Maternal food restriction during pregnancy affects offspring development and swimming performance in a placental live-bearing fish

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Summary statement
Maternal food restriction during pregnancy results in smaller offspring, slower postnatal body fat gain, and an inhibition of postnatal improvement of swimming skills during feeding, possibly leading to lower competitive abilities after birth.

Abstract
How pregnant mothers allocate limited resources to different biological functions such as maintenance, somatic growth, and reproduction can have profound implications for early life development and survival of offspring. Here we examined the effects of maternal food restriction during pregnancy on offspring in the matrotrophic (i.e. mother-nourishment throughout gestation) live-bearing fish species Phalloptychus januarius (Poeciliidae). We fed pregnant females either with a ‘low-food’ or ‘high-food’ ration for six weeks and quantified the consequences for offspring size and body fat at birth and one week after birth. We further measured fast-start escape performance of offspring at birth, as well as swimming kinematics during prey capture at zero, two, and seven days after birth. We found that the length of maternal food restriction during pregnancy negatively affected offspring dry mass and lean dry mass at birth, as well as body fat gain during the first week after birth. Moreover, it impacted the locomotor performance of offspring during prey capture at, and during the first week after, birth. We did not observe an effect of food restriction on fast-start escape performance of offspring. Our study suggests that matrotrophic poeciliid fish are maladapted to unpredictably
fluctuating resource environments, because sudden reductions in maternal food availability during pregnancy result in smaller offspring with slower postnatal body fat gain and an inhibition of postnatal improving swimming skills during feeding, potentially leading to lower competitive abilities after birth.

Introduction
How individuals allocate resources to different biological functions such as maintenance, somatic growth, and reproduction is crucial for their life-history (Stearns, 1992). Different functions compete for limited resources, leading to trade-offs and a limited set of possible life-history strategies (Braendle et al., 2011).

Besides intrinsic trade-offs and constraints, resource allocation, and hence, life-histories are influenced by environmental factors, such as food availability (Boggs, 1992; Santi et al., 2020). Increased maternal food availability enables a higher energy uptake, which can be allocated to any function (van Noordwijk and de Jong, 1986). However, when food is scarce, specific functions may be prioritized over others. In lecithotrophic live-bearing animals, where all resources are allocated to the eggs prior to fertilization, adverse food conditions may reduce maternal growth, fat reserves, and fecundity (Bashey, 2008; Reznick et al., 1996; Wang et al., 2017), yet also increase the relative investment in offspring size (Bashey, 2008; Reznick et al., 1996). In this example, mothers adaptively modified offspring phenotype in preparation for predicted, adverse environmental conditions (low food availability). This response is presumably advantageous, as under competitive adverse food conditions, mothers gain fitness benefits by producing larger offspring (Leips et al., 2013; Parker and Begon, 1986). However, as optimal offspring size is given by the offspring size-performance relationship that depends on the environment, the advantage of producing larger offspring at birth diminishes or disappears in favorable environments (Bashey, 2008; Leips et al., 2013; Parker and Begon, 1986), and the predicted optimal strategy for the mother is to produce more numerous but smaller offspring (Jørgensen et al., 2011). If the maternal environment reliably predicts future environmental conditions, females may evolve the ability to adaptively adjust offspring phenotype at birth, based on environmental cues (Mousseau and Fox, 1998a).

The modulation and timing of nutrient acquisition and allocation also affect life-histories (Zera and Harshman, 2001). In matrotrophic live-bearing animals, experimental manipulations of food availability can impact the pattern of resource allocation to offspring (Banet and Reznick, 2008; Banet et al., 2010; Pollux and Reznick, 2011; Reznick et al., 1996; Van Dyke and Griffith, 2018). Instead of allocating all resources to the eggs prior to fertilization (i.e. lecithotrophy), matrotrophic species transfer nutrients to their embryos throughout gestation via a placenta (Pollux et al., 2014; Reznick et al., 2002; Wourms, 1981). Because matrotrophic species continuously supply embryos with resources,
determination of brood size (i.e. number of embryos per brood) and offspring size are decoupled (Pollux and Reznick, 2011). When resource conditions during pregnancy suddenly deteriorate, matrotrophic species may not be able to optimally provision their embryos. Because females cannot abort embryos in response to food shortage and maternal fat reserves do not fully buffer females during gestation (Banet and Reznick, 2008; Banet et al., 2010; Pollux and Reznick, 2011; Reznick et al., 1996), sudden resource declines inevitably result in smaller, worse-conditioned (i.e. having less fat reserves) offspring at birth. Small offspring size in low food conditions is associated with a lower survival (Bashey, 2008; Leips et al., 2013; Parker and Begon, 1986). Therefore, matrotrophy has been argued to be maladaptive in fluctuating resource environments (Pollux and Reznick, 2011; Reznick et al., 1996; Trexler and DeAngelis, 2003).

Differential resource allocation to offspring size at birth can impact postnatal development and survival (Mousseau and Fox, 1998b). In fish, body size is linked to locomotor performance (Gibb et al., 2006). Smaller offspring are likely to perform worse on fast-start escapes (Dial et al., 2016), presumably decreasing survival probability in environments with high predation risks. Prey-capture abilities immediately after birth also heavily depend on swimming performance. New-born live-bearing fish are super-precocial, having functional prey-capture abilities at birth and relying on active exogenous feeding after birth (Lankheet et al., 2016). Moreover, they rapidly develop the visuo-motor skills required for prey-capture during the first days after birth and effectively improve their success rate, promoting food uptake and survival (Lankheet et al., 2016). Offspring size thus affects locomotor performance and prey-capturing abilities after birth. However, it is still unknown to what extent restricted maternal food availability during pregnancy affects offspring swimming performance in fast-start escapes and in prey-capture after birth.

Here, we examine the effects of maternal food restriction during pregnancy on growth and locomotor performance of offspring after birth in the matrotrophic fish species *Phalloptychus januarius* (family Poeciliidae; Hensel, 1868). If matrotrophy is maladaptive under these circumstances one may expect differences in offspring size as well as locomotor performance. Specifically, we measured: (i) size and body fat of offspring at different ages (i.e. zero and seven days old), (ii) fast-start escape performance at birth, and (iii) swimming kinematics while feeding during the first week after birth, to quantify immediate and early postnatal effects of food restriction during pregnancy on offspring size, quality, and locomotor performance. As a proxy for locomotor performance, we focused on mean and maximum speed and acceleration of fast-starts and feeding actions. By combining the different measurements, we reveal consequences of maternal food restriction for life-history variation and its implications for the quality and performance of newborn fish in a placental species.
Materials and methods

Experimental animals

*Phalloptychus januarius* is endemic to Brazil and is known from coastal drainages in Rio de Janeiro, São Paulo, and Paraná States of Brazil (Lucinda, 2005). The *P. januarius* used in this experiment were laboratory-born and derived from laboratory stocks originally collected in the Rodrigo de Freitas Lagoon, Rio de Janeiro (Brazil) in November 2006 and held at the Pollux lab (Wageningen University, the Netherlands). In the Rodrigo de Freitas lagoon, *P. januarius* co-occurs with a variety of piscivorous fish (Andreata, 2012), birds (Santi et al., 2020), and bats (Luz et al., 2011), which collectively may represent a predation risk. Moreover, in their natural habitat they may experience both intra- and interspecies competition for food.

Maternal food treatment during gestation

The timing and length of maternal food restriction during (and prior to) pregnancy may influence offspring development. To test how, we conducted a 7-week experiment in 2019 to study the effects of maternal resource restriction during pregnancy on offspring. Females received *ad libitum* food for one week, and were then subjected to either a ‘low-food’ (LF) or ‘high-food’ (HF) ration during a 6-week food treatment. Offspring born during the 1st week of the experiment served as a control, since they did not suffer from maternal food restriction. Offspring born during the 2nd week were (indirectly) ‘exposed’ to maternal food restriction only during the last 25% of their development (embryonic development in *P. januarius* takes approximately four weeks until birth; Pollux and Reznick, 2011). Offspring born during the 3rd week were exposed during the second half of their development and offspring born during the 4th week during the last 75% of their development. Offspring born during week 5–7 were exposed during 100% of their development, but differed in the length of additional maternal food restriction prior to the start of their development: i.e. zero weeks for offspring born during the 5th week to two weeks for offspring born during the 7th week.

Prior to the experiment, we set up 50 8-L aquaria, each containing one mature male and female *Phalloptychus januarius*. This is a particularly interesting study species within the context of this study, because it has superfetation (Pollux et al., 2009): the ability to carry seven to 14 temporally overlapping broods at different developmental stages. This means that over the course of the experiment each female will give birth to offspring that have been exposed to maternal food restriction during a different developmental period and for a different length of time. All fish were fed *ad libitum* prior to the start of the experiment, at which time the males were removed. Females were re-mated once overnight, after three weeks, to ensure a sufficient supply of sperm. There was a fourfold difference in food quantity between the LF and HF treatments, consisting of either 25 μl (LF) or 100 μl (HF) liver paste in the morning and 25 μl or 100 μl of newly-hatched brine shrimp (*Artemia*...
salina) in the afternoon. Previous experiments indicated that the LF rations were sufficient to sustain reproduction while the HF rations were close to ad libitum feeding (Pollux and Reznick, 2011). We measured female body length (to the nearest mm) and wet mass (to the nearest 0.1 mg) at the beginning of the food treatment (i.e. after week 1) and at the end of the experiment (i.e. after week 7).

Collection of offspring for various measurements
Experimental tanks were checked daily during the 7-week experiment for the presence of new-born offspring. These offspring were subsequently used for various experimental procedures: one randomly selected subset of offspring was used to measure dry mass and body fat (on day 0 and 7 after birth) using established protocols (Supplementary Methods 1.1). A second subset was used to quantify the fast-start escape performance (on day 0) and swimming kinematics during feeding (on day 0, 2, and 7 after birth) (see below for more details).

Locomotor performance of offspring
Individual fish were isolated in Petri dishes (Ø 55 mm) and stored in an incubator at 24°C. The fish were subsequently transferred to an experimental set-up to record either the (i) fast-start escape performance on day 0, or (ii) swimming kinematics during feeding on day 0, 2, and 7 after birth. The fish were filmed from the dorsal side against an array of LEDs behind a white, translucent plate using a high-speed video camera (Supplementary Methods 1.2). The water level was kept at 5 mm to minimize vertical movements of the fish (average body length of new-born offspring: ~7 mm). Following the performance measurements, the fish were euthanized and preserved to measure dry mass and fat content (Supplementary Methods 1.1).

a) Fast-start escape response
Throughout the experiment up to 15 offspring were collected each Monday and Thursday (if available) to measure escape performance at birth. For this, the fish were transferred to smaller Petri dishes (Ø 35 mm). A maximum of five dishes, each containing a single fish, were simultaneously filmed. The fast-start escape maneuver was initiated after a 5 min acclimation period by dropping a weight on the plate. Fish were allowed to recover for 5 min, before initiating a second and third fast-start.
b) Swimming kinematics during feeding

Throughout the experiment up to 18 offspring were collected each Tuesday and Wednesday (if available) to measure swimming kinematics during feeding. The fish were held in the incubator at a 12L:12D cycle for one week and fed daily *ad libitum* with newly-hatched *Artemia*. Swimming kinematics during feeding were measured on day 0, 2, and 7 after birth. A maximum of nine dishes, each containing a single fish, were simultaneously filmed (Fig. 1A). After an initial 5 min acclimation period, the swimming kinematics were first recorded for 5 min without food present (control). Subsequently, ~30 newly-hatched *Artemia* were added to each Petri dish to trigger feeding responses. The swimming kinematics were recorded for another 5 min. To sufficiently motivate offspring to feed, there was no additional food supplied prior to the feeding trials. The resolution in our videos was too low to track individual *Artemia*, therefore we lack information about prey-capture success of individual fish.

c) Video analysis

We used an in-house developed Python program to track the fish’s silhouette in real-time and to automatically extract the location of the center of mass through time. Using the fish’s position, we calculated swimming speed and linear acceleration (Supplementary Methods 1.3). As a proxy for locomotor performance, we extracted the mean and maximum speed and acceleration for each fast-start and food response. For fast-starts, we used a period after releasing the weight, which was sufficiently long to capture the response (0–0.157 s; Fig. 1B). Response intervals thus included a baseline during the time it took for the weight to hit the plate. Speed profiles were considered as fast-start responses if the speed during a 7 frame (0.103–0.120 s) window around the maximum of the overall mean speed exceeded the threshold of 60 mm s$^{-1}$ (Fig. 1B, C). For food responses, we used the 5 min control period (no *Artemia* present), as well as the 5 min feeding period (Fig. 1D). Because extreme values for speed and acceleration are relatively sensitive to measurement noise, we defined the maximum speed and acceleration as the mean of the values above the 95% quantile, rather than the actual maximum values (Fig. 1E).

Statistical analysis

All estimations were carried out in a Bayesian framework using the *MCMCglmm* package (Hadfield, 2010) in R v 3.5 (R Core Team, 2020). Multivariate models allowed for the covariance between the residuals of all responses. Convergence was assessed by visual examination of the traces and the autocorrelations of the parameter chain was checked to be less than 0.1.
To identify potential effects of maternal food restriction on maternal wet mass and standard length, each trait was fitted in an univariate LMM. Fixed effects included treatment (LF or HF), experimental day (9 or 51), as well as treatment×day. In the case of maternal wet mass, maternal standard length was fitted as an additional covariate to quantify mass changes relative to length. Maternal identity was fitted as random intercept to correct for maternal variance sources not accounted for by the fixed effects (Hagmayer et al., 2018). Females that were not pregnant at the end of the experiment (n=2) or that died before (n=3), were excluded from the analysis. In addition, maternal fecundity was fitted in a GLMM using a log link for the Poisson-distributed response. Fixed effects included treatment, experimental week (1–7), as well as treatment×week. Maternal identity was fitted as random intercept (see above).

The effects of maternal food restriction on offspring phenotypic traits (dry mass, lean mass, and body fat) were analyzed by fitting all traits in a multivariate LMM as a function of treatment (LF or HF). Additional fixed effects included experimental day (day), day², age (0 or 7 days), treatment×day, and treatment×age. Another fixed effect specified whether the offspring were found alive or dead. The probability of finding alive offspring significantly decreased throughout the experiment (β_post.mean=-0.065, P_MCMC=0.001), but did not differ between food treatments (β_post.mean=0.008, P_MCMC=0.732). The cause of the increasing frequency of dead offspring is unclear and warrants further research. Maternal identity was fitted as random intercept (see above). To optimize normality and homoscedasticity of the model residuals, body fat was square-root transformed.

For fast-start escapes we first analyzed the probability of an individual to ‘respond’, which is used as a proxy for the behavioral propensity to react to the startle stimulus. To model the potential effects of treatment, the individual’s response (yes or no) was fitted as a function of treatment (LF or HF), experimental day, as well as treatment×day in a GLMM using a logit link for the binomial-distributed response. Secondly, the mean and maximum speed and acceleration (all ln-transformed) of identified responses were fitted in a multivariate LMM. Fixed effects were treatment, experimental day, as well as treatment×day. In both models, maternal identity and Petri dish position were fitted as random intercepts. The latter accounts for potential effects of the Petri dish position relative to the camera and stimulus (i.e. location of weight drop). Moreover, offspring and replicate trial identity were fitted as random intercepts to account for pseudo-replication and for variation through habituation to the stimulus, respectively.

Likewise, the effects of maternal food restriction on swimming kinematics during feeding were analyzed by fitting the mean and maximum speed and acceleration (all ln-transformed) in a multivariate LMM. To specifically quantify the effect of food supply, the model was fitted to the locomotor performance parameter extracted during the (i) 5 min control period (no food supply) and (ii) 5 min feeding period. To reduce model complexity, data from the two periods were analyzed in
separate models. Fixed effects included treatment (LF or HF), experimental day, age (0, 2, or 7 days), treatment×day, and treatment×age. Maternal and offspring identity, as well as Petri dish position were fitted as random intercepts (see above).

Results

Maternal length, wet mass, and fecundity
At the beginning of the food treatment, maternal standard length (SL) did not significantly differ between LF and HF females ($\beta_{\text{post.mean}}=-0.615$, $P_{\text{MCMC}}=0.296$). However, maternal wet mass was significantly lower for a given length in HF females ($\beta_{\text{post.mean}}=-0.029$, $P_{\text{MCMC}}=0.008$; Fig. S1). Over the course of the experiment, LF and HF females both showed an increase in SL (Fig. S1A). LF females, however, lost significantly more mass for a given length compared to HF females (0.08 vs. 0.03 g; $P_{\text{MCMC}}=0.004$; Fig. S1B). Finally, maternal fecundity did not significantly change throughout the experiment ($\beta_{\text{post.mean}}=0.021$, $P_{\text{MCMC}}=0.374$), and did not differ between food treatments ($\beta_{\text{post.mean}}=0.006$, $P_{\text{MCMC}}=0.882$).

Offspring size and body composition at birth
The dry mass and lean mass of offspring at birth significantly decreased throughout the 6-week food treatment in both LF (dry mass: $\beta_{\text{post.mean}}=-0.010$, $P_{\text{MCMC}}<0.001$; lean mass: $\beta_{\text{post.mean}}=-0.008$, $P_{\text{MCMC}}<0.001$) and HF females (dry mass: $\beta_{\text{post.mean}}=-0.003$, $P_{\text{MCMC}}=0.012$; lean mass: $\beta_{\text{post.mean}}=-0.002$, $P_{\text{MCMC}}=0.038$), however, this decrease was stronger in LF females (Fig. 2A, B, left panels). As a result, the offspring born at the end of the experiment (i.e. experimental week 7) were significantly lighter and leaner for LF than HF females (dry mass: $\beta_{\text{post.mean}}=0.251$, $P_{\text{MCMC}}<0.001$; lean mass: $\beta_{\text{post.mean}}=0.228$, $P_{\text{MCMC}}<0.001$; Fig. 2A, B, left panels). Offspring body fat significantly decreased throughout the food treatment ($\beta_{\text{post.mean}}=-0.001$, $P_{\text{MCMC}}=0.010$; Fig. 2C, left panel), but did not differ between LF and HF females ($\beta_{\text{post.mean}}=-0.001$, $P_{\text{MCMC}}=0.110$).

Offspring size and body composition seven days after birth
Fish held in the laboratory for one week to measure swimming kinematics during feeding were additionally used to study growth after birth. The difference in dry mass, lean mass, and body fat at birth observed at the end of the experiment persisted during the first week after birth (Fig. 2A–C, right panels). The body fat of offspring from HF females slightly increased during the first week after birth relative to offspring from LF females, resulting in a significantly different body fat of one-week-old offspring ($\beta_{\text{post.mean}}=4.1\times10^{-2}$, $P_{\text{MCMC}}=0.040$; Fig. 2C, right panel).
Offspring fast-start escape response at birth

The probability of offspring to respond to the stimulus tended to increase throughout the 7-week experiment, though not significantly ($\beta_{\text{post.mean}} = 0.044$, $P_{\text{MCMC}} = 0.056$). This increase was similar in both food treatments ($\beta_{\text{post.mean}} = -0.010$, $P_{\text{MCMC}} = 0.796$; Fig. 3A). Likewise, the mean and maximum speed and acceleration during the fast-start did not significantly change throughout the experiment, nor did it significantly differ between both food treatments (Fig. 3B–E).

Offspring swimming kinematics while feeding in the first week after birth

Prior to the start of the food treatment (i.e. left of the dashed lines in Figure 4), HF offspring showed a lower mean and maximum speed and acceleration at birth compared to LF offspring (Fig. 4A–D, left panels). As food availability was not yet manipulated, this indicates a random bias in maternal, and hence offspring, phenotypes at the beginning of the experiment. This difference in swimming kinematics during feeding at birth diminished during the experiment. Maternal food restriction during pregnancy, therefore, had a significant effect on swimming kinematics during feeding. Specifically, the mean and maximum speed and acceleration did not significantly change throughout the experiment in LF offspring ($v_{\text{mean}}$: $\beta_{\text{post.mean}} = -0.2 \times 10^{-3}$, $P_{\text{MCMC}} = 0.948$; $v_{\text{max}}$: $\beta_{\text{post.mean}} = -1.0 \times 10^{-3}$, $P_{\text{MCMC}} = 0.584$; $a_{\text{mean}}$: $\beta_{\text{post.mean}} = -1.3 \times 10^{-3}$, $P_{\text{MCMC}} = 0.576$; $a_{\text{max}}$: $\beta_{\text{post.mean}} = -0.8 \times 10^{-3}$, $P_{\text{MCMC}} = 0.686$), yet significantly increased throughout the experiment in HF offspring ($v_{\text{mean}}$: $\beta_{\text{post.mean}} = 0.012$, $P_{\text{MCMC}} < 0.001$; $v_{\text{max}}$: $\beta_{\text{post.mean}} = 0.007$, $P_{\text{MCMC}} = 0.002$; $a_{\text{mean}}$: $\beta_{\text{post.mean}} = 0.008$, $P_{\text{MCMC}} = 0.010$; $a_{\text{max}}$: $\beta_{\text{post.mean}} = 0.005$, $P_{\text{MCMC}} = 0.034$).

Furthermore, there was a clear ontogenetic effect on the swimming kinematics while feeding during the first week after birth, with two- (Fig. 4A–D, middle panels) and seven-day-old (Fig. 4A–D, right panels) offspring of both food treatments showing increasingly higher speeds and accelerations than new-born offspring (Fig. 4A–D, left panels). This ontogenetic effect was further influenced by maternal food availability during pregnancy, with the locomotor performance of HF offspring improving relative to that of LF offspring. This resulted in significant differences in most, but not all, kinematic parameters between both food treatments in one-week-old offspring born at the end of the experiment ($v_{\text{mean}}$: $\beta_{\text{post.mean}} = 1.414$, $P_{\text{MCMC}} = 0.028$; $v_{\text{max}}$: $\beta_{\text{post.mean}} = 1.951$, $P_{\text{MCMC}} = 0.020$; $a_{\text{mean}}$: $\beta_{\text{post.mean}} = 0.340$, $P_{\text{MCMC}} = 0.034$; $a_{\text{max}}$: $\beta_{\text{post.mean}} = 0.646$, $P_{\text{MCMC}} = 0.058$; Fig. 4A–D right panels). Interestingly, the segregation in locomotor performance between LF and HF offspring throughout the experiment and during the first week after birth was only apparent when food was supplied: during the 5 min control period (i.e. before food was supplied), LF and HF offspring showed similar mean and
maximum speed and acceleration (Fig. S2). This suggests that the length of maternal food restriction during pregnancy likely affects the feeding capabilities of offspring.

**Discussion**

We examined to what extent the length of maternal food restriction during pregnancy affects size, quality, and performance of offspring in the matrotrophic live-bearing fish species *Phalloptychus januarius*. Females that were fed a ‘low-food’ (LF) ration during gestation produced offspring with significantly lower dry mass and lean dry mass with a tendency of having fewer fat reserves at birth compared to females fed with a ‘high-food’ (HF) ration. The longer the maternal food treatment during (and prior to) pregnancy, the more pronounced were these phenotypic differences. Furthermore, these differences persisted (dry mass and lean dry mass), or became even more pronounced (body fat), during the first week after birth, suggesting slower postnatal body fat gain of the smaller offspring. Maternal food restriction during pregnancy did not impact the fast-start escape performance of offspring at birth, however, it did influence several swimming kinematic parameters while feeding during their first week after birth. Together, our findings show that maternal food restriction during pregnancy adversely affects offspring size, postnatal body fat gain, postnatal improvement of locomotor performance, and hence possibly their competitive abilities (Bashey, 2006) after birth.

*Effects of maternal food restriction on offspring fast-start escape performance at birth*

The probability of offspring to respond to a startle stimulus tended to increase over the course of the experiment, for both treatments. Fish may vary their neural threshold for triggering a fast-start response (Wakeling, 2006) depending on stress levels or health condition (Chick and Van Den Avyle, 2000). Fast escapes are energetically expensive and cannot be repeated at a high rate (Frith, 1990). The observed increase in response rate might thus reflect the modulation of a neuronal threshold, presumably in the Mauthner neurons that mediate the response, or in neurons that stimulate the Mauthner neurons. However, it is currently unclear why the probability to induce a startle response increased over the course of the experiment.

In studying the fast-start escape behavior, we have at least partially accounted for a change in motivation or threshold by selecting only trials with a clear response to the stimulus. This is important because differences in motivation can introduce noise and variability in the response parameters (Losos et al., 2002). In general, the fast-start escape performance depends on physiological and mechanical muscle properties, as well as muscle activation and body form parameters (Fleuren et al., 2019; Wakeling, 2006). Larger fish typically achieve higher maximum velocities during fast-starts due to larger muscle mass, body length, and production of higher bending moments (Dial et al., 2016; Gibb et al., 2006; Voesenek et al., 2020; Wakeling, 2006). Since LF offspring are born significantly
lighter and leaner at the end of the experiment compared to HF offspring, it is surprising that we do not find an effect on their fast-start escape performance (Dial et al., 2016). This may be due to a low statistical power to detect significant differences, because our response selection removed about 67% of the data (Fig. 1B). Although additional analyses with different response thresholds indicate that these findings are relatively robust (Supplementary Methods 1.4; Figs. S3–S5), we have to be cautious when concluding that maternal food restriction does not impact fast-start escapes at birth. Future studies should try to maximize sample sizes to yield extended measurements of locomotor performance.

Effects of maternal food restriction on offspring swimming kinematics during feeding
New-born poeciliid fish are super-precocial, having functional prey-capture abilities at birth and relying on active exogenous feeding after birth (e.g. *Girardinus metallicus*; Lankheet et al., 2016). Their prey-capturing ability undergoes a rapid integrated development of the visuo-motor system during the first days after birth. Swimming speed and acceleration are key parameters determining prey-capture success rate (Lankheet et al., 2016). In *P. januarius*, these parameters improve rapidly after birth: one-week-old offspring showed higher mean and maximum speeds and accelerations than new-born offspring, both in LF and HF offspring. However, the locomotor performance during feeding improved significantly faster in HF offspring compared to that of LF offspring, during the first week after birth. Interestingly, this difference in locomotor performance cannot be explained by differences in ontogenetic growth between HF and LF offspring, because (i) although HF offspring were larger at birth than LF offspring, they showed similar increases in size during the first week after birth, and (ii) the segregation in locomotor performance between LF and HF offspring was only apparent when food was supplied. This suggests that maternal food availability during pregnancy affects the postnatal maturation of tissues associated with locomotion during feeding, which may cause differently developing feeding capabilities after birth between LH and HF offspring.

We found that LF offspring are smaller at birth and tend to have less body fat. It is likely that they also differed in other morphological and physiological features that can influence their postnatal development of locomotor performance during feeding. For instance, smaller guppy offspring were shown to have a lower degree of skeletal ossification at birth, which is considered a proxy for internal maturity (Dial et al., 2016). Skeletal ossification can directly affect swimming performance (Dial et al., 2016), and may also influence postnatal development of locomotor performance. In addition, locomotor performance depends on muscle fiber type (Rome et al., 1988) that undergoes a distinct shift in composition after birth (Veggetti et al., 1993). Similarly, maximum body curvature during prey-capture was shown to change after birth (Lankheet et al., 2016). Particularly, an increase in muscle mass-specific power output induces a higher body curvature (Wakeling, 2006), and

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consequently improves prey-capture success rate if the motion control is sufficiently matured (Lankheet et al., 2016). Better prey-capturing success enables uptake of more energy (via feeding) per unit of time, which can be allocated to either growth or quality (e.g. body fat). It is thus possible that LF and HF offspring differ in various morphological or behavioral parameters at birth (e.g. degree of skeletal ossification, composition of muscle fiber type, or body curvature) that, furthermore, differently develop between both food treatments after birth. The improved prey-capture success and increased efficiency of resource acquisition in HF offspring compared to LF offspring may also explain why HF offspring gained body fat during the first week after birth, but LF offspring did not.

Compounding effects on offspring growth

Overall, our findings suggest that differential resource allocation of mothers to offspring may not only cause LF offspring to be born smaller than HF offspring, but also ‘worse-conditioned’, i.e. showing a slower postnatal body fat gain and an inhibition of postnatal improvement of swimming capabilities during feeding. Consequently, these smaller offspring are likely to have lower competitive abilities in a resource-limited environment, for two reasons: first, larger offspring that carry more fat reserves have a competitive advantage over smaller offspring if they are born in environments where resources are scarce, allowing them to survive for longer periods of time under low food conditions (Bashey, 2006; Bashey, 2008; Parichy and Kaplan, 1992). Secondly, larger offspring have better swimming performance during feeding, which may translate to superior prey-capture abilities (Lankheet et al., 2016), and hence, faster postnatal body fat gain, compared to smaller offspring.

Compensatory growth could be an adaptive strategy for small offspring to fully or even overcompensate a smaller body size in response to increased food availability following a period of growth restriction (Auer et al., 2010; Metcalfe and Monaghan, 2001). However, although LF and HF offspring were fed ad libitum after birth, we observed no compensatory growth for LF offspring in P. januarius during the first week after birth. The poorer swimming performance during feeding of LF offspring compared to HF offspring, potentially made it more difficult for the smaller LF offspring to catch up in mass during the first week after birth. This may have further exacerbated the differences: rather than displaying catch-up growth to compensate for their smaller size at birth, LF offspring fall further behind despite ad libitum food availability after birth. Interestingly, however, these differences in size and body composition between LF and HF offspring had disappeared at sexual maturity (Supplementary Methods 1.5; Fig. S6), indicating that in the long-term LF offspring may be able to (partly) compensate for their poor nutrition during gestation.
**Contrasting findings in non-placental live-bearing species**

Non-placental (i.e. lecithotrophic) live-bearing females provide all resources required for embryo development as yolk, prior to fertilization. Therefore, brood size and offspring size are determined prior to fertilization based on prior food availability (Reznick et al., 1996). Rather than resulting in smaller, worse-conditioned offspring at birth, adverse food conditions were shown to reduce maternal growth, fat reserves, and fecundity, yet also increase the relative investment in offspring size (Bashey, 2008; Reznick et al., 1996; Riesch et al., 2016). Here, mothers adaptively modified offspring phenotype in preparation for adverse environmental conditions (i.e. low food availability). This response is presumably advantageous, as under competitive adverse food conditions, mothers gain fitness benefits by producing larger offspring (Leips et al., 2013; Molina-Moctezuma et al., 2020; Parker and Begon, 1986).

Placental females, however, continuously supply their developing embryos with nutrients throughout pregnancy. Consequently, the timing of determining brood size and offspring size are likely decoupled (Pollux and Reznick, 2011; Reznick et al., 1996). Whereas brood size is determined prior to fertilization based on current food availability, offspring size is affected by food availability after fertilization, throughout gestation (Pollux and Reznick, 2011; Reznick et al., 1996). As a result, when resource conditions suddenly deteriorate, placental species may not be able to optimally provision their embryos. In contrast to lecithotrophic species, therefore, placental species lack the possibility to adaptively adjust offspring phenotype in response to sudden reductions in food availability.

**Conclusion**

Our study shows that matrotrophy in poeciliids may be a maladaptive strategy in unpredictable fluctuating resource environments, because sudden reductions in maternal food availability during pregnancy result in smaller offspring with slower postnatal body fat gain and an inhibition of postnatal improvement of swimming capabilities during feeding. Future studies should try to explicitly quantify the consequences of maternal food restriction on prey-capture success rate of offspring to better understand the role of compromised postnatal development of locomotion in shaping offspring growth and hence fitness.

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Competing interests
No competing interests declared.

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Ethical statement
All procedures were approved by the Animal Ethics Committee of Wageningen University and Research (permit number 2018.W-0022.002).

Data availability
The data and code that support the findings of this study are available from Dryad Digital Repository.

Authors’ contributions
A.H., M.J.L., and B.J.A.P. conceived the ideas and planned the experiment. A.H. performed the food treatment and M.J.L. built the experimental setup for swimming trials; A.H. and J.B. collected the experimental data; M.J.L. and J.B. developed the software to analyze the swimming trials; A.H. performed all statistical analyses, wrote the first draft of the manuscript and finalized the manuscript with comments from M.J.L., J.B., J.L.vL., and B.J.A.P.

References


Figure 1. A Experimental set-up to record either (i) swimming kinematics during feeding, or (ii) fast-start escape performance. Fish were high-speed filmed from the dorsal side. For (i), a maximum of nine Petri dishes, each with a single fish, were placed on pre-defined positions on the plate (arranged in a 3×3 pattern). For (ii), a maximum of five dishes were placed on the plate (arranged in a 1×5 pattern). Fast-start escapes were initiated by dropping a weight on the plate (*). B Speed profiles of all fast-start escapes. Dashed vertical line indicates the time at which the weight is released to trigger an escape maneuver. Solid black curve shows the overall mean speed. Mean and maximum performance parameters were extracted from a period that was sufficiently long to perform a fast-start (light red
Fish were considered as having ‘responded’ if their speed during a 7 frame window around the maximum of the overall mean speed (thick red line) exceeded the threshold of 60 mm s⁻¹ (thin red line). C Histogram of all instant fast-start speeds observed in the light red rectangle shown in B. The histogram shows two peaks, which correspond to individuals that have either responded to the stimulus or not. The response threshold for a fast-start (thin red line in B and C) was defined as the speed observed at the minimum frequency in between the two peaks (60 mm s⁻¹). D Example time trace of speed during an individual measurement of swimming kinematics while feeding. Mean and maximum locomotor performance parameters were extracted from the 5 min control period (grey line; no food available), and 5 minutes with available food (red line). The period during which food was supplied (grey rectangle) was filtered out to remove disturbances due to the experimenter. E Histogram of all instant speeds observed during the measurements of swimming kinematics while feeding. Grey: 5 min control period (no food present); red: 5 min feeding period. Solid lines correspond to the (i) overall mean speed and (ii) mean of speeds above 95% quantile.
Figure 2. A Dry mass, B lean dry mass, and C body fat (± 95% CI) of offspring ($n_{LF}$=300, $n_{HF}$=287). Left panels: effect of the length of maternal food treatment during pregnancy on offspring traits at birth for offspring born during the 7-week experiment (the first week all females received *ad libitum* food, the dashed vertical line indicates the start of the 6-week food treatment; blue: low food; red: high
Right panels: increase in offspring dry mass, lean dry mass, and body fat during the first week after birth, predicted for offspring that were born at the end of the 7-week experiment for both food treatments. Estimates are based on fish that were held in the laboratory for one week to measure swimming kinematics during feeding. Dashed lines represent linear fits throughout the posterior samples of a given food treatment. Significant codes: $P_{\text{MCMC}} \leq 0.001$ ***, $< 0.01$ **, $\leq 0.05$ *, $> 0.05$ n.s.
Figure 3. The effect of the length of maternal food treatment during pregnancy on offspring fast-start performance at birth. A Probability of new-born offspring to respond to the stimulus during fast-start trials (± 95% CI) \((n_{LF}=94, n_{HF}=113)\). The responsiveness is predicted for both food treatments (blue: low food; red: high food) and throughout the experiment. The dashed vertical line indicates the start of the 6-week food treatment. \(P_{MCMC}\)-value for the interaction between experimental day and food treatment is given at the top. B–E Swimming performance of new-born offspring during fast-start escape response \((n_{LF}=24, n_{HF}=31)\). During the 7-week experiment, new-borns were collected weekly to assess four swimming kinematic parameters: B mean speed, C maximum speed, D mean acceleration, and E maximum acceleration (± 95% CI) at birth. These parameters are predicted for both food treatments (blue: low food; red: high food) and throughout the experiment. The dashed vertical line indicates the start of the 6-week food treatment. \(P_{MCMC}\)-values for the interaction between experimental day and treatment are given at the top.
Figure 4. The effect of the length of maternal food treatment during pregnancy on the swimming kinematics of offspring while feeding ($n_{LF}=183$, $n_{HF}=135$). During the 7-week experiment, new-borns were collected weekly to assess four swimming kinematic parameters: A mean speed, B maximum speed, C mean acceleration, and D maximum acceleration ($\pm 95\%$ CI). These parameters are predicted
throughout the experiment for both food treatments (blue: low food; red: high food) at three different ages: at birth (zero days old, left panels), two days old (middle panels), and seven days old (right panels). The dashed vertical line indicates the start of the 6-week food treatment. Significance codes are given at the top for the difference between both food treatments at the start of the food treatment and at the end of the experiment. Significant codes: $P_{\text{MCMC}} \leq 0.001 ***$, $<0.01 **$, $\leq 0.05 *$, $>0.05$ n.s.
1 Supplementary Methods

1.1 Measurements of dry mass and body fat

Dry mass and body fat was measured after euthanizing the fish with an overdose of MS-222 and preserving in 4% formaldehyde. Dry mass was measured to the nearest 0.01 mg on a Mettler Toledo AE163 Microbalance (Mettler Instruments Corp., Hightstown, New Jersey, USA) after air-drying overnight at 60°C in a drying oven. Lean mass was measured by extracting the fat twice (2× overnight) with anhydrous diethyl ether to remove triglycerides, and by subsequently air-drying and re-weighting the fish. Body fat was then calculated by subtracting lean mass from dry mass.

1.2 Image analysis

Images were recorded at 75 Hz (feeding experiments) or 350 Hz (fast-start experiments) with exposure times of 600 µs and at a spatial resolution of 5 pix-mm⁻¹. The sensitivity of the camera (Basler Ace 2040 90um NIR USB3) was increased by a factor of 4, by binning pixels both horizontally and vertically by a factor of two. This reduced image size to 1024×1024 pixels. Camera images were analyzed on the fly while conducting the experiments, using Python and OpenCV. Noise was removed from images by applying a median filter (3 pixels). After subtraction of a background image (without fish present) we applied a fixed threshold to detect the silhouette of the fish. Difference images were again subjected to a median filter (3 pixels) to remove noise. Fish were then distinguished from false hits by applying lower and upper limits to the size of the object, and lower and upper limits to the length-width ratio. We used Kalman filters to track the individual fish and estimate their path of motion, based on the location of the center of mass of the fish silhouette. To accelerate the analyses and thus to be able to track multiple fish simultaneously at high frame rates, we alternated between full analysis of the total image, as described above, and analyzing only those parts in which fish were previously detected. For the latter we used the Kalman prediction to select an area of 200×200 pixels centered on the predicted, new location. For each frame and each fish detected we saved the location estimated by the Kalman filter, together with the full contour of the silhouette. Exact timing of events defining control and feeding intervals, as well as the timing of the startle stimulus were also automatically saved to file.

1.3 Local polynomial regression smoothing

We used an in-house developed Python program to track the fish’s silhouette in real-time and to automatically extract the location (i.e. X- and Y-position) of the center of mass through time. Swimming speed was calculated by numerically differentiating the fish’s position, and linear acceleration was calculated by differentiating the resulting swimming speed. We performed local polynomial regression smoothing as a function of frame number before each differentiation step using the function `loess` in R v 3.5 [4]. This procedure removed high-frequency noise in the data. The order of the polynomial was 2, and the proportion of the data that was considered to be neighbouring x (i.e. the degree of smoothing, α) was 0.015 for the swimming kinematics during feeding, and ranged between 0.025 and 0.12 for the fast-start escape responses. The reason for a varying α in the latter case was a larger variation in the number of frames (i.e. available data) among individual fast-starts. Rather than having a constant α for all fast-starts, we therefore calculated α for each individual fast-start as:
\[ \alpha_i = \frac{0.025 \cdot F_{\text{max}}}{n_i} \]  

where \( F_{\text{max}} \) is the maximum number of frames recorded during the fast-starts, and \( n_i \) the available number of frames during the \( i \)th fast-start.

1.4 Defining the response threshold during the fast-start escape response

When plotting a histogram of all instant fast-start speeds observed in the light red rectangle shown in Figure 1B (i.e. the period from which the locomotor performance parameters were extracted), the histogram shows two peaks (Fig. S3). These correspond to individuals that have either responded to the stimulus (i.e. by dropping a weight on the plate) or not. We considered fish as having ‘responded’ to the stimulus if their speed during a narrow time window around the maximum of the overall mean speed (± 3 frames) exceeded 60 mm s\(^{-1}\). This response threshold was defined as the speed observed at the minimum frequency in between the two peaks of the histogram (Fig. S3).

The potential effects of maternal food restriction on offspring performance during the fast-start escape response were then analyzed by fitting the mean and maximum speed and acceleration in a multivariate LMM. Here, only individuals that have ‘responded’ to the stimulus were included in the model. Fixed effects included treatment (low food or high food), experimental day, as well as treatment \( \times \) day. Defining a response threshold is important because differences in motivation can introduce noise and variability in the response parameters [2]. However, it can also be biased, as the sample size depends on the threshold: the larger the value, the more fish are considered to have not responded to the stimulus, and thus, more data is removed.

To quantify the sensitivity of our results to different thresholds, we defined five additional threshold values in between the two peaks of the histogram (\( d = 20, 40, 80, 100, \text{ and } 120 \) mm s\(^{-1}\); Fig. S3). For each \( d \), we subsequently extracted the corresponding locomotor performance parameters (i.e. mean and maximum speed and acceleration) and applied the LMM (as described above) to the resulting data sets. To compare the strength of individual relationships, the slopes (\( \beta \)) were standardized by multiplying with the phenotypic standard deviation of the predictor variable (i.e. experimental day) and dividing by the phenotypic standard deviation of the response variable (i.e. mean and maximum speed and acceleration) [5]. The resulting effect sizes (\( \beta^* \)) take values between \(-1\) and \(1\).

The results show that the interaction between treatment and experimental day is not significant for all threshold values and locomotor performance traits. Moreover, the slopes are relatively robust to threshold values between 40 and 100 mm s\(^{-1}\) (Figs. S4, S5). The effect sizes (i.e. standardized slopes) of experimental day on the locomotor performance parameters show a relatively small range for the low food (\( v_{\text{mean}} = -0.03 – 0.10; v_{\text{max}} = -0.12 – 0.02; a_{\text{mean}} = -0.08 – 0.02; a_{\text{max}} = -0.28 – 0.19 \)) and high food treatment (\( v_{\text{mean}} = -0.02 – 0.19; v_{\text{max}} = -0.03 – 0.24; a_{\text{mean}} = -0.20 – 0.08; a_{\text{max}} = -0.22 – 0.04 \)).

By contrast, the output is relatively sensitive to threshold values closer to the two peaks of the histogram (i.e. \( d = 20 \) and 120) (Fig. S4). This is not surprising, as a low threshold causes more noise and variability in the response parameters. The opposite is true for a high threshold. A threshold of \( d = 120 \) includes much less noise, but also removes more data (\( d_{120} = 73\% \) versus \( d_{20} = 60\% \) data loss).

To balance the conflict between maximizing sample size and minimizing noise, we therefore chose a threshold in the middle of two peaks of the histogram. Specifically, we defined the threshold at 60
mm-s$^{-1}$, which is the speed observed at the minimum frequency in between the two peaks of the histogram.

1.5 Effects of maternal food restriction on F$_1$-adult size and body composition

A randomly selected subset of offspring was collected during experimental week 7 and reared from birth to sexual maturity (in 40-L stock tanks under *ad libitum* food). After 9 months, these F$_1$-adults were euthanized and preserved to measure dry mass and body fat. The effects of maternal food restriction during pregnancy on F$_1$-adult phenotypic traits (dry mass, lean mass, and body fat) were then analyzed by fitting all traits in a multivariate LM as a function of treatment (LF or HF). Additional fixed effects were sex (female or male), as well as treatment×sex. To optimize normality and homoscedasticity of the model residuals, body fat was square-root transformed. Whereas, as expected, adult size and body composition differed significantly between F$_1$-males and F$_1$-females (dry mass: $\beta_{\text{post.mean}} = 0.076$, $P_{\text{MCMC}} < 0.001$; lean mass: $\beta_{\text{post.mean}} = 0.046$, $P_{\text{MCMC}} < 0.001$; body fat: $\beta_{\text{post.mean}} = 0.029$, $P_{\text{MCMC}} < 0.001$), the differences in dry mass, lean mass, and body fat at birth observed at the end of the experiment between food treatments had disappeared in the adult stage for both males and females (dry mass$_f$: $\beta_{\text{post.mean}} = 4 \times 10^{-3}$, $P_{\text{MCMC}} = 0.496$; lean mass$_f$: $\beta_{\text{post.mean}} = 0.2 \times 10^{-3}$, $P_{\text{MCMC}} = 0.964$; body fat$_f$: $\beta_{\text{post.mean}} = 3.5 \times 10^{-3}$, $P_{\text{MCMC}} = 0.388$; dry mass$_g$: $\beta_{\text{post.mean}} = 0.5 \times 10^{-4}$, $P_{\text{MCMC}} = 0.980$; lean mass$_g$: $\beta_{\text{post.mean}} = -7.2 \times 10^{-4}$, $P_{\text{MCMC}} = 0.828$; body fat$_g$: $\beta_{\text{post.mean}} = 5.6 \times 10^{-4}$, $P_{\text{MCMC}} = 0.714$; Fig. S6).
2 Supplementary Results

**Fig. S1.** A Maternal standard length (SL) and B wet mass (± 95% CI) at the start of the food treatment (after week 1) and at the end of the experiment (after week 7) (n_{LF} = 22, n_{HF} = 23). Blue: low food; red: high food. Dashed lines correspond to linear fits throughout the posterior samples of a given food treatment. Please note that females of both food treatments showed an increase in standard length during the experiment, and as expected, LF females lost significantly more mass for a given length throughout the experiment (0.08 vs. 0.03 g; P_{MCMC} = 0.004). Whereas one might expect an increase in mass for HF females fed *ad libitum* in *P. januarius* [3], it also decreased, though to a lesser degree. This suggests that both treatments may have been food restricted, albeit differently, towards the end of the experiment. As food rations remained constant, the HF ration may have caused an unintended mild food restriction. Together with other potential stressors (e.g. individual housing, checking tanks for offspring), this may also explain the slight decline in offspring mass at birth in the HF treatment [1]. Despite a smaller difference in food regime than intended, we still see differences in maternal phenotype.
**Fig. S2.** The effect of the length of maternal food treatment during pregnancy on the swimming kinematics of offspring during feeding trials without food supply (i.e. control) \((n_{LF} = 190, n_{HF} = 144)\). During the 7-week experiment, newborns were collected weekly to assess four swimming kinematic parameters: **A** mean speed, **B** maximum speed, **C** mean acceleration, and **D** maximum acceleration (± 95% CI). These parameters are predicted throughout the experiment for both food treatments (blue: low food; red: high food) at three different ages: at birth (zero days old, left panels), two days old (middle panels), and seven days old (right panels). The dashed vertical line indicates the start of the 6-week food treatment. Significance codes are given at the top for the difference between both food treatments at the start of the food treatment and at the end of the experiment. Significant codes: \(P_{MCMC} \leq 0.001^{***}, < 0.01^{**}, \leq 0.05^*, > 0.05\) n.s.
Fig. S3. Histogram of all instant fast-start speeds observed in the light red rectangle shown in Figure 1B. The histogram shows two peaks, which correspond to individuals that have either responded to the stimulus or not. To quantify the sensitivity of our results to different response thresholds, we defined six threshold values in between the two peaks of the histogram ($d = 20, 40, 60, 80, 100, \text{ and } 120 \text{ mm s}^{-1}$; red vertical lines). We finally defined the threshold at 60 mm s$^{-1}$ (thick red vertical line), which is the speed observed at the minimum frequency in between the two peaks of the histogram.
Fig. S4. A–D Swimming performance of new-born offspring during the fast-start escape response depending on different response thresholds ($d = 20, 40, 60, 80, 100, \text{ and } 120 \text{ mm s}^{-1}$). During the 7-week experiment, new-borns were collected weekly to assess four swimming kinematic parameters: A mean speed, B maximum speed, C mean acceleration, and D maximum acceleration at birth. These parameters are predicted for both food treatments (blue: low food; red: high food) and throughout the experiment. The dashed vertical line indicates the start of the 6-week food treatment. We finally defined the threshold at 60 mm s$^{-1}$ (thicker lines), which is the speed observed at the minimum frequency in between the two peaks of the histogram shown in Figure S3.
Fig. S5. Standardized effect size (± sd) of experimental day on the locomotor performance parameters (i.e. mean and maximum speed and acceleration) during the fast-start escape responses. The effect sizes are averaged over the results of four different response thresholds (d = 40, 60, 80, and 100 mm·s⁻¹, i.e. excluding the two extremes d = 20 and 120 mm·s⁻¹) and plotted as a function of treatment (blue: low food; red: high food). The effect sizes take values between −1 and 1.
Fig. S6. A Dry mass, B lean dry mass, and C body fat (± 95% CI) of adult (9 months after birth) F₁-offspring born at the end of the experiment, as a function of sex (female or male) and food treatment (nLF = 19, nHF = 20).
References


