Maternal body condition influences magnitude of anti-predator response in offspring

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Organisms exhibit plasticity in response to their environment, but there is large variation even within populations in the expression and magnitude of response. Maternal influence alters offspring survival through size advantages in growth and development. However, the relationship between maternal influence and variation in plasticity in response to predation risk is unknown. We hypothesized that variation in the magnitude of plastic responses between families is at least partly due to maternal provisioning and examined the relationship between maternal condition, egg provisioning and magnitude of plastic response to perceived predation risk (by dragonfly larvae: *Aeshna* spp.) in northern leopard frogs (*Lithobates pipiens*). Females in better body condition tended to lay more (clutch size) larger (egg diameter) eggs. Tadpoles responded to predation risk by increasing relative tail depth (morphology) and decreasing activity (behaviour). We found a positive relationship between morphological effect size and maternal condition, but no relationship between behavioural effect size and maternal condition. These novel findings suggest that limitations imposed by maternal condition can constrain phenotypic variation, ultimately influencing the capacity of populations to respond to environmental change.

1. Introduction

The phenotype of an organism is derived from genotype by environment interactions. Phenotypic plasticity in response to environmental conditions has been documented in almost every group of organisms [1,2], however, large gaps remain in our understanding of variability in plasticity and the constraints affecting its expression. Inter-family variation in plasticity within populations can be substantial with families even showing opposing trait changes in response to common environmental conditions [3–5]. Plasticity itself is a heritable and adaptive trait under predictably variable environments [2,6]. Variance in plasticity estimated from mothers exceeds that attributable to fathers [3,7], suggesting that sources of variance additional to genetics contribute to inter-family variation in plasticity. As variation provides the raw material upon which natural selection acts, identifying sources of variation is an essential component of understanding the evolutionary potential of populations.

Maternal effects are being increasingly recognized as factors affecting individual fitness [8–10], population dynamics [11,12] and evolutionary trajectories [13]. In species that do not show post-ovipositional parental care, maternal effects are largely limited to egg provisioning [14], including egg size, composition [15,16] and nest location [17]. Maternal influence on size can alter offspring fitness: larger eggs hatch into larger offspring who show advantages in growth and survival [15,18]. Larger larval size can also confer an advantage in coping with both abiotic [19,20] and biotic [9,16] stressors. Maternal influence on egg size can also alter the probability of offspring developing a competitive morphology under crowded conditions [21]. Thus,
maternal provisioning influences the expression of plasticity through variation in offspring body size.

Outside of size, maternal condition can influence offspring response to stress through the transfer of maternal hormones during development [11,22]. Affected offspring then show greater anti-predator behaviours in risky environments [11,23]. Maternal influence also can increase variation through the development of novel traits [13]. Thus, the expression of maternal influence is wide-ranging and dynamic under differing environmental conditions. For populations facing variability in predation risk, influences on defensive plasticity may be more advantageous than, or complementary to, canalized changes in body size, in terms of fitness. Indeed, observed inter-family variation in anti-predator responses suggests differences in survivorship between families under predation pressure [3,4]. Our study is designed to determine whether maternal influence can account for any inter-family variation in anti-predator plasticity.

Tadpoles show plasticity in a number of defensive traits in response to the perception of risk. Tadpoles detect risk via chemical cues released when predators injure and consume conspecifics [24,25]. Northern leopard frog tadpoles (Lithobates pipiens) respond to risk from dragonfly larvae (family: Aeshnidae) by increasing relative tail depth [26,27], decreasing activity levels [26–29] and increasing growth [27,30]. Using northern leopard frogs as a model species, we examined the relationships between maternal condition, reproductive investment and offspring response to perceived predation risk. We hypothesized that the magnitude of a plastic response is at least partly explained by maternal provisioning. We predicted that females in better body condition would invest more heavily in offspring, evidenced by larger eggs, and that tadpoles hatched from those eggs would show a higher magnitude of plasticity, manifested through morphological (relative tail depth, growth and development) and behavioural (activity level) anti-predator responses.

2. Material and methods

(a) Experimental design and data collection
Northern leopard frogs were collected in early April near Peterborough, Ontario, Canada (44°20′N, 78°11′W) in 2011 (10 females, 16 males), 2012 (four females, five males) and 2013 (14 females, 25 males). Frogs were weighed, measured (snout–urostyle length) and brought to a captive facility, where they were housed in 10 l (10 females, 16 males), 2012 (four females, five males) and 2013 (14 females, 25 males). Frogs were weighed, measured (snout–urostyle length) and brought to a captive facility, where they were injected with the AMPHIPLEX cocktail [31,32] to induce breeding. Injected frogs were placed outdoors in a fenced aviary in 568 l tanks furnished with vegetation (cattails: Typha spp.), a basking platform, an aquarium heater (in 2012 and 2013: 200 W submersible) and a mesh screen cover, in a ratio of 2:1 males: females. Turtles were monitored in the morning (9.00) and afternoon (16.00) for breeding. After breeding, frogs were weighed and euthanized by an overdose of tricaine methanesulfonate (MS-222), then females were freeze-dried at −51°C with a vacuum of 0.120 mbar for 24 h (eggs dried in 2013 only). Mean dry egg mass was then calculated from the three subsamples. Embryo diameter (mean of 15 eggs—five per subsample) was measured from photographs using ImageJ [33] and clutch size was estimated from the wet masses of the three-counted subsamples compared against whole clutch mass.

Upon reaching Gosner stage 25 [34], tadpoles were moved indoors where they were housed in 10 l (n = 30 per tank) of either aged tap water (2011, 2012) or reconstituted reverse-osmosis water (2013). Water temperatures were approximately 19°C (range 18–21°C) with natural lighting conditions (2011, 2012) or a 12 L:12 D (2013). Initially, year was included as a factor in our analyses, but we found no evidence of interactions between year and treatment on any response variable (p > 0.10, in all cases). Therefore, we do not include year in our reported analyses. Each tank contained a floating plastic cage equipped with a dragonfly larva perch (rubber dowelling). Half of the tanks held a late-instar dragonfly larva (Aeshna spp.), fed three L. pipiens tadpoles twice weekly (predator), while the other half were empty (control), with three replicates per treatment per clutch. Tadpoles were fed ground algae discs ad libitum, with weekly-water changes. Number of active tadpoles (any movement shown) were counted during 30 s tank scans, 4 days per week. Tadpole morphology was recorded weekly by photographing five tadpoles per tank using a Nikon D70 digital SLR camera equipped with a Tamron 90 mm macro lens against a scale bar. Tadpoles were then weighed, staged using a dissecting microscope [35] and returned to their tank. At the end of three weeks, tadpoles were euthanized with an overdose of MS-222.

(b) Statistical analyses
Maternal body condition was calculated as the residuals from a log–log least-squares (OLS) regression [34,36,37] of post-laying wet mass on snout–urostyle length (r² = 0.58, F₁,₁₇ = 23.5, p < 0.001). We assessed relationships between maternal body condition and egg size (diameter, dry mass) and clutch size (egg number) using OLS regressions. Tank means were used as the experimental unit to avoid pseudoreplication, as individuals were returned to tanks after weekly samples. To analyse treatment effect on growth and development, we used general linear models with mean tank mass (g) or stage (treated as a continuous variable [38,39]) at week 3 as the response variable and treatment (predator or control) as a fixed factor nested within maternal ID (random factor).

Tadpole behaviour was analysed using a repeated-measures ANOVA, with maternal ID and treatment as fixed factors and the arcsine square-root transformed proportion of active tadpoles per tank each day as the repeated measure (n = 11 days). As there was no significant interaction between day and treatment (F₀,₀₉₀ = 1.67, p = 0.09), we calculated behavioural effect size (Cohen’s D with a pooled standard deviation [40]) using mean daily activity per tank.

For morphology, we measured tail fin depth, tail length, body length and depth, and tail muscle depth at week 3, from digital photographs using ImageJ [33]. We conducted a principal components (PC) analysis using standardized morphological measurements excluding tail fin depth. PC1 explained over 90% of the variation, with an eigenvalue of 3.89. As each measurement contributed equally to the variation explained by PC1 (eigenvectors = −0.50, in all cases), we interpret PC1 as a size axis. We then used PC1 scores as an independent measurement of size and regressed tail fin depth on PC1 (r² = 0.91, p < 0.001). The residuals, averaged by tank, were used as the dependent variable (relative tail depth [26,41]) in a general linear model with treatment (fixed) nested within maternal ID (random). Mean relative tail depth was also used to calculate morphological
effect size (Cohen’s $D$ with a pooled standard deviation [40]). Analyses were performed using STATISTICA v. 7.0 (StatSoft 2007), with significance set at $\alpha < 0.05$.

3. Results

(a) Maternal provisioning

Females in better condition invested more in reproduction, as evidenced by larger eggs (egg diameter: $r^2 = 0.32$, $F_{1,16} = 7.61$, $p = 0.01$; dry egg mass: $r^2 = 0.33$, $F_{1,16} = 4.82$, $p = 0.05$) and larger clutches (number of eggs: $r^2 = 0.23$, $F_{1,16} = 4.90$, $p = 0.04$) among better conditioned females (figure 1).

(b) General tadpole response to predation risk

(i) Growth and development

Both tadpole mass and developmental stage varied significantly between clutches ($F_{14,65} = 65.96$, $p < 0.01$ and $F_{14,65} = 5.64$, $p < 0.01$, respectively), however, there was no effect of exposure to predation risk on either (mass: $F_{15,63} = 0.71$, $p = 0.77$, stage: $F_{15,63} = 0.28$, $p = 1.00$).

(ii) Behaviour

Tadpoles reduced activity in response to predation risk ($F_{1,67} = 7.44$, $p < 0.01$). Mean tank activity varied between clutches ($F_{14,67} = 16.07$, $p < 0.01$), although there was no interaction between maternal ID and treatment ($F_{14,67} = 0.19$, $p = 0.10$; figure 2a). Activity also varied by day ($F_{10,670} = 6.82$, $p < 0.01$), with no significant interactions between day, treatment or maternal ID ($p > 0.08$, in all cases).

(iii) Morphology

Tadpoles increased relative tail depth in response to predation risk ($F_{15,66} = 2.70$, $p < 0.01$), and tail depth also varied by clutch ($F_{14,66} = 7.63$, $p < 0.01$; figure 2b).

(c) Maternal body condition and magnitude of tadpole response

There was no relationship between behavioural effect size and maternal body condition ($r^2 = 0.04$, $F_{11,3} = 0.47$, $p = 0.50$), however, morphological effect size was positively correlated with maternal body condition ($r^2 = 0.35$, $F_{11,3} = 7.01$, $p = 0.02$). Mothers in better body condition had tadpoles that mounted greater morphological, but not behavioural, responses to predation risk, when compared with tadpoles from mothers in poorer body condition (figure 3).

4. Discussion

We found that maternal phenotype (body condition) is positively correlated with the magnitude of offspring morphological response to predation risk, suggesting the influence of pre-partum investment on offspring survivorship can be manifested through changes in the expression of inducible defences. Inter-family variation in plastic responses to predation risk has been previously documented, although these studies do not distinguish between genetic and maternal effects [3,4]. In the only study we could find that directly examined narrow-sense heritability of predator-induced traits, trait variance estimated from mothers exceeded the variance measured in fathers [3], suggesting maternal effects as an additional source of variation. Genetic contributions to larval traits such as growth and size at metamorphosis can be masked by maternal effects acting through egg size [42]. Maternal effects acting largely through size can also influence plasticity in response to abiotic [19,20] and biotic [21] environmental conditions other than predation risk. Our study provides critical evidence that maternal provisioning can, independent of size effects, contribute to variation in phenotypic plasticity.

Contrary to our prediction, offspring behavioural response to predation risk was not related to maternal body condition. In retrospect, this could be explained by differences in the reversibility and timing of investment in behavioural versus morphological plasticity. Morphological responses to predation risk in tadpoles appear limited to an early developmental window [43,44], with increases in relative tail depth only occurring within two or three weeks of reaching Gosner stage 25 (free-swimming, feeding larvae) [43,45]. Behavioural responses, however, are highly reversible and do not appear to be as limited in timing [44,45]. Our inference, then, is that the morphological response is more limited by egg provisioning from maternal investment, whereas behavioural response is limited by current environmental conditions, such as resource availability.

One potential source of uncertainty is our interpretation of female body condition. We used post-ovipositional weight and snout–urostyle length to calculate condition, under the presumption that post-laying condition is representative of pre-hibernation condition, when egg production begins. Therefore, females emerging from breeding in better condition had more energy to invest into reproduction. However, it is also possible that females in poor condition after oviposition are in such a state precisely because of their energetic investment in reproduction. This might be especially true for older frogs, where survivorship in the next year may be sufficiently low to alter the pay-off between reproduction and somatic maintenance. To overcome this uncertainty, we confirmed a positive relationship between

![Figure 1](http://rspb.royalsocietypublishing.org/Downloaded from http://rspb.royalsocietypublishing.org/)
post-laying condition and investment (egg size and number) and are thus satisfied with the assumption that post-oviposition condition reflects reproductive investment.

This research is, to our knowledge, the first to demonstrate a relationship between offspring plasticity in response to predation risk and maternal condition. Plastic responses to risk increase survivorship under predation pressure. Our study suggests that maternal body condition affects the magnitude of response offspring are mounting, thus altering survival and fitness. Therefore, maternal influence may play a significant role in the adaptability and persistence of populations, altering the amount of variation on which natural selection can act. In this study, we provide empirical evidence of a positive relationship between offspring anti-predator response and maternal condition; future research should quantify the relative contribution of genetics versus maternal influence, across multiple populations and geographical gradients. Understanding the importance of maternal condition on trait variance in populations speaks not only to our knowledge of evolutionary history but can also provide important information on future trajectories in the face of changing environmental conditions.

**Ethics statement.** This study was approved by the Trent University Animal Care Committee (Protocol nos. 22022 (2011), 12007 (2012), 22828 (2013)) and strictly followed the Canadian Council on Animal Care’s guidelines for ethical animal use. Collection of adult northern leopard frogs and dragonfly larvae was approved through the Ontario Ministry of Natural Resources’ Wildlife Scientific Collectors Authorization (authorization nos. 1062294 (2011), 1067642 (2012), 1073024 (2013)) and was carried out with landowner permission. Collection did not involve endangered or specially protected species.

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