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Exploration of conditions favoring the evolution of superfetation using a state dependent life history model

Ashley Edwards
Department of Biology
The University of North Carolina Asheville
One University Heights
Asheville, NC 28804

Faculty Advisor: Dr. Rebecca Hale

Abstract

Superfetation, the ability of a female to fertilize and carry simultaneous broods of embryos, has been documented in a variety of organisms, but most notably in the live-bearing fish, *Poeciliidae*. Current hypotheses for the evolution of superfetation include that superfetation evolved in response to morphological constraints on female body shape, and that superfetation may have evolved in response to resource availability. Further, there is increasing evidence that superfetation may be derived from matrotrophy, where at least some of the embryonic nourishment is supplied maternally, rather than by a yolk. Previous authors have developed analytical models that describe the conditions under which matrotrophy is favored over lecithotrophy (nourishment entirely from yolk) by manipulating resource availability. Similarly, this paper investigates the conditions that favor superfetation over non-superfetation by manipulating mean resource availability and variance in a state-dependent life history model. An increase in mean available resources decreased the gestational age at which individual offspring were born, but was not shown to affect the size of individuals at birth. Increasing variance resulted in an increased range of offspring sizes. An increase in mean resource availability resulted in an increase in total offspring born during the reproductive season. Increasing mean resources and variance had no effect on the number of offspring a female would birth at one time, but the number of gestating offspring was greater with high mean resource availability. Superfetation was not observed in any of the forward simulated females, regardless of the mean resource and variance.

1. Introduction

Superfetation, the ability of a female to fertilize and carry simultaneous broods of embryos, has been documented in a variety of organisms including American minks[1,2], European badgers[3], and common pigeons[4], but perhaps most prominently in the live-bearing fish family, *Poeciliidae*[5–10]. The *Poeciliidae* family exhibits a wide variety of reproductive adaptations, with the most widely studied strategy being matrotrophy, in which at least some embryonic nourishment comes from directly from the mother after fertilization. There is increasing evidence that lecithotrophy (embryo feeding solely on yolk supplied before fertilization) is the ancestral state of embryonic nourishment in poeciliid fish and that matrotrophy has independently evolved several times[11]. In much the same way, superfetation may be a trait derived from matrotrophy. In fact, phylogenetic analysis of the poeciliid fishes suggests that this trait has independently evolved at least four times and may be co-evolving with the development of placentation in matrotrophic fish[8,12]. Although superfetation has evolved repeatedly, both across vertebrates and within Poeciliids, the conditions under which this trait may have evolved are not well characterized.

One hypothesis for the conditions favoring the evolution of superfetation is that superfetation evolved in response to morphological constraints on female body shape[5,7]. Pregnant poeciliids can have very enlarged abdomens when carrying large, later-stage embryos and may be poorly able to maneuver in high water flow streams. The morphological constraints hypothesis proposes that fish living in environments with high water-velocity will exhibit higher degrees of superfetation than fish living in slow moving waters because superfetation reduces the proportion of embryos that are in larger, late developmental stages[5]. Indeed, among *Poeciliopsis turrubarensis* populations there is a great deal of variation in the degree of brood overlap, including superfetation with a maximum of four simultaneous broods observed in higher water-velocity environments[5]. While variation in water velocity may explain variation in superfetation in *P. turrubarensis*, morphological constraints probably do not explain the evolution of superfetation in all Poeciliids in which it is observed. For example, the least killifish (*Heterandria formosa*) displays a high level of superfetation and is found in environments that harbor slow moving waters such as freshwater ponds and lakes to brackish marshes[10,13]. In this case, the morphological constraints hypothesis does not provide sufficient evidence for the evolution of superfetation.

A second hypothesis that has been explored is that superfetation evolved in response to resource availability. Thibault and Schultz[7] found that even in a non-superfetating fish (*Poecilia reticulata*), variation in size is common among embryos within a brood; by staggering egg fertilization, the female is able to accumulate the resources necessary for allocating yolk to eggs over a longer period of time (in this case, two to six days over which all eggs are yolked and fertilized). Superfetation can be viewed as an extension of staggered egg development, as it provides a more elaborate method to circumvent the resource-availability problem of fertilizing many embryos in a single brood. Burley[4] reasoned that if a reproductive season is sufficiently long to rear successive broods, then producing more frequent, slightly overlapping broods of fewer embryos will yield at least as many offspring in a reproductive season than non-overlapping broods. This resource availability hypothesis emphasizes the constraint imposed on the mother during the period of time when reproduction is most costly, usually at the end of development when offspring are large. The resource demand from younger broods is typically not as great as that of older broods; therefore, the probability of offspring mortality is lower if resources suddenly become limited[4]. Consistent with this hypothesis, Travis et al.[10] reported that superfetation increased offspring production rates without compromising brood size or individual offspring size. Furthermore, superfetation was found to occur more frequently when resource levels were high, implying superfetation may be a means to increase offspring production during periods of excess resources.

Much like Burley[4], we propose superfetation may be a means of reducing the amount of resources required to sustain a large number of developing embryos. Trexler and DeAngelis'[14] analytical model of the evolution of matrotrophy may help us understand the evolution of superfetation. In their model, daily available resources were stochastic variables to determine the conditions that will favor matrotrophy over lecithotrophy (nourishment via yolk, only). They compared the fitness of an individual exhibiting lecithotrophy to that of an individual with matrotrophy while varying the mean daily resources. The lecithotrophic individual was only able to initiate a brood when she had sufficient resources for yolk that would carry embryos to birth. The matrotrophic individual could initiate a larger brood with fewer resources. Further, if the resources on a given day were below the minimum to sustain her embryos, the female would abort or resorb embryos down to a number that could be maintained on the current resource level. They found that matrotrophy conferred higher fitness when mean resource levels were high and the female had a high level of embryo resorption efficiency. They suggest, therefore, that matrotrophy may have evolved in environments that consistently provided more resources than needed for embryonic development.

From Trexler and DeAngelis' conclusions, it can be inferred that matrotrophic females would then become restricted to living in these high resource environments, and therefore be disadvantaged if rapid changes in resource availability occurred. We apply a similar premise with respect to stochastic resources, but a different modeling approach to explore how mean and variability in resource level influence the benefit of superfetation over non-superfetation. We hypothesize that superfetation evolves as a way for matrotrophic females to maximize offspring production over the reproductive season when resources are relatively low. We expect that superfetation will be favored over non-overlapping broods when the expected resource levels are low, and daily resource variability is high.

2. Methods

We develop a model of progressive provisioning of parental care to offspring in which decisions are made daily that determine how many offspring a female will birth and whether or not she will fertilize a new brood of embryos. The parent can regulate the size of the oldest brood by birthing all or some of the embryos at any time during the reproductive season.

2.1 Approach

We develop a state-dependent life history model[15–17] of progressive provisioning in which a female can reproduce multiple times during a reproductive season of length, T. We consider a female that begins the reproductive season (at $t = 1$) gestating one brood of n_0 embryos. At the start of each time step, t (e.g., day), the female acquires an amount of resources determined stochastically and drawn from a normally distributed range of possible resource levels. Given that amount of resources, the female simultaneously decides how many offspring to birth and whether or not to fertilize a new brood of n_0 younger offspring, both of which will alter the number of embryos she is carrying. Following adjustment of her embryo counts, she evenly distributes among all offspring the resources that she acquired that day. At the end of the reproductive season, she does not gain additional fitness from unbirthed offspring. The fitness gained by birthing an individual embryo is a monotonically increasing function of offspring size at birth[16,18]; in other words, the longer the female gestates an embryo, the more fitness she gains at its birth.

We use the rate-maximizing model developed by Mangel[19] to determine the number of embryos to birth, as well as the fertilization decision, that will maximize her cumulative fitness from that day to the end of the reproductive season. This model requires that we start at T-1 and continue backward to t, determining the immediate fitness gain and expected future fitness gain of each possible birthing and fertilization decision, and then comparing them to identify the optimal birthing and fertilization decisions. Following this backward iterative process, we simulate 100 females progressing through a reproductive season, each receiving stochastically determined resource amounts each day and making the optimal birthing and fertilization decisions given the resources received. We then manipulate the mean and variance in daily resources and examine their effects on offspring size at birth, offspring age at birth, number offspring born simultaneously, number embryos carried simultaneously, total offspring born over the season, and the incidence of superfetation.

2.2 The Model

We consider a female who begins at time t, carrying n_{old} identical offspring of size, s_{old}. If she births b of these offspring, then the number of embryos at time $t+1$ is

$$N_{old}(t+1) = N_{old}(t) - b, \tag{1}$$

and she accrues fitness that is the product of the number of embryos birthed and their probability of survival to maturity, $W_o(s)$. $W_o(s)$ is a function of offspring size (s) given by

$$W_o(s|k,I) = max\{1 - e^{-k(s - I)},0\} \tag{2}$$

where k influences the slope of the function, and I is the minimum size requirement for offspring survival to maturity (Figure 1). Thus, for $s < I$, $W_o(s|k, I) = 0$. In this model, I is defined as a constant and is the same for all offspring. If offspring are born at a size that is less than I, parents gain no fitness; these embryos are equivalent to underdeveloped, aborted young.

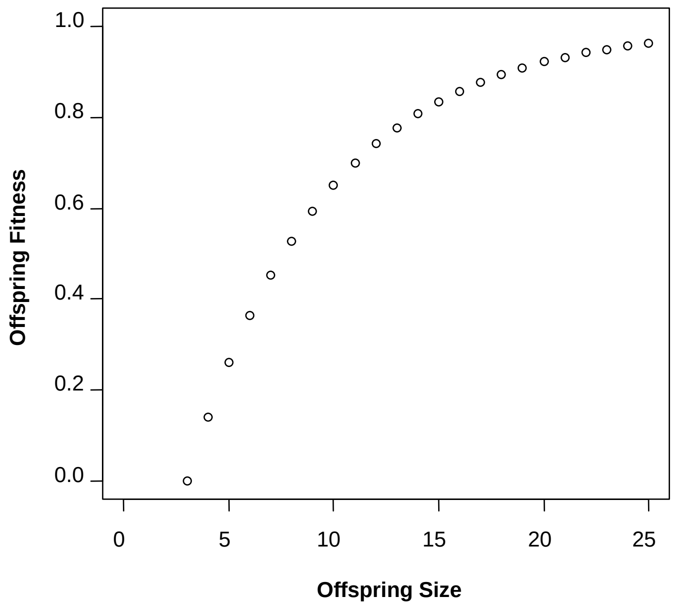


Figure 1. Offspring fitness as a function of size[18]. Offspring fitness is a function of size at birth, which is determined by the accumulation of resources provided by the female. *I* is the minimum size requirement for embryonic viability and *k* determines the rate of increase in the function; each are held constant for the simulations at 3 and 0.15, respectively.

We now consider the decision to fertilize, *f*, a second brood of n_0 embryos. For this model, *f* is a binary term assuming values of 0 or 1 for decisions *not to* fertilize or *to* fertilize a second brood, respectively. We assume that females can only give birth to embryos in the oldest brood and that she can carry a maximum of two broods simultaneously. Therefore, the number of gestating embryos at any point during the reproductive season can be described by rewriting Eq. (1) to include the presence of a younger brood:

$$N_{total}(t+1) = (N_{old}(t) - b) + N_{young}(t). \tag{3}$$

All embryos not birthed continue to grow during time *t*. Let *E(t)* denote the resource available for offspring growth during the reproductive period, *t*. For simplicity, we assume that each embryo, regardless of brood, is allocated an equal portion of resources. Thus, the size increment by which embryos grow each day is described by

$$G(t) = \frac{E(t)}{Nold\ (t) + Nyoung(t)}. \tag{4}$$

Then, the size of the older brood is given by

$$S_{old}(t+1) = S_{old}(t) + G(t), \tag{5}$$

and similarly the size of the younger brood is given by

$$S_{young}(t+1) = S_{young}(t) + G(t), \tag{6}$$

under the implication that growth for the older and younger broods occurs when $N_{old}(t) - b > 0$, and $N_{young}(t) > 0$. Because $N_{old}(t+1)$, $N_{young}(t+1)$, $S_{old}(t+1)$, and $S_{young}(t+1)$ will depend on whether a female has birthed her entire older brood, is currently carrying a younger brood, and chooses to fertilize a new brood, we define three indicator functions, D_x, Y_y, and F_f, such that

$$D_x = \begin{cases} 0, & \text{if } x = 0 \\ 1, & \text{if } x > 0 \end{cases}$$

$$Y_y = \begin{cases} 0, & \text{if } y = 0 \\ 1, & \text{if } y > 0 \end{cases}$$

$$F_f = \begin{cases} 0, & \text{if } f = 0 \\ 1, & \text{if } f > 0. \end{cases} \tag{7}$$

If the female is not carrying a younger brood, $Y_y = 0$ and N_{old} and S_{old} will be as in Eq. (1) and (5), respectively. However, N_{young} and S_{young} will depend on whether she fertilizes a new brood.

$$N_{young}(t+1) = \begin{cases} 0, & \text{if F} = 0 \\ n_0, & \text{if F} = 1, \end{cases}$$

$$S_{young}(t+1) = \begin{cases} 0, & \text{if F} = 0 \\ s_0, & \text{if F} = 1. \end{cases} \tag{8}$$

When $b = N_{old}(t)$ (i.e., all embryos are birthed), $D_x = 0$ and the number and size of embryos in both the older and younger brood depend on whether there is a younger brood already and whether a new brood is fertilized. If no younger brood is present, then a new brood is fertilized and immediately becomes the older brood. If a younger brood is present, it will assume the older brood position and the female must decide whether to fertilize a new brood. Therefore,

$$N_{old}(t+1) = \begin{cases} n_0, & \text{if Y} = 0 \\ n_{young,} & \text{if Y} = 1, \end{cases}$$

$$S_{old}(t+1) = \begin{cases} s_0, & \text{if Y} = 0 \\ s_{young} + G(t), & \text{if Y} = 1, \end{cases}$$

$$N_{young}(t+1) = \begin{cases} 0, & \text{if F} = 0 \\ n_0, & \text{if F} = 1, \end{cases}$$

$$S_{young}(t+1) = \begin{cases} 0, & \text{if F} = 0 \\ s_0, & \text{if F} = 1. \end{cases} \tag{9}$$

Each day, the female consumes a finite amount of resources drawn from a stochastic random variable with mean, μ, and variance, σ^2. We assume a finite set of resource states, J, for the environment such that

$$Pr\ \{\mathrm{E}(t) = E_j\} = p_j\,, \tag{10}$$

where p_j is a discrete normal distribution and

$$p_j = \mathrm{c}e^{-\left(\frac{(Ej-\mu)^2}{2\sigma^2}\right)} \tag{11}$$

with c chosen so that $\sum_{j=1}^{J} \mathrm{p_j} = 1$.

We let $W_p\ (n_{old}, n_{young}, s_{old}, s_{young}, t \mid \mu,\sigma^2)$ represent the female's maximum expected fitness during t, where $n_{old} = N_{old}(t)$, $n_{young} = N_{young}(t)$, $s_{old} = S_{old}(t)$, $s_{young} = S_{young}(t)$, and equal the sum of the fitness gained from birthing embryos at t and the residual fitness gained by continuing to brood the remaining embryos to $t+1$. No additional fitness can be accumulated after the reproductive season ends; therefore,

$$W_{\mathrm{p}}\,(n_{old}, n_{young}, s_{old}, s_{young}, T \mid k\,, I) = 0.$$

Then, for previous times ($t < T$),

$$W_P(n_o, n_y, s_o, s_y, t \mid \mu, \sigma^2)$$
$$= \sum_{j=1}^{J} p_j \max_{b,f} \left\{ \begin{aligned} & bW_O(s) \\ & + (1-D_{n_o-b})Y_{n_y} e^{-m} W_P\left(n_o-b, n_y, s_o+g(s_o, n_o-b, E_j), s_y + \frac{E_j - g(s_o, n_o-b, E_j)}{n_y}, t+1\right) \\ & + (1-D_{n_o-b})(1-Y_{n_y})F_f e^{-m} W_P\left(n_o-b, n_0, s_o+g(s_o, n_o-b, E_j), s_0 + \frac{E_j - g(s_o, n_o-b, E_j)}{n_0}, t+1\right) \\ & + (1-D_{n_o-b})(1-Y_{n_y})(1-F_f)e^{-m} W_P(n_o, 0, s_o+g(s_o, n_o-b, E_j), 0, t+1) \\ & + D_{n_o-b}Y_{n_y}F_f e^{-m} W_P\left(n_y, n_0, s_y+g(s_y, n_y, E_j), s_0 + \frac{E_j - g(s_o, n_y, E_j)}{n_0}, t+1\right) \\ & + D_{n_o-b}Y_{n_y}(1-F_f)e^{-m} W_P(n_y, 0, s_y+g(s_y, n_y, E_j), 0, t+1) \\ & + D_{n_o-b}(1-Y_{n_y})F_f e^{-m} W_P(n_0, 0, s_0+g(s_0, n_0, E_j), 0, t+1) \end{aligned} \right\} \tag{12}$$

The summation on the right-hand side indicates that the sum is taken over all possible resource values (multiplied by the probability of obtaining that resource value, p_j) encountered by the female. Each term describes the fitness gained by each of the decisions the female can make during the reproductive time horizon. The first term inside the bracket is the immediate maternal fitness gained by the embryos that were born during t. The remaining terms are the expected fitnesses gained from t +1 to T, given her possible states at t +1, where m is the mortality rate during t. Therefore, the second term indicates a female's expected fitness if she births none or only part of her older brood, has a younger brood, and necessarily does not fertilize any additional broods; the third term is the expected fitness if the female births none or only part of her older brood, has no younger brood present, and fertilizes a second brood of n_0 eggs of size s_0; etc.

We identify the optimal number of offspring to birth, $b^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$, and the optimal fertilization decision, $f^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$, given each possible combination of older brood size, younger brood size, and the corresponding embryo sizes at each step through the gestational period using equation (12). We begin at T - 1 and solve backwards through time to t = 1.

Once b^* and f^* have been determined across all values of n_{old}, n_{young}, s_{old}, s_{young}, and E_j, we iterate the model forward to simulate the reproductive strategy of a gestating female in an environment in which the daily resource availability is stochastic. To incorporate stochasticity in resource availability, we generate a uniform random variable X and find the j satisfying

$$\sum_{j'}^{j-1} p_{j'} < X < \sum_{j'}^{j} p_{j'} \qquad (13)$$

We then determine E_j (equation 10) and use that value to identify $b^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$ and $f^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$. These decisions are used to calculate $N_{\text{old}}(t+1)$, $N_{\text{young}}(t+1)$, $S_{\text{old}}(t+1)$, and $S_{\text{young}}(t+1)$ (equation 9). During forward iteration, the size values $S_{\text{old}}(t)$, and $S_{\text{young}}(t)$ are continuous variables and can assume non-integer values. Since the backward iteration solved for discrete values of brood and embryonic sizes, values for $b^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$ and $f^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$ are not available for non-integer combinations, and thus linear interpolation[17] would not provide results that are biologically valid. We adjust embryonic sizes by identifying the r' and v' that satisfy

$$s_{r'} \leq S_{old} < s_{r'+1},$$

$$s_{v'} \leq S_{young} < s_{v'+1} \qquad (14)$$

We then identify $b^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$ and $f^*(n_{old}, n_{young}, s_{old}, s_{young}, E_j, t \mid \mu,\sigma^2)$, where

$$Wp = \max\{Wp(n_{old}, n_{young}, s_{r'}, s_{v'}, t), Wp(n_{old}, n_{young}, s_{r'+1}, s_{v'+1}, t)\} \qquad (15)$$

We assume that resources are allocated equally to each of the embryos in both the older and younger broods. We assume that a female can carry no more than two simultaneous broods during t. For each of 100 simulations, we identify the total number of offspring birthed, the average number of embryos a female is carrying at a given time t, how many times a second brood is initiated during the reproductive season, and parental fitness for the female, Wp, for a range of resource availability mean, μ, and variance, σ^2. A summary of the variables, definitions, and their ranges is provided in Table 1.

Table 1. Model State Variables and Parameters.

Variable	Definition	Value or range
T	Time horizon; duration of gestation	10 days
E	Daily energy intake, stochastic variable	$\sim N(\mu, \sigma^2)$
n_{old}	Number of embryos in older brood, state variable	1-5
n_{young}	Number of embryos in younger brood, state variable	0 or 5
s_{old}	Body size of embryos in older brood, state variable	0-25
s_{young}	Body size of embryos in younger brood, state variable	0-25
m	Mortality rate of female during time t	0.1
k	Slope parameter of offspring fitness function	0.15
I	Minimum size at birth necessary for survival	3
b	Number of embryos born during time t	0 - n_{old}

*b**	Maternal optimum number of embryos to birth	
f	Decision regarding fertilization of second brood, binary	0 (do not fertilize) or 1 (fertilize)
*f**	Maternal optimum decision regarding fertilization of second brood	

3. Results

Manipulating the mean available resources affected gestating females and their offspring in several ways. An increase in mean available resources decreased the gestational age at which individual offspring were born, but was not shown to affect the size of individuals at birth (Figure 2). Increasing variance resulted in an increased range of sizes (increased standard deviation) for all mean values of resources, particularly when the mean resource level was low ($\mu = 2$). Variance in the resource levels only affected the gestational age at birth when the mean resources levels were low ($\mu = 2$), and resulted in a decrease in age as variability increased.

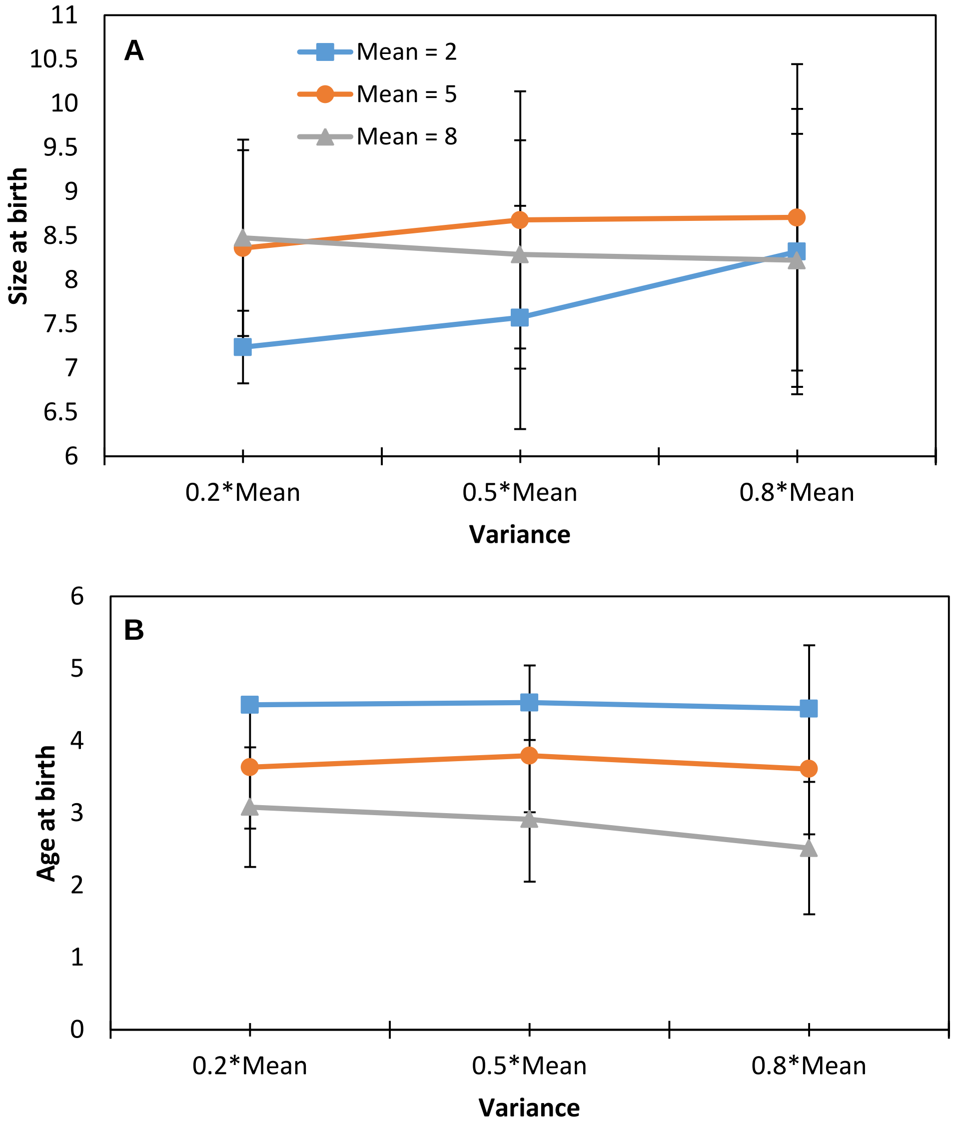
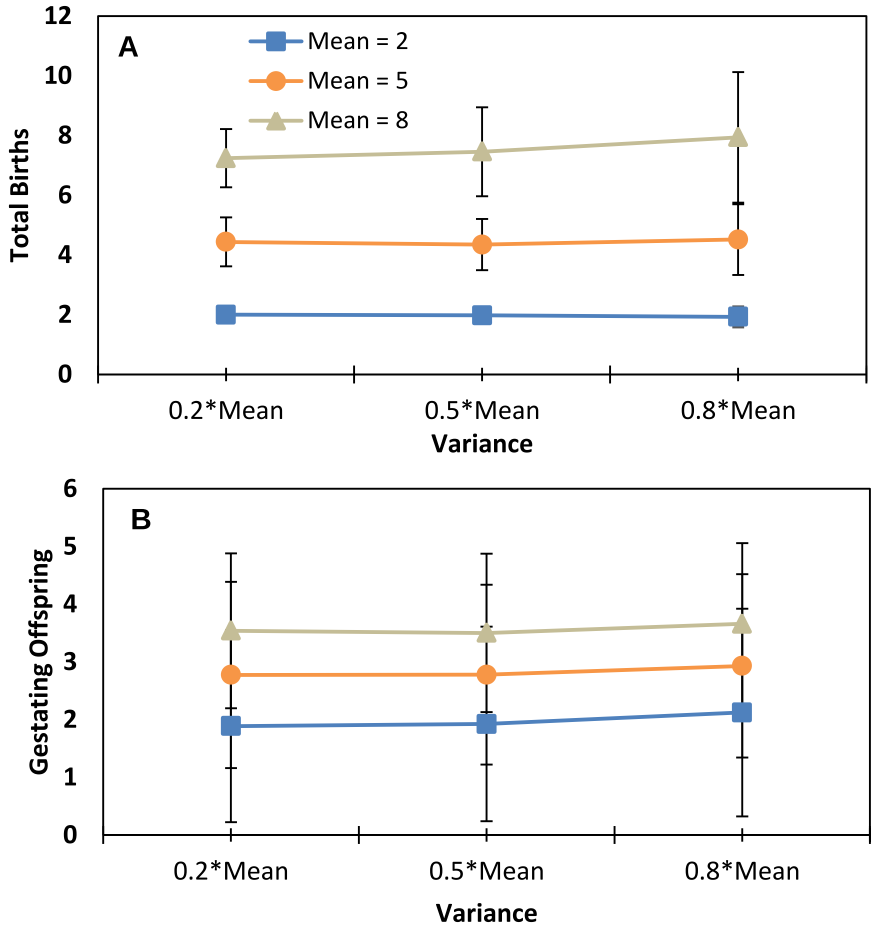


Figure 2. Effects of energy resources on individual offspring during the reproductive season. Daily energy resource level is a stochastic variable with mean = 2 (squares), 5 (circles), and 8 (triangles), and variance equal to 20%, 50%, 80% of the total energy resources available. **A**, Mean ± SD size of individuals at birth. **B**, Mean ± SD age of individuals at birth.

An increase in mean resource availability resulted in an increase in total offspring born during the reproductive season, while variance had only a slight effect on the number of offspring born when the mean resource levels were high (μ = 8), and little to no effect on the remaining mean resource levels (μ = 2, μ = 5). Increasing mean resources and variance had no effect on the number of offspring a female would birth at one time, but the number of gestating offspring was greater with high mean resource availability (μ = 8) than with low mean (μ = 2). Variance in mean resources had little to no effect on the number of gestating offspring or individuals born at once time during the reproductive season (Figure 3).

Superfetation was not observed in any of the forward simulated females, regardless of the mean resource and variance.



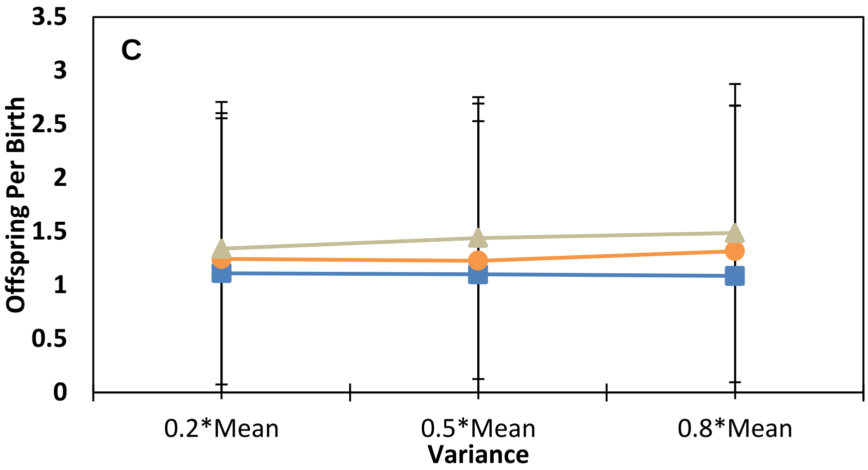


Figure 3. Effects of energy resources on gestating females during the reproductive season. Daily energy resource level is a stochastic variable with mean = 2 (squares), 5 (circles), and 8 (triangles), and variance equal to 20%, 50%, 80% of the total energy resources available. **A**, Mean ± SD of total number of viable embryos birthed. **B**, Mean ± SD of number of gestating embryos a female has at one time. **C**, Mean ± SD of the number of embryos born at one time.

4. Discussion

We found that increasing the mean and variance of resource availability in an environment can influence the reproductive strategy of a gestating female. When resource levels are high, a female can apportion a greater allowance of resources to individual offspring, allowing for more rapid growth when compared to offspring counterparts in low resource environments. This is an important factor as we consider why females with access to more resources produce more total offspring over the reproductive season. Because W_o, the function relating offspring size at birth to offspring fitness, is the same for all levels of resources, there exists a single optimal offspring size at birth. This likely explains why neither resource mean nor variance influenced offspring size at birth. However, resource mean greatly influences how long it takes for embryos to reach this optimal size. Females in low mean resource environments allocate fewer resources to offspring on a given day, and there is a longer duration necessary for the offspring to reach the optimal size. Similarly, when resources are high, offspring will reach this optimal size sooner. This allows a female to initiate new cohorts of embryos more frequently and subsequently produce a greater number of total offspring. This pattern is consistent with previous research on poeciliid fish in which females with access to more resources had higher fecundity at over the reproductive season[6].

The ability for a female to reduce her brood size becomes increasingly more important as resource levels decline. This is evidenced by a decrease in the number of offspring that a female was gestating at any time during the time horizon under low resource levels. The number of aborted offspring in our model is analogous to the results produced by resorption in the Trexler and DeAngelis[14] model. In that model, matrotrophic females were able to provide nourishment during times of low resource availability, by selectively aborting embryos and reallocating the energy from the aborted embryos among the remaining offspring. Resorption and abortion by early birth each occur as a means to ensure that at least some embryos get enough resources to survive until the next resource is encountered. These patterns suggest that a female will adjust her brood to a size that increases her overall fitness given the expected resource level.

Superfetation was not observed in any of the resource level variations. This can be partially explained by the simplification of energy allocation to offspring used in our model. As offspring continue to grow during the reproductive season, the amount of resources needed for continued growth increases[14,16,18,20]. A key assumption of our model is that resources are divided evenly among the offspring, regardless of brood (older vs. younger). We made this assumption for computational efficiency. A more biologically realistic mode of allocation is that a female will provide the minimum resources needed to her older offspring first and divide the remainder among the younger brood. Indeed, our hypothesis that superfetation will evolve when resource levels are low is based on the idea that younger embryos have lower resource requirements. Future work will include an expansion of the current model to include this uneven distribution of resources based on size-dependent resource demand from offspring.

5. Acknowledgements

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