TECHNICAL REPORT

Offspring size–number strategies: experimental manipulation of offspring size in a viviparous lizard (*Lacerta vivipara*)

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Summary

1. Aspects of female 'offspring size *vs* number' strategies were studied in the viviparous Common Lizard, *Lacerta vivipara* Jacquin, by surgical yolk extraction from ovarian follicles and ovulated eggs in the oviduct ('allometric engineering').

2. This method has previously been applied exclusively to oviparous lizards but here it is applied to a viviparous taxon.

3. There was a strong negative relationship between the amount of yolk removed and the size of offspring at birth, and yolk-removed young were smaller and in poorer condition at birth than their control counterparts from within the same litters.

4. In yolk-removed young physiological performance was positively correlated with body mass.

5. Maternal resource provisioning thus influences offspring physiological performance, which suggests ongoing selection on female optimization of the offspring size–number trade-off.

Introduction

The neonate's phenotype is a compromise. It is the net outcome of a set of proximate and ultimate factors, starting with maternal allocation of resources to the developing zygote. These resources are used by the embryo for development along species-specific trajectories into a shape and form determined by its genotype (e.g. Sinervo & Huey 1990). The life of this genotype ends after a relatively predictable time from hatching/parturition after 'repairs' and maintenance of its body and one or several bouts of reproduction. Life-history theory attempts to explain why there is predictability in these events (Lack 1947; Smith & Fretwell 1974; Brockelman 1975; Stearns 1976; Roff 1992), or more precisely (i) what are the selection pressures and constraints that determine female resource allocation to one or several offspring, and (ii) how does selection mould 'decision rules' of resource allocation into current *vs* future reproduction.

Specifically, this study deals with the first of these problems, namely offspring size *vs* number strategies

(heretoforth OSN). 'While a trade-off between egg and clutch size seems an inevitable consequence of limited resources for egg laying, the measurement of this trade-off is not easy. Neither clutch size nor egg size can be directly manipulated' (Lessells 1991, p. 44). Since Lessells' pessimistic reflection, a host of experimental techniques has been developed that admits such manipulations. In particular, Sinervo and colleagues developed 'allometric engineering' techniques (e.g. Sinervo & McEdward 1988; Sinervo 1990; Sinervo & Huey 1990; Sinervo & Licht 1991; Sinervo *et al.* 1992; see Sinervo 1998 for review of techniques), a set of ingenious experimental manipulation 'tools' whereby clutch size and the size and the number of offspring can be manipulated independently of each other. This has the crucial advantage that, unlike in early studies, offspring size does not rely on maternal genotypic or phenotypic effects. For similar reasons, it may be advantageous to perform OSN studies on species that lack parental care, such as many species of reptiles (Shine 1988). In the present study we exploit this by applying the successful 'allometric engineering' techniques developed by Sinervo and colleagues (e.g. Sinervo 1990; Sinervo & Huey 1990; Sinervo *et al.* 1992) to the Common Lizard, *Lacerta vivipara* Jacquin. The present study has two major objectives: (i) to explore

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allometric engineering techniques on a viviparous species (for the first time) and (ii) to estimate physiological costs and benefits of maternal resource allocation to the offspring.

Materials and methods

Allometric engineering involves three main manipulation techniques: (i) hormonal manipulations can change clutch size; (ii) follicle ablation can change both clutch size (decreases) and offspring size (increases); and (iii) yolkectomy reduces offspring size (e.g. Sinervo 1990; Sinervo & Huey 1990; Sinervo & Licht 1991; Sinervo & DeNardo 1996; Sinervo & Doughty 1996). In our present study we primarily exploit yolkectomy, while also exploring the effects of follicle ablation in a single case.

FOLLICLE ABLATION

Prior to ovulation, a number of secondary follicles (i.e. primary follicles being yolked; Sinervo & Licht 1991; Sinervo & DeNardo 1996) were ruptured with a blunt probe at a stage when the female had committed herself to a given clutch size. This results in a channelling of resources into the remaining follicles, thereby increasing their resource allocation with a factor that is approximately linearly related to the number of ablated follicles.

YOLKECTOMY

This technique simply involves a reduction of the yolk content in the egg, in order to reduce the energy provisioning and, hence, the size of the hatchling. This can be accomplished by suction of yolk from the egg in oviparous species, using a syringe inserted into the yolk sac on the opposite side of the egg to where the embryonic disk is situated (Sinervo & Huey 1990; Sinervo & Licht 1991). In the viviparous Common Lizard yolk was extracted from ovulated eggs in the female oviduct.

STUDY SPECIES AND MODIFICATION OF METHODOLOGY

The Common Lizard is a small (<6 g, 60–70 mm snout–vent length), ground-dwelling species with a wide distribution range comprising a wide set of climatic regimes from S. France to N. Scandinavia. In the northern part of the distribution range (i.e. where the current study took place), females emerge from hibernation in March (depending on thermal conditions). Subsequent to the mating period, females carry their young until approximately August when parturition follows (van Nuland & Strijbosch 1981).

In the present study, Common Lizards of both sexes were captured in three populations on the Swedish West Coast (20–50 km south of Gothenburg) in

mid-April to the first week in May 1999. The lizards were captured by noose or by hand, brought to the Department of Zoology, University of Gothenburg, and kept in temperature-controlled rooms on a 14 : 10 h light : dark regime. During the day, the ambient temperature was set to 22 °C and the night temperature to 18 °C. Females were mated at capture (evident by the presence of mating scars (van Nuland & Strijbosch 1981). However, to reduce the risk of infertility, females were initially kept together with males to allow additional mating. A thermal gradient was arranged in the cage using a 40-W spotlight, allowing thermoregulation to the preferred body temperature (PBT; *c*. 29·9 °C, van Damme, Bauwens & Verheyen 1987), a shelter site was provided using two stacked tiles, and water was available *ad libitum*. The lizards were fed crickets (*Gryllus* sp.) and mealworms (*Tenebrio* larvae) dusted with multivitamins and calcium *ad libitum* (Nekton-Rep, Nekton-product). Two weeks postcapture, the males were released back into their natural populations while the females were kept in separate cages in the laboratory under standard conditions.

At the time of surgery, all females except one had ovulated. On the female not yet ovulated, a pilot test was performed of the effects of follicle ablation on offspring size. On the remaining females yolkectomies were performed as follows. Prior to surgery, 0·0083 mg g^{-1} body mass of Brietal (Lilly, VL-660) was injected subcutaneously, resulting in a *c*. 40 min general anaesthesia (M. Olsson, unpublished data). The anaesthetized female was taped to a sterile surgery board and had her abdomen sterilized with alcohol. Thereafter a 10–15 mm incision was made 2–3 mm laterally of the mid-ventral line using a pair of surgical scissors while 'lifting' the abdominal skin with a pair of tweezers. Using a blunt probe bent into the desired shape, one oviduct at a time was then lightly lifted/pulled out of the incision. A syringe, bent into a fine hook, was used to cause a minute rupture in the membrane on the opposite side to which the disk (i.e. the embryo) was clearly discernible through the transparent membranes. A capillary tube attached to a rubber tube was then fitted to the hole in the membranes and yolk was sucked into the capillary tube (the experimenter applied suction via the rubber tube using his mouth). The operation took *c*. 20 min per female. Healing and recovery of the ruptured tissue were evident from successful reproduction of these females in the following year (2000).

At the onset of the study, two factors were potentially strong confounders of our manipulations: maternal placental transfer of nutrients subsequent to the yolkectomy, and leakage of yolk through the small hole in the ruptured membranes. There was no information on the severity of these two problems while the experiment was planned. Furthermore, there was no way of knowing the volume of each egg or the exact proportion of yolk that was withdrawn from

each egg. This was because the length of the incision in the female abdomen was kept to a minimum, in many cases smaller than the length of the egg's longest axis. By judging the shrinkage of the egg resting on the female's abdomen, it was possible, however, to approximate how much yolk was left in the egg subsequent to the yolkectomy. The largest yolkectomies (*c.* +100 µl) resulted in an approximate halving of the yolk volume. Thus, egg size can be estimated to *c.* 200–250 µl. We deliberately varied the withdrawn yolk volume between follicles, position of the egg in the oviduct and females in order to create variation in offspring size at birth and to look for covariation with physiological performance. Subsequent to the yolkectomy, the females were closed using three stitches of suture (Ethicon, coated vicryl 5–0) and a sterile surgical tape to cover the incision. Subsequent to closure, the females spent 24–48 h in a 'wake-up' cage with paper as bottom substrate. They were then transferred to their home cages under standard conditions to wait for parturition.

Ideally, the life-history consequences of our manipulations (i.e. concomitant size-effects on, e.g. growth rate, time and size at maturation, juvenile *vs* adult survival, lifetime reproductive success, etc.) should have been tested. However, in this relatively long-lived species (*c.* 5–6 years, van Nuland & Strijbosch 1981), this was not possible within the time frame assigned to this project. Therefore, it was assumed that aspects of physiological performance, such as 'endurance', would be likely to covary with survival. Previous studies have found consistent, repeatable patterns between several such performance estimates and fitness estimates (e.g. Garland, Hawkins & Huey 1990).

Because Swedish *L. vivipara* inhabits peat bogs interspersed with pools of water and therefore swims regularly, lizard neonates were allowed to swim in a water-filled, thermally insulated aquarium, heated to their preferred body temperature (30·0 °C, physiological performance is temperature dependent in these ectotherms). The neonate was placed in the water, which immediately triggered swimming, predominantly by using strong tail beats, and was kept away from the periphery of the container by the experimenter using a plastic ruler. When the lizard stopped swimming, it was encouraged to continue by a light tap of the plastic ruler. When it did not respond to three taps with resumed swimming, the trial was interrupted and accumulated time swimming used to index endurance.

Results

EFFECTS OF YOLK MANIPULATION ON OFFSPRING TRAITS

During the series of surgical manipulations, it became apparent that water resorption early in development made yolk runnier and leakage of yolk from the

ruptured egg and oviducal membranes harder to control. Further evidence of such a change in yolk viscosity was a change in the colour of the yolk, from relatively darker to lighter as the yolk became more watery. Because yolk leakage in females late in gestation could not be controlled, the volume of yolk removed became too large in some cases. The critical viable offspring mass was *c.* 0·08 g; below this threshold the young tended to die in late stages of development (still-born).

Since 'marking' the developing embryo was not feasible, two observations were relied on to assign new-borns into treatment categories (manipulated *vs* control): (i) the exact number of manipulated eggs in the oviduct and the total clutch size were known, and (ii) there is an approximately linear negative relationship between the volume of the removed yolk and the rank order of the size of the new-born (Sinervo *et al.* 1992). This assumption was tested by examining the relationship between extracted yolk volume and neonatal size in a female sacrificed just prior to parturition. Since the treatment of each egg in the oviduct of all females (i.e. manipulated *vs* control) was known, the relationship between the removed yolk volume and the neonatal size could be analysed. This relationship was highly significant (Spearman's rank order correlation analysis, $r_s = -0·89$, $P = 0·0006$, $N = 10$), giving strong support to the assumption that there was a direct effect of yolk removal on offspring size and that, indeed, there was a rank order effect between the volume of the removed yolk and the size of the offspring.

Additional support for yolk removal effects would come from an increase in the coefficient of variation in offspring mass in clutches in which different amounts of yolk were removed while controlling for mean offspring mass which varies among females. Thus, the directional prediction can be made that clutches containing manipulated young should vary more in offspring mass than clutches where no manipulation was performed. This prediction was confirmed. The mean CV of control clutches was 7·6 (±2·6 SE, $N = 4$), compared with 19·1 for manipulated clutches (±3·9 SE, $N = 17$), yielding a statistically significant difference (*t*-test, $t = 0·3$, $P = 0·013$).

Pooling all young, mean body mass of control young was 0·21 g (±0·004 SE, $N = 59$) and 0·15 g for yolk-removed young (±0·007 SE, $N = 39$). Although this difference is statistically significant (*t*-test, $t = 7·7$, $P < 0·0000$), the validity of this test may be compromised by two factors. Firstly *F*-tests showed that the variances between controls and yolk-removed young differed significantly ($F = 2·1$, df = (38, 58), $P = 0·01$). Secondly, in the closely related Sand Lizard (*L. agilis*), there is a strong relationship between offspring size and clutch size (Olsson & Shine 1997), suggesting that among-clutch differences in offspring phenotypes is in part explained by maternal effects.

Offspring traits were therefore standardized by maternal identification number (setting the mean to

zero and the standard deviation to unity) and the mean scores of control and yolk-removed young within clutches were calculated. The difference in mass between control and yolk-removed young was still highly significant (mean scores 0·60 and −0·89, respectively, *t*-test, *t* = 11·1, df = 30·0, *P* < 0·0001). Furthermore, there was a corresponding reduction in snout–vent length in yolk-removed young compared with controls (mean scores −0·56 and 0·22, respectively; *t*-test, *t* = 3·8, df = 26·0, *P* = 0·0007). There was also a highly significant difference in body condition between the two treatment groups (i.e. in residuals from the mass – snout–vent length regression), with control young being in better condition than yolk-removed ones (mean scores 0·40 and −0·68, respectively; *t*-test, *t* = 6·5, df = 26·0, *P* < 0·0001).

FOLLICLE ABLATION

The single female on which two out of four follicles were ablated produced two viable young from the follicles left untouched. These young weighed on average 0·25 g (±0·02 SE, *N* = 2) compared with the mean offspring mass of 0·20 g of all new-borns from unmanipulated females (±0·003 SE, *N* = 89). This difference in offspring size is statistically significant (*t*-test, *t* = 1·77, *P* < 0·05, one-tailed *t*-test, a single observation compared to a sample mean).

PHYSIOLOGICAL PERFORMANCE OF NEW-BORNS

Average swim 'endurance' for all young pooled together was 21·7 ± 9·0 s (*N* = 74). Mean swim endurance was significantly higher in controls than in yolk-removed young (Wilcoxon two-sample test, *Z* = 3·1, *P* = 0·004; mean scores 0·17 ± 0·12 SE, and −0·41 ± 0·11, respectively, medians 0·21 and −0·51, respectively, standardized scores). This difference in mean swim endurance between treatments was due to the effects of our manipulations as evidenced by a positive correlation between mean swim endurance and mean offspring mass and body condition (r_s = 0·49, *P* = 0·008, and 0·54, *P* = 0·003, respectively, *N* = 28). However, pooling of the data may be questionable owing to the differences between the two treatment groups in several respects, such as body mass. Correlations within groups were therefore also looked for between offspring phenotype and mean swim endurance. In the control group there was no correlation between mean body mass or condition and mean swim endurance (r_s = −0·203, *P* = 0·91, and r_s = −0·20, *P* = 0·48, respectively, *N* = 15). In the yolk-removed group, however, there was a strong correlation between mean mass and mean swim endurance whereas the corresponding positive correlation with body condition was not statistically significant (r_s = 0·64, *P* = 0·019 and r_s = 0·46, *P* = 0·12, respectively, *N* = 13; see Fig. 1).
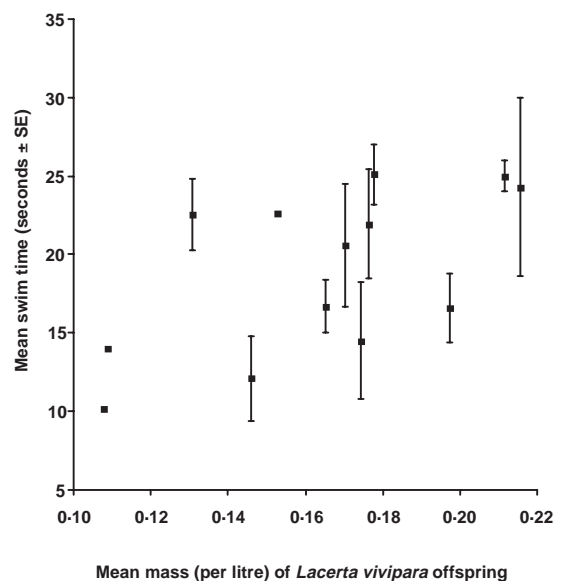

Fig. 1. Mean (per clutch) swim time (s) in 'yolk removed' *Lacerta vivipara* offspring depending on the mean mass of the offspring.

Discussion

EFFECTS OF YOLK MANIPULATION ON OFFSPRING TRAITS

Sinervo and coworkers' allometric engineering techniques (e.g. Sinervo & Huey 1990; Sinervo & Licht 1991; Sinervo *et al*. 1992; see Sinervo 1998 for review) have indeed revolutionized empirical life-history biology (Lessells 1991; Stearns 1992). The question then becomes 'How applicable is allometric engineering to taxa other than the oviparous lizard species on which Sinervo *et al*. developed the techniques?'

In this work we present the first attempt to manipulate offspring size in a viviparous species, the Common Lizard *Lacerta vivipara*. For enlargement of young via follicle ablation the technique is no different from that used by Sinervo & Licht (1991); follicles are simply surgically ablated, forcing the female to allocate postsurgery investments into a smaller number of follicles than she originally committed herself to. Although this could only be done in one female in the present study, the young from this female were larger than the average young in the population. Thus, follicle ablation holds great promise also for work on viviparous species.

The major difference in applying allometric engineering to a viviparous species rather than an oviparous one, lies in yolk removal. In the oviparous species this can be done subsequent to oviposition, whereas in the viviparous species the yolk has to be removed while the egg is still in the oviduct. In retrospect, this posed no problem close to ovulation. However, water absorption of the egg in the oviduct negatively influenced the accuracy of the technique later in the cycle. In future studies there are several ways in which this potentially confounding effect can be much better controlled,

should such manipulation be desired by the experimenter. For example, we experimented with cauterizing the ruptured membranes and experienced some success using this technique.

Superficially, the reduction in the size of the offspring following yolkectomy may seem an inevitable outcome of the manipulation based on Sinervo's previous reports. However, this is not necessarily so since *L. vivipara* has a primitive placenta (Shine 1985) and, hence, there is the possibility that a female may compensate for yolk loss by additional allocation of resources via placental transfer, or that a 'starved' egg somehow is more likely to 'absorb' vitellogenin circulating in the plasma than is a fully provisioned egg. To what degree this took place cannot be deduced from the present study. Nevertheless, it is clear that any compensatory effects via maternal and/or embryonic mechanisms could not cancel the effects of our yolkectomies.

An additional problem using a viviparous species in which parturition is (almost) synchronous is indisputable identification of the manipulated *vs* control offspring, and within the manipulation group, linking the extent of the yolk withdrawal to the correct individual postparturition. In the present study we had to resort to testing the assumption that yolk volume affects offspring size and then use this information to retrospectively assign treatment. We feel confident that this could be done correctly because the relative numbers of yolkectomized *vs* control young in each clutch were known and our yolkectomies were sufficiently large to result in considerable differences in offspring size. However, this technique would be increasingly unsatisfactory if more fine-grained differences in offspring size were desired by the experimenter. In future work, positive postparturition identification of individual offspring could possibly be made by radioactive marking of the embryos *in situ*.

The link between morphology and endurance seems straightforward. Larger offspring had better physiological performance whereas there was no significant relationship between an offspring's body condition and its endurance score. Furthermore, with follicle ablation it is possible to produce offspring at the upper tail end of the offspring size distribution. Thus, most females produce *smaller* offspring than they are physically capable of. Furthermore, using yolkectomy it is possible to demonstrate that females generally produce offspring that are *larger* than the minimum size that survive through embryogenesis.

In summary, this study explores the possibility of using 'allometric engineering' to manipulate life-history traits in a viviparous squamate, the Common Lizard. Using two techniques, follicle ablation (in only one female) and yolkectomy, offspring size was successfully manipulated, resulting in enlargement and reduction of offspring size. In physiological performance trials larger rather than 'chubby' offspring had more sustained endurance. Using allometric engineering, surviving smaller and larger young can be produced than the female originally committed to. Thus, females appear to be under selection to produce offspring of an 'optimal' rather than 'maximal' size intermediate to what the female (and offspring) is physically capable of, in accordance with classic life-history theory (Smith & Fretwell 1974; Brockelman 1975).

Acknowledgements

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