation to offspring increases with environmental quality in house mice. American Naturalist 173:831–840

Schultz DL (1991) Parental investment in temporally varying environments. Evolutionary Ecology 5:414–427

Schwarzkopf L, Blows MW, Caley JM (1999) Life-history consequences of divergent selection on egg size in Drosophila melanogaster. American Naturalist 154:333–340

Simons AM (2009) Fluctuating natural selection accounts for the evolution of diversification bet hedging. Proceedings of the Royal Society B: Biological Sciences 276:1987–1992

Sinervo B, Doughty P (1996) Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal and quantitative genetic aspects. Evolution 50:1314–1327

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

Sinervo B, Svensson E (2002) Correlational selection and the evolution of genomic architecture. Heredity 89:329–338

Sinervo B, Zamudio K, Doughty P, Huey RB (1992) Allometric engineering: a causal analysis of natural selection on offspring size. Science 258:1927–1930

Smith C, Fretwell S (1974) The optimal balance between size and number of offspring. American Naturalist 108:499–506

Staub NL, De Beer M (1997) The role of androgens in female vertebrates. General and Comparative Endocrinology 108:1–24

Steen RP, Quinn TP (1999) Egg burial depth by sockeye salmon (Oncorhynchus nerka): implications for survival of embryos and natural selection on female body size. Canadian Journal of Zoology 77:836–841

Steingrimsson SO, Grant JWA (2003) Patterns and correlates of movement and site fidelity in individually-tagged young-of-the-year Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 60:193–202

Stevens WL (1951) Asymptotic regression. Biometrics 7:247–267

Suttle KB, Power ME, Levine JM, McNeely C (2004) How fine sediment in riverbeds impairs growth and survival of juvenile salmonids. Ecological Applications 14:969–974

Svensson E, Sinervo B (2000) Experimental excursions on adaptive landscapes: density-dependent selection on egg size. Evolution 54:1396–1403

Svärdson G (1949) Natural selection and egg number in fish. Pages 115–122 *in* Report 29. Institute of Freshwater Research, Drottningholm, Sweden.

Taborsky B (2006) Mothers determine offspring size in response to own juvenile growth conditions. Biology Letters 2:225–228

Tappel PD, Bjornn TC (1983) A new method of relating size of spawning gravel to salmonid embryo survival. North American Journal of Fisheries Management 3:123–135

Teletchea F, Gardeur J-N, Kamler E, Fontaine P (2009) The relationship of oocyte diameter and incubation temperature to incubation time in temperate freshwater fish species. Journal of Fish Biology 73:652–668

Tyler C, Sumpter J (1996) Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6:287–318

Tymchuk WV, O'Reilly P, Bittman J, Macdonald D, Schulte P (2010) Conservation genomics of Atlantic salmon: variation in gene expression between and within regions of the Bay of Fundy. Molecular Ecology 19:1842–1859

Vehanen T (2003) Adaptive flexibility in the behaviour of juvenile Atlantic salmon: short-term responses to food availability and threat from predation. Journal of Fish Biology 63:1034–1045

Venable LD (1992) Size-number trade-offs and the variation of seed size with plant resource status. American Naturalist 140:287–304

Vijendravarma RK, Narasimha S, Kawecki TJ (2010) Effects of parental larval diet on egg size and offspring traits in Drosophila. Biology letters 6:238–241

Webb JH, Fryer RJ, Taggart JB, Thompson CE, Youngson AF (2001) Dispersion of Atlantic salmon (Salmo salar) fry from competing families as revealed by DNA profiling. Canadian Journal of Fisheries and Aquatic Sciences 58:2386–2395

Weibull W (1951) A statistical distribution function of wide applicability. Journal of Applied Mechanics 18:293–297

West GB, Brown JH, Enquist BJ (1997) A General Model for the Origin of Allometric Scaling Laws in Biology. Science 276:122–126

West GB, Brown JH, Enquist BJ (2001) A general model for ontogenetic growth. Nature 413:628–31

White H (1942) Atlantic salmon redds and artificial spawning beds. Journal of the Fisheries Research Board of Canada 6:37–44

Wiklund C, Karlsson B, Forsberg J (1987) Adaptive versus constraint explanations for egg-to-body size relationships in two butterfly families. American Naturalist 130:828–838

Winkler DW, Wallin K (1987) Offspring size and number: a life history model linking effort per offspring and total effort. American Naturalist 129:708–720

Witzel LD, MacCrimmon HR (1981) Role of gravel substrate on ova survival and alevin emergence of rainbow trout, Salmo gairdneri. Canadian Journal of Zoology 59:629–636

Witzel LD, MacCrimmon HR (1983) Embryo survival and alevin emergence of brook charr, Salvelinus fontinalis, and brown trout, Salmo trutta, relative to redd gravel composition. Canadian Journal of Zoology 61:1783–1792

Zar JH (1984)Biostatistical Analysis, 2nd edn. Prentice-Hall, Inc., London

Zimmermann EM, Death RG (2002) Effect of substrate stability and canopy cover on stream invertebrate communities. New Zealand Journal of Marine and Freshwater Research 36:537–545

Zimmermann AE, Lapointe M (2005) Intergranular flow velocity through salmonid redds: sensitivity to fines infiltration from low intensity sediment transport events. River Research and Applications 21:865–881