

The causes and consequences of variation in offspring size: a case study using *Daphnia*

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Abstract

Offspring size can have large and direct fitness implications, but we still do not have a complete understanding of what causes offspring size to vary. *Daphnia* (water fleas) generally produce fewer and larger offspring when food is limited. Here, we use a mathematical model to show that this could be explained by either: (1) an advantage of producing larger eggs when food is limited; or (2) a lower boundary on egg volume (below which eggs do not have sufficient resources to be viable), that is similar in volume to the evolutionarily stable egg volume predicted by standard clutch size models. We tested the first possibilities experimentally by placing offspring from mothers kept at two food treatments (high and low – leading to relatively small and large eggs respectively) into two food treatments (same as maternal treatments, in a fully factorial design) and measuring their fitness (reproduction, age at maturity, and size at maturity). We also tested survival under starvation conditions of offspring produced from mothers at low and high food treatments. We found that (larger) offspring produced by low-food mothers actually had lower fitness as they took longer to reproduce, regardless of their current food treatment. Additionally, we found no survival advantage to being born of a food-stressed mother. Consequently, our results do not support the hypothesis that there is an advantage to producing larger eggs when food is limited. In contrast, data from the literature support the importance of a lower boundary on egg size.

Introduction

Life history theory attempts to provide evolutionary explanations for the way organisms live their lives (Roff, 1992; Stearns, 1992; Charnov, 1993). The study of how mothers allocate resources to offspring has provided one of the key areas in life history research as offspring size can have large and direct fitness implications (Smith & Fretwell, 1974; Schaffer & Gadgil, 1975; Parker & Begon, 1986; Stearns, 1992; Bernardo, 1996; Mousseau & Fox, 1998). However, we still do not have a complete understanding of what causes offspring size to vary within and between clutches (McGinley *et al.*, 1987; Roff,

1992; Charnov & Downhower, 1995; Charnov *et al.*, 1995; Einum & Fleming, 2000).

Standard clutch size models assume parents have a fixed, finite amount of resources to allocate to offspring production and that better-provisioned offspring are fitter (Smith & Fretwell, 1974; Parker & Begon, 1986; Godfray, 1987; Lloyd, 1987; Wilson & Lessells, 1994). Parents therefore face a trade-off between the number of offspring they can produce (clutch size) and the size of those offspring (I). There will exist an evolutionarily stable (ES; Maynard Smith, 1982) amount of resources per offspring (I^*), and the number of offspring produced (C) should equal the amount of resources a mother puts into a clutch (R) divided by I^* (R/I^* ; Smith & Fretwell, 1974). This leads to the prediction that variation in the resources available to a given individual will lead to variation in clutch size, and not offspring size (where offspring size is a measure of resources). Additionally, all

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offspring within a clutch will receive equal resources, a result that has been supported empirically in *Daphnia magna* (Guinnee *et al.*, 2004) and a fish (Downhower & Charnov, 1998).

However, the amount of resources a mother has (R) will rarely be an exact integer multiple of the optimum egg size (I^*). At larger clutch sizes, this is relatively inconsequential because any excess or deficit in resources is divided over many eggs. In contrast, for small clutches, where such an excess or deficit must be somehow divided over a very small number of eggs, allocation choices may be more complicated. Imagine a mother that has sufficient resources for one and a half standard-sized eggs (i.e. $R = 1.5 \times I^*$). She will have to produce either one egg that has 50% greater than ES resources, or two eggs, each with 25% less than ES resources. Alternatively, a mother that has resources sufficient for ten and a half eggs (i.e. $R = 10.5 \times I^*$) will produce either 10 eggs, each with 5% greater than ES resources, or 11 offspring, each with approximately 5% less than ES resources. Therefore, as recognized by Ebert (1994) and Charnov & Downhower 1995; Charnov *et al.* 1995, as clutch size increases, the possible amount of variation in offspring size between producing a clutch size of C and a clutch size of $C + 1$ decreases. This process can lead to more complicated relationships between the number and size of offspring when clutch sizes are small.

Models specifically developed to deal with this small integer problem predict that: (1) the variance in offspring size decreases with increasing clutch size (Ebert, 1994; Charnov & Downhower, 1995; Charnov *et al.*, 1995); and (2) the range of offspring size decreases at a rate that is not dependent on the specific details of the underlying trade-offs (Charnov & Downhower, 1995; Charnov *et al.*, 1995). Prediction 1 has been supported empirically in a number of species, including a fish (Charnov *et al.*, 1995), parasitic wasps (Mayhew, 1998; West *et al.*, 2001; Guinnee *et al.*, 2005), and the

species of *Daphnia* used here (Guinnee *et al.*, 2004). However, in experiments specifically designed to test these predictions with *Daphnia*, we did not find support for prediction 2. Instead, we found that the rate at which the range of egg volumes decreased with clutch size did not show a strong fit to the predictions of Charnov and colleagues' model. Additionally, individuals with greater resources (higher food availability) produced both a larger clutch size and smaller eggs (Guinnee *et al.*, 2004; see below). The production of smaller eggs under conditions of greater food availability has also been observed in several other studies on *Daphnia* (Table 1).

Here we extend Charnov and colleagues' model to examine two possible explanations for why the ES egg size should decrease with increasing clutch size. One possibility is that there exists a lower boundary on egg volume, below which eggs are not viable (Glazier, 1992; Guinnee *et al.*, 2004), that is similar in volume to the ES egg size predicted by standard clutch models (I^*). Charnov and colleagues' invariant model predicts that the greatest variation in egg size will occur at small clutch sizes – and therefore also predicts that the smallest eggs (as well as the largest) will occur at small clutch sizes. However, if there exists a lower limit on egg size that is within the range of egg sizes predicted by the model, small eggs (which are predicted by Charnov and colleagues' model to be more prevalent at small clutch sizes) will not be produced, because they will not be viable. This will lead to an increased average egg size at small clutch sizes.

Alternatively, it might be that the ES egg size varies with resource availability as was suggested by Ebert (1994). For example, if the ES offspring size decreases with food availability, we would expect individuals with more food to produce more and smaller offspring (Glazier, 1992; Ebert, 1994; Guinnee *et al.*, 2004). Here, we report the results of an experiment on *Daphnia* designed to test this idea empirically. Specifically, we studied the fitness

Table 1 Studies examining the relationship between resources available to a mother and change in egg or offspring size in *Daphnia*.

Source	Species	Measure of resource investment	Change in egg/offspring size with increase in food	No. of treatments
Boersma (1995)	<i>D. galeata</i>	Egg carbon content	Increase, then decrease	4
Gliwicz & Guisande (1992)	<i>D. hyalina</i>	Neonate length	Decrease	3
Enserink <i>et al.</i> (1990)	<i>D. magna</i>	Neonate length	Decrease	4
Glazier (1992)	<i>D. magna</i>	Egg mass	Decrease or no relationship (2 clones)	2
Ebert (1993)	<i>D. magna</i>	Neonate length	Decrease	13
Enserink <i>et al.</i> (1993)	<i>D. magna</i>	Neonate length	Decrease	3
Trubetskova & Lampert (1995)	<i>D. magna</i>	Egg length and egg mass	Decrease	2
Boersma (1997)	<i>D. magna</i>	Neonate mass	Decrease	2
Taylor (1985)	<i>D. pulex</i>	Egg mass	Decrease	2
Lynch (1989)	<i>D. pulex</i>	Egg mass	Increase	9
Tessier & Consolatti (1991)	<i>D. pulex</i>	Neonate length	Increase	3
LaMontagne & McCauley (2001)	<i>D. pulex</i>	Neonate length	Increase	2
Taylor (1985)	<i>D. pulicaria</i>	Egg mass	No change	2
Tessier & Consolatti (1991)	<i>D. pulicaria</i>	Neonate length	Increase, then decrease	3
Gliwicz & Guisande (1992)	<i>D. pulicaria</i>	Neonate mass	Decrease	3

consequences of hatching from small vs. large eggs at different food treatments. Our experiments were carried out on a single clonally reproducing strain of *Daphnia* to avoid complications caused by genetic variation or genotype \times environment interactions.

The model

Dimensionless clutch size invariants

In this section, we first describe the small integer invariant clutch size model developed by Charnov & Downhower (1995) and Charnov *et al.* (1995) and then extend it to investigate two possible explanations for the observed lack of fit between theory and data from *D. magna*. The invariant model discussed here does not use log-log plots, which can suffer from limitations (Nee *et al.*, 2005). Table 2 outlines the notation used in this section. Assume that offspring survival is equivalent to offspring fitness and increases with offspring size according to:

$$S = S_m \{1 - \exp[-d(I - I_0)]\}, \quad (1)$$

where I is offspring size, I_0 is minimum viable offspring size, S_m is the asymptotic survival approached as $I \rightarrow \infty$, and the parameter d determines the shape of the survival curve. The diminishing returns from investment into a particular offspring means that, for a given clutch size (C), total offspring fitness is maximized by allocating total resources (R) equally within the clutch, i.e. each offspring is of size $I = R/C$. The fitness function to be maximized by natural selection is thus:

$$w = CS \propto \left(\frac{R}{I}\right) \{1 - \exp[-d(I - I_0)]\}. \quad (2)$$

Note that the value of S_m is irrelevant, as it merely provides a constant rescaling of absolute fitness. We can re-arrange this fitness function so as to describe the maximization problem in a dimensionless way:

$$w \propto \left(\frac{R/I_0}{I/I_0}\right) \left\{1 - \exp\left[-dI_0\left(\frac{I}{I_0} - 1\right)\right]\right\}. \quad (3)$$

Here, the units of d (size⁻¹) and I_0 (size) cancel in the product dI_0 , which is the key dimensionless parameter

Table 2 A summary of notation used in this article.

Symbol	Definition
I	Offspring size
I_0	Minimum viable offspring size
I^*	Evolutionarily stable offspring size
C	Clutch size; number of offspring in a clutch
R	Resource availability; total offspring size of a clutch
S	Offspring survival probability
S_m	Maximum offspring survival probability
d	Shape parameter relating offspring size to survival
W	Mother's fitness
λ	Parameter relating resource availability to offspring survival

describing the system. It is the shape of the function relating (relative) investment into individual offspring to the (relative) survivorship of individual offspring. The units of R (size) and I_0 (size) cancel in the dimensionless fraction R/I_0 , which is the (relative) amount of resources available to a particular mother, and the units of I (size) and I_0 (size) cancel in the dimensionless fraction I/I_0 , which is the (relative) allocation of resources to each offspring. There are three immediate advantages of re-phrasing the maximization problem in this way. First, the number of parameters governing a mother's clutch size decision is reduced from three (d , I_0 and R) to two (dI_0 and R/I_0), making analysis simpler. Secondly, unit measures of offspring size are entirely arbitrary. A researcher measuring offspring size in milligrams will have different values of d and I_0 than a researcher who measures in ounces, although the pair should agree on the value of the dimensionless quantity dI_0 . Thirdly, it removes (unimportant) differences in scale. Any two mothers whose offspring provisioning decision is captured by eqn 3 and who share the same values of dI_0 and R/I_0 will maximize their fitness with the same value of I/I_0 (which we shall denote I^*/I_0), regardless of whether one belongs to a species whose minimum viable egg size (I_0) is orders of magnitude larger than for the other species. In this respect, different populations or species with similar dI_0 values can be regarded as closely equivalent, despite potential differences in absolute egg size.

For small clutches, I/I_0 is constrained because $C = R/I$ necessarily takes integer values. Charnov & Downhower (1995) and Charnov *et al.* (1995) have used heuristic arguments to show that this leads to several predictions, one of which is relevant here. The variation in offspring size (between clutches) is predicted to decrease with increasing clutch size, following the invariant rule:

$$\frac{I_{\max,C} - I_{\min,C}}{I_{\max,C-1} - I_{\min,C-1}} \approx \frac{C-1}{C}, \quad (4)$$

where $I_{\max,C}$ and $I_{\min,C}$ are respectively the maximum and minimum offspring sizes observed among clutches of C offspring. However, data from *D. magna* showed that although the variation in offspring size (between clutches) decreased with increasing clutch size, it did not do so as predicted by eqn 4. Interestingly, mean offspring size also decreased with increasing clutch size in that study (Fig. 1; Guinnee *et al.*, 2004). We will investigate to what extent this can be explained by: (1) exploring how the invariant pattern varies with offspring fitness as determined by the shape of the offspring survival function; and (2) looking at the consequences of offspring fitness varying with resources and maternal resources.

Shape of the survival function

The predictions of Charnov & Downhower (1995) and Charnov *et al.* (1995) are only approximate, and may

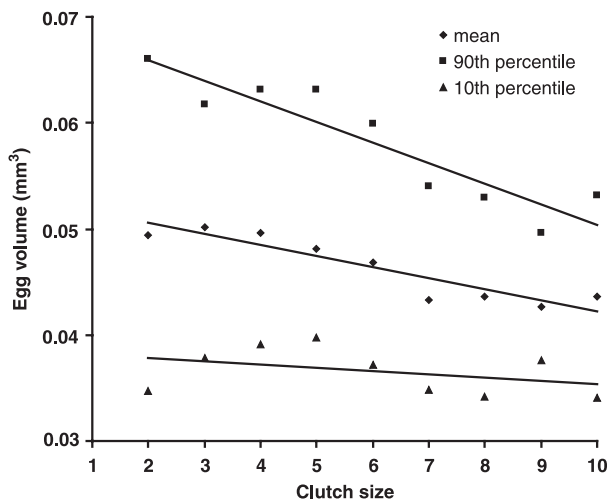


Fig. 1 Results of egg size experiment on *Daphnia magna* described by Guinnee *et al.* (2004). Note that there are no data for clutch sizes = 1. Mean (diamonds), 90th percentile (triangles) and 10th percentile (squares) for egg volume for clutches of size 2–10. Linear trend line: mean, $y = -0.001x + 0.05$, $r^2 = 0.86$; 90th percentile, $y = -0.002x + 0.07$, $r^2 = 0.85$; 10th percentile, $y = -0.0003x + 0.04$, $r^2 = 0.15$. Variation in egg size decreased with increasing clutch size (see original publication for statistics), but the decrease was nonsymmetrical. The 90th percentile of mean egg size decreased with increasing clutch size (slope = $-0.0019 \text{ mm}^3/\text{clutch size}$, $r_s = -0.92$, $n = 10$, $P = 0.0002$), but the 10th percentile did not increase with clutch size (slope = $0.0003 \text{ mm}^3/\text{clutch size}$; $n = 10$, $r_s = -0.61$, $P = 0.060$). Data and statistics were first reported in Guinnee *et al.* (2004).

vary with the shape of the offspring survival function. One potential explanation for a negative relationship between mean offspring size and clutch size is that there is a lower boundary on egg volume below which eggs do not have the minimum necessary amount of resources to be viable. If this is the case, the possible egg sizes will be limited, and this will affect the predictions of Charnov and colleagues' model.

We investigate the possibility that the departure from the predictions of Charnov and colleagues' model among the *D. magna* data (Fig. 1; Guinnee *et al.*, 2004) results from a minimum viable egg size by exploring how the model predictions vary with dI_0 . Larger values of dI_0 correspond to a steep increase in offspring survival with increasing offspring size close to the point at which offspring are only just viable (I_0), so that maximum survival (S_m) is approached with relatively little investment, and further investment into offspring size translates into only a very small improvement in survival. Smaller values of dI_0 correspond to a much shallower survival slope, so that maximum survivorship (S_m) is approached only for offspring many times larger than the minimum viable size (I_0 ; Fig. 2). We find that varying dI_0 translates into variation in the invariant pattern. Charnov and colleagues' model predicts that the range of

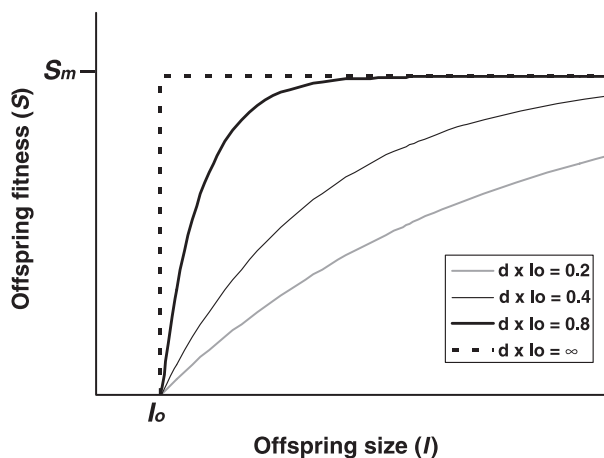


Fig. 2 The relationship between offspring size (I) and offspring fitness (S) can be described by a single dimensionless number, dI_0 . This is the product of minimum viable offspring size (I_0 ; units of size) and the shape parameter of the survival eqn 1 (d ; units of size^{-1}). Survival increases from zero and asymptotes at a maximum (S_m) as size increases from I_0 . Low values of dI_0 correspond to slow increase of survival with size, whereas higher values of dI_0 correspond to steep increase close to I_0 , rapidly levelling off at the maximum, S_m . In the limit as $dI_0 \rightarrow \infty$, survival steps from zero to maximum survival at $I = I_0$.

offspring sizes should approximately follow the pattern described in eqn 4, and we find that this holds well for $dI_0 \approx 1$ and less well for smaller or larger values of dI_0 , although for very large dI_0 the prediction is again very accurate. Additionally, mean egg size decreases with increasing values of dI_0 . The pattern observed in the *D. magna* data (Fig. 1; Guinnee *et al.*, 2004), i.e. that mean offspring size is a decreasing function of clutch size, appears to correspond to high values of dI_0 (see $dI_0 \rightarrow \infty$ in Fig. 3). This suggests that *D. magna* might have a relatively large minimum viable egg size (I_0 ; relative to I^*), and beyond this size there is little increase in the probability of survival.

Offspring survival varies with resource (food) availability

Another possible explanation for a negative correlation between mean offspring size and clutch size is that the ES offspring size decreases with clutch size (Glazier, 1992; Ebert, 1994; Guinnee *et al.*, 2004). This would be the case if greater resource (food) availability (higher R/I_0) lead to both increased clutch sizes and a lower ES offspring size (lower I^*/I_0), i.e. the fitness benefit of being a large offspring is greater in low-food environments (Hutchinson, 1951; Green, 1966; Goulden *et al.*, 1987; Gliwicz & Guisande, 1992), and maternal environment is indicative of offspring environment. This could be encapsulated in our model by a positive correlation between dI_0 and a mother's relative resources (R/I_0). For

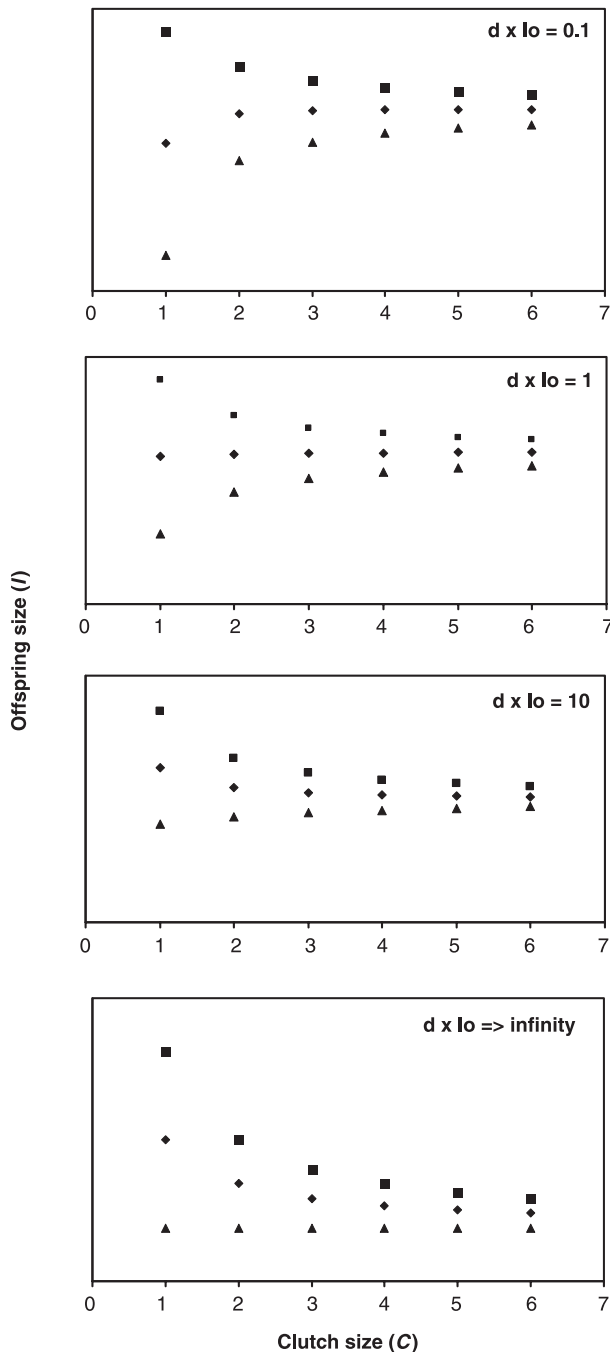


Fig. 3 Switch point patterns for different values of the dimensionless number dI_0 . The squares represent the largest offspring size, the diamonds the mean offspring size, and the triangle the minimum offspring sizes, predicted for that clutch size.

simplicity, we assume a proportional relationship: $dI_0 = \lambda R/I_0$, where the dimensionless parameter λ describes how dependent the size/survival relationship is on relative resource availability (R/I_0). High maternal resources indicate a comfortable environment, so that

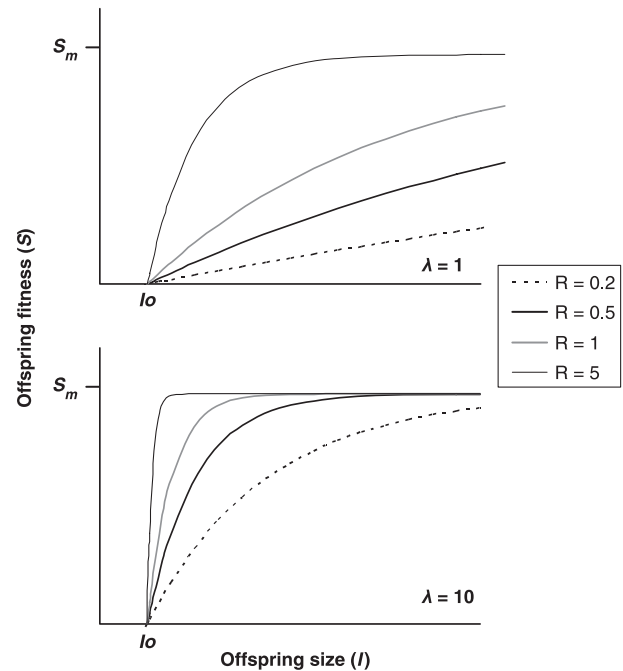


Fig. 4 The steepness of the fitness curve varies with resources (R) and λ . The more resources (R) available to an individual, and the larger the value of λ , the faster the function relating offspring size to offspring fitness approaches an asymptote, and the smaller the optimum offspring size (I^*).

the offspring size/survival relationship is flattened, and thus the ES offspring size is decreased. The parameter λ determines how rapidly the shape of the survival curve changes with R/I_0 (Fig. 4). Implementing this change in the model significantly disturbs the invariant relationship only for small clutch sizes, and the observed data (Fig. 1) is most consistent with larger values of λ (Fig. 5).

Materials and methods

Study organisms

The clone of *D. magna* used in these experiments originated in the Gaarzerfeld pond, Northern Germany. Previously, we found that this clone produces larger eggs at smaller clutch sizes, that variation in clutch size decreases with increasing clutch size, and that resources are divided (relatively) equally within a clutch (Fig. 1; Guinnee *et al.*, 2004). *Daphnia* were kept in 200-mL jars containing a modified version of the Aachener Daphnien Medium (Klüttgen *et al.*, 1994), and fed on a chemostat-grown culture of the green algae *Scenedesmus* sp. The jars were in trays, 12 jars to a tray, and housed in incubators (20 °C, 14 : 10 light/dark cycle). Jars were randomly assigned to trays, and trays were randomly assigned to a location in the incubator, so that any jar, containing any treatment, had an equal chance of being placed in any particular location in the incubator. We systematically

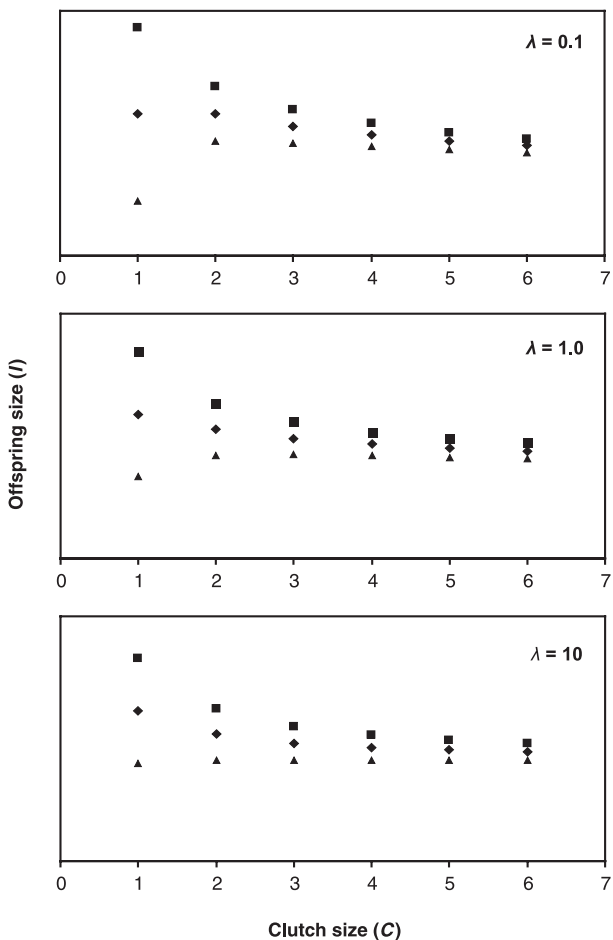


Fig. 5 Switch point patterns for varying values of λ , for the model where the shape of the size–survival curve depends on a mother’s resources ($dI_0 = \lambda R/I_0$, see text for details). Squares represent the largest offspring size, and triangles represent the smallest offspring size, predicted for that clutch size.

rotated the jars within the trays, and the trays within the incubator, each day.

Experiment 1: reproduction

Methods

We raised three generations of *Daphnia* to equilibrate conditions. The first two generations contained five *Daphnia* per jar and were fed 1.5×10^7 algal cells per day per jar (for 72 jars \times 5 *Daphnia*/jar = 360 *Daphnia*). The third generation comprised one *Daphnia* per jar, fed 1.2×10^6 cells/*Daphnia*/day, for 120 *Daphnia*. To start a new generation, all neonates produced over a 24-h period were combined, then randomly allocated to jars. Throughout, *Daphnia* media was changed every-other day.

Figure 6 outlines the experimental design. When the third generation produced their third clutch of offspring,

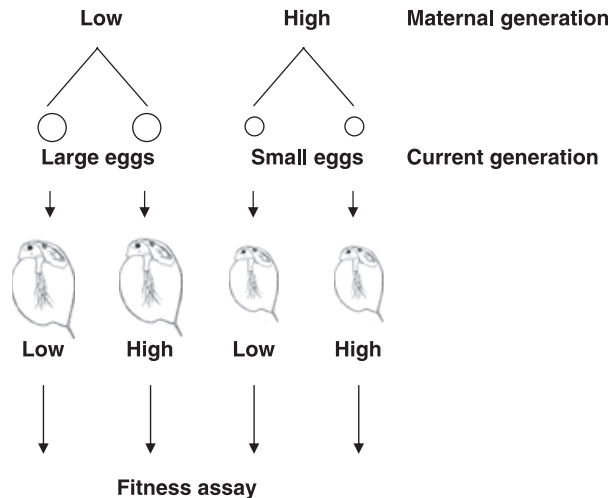


Fig. 6 Experimental design. Food treatments were low (L, 4×10^5 cells/*Daphnia*/day) and high (H, 32×10^5 cells/*Daphnia*/day). Sample sizes for the maternal generations were $L = 51$, and $H = 33$. When a *Daphnia* in the maternal generation produced her second clutch, two of her offspring were randomly chosen and one was placed into each of two treatments, H or L, which were identical to the maternal treatments. Age at maturity, size at maturity, and reproduction were monitored for the current generation.

one offspring from each mother was randomly assigned to one of two treatments: high food (H, 32×10^5 cells/*Daphnia*/day) or low food (L, 4×10^5 cells/*Daphnia*/day). We refer to these *Daphnia* as the ‘maternal generation’. When mothers in the maternal generation produced their second clutch of eggs, we used a camera attached to a dissecting microscope to photograph three eggs (or all eggs, if ≤ 3) from each mother through the mother’s carapace. All eggs were in stage 1 of development as described by Threlkeld (1979). When these second clutch offspring hatched, two offspring (≤ 14 h old) from each *Daphnia* were photographed and then placed (randomly) into a food treatment: H or L (these food treatments were identical to those in the maternal generation). We refer to these *Daphnia* as the ‘current generation’.

We checked the current generation *Daphnia* twice per day, morning and evening, and recorded deaths. Age at maturation was defined as when eggs first became visible in the brood chamber. We photographed the current generation *Daphnia* when they reached maturation. Photographs were later used to measure body length (top of head to base of tail spine) and egg length (length of longest axis). All offspring produced by the current generation were counted. After a (current generation) *Daphnia* produced three clutches, she was removed from the experiment.

Statistics

We used the SAS system (Release 8.0; SAS Institute, Cary, NC, USA) for all data analyses. All linear regression

analyses and mixed effects models were performed using the PROC MIXED procedure. Egg length and offspring length were averaged over mother to avoid pseudo-replication (Hurlbert, 1984). Nonsignificant interaction terms ($P > 0.05$) were eliminated from models using backwards elimination (Crawley, 1993).

To confirm that egg size was dependent on maternal food availability, we tested for a relationship between the length of eggs produced by the maternal generation (averaged over mother) and food treatment using linear regression with the equation: egg length = food treatment. We determined whether offspring size was correlated with egg size using a Spearman's rank test to compare egg length (averaged over clutch) to offspring length (averaged over clutch). As eggs were not isolated for hatching, we could not match particular eggs to particular offspring. However, we have found previously in this clone that eggs from the same clutch are more similar in size relative to eggs from different clutches (Guinnee *et al.*, 2004).

We tested whether the current and the maternal food environment affected age at maturity, size at maturity, and number of offspring produced in the first three clutches using mixed effects models. Age at maturity was square-root transformed to correct for heterogeneity of variances. The equations were: response variable (for current generation *Daphnia*) = current treatment + maternal treatment + current \times maternal treatment. Maternal *Daphnia* was included as a random factor.

Experiment 2: survival

Methods

The preparation of the maternal generation was identical to that described above, i.e. there was a high and a low food maternal generation. When the *Daphnia* in the maternal generation produced a clutch, one randomly chosen offspring was placed in a well of a 6-well, multiwell plate (Corning Life Sciences, New York, NY, USA), flat bottomed wells, approximately 3.5 cm diameter) containing 6 mL *Daphnia* media. Placement of the *Daphnia* in the wells, and the wells within the incubator, were randomized. *Daphnia* were examined once per day, and lifespan recorded in days. This was carried out for

each of the first three clutches produced by each *Daphnia*. No food was provided and thus measurements represent survivorship under starvation conditions.

Statistics

We carried out a Cox proportional hazard model analysis using the PROC TPHREG procedure in the SAS system to determine whether maternal food treatment affects offspring survival. Clutches 1, 2 and 3 were analysed separately.

Results

Experiment 1: reproduction

A total of 84 maternal generation and 138 current generation *Daphnia* were included in the experiment. A total of 151 eggs were measured, and 1833 neonates (produced by the current generation) were recorded. There was little mortality during the experiment (deaths in the current generation: 0 LL, 0 LH, 1 HL, 1 HH), and all surviving *Daphnia* reproduced.

Eggs produced by (maternal generation) mothers in the low food treatment were larger than eggs from (maternal generation) mothers in the high food treatment (mean \pm SD, High: 0.36 ± 0.022 mm, Low: 0.39 ± 0.033 mm; $F_{1,70} = 12.1$, $P < 0.001$). Egg length was positively correlated with neonate length, meaning that big eggs hatched into big offspring ($n = 72$, $r = 0.40$, $P < 0.001$).

High food, be it in the maternal or current generation, was associated with early maturation (Table 3). *Daphnia* (current generation) were larger at maturation when current treatment = high, and when maternal treatment = low (Table 3). The current \times maternal food interaction did not affect age at maturation or size at maturation (in current generation *Daphnia*; $P > 0.05$). The number of offspring produced over the first three clutches was positively correlated with current food treatment, but not affected by maternal food treatment or the interaction between the two (mean \pm SD, current treatment = L: 6.8 ± 1.3 , current treatment = H: 25.0 ± 4.9 ; current treatment: $F_{1,81} = 457.8$, $P < 0.0001$; maternal treatment: $F_{1,80} = 1.1$, $P = 0.30$, current \times maternal

Table 3 ANOVA table outlining the effect of current and maternal treatments on life history traits in *Daphnia magna*. As current \times maternal treatment was nonsignificant ($P > 0.05$), it was not included in the final model. Least squares mean (LSM) values for age at maturity are back-transformed from square-root transformed data.

Trait	Current treatment				Maternal treatment				Current \times maternal treatment				
	Relationship	LSM	d.f.	F	P	Relationship	LSM	d.f.	F	P	d.f.	F	P
Age at maturity (days) LSM	H matured earlier L 13.12, H 7.56		1, 50	650.9	< 0.0001	H matured earlier L 10.81, H 9.54		1, 50	33.3	< 0.0001	1, 49	1.8	0.35
Size at maturity (mm) LSM	H were larger L 2.48, H 2.80		1, 47	175.1	< 0.0001	L were larger L 2.69, H 2.60		1, 47	12.5	0.0009	1, 46	0.03	0.85

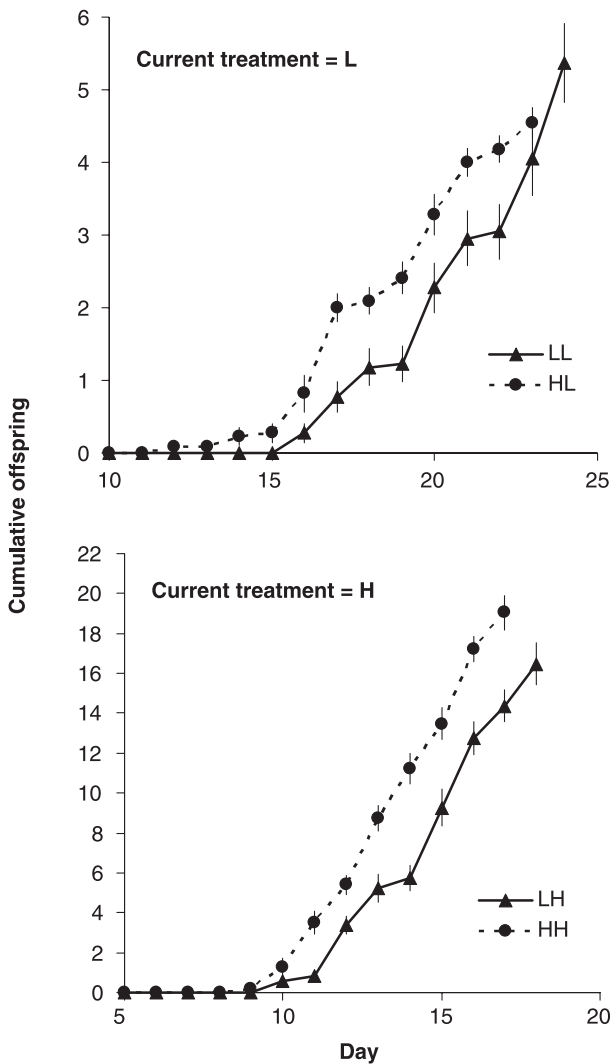


Fig. 7 Offspring production differed with current and maternal food treatment. The top graph is for current treatment = L, the bottom is for current treatment = H. Within each current food treatment, *Daphnia* from low-food mothers had produced fewer offspring at any day post-hatching than *Daphnia* from high-food mothers. Error bars are \pm SEM. Endpoints are the median day on which *Daphnia* for that treatment had their third clutch. *Daphnia* that did not produce three clutches were excluded.

treatment: $F_{1,79} = 0.1, P = 0.81$). Within either current food treatment, *Daphnia* (current generation) from low-food mothers produced fewer offspring than *Daphnia* (current generation) from high-food mothers (when compared with *Daphnia* in the same current food treatment; Fig. 7) because they began reproducing later.

Experiment 2: survival

Survivorship was similar among treatments (mean \pm SD, clutch 1: L 9.7 ± 2.4 days, H 9.4 ± 2.2 days; clutch 2: L

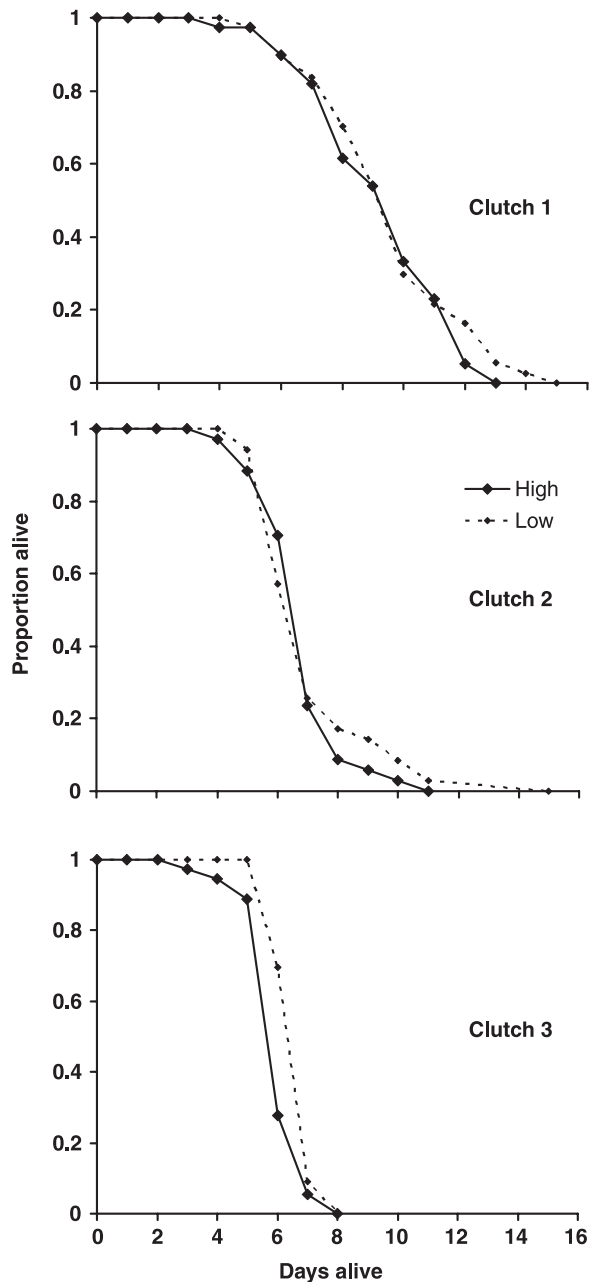


Fig. 8 Survival curves for the first three clutches. There was no statistically significant difference ($P > 0.05$) in survival for those in the different treatments, high (solid lines) and low (dotted lines), for any clutch.

7.3 ± 2.0 days, H 7.0 ± 1.4 days; clutch 3: L 6.8 ± 0.6 days; H 6.1 ± 0.9 days). Thus, maternal food treatment did not affect offspring survival under starvation conditions for any of the first three clutches (Fig. 8; clutch 1: Wald $\chi^2_1 = 0.38, P = 0.55$; clutch 2: Wald $\chi^2_1 = 0.10, P = 0.76$; clutch 3: Wald $\chi^2_1 = 3.63, P = 0.06$).

Discussion

Several studies have shown that in poor environments with low food availability, *Daphnia* produce larger offspring and smaller clutch sizes (Guinnee *et al.*, 2004; Table 1; Fig. 1). We expanded upon the model of Charnov & Downhower (1995) and Charnov *et al.* (1995) to investigate possible explanations for this. We found that the observed pattern of mean egg volume decreasing with greater food availability, when larger clutch sizes are produced, can be theoretically explained by either: (1) the ES egg volume decreasing with food availability (Fig. 4; see also Ebert, 1994); or (2) a lower limit on egg size that is close to the ES egg size (Fig. 2). We then tested the first of these possibilities experimentally with *D. magna*. We found no evidence for the required interaction between resource availability and the advantage of being large, or indeed any evidence that larger offspring are more fit (Figs 7 and 8).

In contrast, data from the literature supports the idea that the lower limit on egg size is similar to the ES egg size. In the present study, we have demonstrated theoretically that the rate of decrease in egg size can be determined by the dimensionless number dI_0 , where I_0 is the minimum viable egg size and d controls the shape of the offspring size/survival curve. A large dI_0 means that I^* (the optimal egg size) is very similar to the minimum viable egg size I_0 , because offspring survival increases sharply then levels off very rapidly with increases in egg size. This provides a potential explanation for the results of Guinnee *et al.* (2004), with the observed data (Fig. 1) consistent with an offspring survival function which steps up from zero to maximum survival as egg size reaches the minimum viable size, indicating that increased provisioning does not appear to benefit already-viable eggs. But could the offspring survival curve be steep enough for this to be true?

Bell (1983) found a steep function relating egg size and likelihood of hatching in *D. pulex*, and that eggs below a certain size never hatched (Fig. 9; a difference in size of 25% can lead to a > 50% increase in survival). If this represents a general pattern across *Daphnia* species, this suggests that I^* might be very similar to the minimum egg size (I_0). Interestingly, if this is the case, *Daphnia* have little room for error – if they produce eggs that are the slightest bit smaller than the ES, the fitness of the clutch will be zero.

In agreement with Ebert (1994), we have shown theoretically that selection for smaller eggs when resources are abundant is a possible explanation for the observed pattern of decreasing mean egg size with increasing clutch size. This prediction of large offspring at low food should evolve if three conditions are met: (1) maternal food is indicative of offspring environment; (2) the environment is variable enough that plasticity in offspring size will have been favoured; and (3) the benefit of being large is greater in low-food

environments as opposed to high-food environments. *Daphnia* have a short generation time, therefore it is reasonable to expect that maternal food availability is indicative of offspring food availability (Boersma, 1997), supporting condition 1. Food availability in the wild is likely to be highly variable (Gliwicz & Guisande, 1992), and *Daphnia* have extremely plastic life-history characteristics in response to food availability (Table 1; Green, 1956; Lynch & Ennis, 1983; Ebert, 1994; LaMontagne & McCauley, 2001), supporting condition 2.

Our experimental work sought to test whether condition 3 held. Specifically, we explored how the fitness consequences of being born from a large egg (produced by mothers in a low-food environment) or small egg (produced by mothers in a high-food environment) varied with food availability. We expected that larger offspring should be fitter than smaller offspring, and that this fitness advantage should be most apparent in stressful environments where food availability to these offspring is low. Thus there should be an interaction between maternal food treatment (which determines offspring size) and current food treatment. We found no evidence that *Daphnia* from mothers in low food were fitter than those produced by mothers at high food, as (larger) offspring produced by mothers in low food took longer to reproduce, and they produced fewer offspring per unit time regardless of current conditions (Table 3; Fig. 7). We found no maternal \times current food interaction for any of our fecundity-related fitness measures. Furthermore, we found that larger offspring, produced by mothers in a limited food environment, had no statistically significant survival advantage under starvation conditions, although in our experiments offspring from low-food mothers did live slightly longer (Fig. 8).

Fitness, however, is notoriously difficult to measure. It is, for example, highly context-dependent, and there may be food conditions or life-history features we did not study under which a fitness advantage for large offspring would be revealed. However, our theoretical results indicate that offspring fitness must be tightly dependent on maternal resources (Fig. 4, where $\lambda = 10$) for the hypothesis that large eggs are adaptive in low food levels to fit the *D. magna* data (Fig. 1). Given this, we did not expect the advantage of being larger to be difficult to detect with our measures of fecundity. It was equally surprising that (large) *Daphnia* from low-food mothers did not have improved survivorship under starvation conditions.

Two previous studies have explored the effect of maternal food treatment on *Daphnia* reproduction, although these studies used *D. pulex* (Lynch & Ennis, 1983; LaMontagne & McCauley, 2001). Similar to our results, Lynch & Ennis (1983) found that offspring produced by low-food mothers had less early reproduction (for a given current treatment). We additionally found that offspring produced by low-food mothers were larger at maturity (for a given current food treatment), as

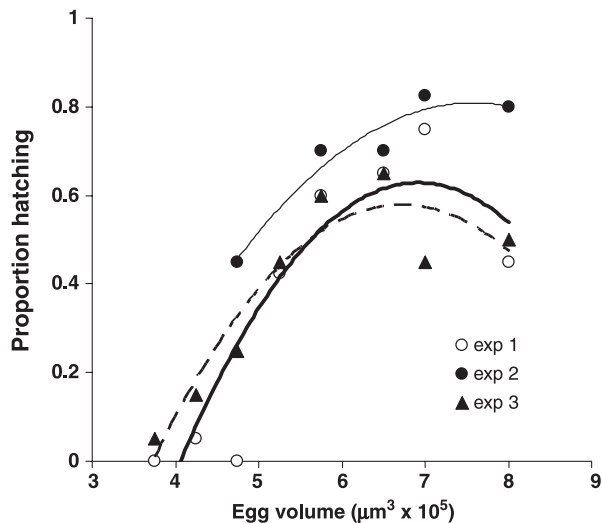


Fig. 9 Proportion hatching increases with egg volume. This figure is redrawn from Bell (1983), and shows data from three separate experiments using *Daphnia pulex*. Polynomial (incorporating a squared term) lines of best fit: experiment 1, $y = -0.077x^2 + 1.06x - 3.05$, $r^2 = 0.81$; experiment 2, $y = -0.044x^2 + 0.66x - 1.71$, $r^2 = 0.93$; experiment 3, $y = -0.065x^2 + 0.87x - 2.34$, $r^2 = 0.88$. See original publication for details on materials and methods.

was also found by LaMontagne & McCauley (2001; under food-limited conditions). It might be that simple resource provisioning differences cannot explain the maternal effects observed in *Daphnia*. Perhaps offspring from mothers in low-food environments are programmed to be cautious, and save resources for later reproduction. Future experiments might gain useful insight into the trade-off between reproduction and survival by studying survivorship under a more comprehensive range of conditions.

Our experiments and theoretical results assume that egg size is an accurate measure of resources allocated to an egg, an assumption that has been made previously (see Charnov *et al.*, 1995; Guinnee *et al.*, 2004). While it has been demonstrated in *Daphnia* that egg size is predictive of offspring size (the present study; Goulden *et al.*, 1987; Ebert, 1993; Lampert, 1993), and, moreover, Bell's (1983; Fig. 9) results support a link between egg size and fitness, it is nevertheless possible that eggs produced by mothers experiencing different environmental conditions differ in some important way not measured by size. For example, maternal food condition has been shown to affect carbon : nitrogen ratios in *D. pulex* (Tessier & Consolatti, 1991). Interestingly, first clutch eggs in *Daphnia* are generally the smallest (egg sizes were not compared among clutches in the present study, but see Glazier, 1992; Boersma, 1997 and references therein), and here first clutch eggs had the highest survival (Fig. 8), suggesting that egg size may not be the only factor influencing egg quality. Additionally, the

models we tested also assume that the amount of resources a mother has for reproduction (R) is fixed. If this is not the case, any extra resources above and beyond that needed for producing the optimal size (or resource-laden) eggs might be invested in growth, future reproduction, or other functions. In sum, if eggs differ chemically, or if R is not fixed, optimal clutch size models could become significantly more complicated, and these are interesting topics in need of further investigation.

To conclude, *Daphnia* often produce larger eggs and/or offspring when food is limited (Table 1). This pattern might be adaptive if being a larger offspring is more important when food is limited (Hutchinson, 1951; Green, 1966; Goulden *et al.*, 1987; Gliwicz & Guisande, 1992; Ebert, 1994). Our theoretical results are consistent with this hypothesis, but our experiments found that offspring produced by low-food mothers did not have higher fitness, and there was no evidence of a maternal \times current food environment interaction. Our theoretical results also suggest that a negative relationship between egg size and clutch size could be the result of a minimum viable egg size that is similar to the I^* egg size. This hypothesis is consistent with the literature which finds the likelihood of hatching is strongly dependent on egg size (Bell, 1983; Fig. 9), and it would be extremely useful to investigate this further. These results suggest that a negative relationship between food availability and egg size need not result solely from an adaptive switch to larger offspring under food-limited conditions, but instead arise from the combination of a minimum viable egg size and egg-size optimization. These patterns remain qualitatively well described by the small clutch model of Charnov & Downhower (1995) and Charnov *et al.* (1995).

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