

Is the Evolution of Viviparity Accompanied by a Relative Increase in Maternal Abdomen Size in Lizards?

Yan-Yan Sun · Yu Du · Jing Yang ·
Tian-Bao Fu · Chi-Xian Lin · Xiang Ji

Received: 23 November 2011 / Accepted: 4 February 2012 / Published online: 24 February 2012
© Springer Science+Business Media, LLC 2012

Abstract Female reptiles with viviparous reproduction should leave space for their eggs that reach the maximum mass and volume in the oviducts. Is the evolution of viviparity accompanied by a relative increase in maternal abdomen size, thus allowing viviparous females to increase the amount of space for eggs? To answer this question, we compared morphology and reproductive output between oviparous and viviparous species using three pairs of lizards, which included two *Eremias*, two *Eutropis* and two *Phrynocephalus* species with different reproductive modes. The two lizards in each pair differed morphologically, but were similar in the patterns of sexual dimorphism in abdomen and head sizes and the rates at which reproductive output increased with maternal body and abdomen sizes. Postpartum females were heavier in viviparous species, suggesting that the strategy adopted by females to allocate energy towards competing demands differs between oviparous and viviparous species. Reproductive output was increased in one viviparous species, but decreased in the other two, as compared with congeneric oviparous species. The space requirement for eggs did not differ between

oviparous and viviparous females in one species pair, but was greater in viviparous females in the other two pairs greater in relative clutch mass and relative litter mass. In the two *Phrynocephalus* species, viviparous females produced heavier clutches than did oviparous females not by increasing the relative size of the abdomen, but by being more full of eggs. In none of the three species pairs was the maternal abdomen size greater in the viviparous species after accounting for body size. Our data show that the evolution of viviparity is not accompanied by a relative increase in maternal abdomen size in lizards. Future work could usefully investigate other lineages of lizards to determine whether our results are generalisable to all lizards.

Keywords Lizards · Reproductive mode · Morphology · Reproductive output · Body-volume constraint · Sexual dimorphism

Introduction

Life-history traits differ among species, among populations, and among individuals within a population. Such differences are assumed to have been shaped by natural selection, favoring the evolution of mechanisms and traits that maximize fitness, thus resulting in optimization or adaptation to the prevailing environmental conditions (Stearns 1992; Roff 2002; Pigliucci 2003). Studies of life-history variation at different levels address distinct but complementary questions, and can provide a basis for understanding the causes and evolutionary processes for such variation. Reproductive output, measured as a product of offspring size and number, which are both tightly linked to fitness, is a fundamental life-history trait that is subject to trade-offs and constraints (Williams 1966; Hirshfield and

Y.-Y. Sun · J. Yang · T.-B. Fu · X. Ji (✉)
Jiangsu Key Laboratory for Biodiversity and Biotechnology,
College of Life Sciences, Nanjing Normal University,
Nanjing 210046, Jiangsu, China
e-mail: xji@mail.hz.zj.cn

Y.-Y. Sun
Hangzhou Key Laboratory for Animal Adaptation and
Evolution, School of Life Sciences, Hangzhou Normal
University, Hangzhou 310036, Hangzhou, China

Y. Du · C.-X. Lin
Hainan Key Laboratory for Herpetological Research, School
of Life Sciences, Qiongzhou University, Wuzhishan 572200,
Hainan, China

Tinkle 1975; Stearns 1992; Roff 2002; Shine 2005). Body size and shape are among the key determinants of reproductive output in diverse animal taxa where selection on these two morphological variables yields a strong correlated response in reproductive output (Kaplan and Salthe 1979; Wickman and Karlsson 1989; Isaac 2005; Lourdaï et al. 2006; Goodman et al. 2009).

Like many other animal taxa, lizards can be sexually dimorphic in body size and shape, and in particular, two aspects differing between the sexes are the greater relative head size in males and the greater relative abdomen size in females (Braña 1996; Olsson et al. 2002a; Cox et al. 2003; Kratochvíl et al. 2003; Pincheira-Donoso and Tregenza 2011). The causes of sexual dimorphism are complex, but most sexually dimorphic traits are believed to be directly linked to the reproductive role of each sex (Bulté et al. 2008). In lizards, for example, the greater relative head size benefits males in bouts of male–male combat (Huyghe et al. 2005; Lappin and Husak 2005; Shine 2005; see also Kratochvíl and Frynta 2002), and may have a secondary role in amplifying food niche divergence between the sexes and thus reducing intersexual resource competition (Braña 1996; Zhang and Ji 2004). In females, increased reproductive output may result from a relative increase in abdomen size, which causes an increase in the amount of abdominal space available to hold eggs (Vitt and Congdon 1978; Shine 1992; Olsson et al. 2002a; Goodman et al. 2009; Bleu et al. 2012).

Most oviparous lizards lay eggs at embryonic stages grouping around Stage 30 in Dufaure and Hubert's (1961) developmental series (Shine 1983; Andrews and Mathies 2000). Pliable-shelled eggs laid by oviparous lizards and eggs retained in the oviducts both take up substantial amounts of water, and expand accordingly, during embryonic development (Vleck 1991; Qualls and Shine 1995; Qualls and Andrews 1999), with rapid water uptake taking place after Stage 30 (Shadrix et al. 1994; Ji and Zhang 2001; Ji et al. 2002; Qu et al. 2011b). Although eggs in the oviducts absorb less water than eggs in the nests because the ability of eggs to absorb water is constrained physically in the oviducts (Sun 2009), viviparous females should leave space for their eggs that reach the maximum mass and volume in the oviducts. This increase in space requirement reduces the maximum level of reproductive output at which females are physically full of eggs. This raises the question: is the evolution of viviparity accompanied by a relative increase in maternal abdomen size, thus allowing viviparous females to increase the amount of space available to hold eggs? If so, one may hypothesize that body plans should differ between oviparous and viviparous females, and in particular, selection should favor the evolution of a larger abdomen size in viviparous females. To test this hypothesis, one needs to compare

morphology and reproductive output between oviparous and viviparous species, better by using related species to minimize bias from phylogenetic and/or ecological differences (Qualls and Shine 1995).

In the present study, we used three pairs of lizards of which each included two congeneric species with different reproductive modes to test the above hypothesis. The first pair included two *Eremias* lizards (Lacertidae), *E. argus* (oviparous) and *E. multiocellata* (viviparous) coexisting in Inner Mongolia, northern China. The second pair included two *Eutropis* (formerly the genus *Mabuya*) lizards (Scincidae), *Eu. longicaudata* (oviparous) and *Eu. multifasciata* (viviparous) coexisting in Hainan, southern China. The third pair included two *Phrynocephalus* lizards (Agamidae), *P. axillaris* (oviparous) and *P. forsythii* (viviparous) coexisting in Xinjiang, northwestern China. The two species in each pair are similar ecologically in terms of habitat and thermal preferences, food habits and general behavior (Huang 1998; Zhao 1998a, b). For example, both *Eu. longicaudata* and *Eu. multifasciata* are insectivorous, use lowland habitats in forest edges, and mostly occur in places with direct sun exposure (Huang 2006; Ji et al. 2006). Among these six species, only *E. argus* is a multi-clutched species (Wang et al. 2011), females of the two *Eutropis* species often reproduce once within a breeding season (Huang 2006; Ji et al. 2006), and females of the remaining three species always reproduce once in a single year (Li et al. 2006, 2011; Wang 2011).

Materials and Methods

Adults *Eremias argus* (82 females and 92 males) and adult *E. multiocellata* (70 females and 75 males) were collected between April and May of 2007–2008 in an area between Wulatehouqi (41°27'N, 106°59'E) and Baotou (41°34'N, 108°31'E) in Inner Mongolia. Adult *Eutropis longicaudata* (24 females and 24 males) and adult *Eu. multifasciata* (78 females and 44 males) were collected between March and July of 2010–2011 in an area between Lingshui (18°48'N, 110°02'E) and Ledong (18°73'N, 109°17'E) in Hainan. Adult *Phrynocephalus axillaris* (34 females and 34 males) and adult *P. forsythii* (39 females and 30 males) were collected between May and June of 2010–2011 in an area between Aksu (41°15'N, 80°29'E) and Luntai (41°77'N, 84°25'E) in Xinjian. Males were released at their point of capture following the collection of morphological data with Mitutoyo digital calipers. Measurements taken for each individual included snout-vent length (SVL), abdomen length (AL; the distance between the points of insertion of the fore- and hind-limbs), head length (HL; from the snout to the posterior edge of the skull for *Phrynocephalus* lizards without the external auditory meatus, and to the

anterior edge of the external auditory meatus for other lizards) and head width (HW, taken at the posterior end of the mandible).

We transported *Eutropis* females to Wuzhishan, and other females to Nanjing. Between 6 and 10 individuals were housed in each 900 × 650 × 600 mm (length × width × height) communal cage, in an indoor animal holding facility. The cages contained a substrate of moist soil (*Eutropis* females) or sand (other females), with litter layers and pieces of clay tiles provided as shelter and basking sites. Thermoregulatory opportunities were provided between 07:00 and 19:00 h by a 100 W full-spectrum lamp suspended over one end of the cage; overnight temperatures followed indoor temperatures varying from 21 to 28°C in Wuzhishan, and from 18 to 26°C in Nanjing. Mealworms (*Tenebrio molitor*) and house crickets (*Achetus domestica*) dusted with multivitamins and minerals and water were provided daily, so that excess food was always available in the communal cages.

Females were isolated from each other using dividers that created 400 × 300 × 400 mm (*Eutropis* females) or 200 × 200 × 200 mm (other females) chambers in the case that they laid eggs or gave birth during the same time period in the same cage, such that eggs or neonates (= hatchlings in oviparous species) could be accurately allocated to the mother. None of these females was isolated for more than 48 h, and a 25 W heating light was mounted in each divider to allow thermoregulation. Eggs were collected, measured and weighed less than 6 h post-laying, and were then incubated at temperatures varying from 26 to 30°C. Hatchlings were collected, weighed and measured less than 6 h post-hatching or post-parturition. Postpartum females were weighed and measured, and were then released at their point of capture, usually within 1 month after oviposition or parturition. Relative clutch mass (RCM, for oviparous species) or relative litter mass (RLM, for viviparous species) was calculated by dividing clutch or litter mass by the postpartum body mass (Shine 1992). To make data comparable between oviparous and viviparous species, we defined reproductive output as the total wet mass of hatchlings produced in a single reproductive episode.

We used linear regression analysis, one-way analysis of variance (ANOVA), one-way analysis of covariance (ANCOVA), multivariate analysis of variance (MANOVA) and partial correlation analysis to analyze corresponding data. The homogeneity of slopes was checked prior to using ANCOVA to examine differences in the adjusted means. Regression residuals were calculated and analyzed when all slopes differed from zero but were unequal. Prior to parametric analyses, we tested data for normality using the Kolmogorov–Smirnov test, and for homogeneity of variances using the Bartlett's test (univariate level) or the Box's M test (multivariate level). Statistical analyses were

performed with Statistica 6.0 (StatSoft, Tulsa, USA). Throughout this paper, values were presented as mean ± standard error (SE), and the significance level was set at $\alpha = 0.05$.

Results

Females did not differ from males in mean SVL in four of the six species (ANOVA; all $P > 0.185$); females were the smaller sex in *P. axillaris* ($F_{1, 66} = 27.67$, $P < 0.0001$) but the larger sex in *P. forsythii* ($F_{1, 67} = 62.16$, $P < 0.0001$) (Table 1). AL, HL and HW were positively related to SVL in each species × sex combination (linear regression analysis; all $P < 0.0001$). AL, HL and HW differed between the sexes (MANOVA on regression residuals of the three variables against SVL; all $P < 0.0001$), and between the two species (all $P < 0.0001$), in each species pair. The sex × species interaction was a significant source of variation in these three variables in *Eremias* (Wilks' $\lambda = 0.80$, $df = 3, 313$, $P < 0.0001$) and *Eutropis* (Wilks' $\lambda = 0.90$, $df = 3, 164$, $P < 0.001$) lizards, but not in *Phrynocephalus* lizards (Wilks' $\lambda = 0.97$, $df = 3, 131$, $P = 0.339$). Females were larger in AL but smaller in HL and HW than males of the same SVL, and sexual differences were more pronounced in AL than in HL and HW, in all species (Fig. 1). Neither in males nor in females did AL differ between *P. axillaris* and *P. forsythii* after accounting for body size (ANCOVA with SVL as the covariate; both $P > 0.141$) (Fig. 2). In the other two species pairs, AL was longer in the oviparous species in both females and males after accounting for body size (ANCOVA with SVL as the covariate; all $P < 0.001$) (Fig. 2). Setting SVL at the overall mean level for single species pairs, we found that: (1) adult *E. argus* were longer in AL than adult *E. multiocellata* by an average of 2.1 mm in males, and by an average of 0.6 mm in females; and (2) adult *Eu. longicaudata* were longer in AL than adult *Eu. multifasciata* by an average of 5.6 mm in males, and by an average of 2.8 mm in females.

Female *E. argus* laid the first clutch of 2–6 eggs between May and June; female *E. multiocellata* produced a single litter of 2–5 young between June and August. Of the 24 female *Eu. longicaudata*, 15 laid a single clutch of 5–9 eggs between April and July and the remaining nine did not lay eggs for unknown reason; female *Eu. multifasciata* produced a single litter of 3–9 young between March and July. Female *P. axillaris* laid a single clutch of 2–4 eggs between May and July; female *P. forsythii* produced a single litter of 2–8 young between June and July (Table 2).

Postpartum body mass was greater in the viviparous species in each of the three species pairs after accounting for body size (ANCOVA with SVL as the covariate; all

Table 1 Descriptive statistics, expressed as mean ± SE and range, for morphological variables measured for the six lizards species

	Eremias lizards				Eutropis lizards				Phrynocephalus lizards			
	<i>E. argus</i>		<i>E. multiocellata</i>		<i>Eu. longicaudata</i>		<i>Eu. multifasciata</i>		<i>P. axillaris</i>		<i>P. forsythii</i>	
	Females	Males	Females	Males	Females ^a	Males	Females	Males	Females	Males	Females	Males
<i>N</i>	82	92	70	75	24	24	78	44	34	34	39	30
Snout-vent length (mm)	56.6 ± 0.4	56.8 ± 0.4	61.6 ± 0.5	60.8 ± 0.5	112.3 ± 1.6	114.7 ± 1.3	109.1 ± 0.6	109.2 ± 0.9	48.4 ± 0.4	51.6 ± 0.4	53.3 ± 0.6	47.7 ± 0.3
Abdomen length (mm)	47.3–64.3	47.1–67.6	55.1–72.7	55.0–77.1	100.1–128.0	101.3–127.9	98.5–125.2	98.4–120.5	43.1–53.2	46.1–56.5	45.2–60.7	45.0–52.4
Head length (mm)	29.8 ± 0.2	28.4 ± 0.2	32.1 ± 0.3	28.5 ± 0.3	57.5 ± 0.9	57.0 ± 0.8	53.0 ± 0.5	48.7 ± 0.5	25.9 ± 0.4	25.5 ± 0.3	29.9 ± 0.4	23.6 ± 0.3
Head width (mm)	24.2–33.6	21.4–36.5	26.2–38.9	24.2–37.3	49.7–65.5	49.2–62.8	45.3–63.9	43.2–55.4	21.5–30.2	22.0–28.6	24.0–34.7	21.5–28.6
	11.9 ± 0.1	13.3 ± 0.1	12.9 ± 0.1	14.6 ± 0.1	20.7 ± 0.2	22.7 ± 0.3	19.5 ± 0.1	20.7 ± 0.2	12.3 ± 0.1	13.8 ± 0.1	12.1 ± 0.1	12.4 ± 0.1
	10.5–13.3	10.8–15.9	11.9–14.4	11.1–16.4	18.7–22.7	19.2–25.7	17.6–22.3	18.7–22.9	11.0–13.9	11.3–15.9	10.9–13.2	11.4–14.0
	8.4 ± 0.03	9.0 ± 0.1	9.1 ± 0.1	10.0 ± 0.1	12.9 ± 0.2	14.3 ± 0.2	11.8 ± 0.1	12.8 ± 0.1	10.6 ± 0.1	11.8 ± 0.2	10.7 ± 0.1	10.6 ± 0.1
	7.5–9.2	7.6–11.0	8.2–10.1	8.7–11.7	11.0–14.4	11.7–16.6	10.8–13.3	11.4–14.0	9.0–12.3	10.1–14.4	9.6–11.8	9.9–12.2

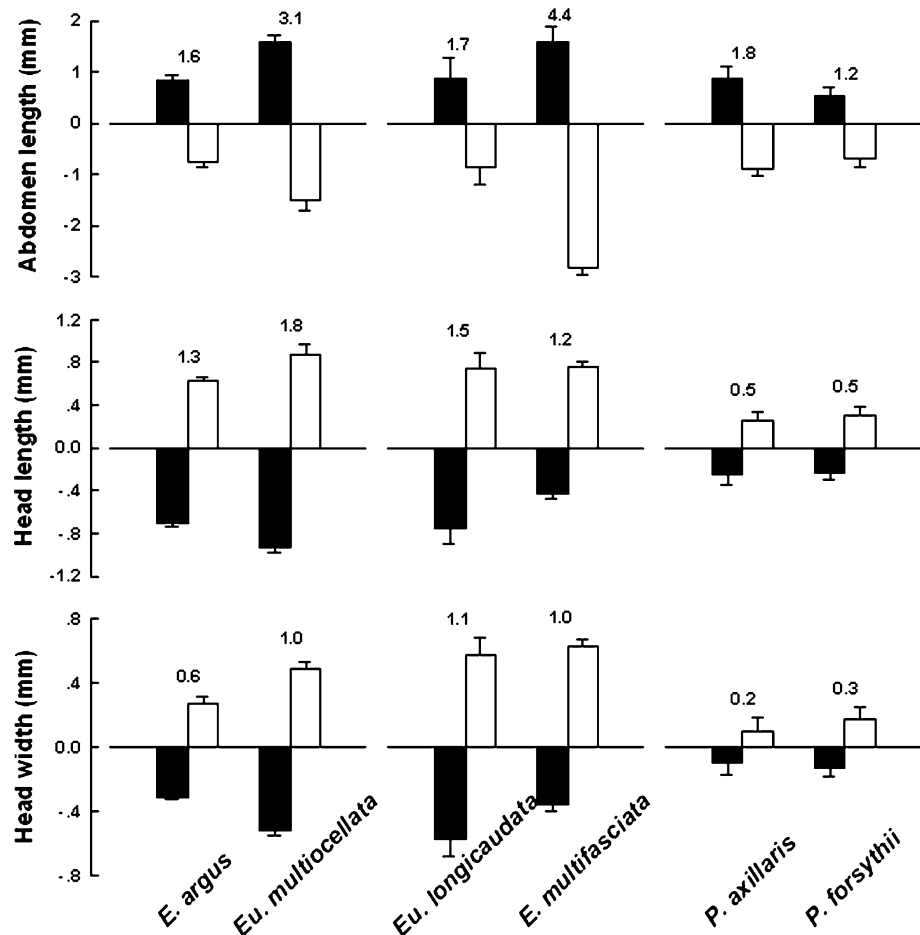
^a Of the 24 female *Eu. Longicaudata*, 15 laid eggs in the laboratory

$P < 0.03$). Fecundity (clutch or litter size) was positively related to maternal SVL (Fig. 3), and to maternal AL (Fig. 4), in all species (linear regression analysis; all $P < 0.021$) except *E. multiocellata* (both $P > 0.462$). Fecundity was higher in *E. multiocellata* than in *E. argus* in absolute terms (ANOVA; $F_{1, 150} = 6.65$, $P = 0.011$). Fecundity was higher in the oviparous species in the two *Eutropis* lizards, but lower in the oviparous species in the two *Phrynocephalus* lizards, in both absolute (ANOVA; both $P < 0.004$) and relative (ANCOVA with SVL as the covariate; both $P < 0.03$) terms.

Reproductive output was positively related to maternal SVL (Fig. 3), and to maternal AL (Fig. 4), in all species (linear regression analysis; all $P < 0.005$). Reproductive output was higher in the viviparous species in the two *Phrynocephalus* lizards, but lower in the viviparous species in other two species pairs, in both absolute (ANOVA; all $P < 0.02$) and relative (ANCOVA with SVL as the covariate; all $P < 0.01$) terms. ANCOVAs with reproductive output as the covariate showed that: (1) maternal SVL ($F_{1, 149} = 116.99$, $P < 0.0001$) and AL ($F_{1, 149} = 62.30$, $P < 0.0001$) were greater in the viviparous species in the two *Eremias* lizards; (2) maternal SVL ($F_{1, 90} = 0.07$, $P = 0.788$) and AL ($F_{1, 90} = 3.86$, $P = 0.053$) did not differ significantly between the two *Eutropis* lizards; and (3) maternal SVL did not differ significantly between the two *Phrynocephalus* lizards ($F_{1, 70} = 3.49$, $P = 0.066$), whereas maternal AL was greater in the viviparous species ($F_{1, 70} = 4.56$, $P = 0.036$) (Fig. 5). ANCOVA with post-partum body mass as the covariate and clutch or litter mass as the dependent variable showed that: (1) RCM did not differ from RLM in the two *Eremias* lizards ($F_{1, 149} = 0.05$, $P = 0.826$); (2) RCM was greater than RLM in the two *Eutropis* lizards ($F_{1, 90} = 74.20$, $P < 0.0001$); and (3) RCM was smaller than RLM in the two *Phrynocephalus* lizards ($F_{1, 70} = 20.77$, $P < 0.0001$) (Table 2).

Hatchling mass was dependent on egg mass at laying in three oviparous species (linear regression analysis; all $P < 0.0001$). The mean hatchling mass outweighed the mean egg mass by 0.11 g in *E. argus*, and by 0.08 g in *P. axillaris* (Table 2). On the contrary, the mean egg mass outweighed the mean hatchling mass by 0.24 g in *Eu. longicaudata* (Table 2). Hatchling mass was positively related to maternal SVL in three viviparous species (linear regression analysis; all $P < 0.004$), but not in three oviparous species (all $P > 0.107$) (Fig. 3). Hatchling mass was positively related to maternal AL in three viviparous and one oviparous (*Eu. longicaudata*) species (linear regression analysis; all $P < 0.04$), but not in the other two oviparous species (both $P > 0.594$) (Fig. 4). The mean hatchling mass did not differ between the two *Eremias* lizards (ANOVA; $F_{1, 150} = 0.01$, $P = 0.913$), nor between the two *Phrynocephalus* lizards (ANOVA; $F_{1, 71} = 2.38$,

Fig. 1 Mean values (\pm SE) for regression residuals of abdomen length, head length and head width against snout-vent length. Solid bars females; and open bars males. Numbers in the figure are differences in mean regression residual between the sexes



$P = 0.128$). In the two *Eutropis* lizards, the mean hatchling mass was greater in the oviparous species (ANOVA; $F_{1, 91} = 10.89$, $P < 0.002$) (Table 2). Holding maternal SVL constant with a partial correlation analysis, we found a significant negative correlation between size (mass) and number of offspring in all species (all $r < -0.24$, and all $P < 0.04$) except *Eu. longicaudata* ($r = -0.02$, $t = 0.06$, $df = 12$, $P = 0.956$).

Discussion

This study demonstrated for the first time male-biased sexual size dimorphism (SSD) in *P. axillaris* and female-biased SSD in *P. forsythii*, and confirmed the lack of SSD in the two *Eremias* lizards (Li et al. 2006; Liu 2006). Male-biased SSD as reported previously for the two *Eutropis* lizards (Huang 2006; Ji et al. 2006) was not detected in this study. Morphological data were collected previously from 153 adult *Eu. longicaudata* (82 females and 71 males; Huang 2006) and 313 adult *Eu. multifasciata* (189 females and 124 males; Ji et al. 2006), far more than the number of individuals measured in this study, thus allowing more accurate determination of the SSD pattern. Females were

the larger sex in abdomen size (AL) and males were the larger sex in head size (HL and HW), and AL was sexually more dimorphic than HL and HW, in all species (Fig. 1). Sexual differences in body-volume primarily result from differences in AL, as in no species involved in this study do the width and height of the abdomen differ between males and non-reproductive females after accounting for SVL (Li 2009; Sun 2009; Wang 2011). These observations suggest that AL provides a good index of room available for eggs in both oviparous and viviparous species. The trade-off between size and number of offspring was significant in all species except *Eu. longicaudata*. The lack of such a trade-off in *Eu. longicaudata* where females rarely reproduce twice in a single year might be due to the limited sample size (Table 2), as female lizards reproducing less frequently are often found to tradeoff offspring size against number (Sinervo and Licht 1991; Olsson et al. 2002b; Li et al. 2009, 2011; Qu et al. 2011a; Wang et al. 2011).

Postpartum females were heavier in the viviparous species in each of the three species pairs. The finding suggests that the strategy adopted by reproducing females to allocate energy towards competing demands differs between oviparous and viviparous species. Energy available to a female should be allocated towards three main competing demands,

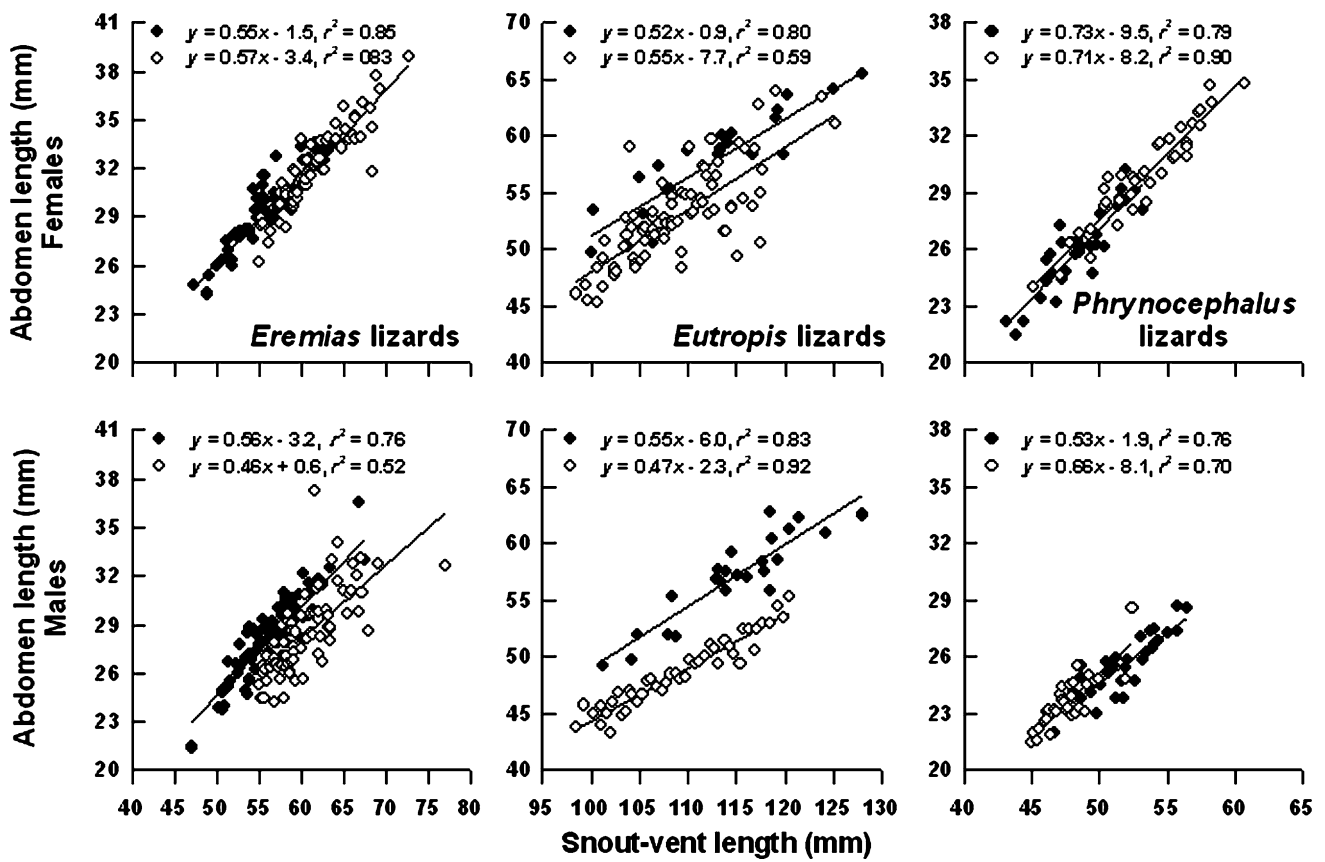


Fig. 2 Linear regressions of abdomen length against snout-vent length for females and males. *Solid dots* oviparous species; *open dots* viviparous species; plots on the *left*: *Eremias* lizards; plots in the *middle*: *Eutropis* lizards; and plots on the *right*: *Phrynocephalus* lizards. The regression equations and coefficients are given in the figure

Table 2 Descriptive statistics, expressed as mean \pm SE and range, for female reproductive traits of the six lizard species

	<i>Eremias</i> lizards		<i>Eutropis</i> lizards		<i>Phrynocephalus</i> lizards	
	<i>E. argus</i>	<i>E. multiocellata</i>	<i>Eu. longicaudata</i>	<i>Eu. multifasciata</i>	<i>P. axillaris</i>	<i>P. forsythii</i>
<i>N</i>	82	70	15	78	34	39
Maternal snout-vent length (mm)	56.5 \pm 0.4 47.3–64.3	61.6 \pm 0.5 55.1–72.7	113.6 \pm 2.1 100.1–128.0	109.1 \pm 0.6 98.5–125.2	48.4 \pm 0.4 43.1–53.2	53.3 \pm 0.6 45.2–60.7
Postpartum body mass (g)	3.5 \pm 0.07 2.1–5.4	4.6 \pm 0.1 2.8–7.0	27.5 \pm 1.9 15.2–37.3	29.3 \pm 0.6 19.9–42.5	3.3 \pm 0.1 1.8–4.6	4.6 \pm 0.1 3.1–6.8
Clutch or litter size	3.5 \pm 0.1 2–6	3.1 \pm 0.1 2–5	6.5 \pm 0.3 5–9	5.2 \pm 0.2 3–9	2.6 \pm 0.2 2–4	4.8 \pm 0.2 2–8
Egg mass (g)	0.40 \pm 0.01 0.24–0.63	–	1.40 \pm 0.05 1.11–1.91	–	0.53 \pm 0.02 0.32–0.71	–
Hatchling mass (g)	0.51 \pm 0.01 0.30–0.80	0.51 \pm 0.01 0.33–0.69	1.16 \pm 0.05 0.92–1.65	1.05 \pm 0.01 0.77–1.35	0.61 \pm 0.02 0.37–0.82	0.57 \pm 0.01 0.46–0.70
Relative clutch or litter mass	0.40 \pm 0.01 0.20–0.86	0.34 \pm 0.01 0.16–0.63	0.34 \pm 0.02 0.20–0.54	0.19 \pm 0.01 0.09–0.30	0.42 \pm 0.02 0.22–0.78	0.61 \pm 0.03 0.30–1.01
Reproductive output ^a (g)	1.71 \pm 0.05 0.93–3.10	1.53 \pm 0.05 0.89–2.44	7.54 \pm 0.51 4.92–12.01	5.49 \pm 0.21 2.32–10.83	1.58 \pm 0.08 0.84–2.55	2.74 \pm 0.13 1.00–4.20

^a Reproductive output is defined as the total mass of hatchlings produced in single reproductive episode

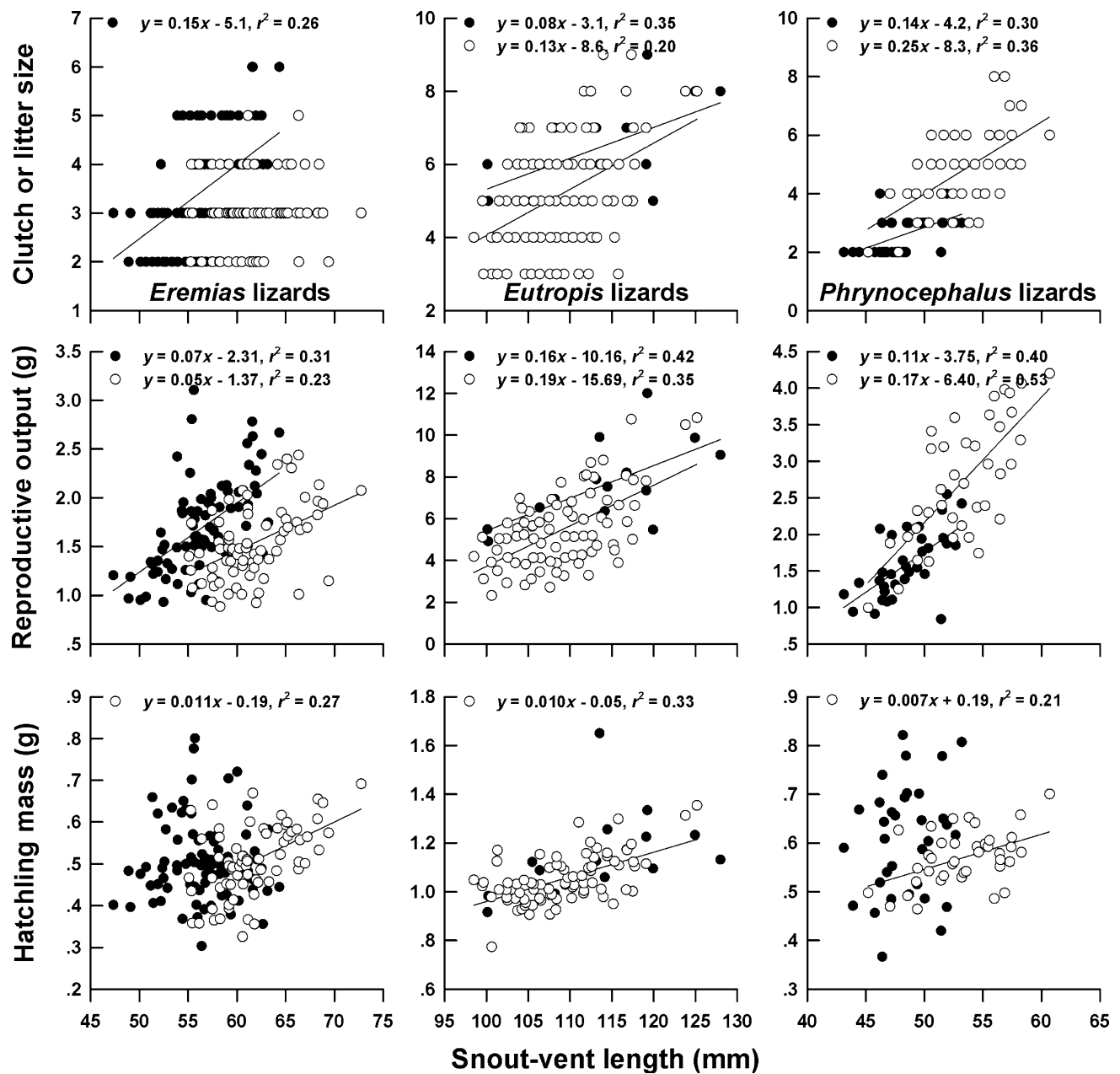


Fig. 3 Linear regressions of fecundity (clutch or litter size), reproductive output and hatchling mass against maternal snout-vent length. Solid dots oviparous species; open dots viviparous species; plots on the left: *Eremias* lizards; plots in the middle: *Eutropis* lizards; and

plots on the right: *Phrynocephalus* lizards. For the relationships that are significant, the regression equations and coefficients are given in the figure

maintenance, growth and reproduction. Reproduction has a lower priority in energy allocation in many animals, and only is allowed after meeting the energetic requirements for maintenance and growth (Congdon et al. 1982; Lucas 1996; McNab 2002; Kubička and Kratochvíl 2009; Luo et al. 2010). As the total energy available to any female is finite, she cannot improve her postpartum body condition without a concomitant reduction in the amount of energy allocated towards reproduction. Thus, a female's decision to improve

postpartum body condition at the expense of current reproductive investment, or devote more energy to fuel reproduction at the expense of postpartum body condition, likely reflects a strategy adopted by her to maximize lifetime reproductive success. Our data show that viviparous females are more likely to adopt the former strategy, presumably due to the importance of postpartum body condition for maternal fitness in viviparous females with more restricted opportunities to acquire energy, either as a consequence of the

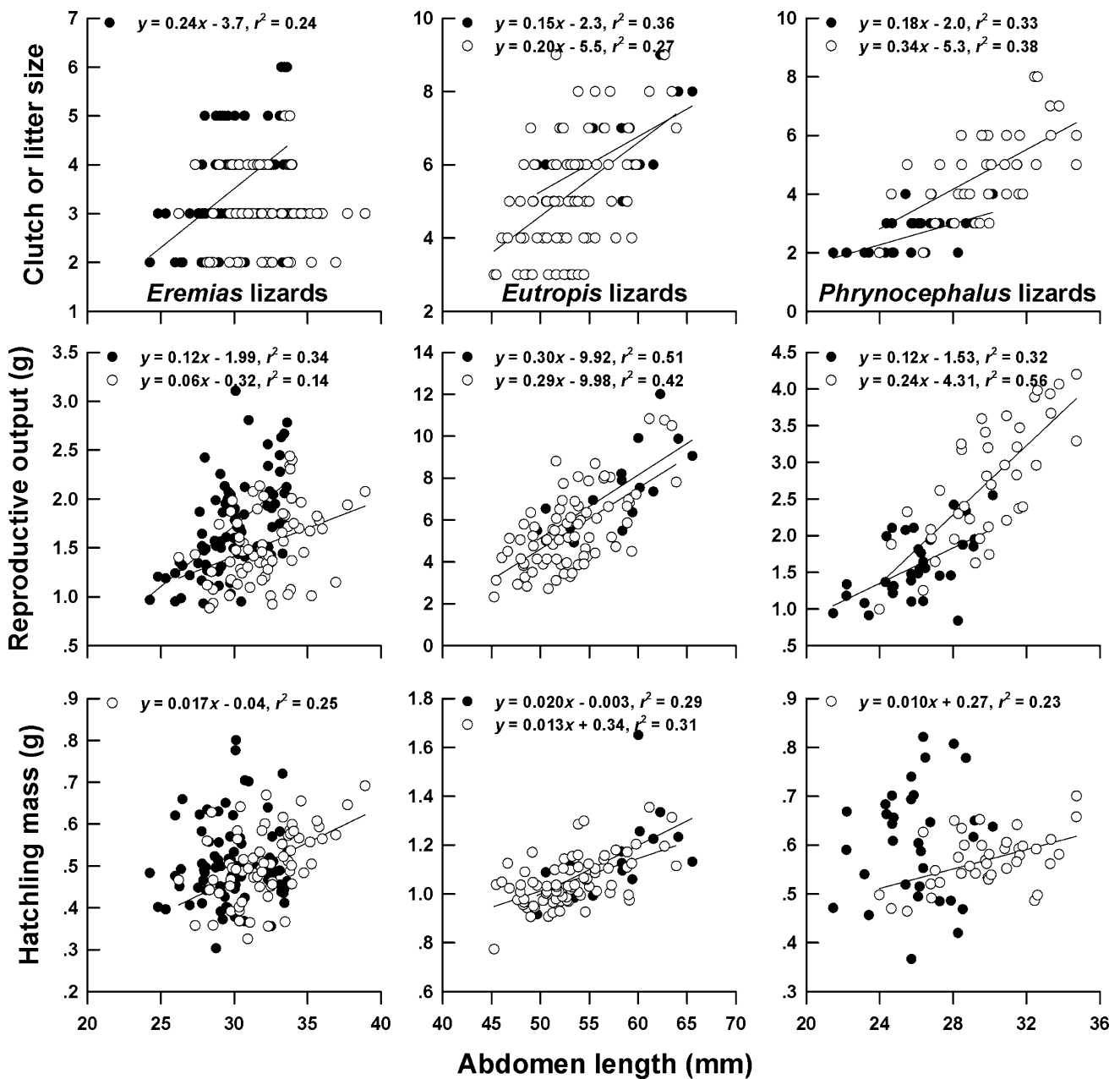


Fig. 4 Linear regressions of fecundity, reproductive output and hatchling mass against maternal abdomen length. *Solid dots* oviparous species; *open dots* viviparous species; plots on the *left*: *Eremias*

lizards; plots in the *middle*: *Eutropis* lizards; and plots on the *right*: *Phrynocephalus* lizards. For the relationships that are significant, the regression equations and coefficients are given in the figure

reduced locomotor capacity when they are gravid (Bauwens and Thoen 1981; Miles et al. 2000; Shine 2003; Kratochvíl and Kubička 2007; Lin et al. 2008), or because gestation shortens the season suitable for foraging.

Under the body-volume constraints hypothesis, females are expected to be unlikely to increase reproductive output without increasing body size overall and/or the relative size of the abdomen (Qualls and Shine 1995; Goodman et al. 2009; Griffith 2009). Our data show that larger females produce heavier clutches (offspring heavier in total mass)

than smaller ones in both oviparous and viviparous species (Fig. 3), thus validating the expectation that females increase reproductive output by increasing body size overall. However, contrary to what was expected in none of the three species pairs did we find that viviparous females increased reproductive output by increasing the relative size of the abdomen.

We found in the two *Phrynocephalus* lizards that reproductive output was greater in the viviparous species in both absolute (Table 2) and relative (Fig. 3) terms.

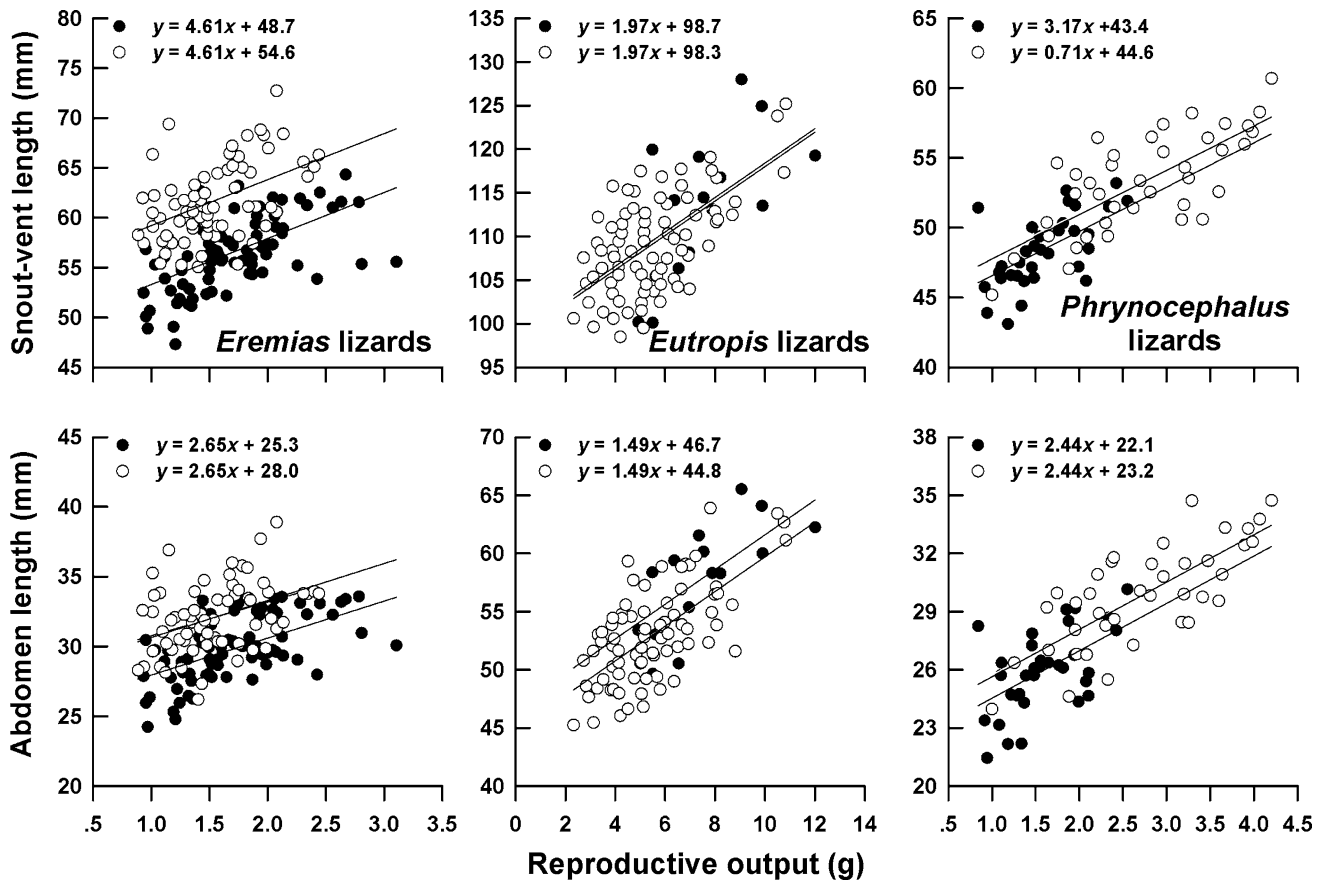


Fig. 5 Linear regressions of maternal snout-vent length and abdomen length against reproductive output. *Solid dots* oviparous species; *open dots* viviparous species; plots on the *left*: *Eremias* lizards; plots in the *middle*: *Eutropis* lizards; and plots on the *right*: *Phrynocephalus*

lizards. Regression lines in each plot were adjusted for two species with a common slope to facilitate comparisons. The corrected regression equations are given in the figure

This result is similar to that reported for *Lerista bougainvillii*, a reproductively bimodal scincid lizard where viviparous females produce heavier clutches than their conspecific oviparous females (Qualls and Shine 1995). However, reproductive output could have been overestimated in the study of *L. bougainvillii* where it was calculated by subtracting the mass of a female after parturition from her mass before parturition, including the mass of fluids lost in the course of parturition (Qualls and Shine 1995). Our study avoided this problem by defining reproductive output as the total mass of hatchlings produced in a single reproductive episode, thus providing stronger evidence that the evolution of viviparity can be accompanied by an increase in reproductive output in lizards (Qualls and Shine 1995). In viviparous lizards such as *Sphenomorphus indicus* (Ji and Du 2000) and *Phrynocephalus vlangalii* (Zhang et al. 2005), female-biased SSD may result in increased reproductive output. We did find female-biased SSD in *P. forsythii*, and that female *P. forsythii* were larger than female *P. axillaris* in SVL (Table 1). However, this increase in overall body size in female *P. forsythii* cannot

be viewed as a way of allowing them to produce offspring heavier in total mass than female *P. axillaris*, as the linear slope of reproductive output on maternal SVL did not differ between the two species (ANCOVA; $F_{1, 69} = 2.28$, $P = 0.136$; Fig. 3). Sexual dimorphism in AL was less evident in *P. forsythii* than in *P. axillaris* (Fig. 1), and in no sex did the relative AL differ between *P. forsythii* and *P. axillaris* (Fig. 2). These observations suggest that female *P. forsythii* produce offspring heavier in total mass than female *P. axillaris* not by increasing the relative size of the abdomen. Maternal AL was greater in *P. forsythii* than in *P. axillaris* after accounting for reproductive output (Fig. 5). This result together with the lack of a difference in the relative maternal AL between the two species suggest that female *P. forsythii* produce heavier clutches than female *P. axillaris* by being more full of eggs.

Reproductive output was reduced in the viviparous species in the other two species pairs (Table 2; Fig. 3). Do viviparous females in these two species pairs compensate for reduced reproductive output by increasing body size overall? Our answer to this question is no for three reasons.

First, if they could do so, the SSD pattern would differ between two *Eremias* lizards, and male-biased SSD would be less evident in *Eu. multifasciata* as compared with *Eu. longicaudata*. In opposition to these expectations, adults are not sexually dimorphic in body size in the two *Eremias* lizards (Li et al. 2006; Liu 2006; Table 1), and the two *Eutropis* lizards display almost the same degree of male-biased SSD in both absolute (males are larger than females by an average of 5 mm SVL in each species) and relative (1.044 in *Eu. multifasciata*, and 1.049 in *Eu. longicaudata*) terms (Huang 2006; Ji et al. 2006). Second, the proportions of variation in reproductive output explained by maternal SVL were lower in *E. multiocellata* (23%; Fig. 3) and *Eu. multifasciata* (35%; Fig. 3) than in *P. vlangalii* (49%; Zhang et al. 2005), *P. forsythii* (53%; Fig. 3) and *S. indicus* (40%; Ji and Du 2000) with female-biased SSD, suggesting that reproductive output is comparatively less tightly related to maternal SVL in the former two species. Third, as we found in the two *Phrynocephalus* lizards, neither in the two *Eremias* lizards (ANCOVA; $F_{1, 148} = 2.30$, $P = 0.132$) nor in the two *Eutropis* lizards (ANCOVA; $F_{1, 89} = 0.44$, $P = 0.508$) did the linear slope of reproductive output on maternal SVL differ between oviparous and viviparous species (Fig. 3). What can be inferred from these observations is that the rate at which reproductive output increases with increasing maternal SVL does not differ significantly between oviparous and viviparous species in the species pairs compared.

Evidence from *Eremias* and *Eutropis* lizards shows also that viviparous females do not compensate for reduced reproductive output by increasing the relative size of the abdomen. If they could do so, we would expect that maternal AL at a given body size would be greater in viviparous species than in their congeneric oviparous species. However, in opposition to our expectation, neither in the two *Eremias* lizards nor in the two *Eutropis* lizards was the relative AL larger in the viviparous species in both males and females over the range of adult body sizes (Fig. 2). We did find in the two species pairs that sexual dimorphism in AL was more pronounced in the viviparous species (Fig. 1). However, in none of these two species pairs was the inter-specific difference in AL greater in females and, in fact, the degree of sexual dimorphism in AL was more heavily dependent on male AL in these two species pairs (Fig. 2). Maternal AL was greater in *E. multiocellata* than in *E. argus*, but did not differ significantly between the two *Eutropis* lizards, after accounting for reproductive output (Fig. 5). These results suggest that the amount of space required by females to hold eggs does not differ significantly between the two *Eutropis* lizards, but is greater in the viviparous species in the two *Eremias* lizards. Relative clutch mass was lower in *Eu. longicaudata* (0.33) than in *E. argus* (0.40) and

P. axillaris (0.42), and relative litter mass was lower in *Eu. multifasciata* (0.19) than in *E. multiocellata* (0.34) and *P. forsythii* (0.61). Thus, the lack of a significant difference in the space requirement for eggs between the *Eutropis* lizards could be attributable to their lower levels of reproductive investment.

In summary, our data show that the two lizards in each species pair differ morphologically, but are similar in the patterns of sexual dimorphism in abdomen and head sizes and the rates at which reproductive output increases with maternal body and abdomen sizes. Maternal AL (and thus, body-volume) acts an important constraint on reproductive output in both oviparous and viviparous species. Reproductive output is increased in *P. forsythii*, but is reduced in *E. multiocellata* and *Eu. multifasciata* as compared with congeneric oviparous species. The three species pairs compared belong to different lineages and occur in different climatic regions, thus allowing us to draw some general conclusions of which the most interesting one is that the evolution of viviparity is not accompanied by a relative increase in maternal abdomen size. Future work could usefully investigate other lineages of lizards with both oviparous and viviparous species in a phylogenetic context to determine whether our results are generalisable to all lizards.

Acknowledgements The work was carried out in compliance with the current laws on animal welfare and research in China, and was supported by grants from the Natural Science Foundation of China (30670281, 31060064 and 31071910), Innovative Team Project of Nanjing Normal University (0319PM0902) and Priority Academic Program Development of Jiangsu Higher Education Institutions (CXLX11_0885 and 2010BS0040) to Ji's group. We thank Hong Li, Zheng-Cheng Li, Hong-Liang Lu, Li Ma, Qing-Bo Qiu, Zheng Wang, Yan-Qing Wu, Hui-Qin Yin and Zong-Shi Zhou for help during the research.

References

- Andrews, R. M., & Mathies, T. (2000). Natural history of reptilian development: Constraints on the evolution of viviparity. *BioScience*, *50*, 227–238.
- Bauwens, D., & Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *The Journal of Animal Ecology*, *50*, 733–743.
- Bleu, J., Massot, M., Haussy, C., & Meylan, S. (2012). Experimental litter size reduction reveals costs of gestation and delayed effects on offspring in a viviparous lizard. *Proceedings of the Royal Society. Section B*, *279*, 489–498.
- Braña, F. (1996). Sexual dimorphism in lacertid lizards: Male head increase vs female abdomen increase? *Oikos*, *75*, 511–523.
- Bulté, G., Irschick, D. J., & Blouin-Demerset, G. (2008). The reproductive role hypothesis explains trophic morphology dimorphism in the northern map turtle. *Functional Ecology*, *22*, 824–830.
- Congdon, J. D., Dunham, A. E., & Tinkle, D. W. (1982). Energy budgets and life histories of reptiles. In C. Gans (Ed.), *Biology of reptilia* (Vol. 13, pp. 233–271). New York: Academic Press.

- Cox, R. M., Skelly, S. L., & John-Alder, H. B. (2003). A comparative test of adaptive hypothesis for sexual size dimorphism in lizards. *Evolution*, *57*, 1653–1669.
- Dufaure, J. P., & Hubert, J. (1961). Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* Jacquin. *Archives D'anatomie Microscopique et de Morphologie Expérimentale*, *50*, 309–328.
- Goodman, B. A., Hudson, S. C., Isaac, J. L., & Schwarzkopf, L. (2009). The evolution of body shape in response to habitat: Is reproductive output reduced in flat lizards? *Evolution*, *63*, 1279–1291.
- Griffith, H. (2009). Body elongation and decreased reproductive output within a restricted clade of lizards (Reptilia: Scincidae). *Journal of Zoology*, *233*, 541–550.
- Hirshfield, M. F., & Tinkle, T. W. (1975). Natural selection and the evolution of reproductive effort. *Proceedings of the National Academy of Sciences of the United States of America*, *72*, 2227–2231.
- Huang, Q. Y. (1998). *Mabuya fitzinger*, 1826. In E. M. Zhao, K. T. Zhao, & K. Y. Zhou (Eds.), *Fauna Sinica, Reptilia (Squamata, Lacertilia)* (Vol. 2, pp. 304–309). Beijing: Science Press.
- Huang, W. S. (2006). Ecological characteristics of the skink, *Mabuya longicaudata*, on a tropical East Asian island. *Copeia*, *2006*, 293–300.
- Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M., & Van Damme, R. (2005). Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Functional Ecology*, *19*, 800–807.
- Isaac, J. L. (2005). Potential causes and life history consequences of sexual size dimorphism in mammals. *Mammal Review*, *35*, 101–115.
- Ji, X., & Du, W. G. (2000). Sexual dimorphism in body size and head size and female reproduction in a viviparous skink, *Sphenomorphus indicus*. *Zoology Research*, *21*, 349–354.
- Ji, X., Lin, L. H., Lin, C. X., Qiu, Q. B., & Du, Y. (2006). Sexual dimorphism and female reproduction in the many-lined sun skink (*Mabuya multifasciata*) from China. *Journal of Herpetology*, *40*, 353–359.
- Ji, X., Qiu, Q. B., & Diong, C. H. (2002). Influence of incubation temperature on hatching success, embryonic use of energy, and size and morphology of hatchlings in the oriental garden lizard, *Calotes versicolor* (Agamidae). *Journal of Experimental Zoology*, *292*, 649–659.
- Ji, X., & Zhang, C. H. (2001). Effects of thermal and hydric environments on incubating eggs, hatching success, and hatchling traits in the Chinese skink (*Eumeces chinensis*). *Acta Zoologica Sinica*, *47*, 250–259.
- Kaplan, R. H., & Salthe, S. N. (1979). The allometry of reproduction: An empirical view in salamanders. *American Naturalist*, *113*, 671–689.
- Kratochvíl, L., Fokt, M., Reháč, I., & Frynta, D. (2003). Misinterpretation of character scaling: A tale of sexual dimorphism in body shape of common lizards. *Canadian Journal of Zoology*, *81*, 1112–1117.
- Kratochvíl, L., & Frynta, D. (2002). Body size, male combat and the evolution of sexual dimorphism in eublepharid geckos (Squamata: Eublepharidae). *Biological Journal of the Linnean Society*, *76*, 303–314.
- Kratochvíl, L., & Kubička, L. (2007). Why reduce clutch size to one or two eggs? Reproductive allometries reveal different evolutionary causes of invariant clutch size in lizards. *Functional Ecology*, *21*, 171–177.
- Kubička, L., & Kratochvíl, L. (2009). First growth, then breed and finally get fat: Hierarchical allocation to life-history traits in a lizard with invariant clutch size. *Functional Ecology*, *23*, 595–601.
- Lappin, A. K., & Husak, J. F. (2005). Weapon performance, not size, determines mating success and potential reproductive output in the collard lizard (*Crotaphytus collaris*). *American Naturalist*, *166*, 426–436.
- Li, H. (2009). *The evolution of reptilian viviparity and its adaptive significance using lizards as the model systems*. Ph.D. Dissertation, Nanjing Normal University, Nanjing, China.
- Li, H., Ji, X., Qu, Y. F., Gao, J. F., & Zhang, L. (2006). Sexual dimorphism and female reproduction in the multi-ocellated racerunner, *Eremias multiocellata* (Lacertidae). *Acta Zoologica Sinica*, *52*, 250–255.
- Li, H., Qu, Y. F., Ding, G. H., & Ji, X. (2011). Life-history variation with respect to the experienced thermal environments in a lizard, *Eremias multiocellata* (Lacertidae). *Zoological Science*, *28*, 332–338.
- Li, H., Qu, Y. F., Hu, R. B., & Ji, X. (2009). Evolution of viviparity in cold-climate lizards: Testing the maternal manipulation hypothesis. *Evolutionary Ecology*, *23*, 777–790.
- Lin, C. X., Zhang, L., & Ji, X. (2008). Influence of pregnancy on locomotor performances of the skink, *Mabuya multifasciata*: Why do females shift thermal preferences when pregnant? *Zoology*, *111*, 188–195.
- Liu, H. X. (2006). Geographic patterns of variation in life-history traits in the Mongolian racerunner, *Eremias argus*. Master Thesis, Nanjing Normal University, Nanjing, China.
- Lourdais, O., Shine, R., Bonnet, X., & Bricoux, F. (2006). Sex differences in body composition, performance and behaviour in the Columbian rainbow boa (*Epicrates cenchria maurus*, Boidae). *Journal of Zoology*, *269*, 175–182.
- Lucas, A. (1996). *Bioenergetics of aquatic animals*. London: Taylor and Francis Ltd.
- Luo, L. G., Ding, G. H., & Ji, X. (2010). Income breeding and temperature-induced plasticity in reproductive traits in lizards. *Journal of Experimental Biology*, *213*, 2073–2078.
- McNab, B. K. (2002). *The physiological ecology of vertebrates: A view from energetics* (Vol. 1). Cornell: Comstock.
- Miles, D. B., Sinervo, B., & Frankino, W. A. (2000). Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution*, *54*, 1386–1395.
- Olsson, M., Shine, R., Wapstra, E., Ujvari, B., & Madsen, T. (2002a). Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. *Evolution*, *56*, 1538–1542.
- Olsson, M., Wapstra, E., & Olofsson, C. (2002b). Offspring size-number strategies: Experimental manipulation of offspring size in a viviparous lizard (*Lacerta vivipara*). *Functional Ecology*, *16*, 135–140.
- Pigliucci, M. (2003). Phenotypic integration: Studying the ecology and evolution of complex phenotypes. *Ecological Letters*, *6*, 265–272.
- Pincheira-Donoso, D., & Tregenza, T. (2011). Fecundity selection and the evolution of reproductive output and sex-specific body size in the *Liolaemus* lizard adaptive radiation. *Evolutionary Biology*, *38*, 197–207.
- Qu, Y. F., Gao, J. F., Mao, L. X., & Ji, X. (2011a). Sexual dimorphism and female reproduction in two sympatric toad-headed lizards, *Phrynocephalus frontalis* and *P. versicolor* (Agamidae). *Animal Biology*, *61*, 139–151.
- Qu, Y. F., Li, H., Gao, J. F., & Ji, X. (2011b). Embryonic thermosensitivity and hatchling morphology differ between two coexisting lizards. *Acta Oecologica*, *37*, 375–380.
- Qualls, C. P., & Andrews, R. M. (1999). Maternal body volume constrains water uptake by lizard eggs in utero. *Functional Ecology*, *13*, 845–851.

- Qualls, C. P., & Shine, R. (1995). Maternal body-volume as a constraint on reproductive output in lizards: Evidence from the evolution of viviparity. *Oecologia*, *103*, 73–78.
- Roff, D. A. (2002). *Life history evolution*. Sunderland: Sinauer Associates.
- Shadrix, C. A., Crotzer, D. R., McKinney, S. L., & Stewart, J. R. (1994). Embryonic growth and calcium mobilization in oviposited eggs of the scincid lizard, *Eumeces fasciatus*. *Copeia*, *1994*, 493–498.
- Shine, R. (1983). Reptilian reproductive modes: The oviparity-viviparity continuum. *Herpetologica*, *39*, 1–8.
- Shine, R. (1992). Relative clutch mass and body shape in lizards and snakes: Is reproductive investment constrained or optimized? *Evolution*, *46*, 828–833.
- Shine, R. (2003). Locomotor speeds of gravid lizards: Placing ‘costs of reproduction’ within an ecological context. *Functional Ecology*, *17*, 526–533.
- Shine, R. (2005). Life-history evolution in reptiles. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 23–46.
- Sinervo, B., & Licht, P. (1991). Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. *Science*, *252*, 1300–1302.
- Stearns, S. C. (1992). *The evolution of life histories*. Oxford: Oxford University Press.
- Sun, Y. Y. (2009). Life-history traits, thermal dependence of offspring phenotype and costs of tail loss in the many-lined sun skink, *Mabuya multifasciata*. Master thesis, Hangzhou Normal University, Hangzhou, China.
- Vitt, L. J., & Congdon, J. D. (1978). Body shape, reproductive effort and relative clutch mass in lizards: Resolution of a paradox. *American Naturalist*, *112*, 595–608.
- Vleck, D. (1991). Water economy and solute regulation of reptilian and avian embryos. In D. C. Deeming & M. W. J. Ferguson (Eds.), *Egg incubation: Its effects on embryonic development in reptiles and birds* (pp. 245–259). Cambridge: Cambridge University Press.
- Wang, Z. (2011). Adapting to extreme climate: The evolution of viviparity in *Phrynocephalus* lizards. Ph.D. Dissertation, Nanjing Normal University, Nanjing, China.
- Wang, Z., Xia, Y., & Ji, X. (2011). Clutch frequency affects the offspring size-number trade-off in lizards. *PLoS One*, *6*, e16585.
- Wickman, P. O., & Karlsson, B. (1989). Abdomen size, body size and the reproductive effort of insects. *Oikos*, *56*, 209–214.
- Williams, G. C. (1966). Natural selection, the costs of reproduction and a refinement of Lack’s principal. *American Naturalist*, *100*, 687–690.
- Zhang, Y. P., & Ji, X. (2004). Sexual dimorphism in head size and food habits in the blue-tailed skink *Eumeces elegans*. *Acta Zoologica Sinica*, *50*, 745–752.
- Zhang, X. D., Ji, X., Luo, L. G., Gao, J. F., & Zhang, L. (2005). Sexual dimorphism and female reproduction in the Qinghai toad-headed lizard *Phrynocephalus vlangalii*. *Acta Zoologica Sinica*, *51*, 1006–1012.
- Zhao, K. T. (1998a). *Phrynocephalus* Kaup, 1825. In E. M. Zhao, K. T. Zhao, & K. Y. Zhou (Eds.), *Fauna Sinica, Reptilia (Squamata, Lacertilia)* (Vol. 2, pp. 151–192). Beijing: Science Press.
- Zhao, K. T. (1998b). *Eremias* Wiegmann, 1834. In E. M. Zhao, K. T. Zhao, & K. Y. Zhou (Eds.), *Fauna Sinica, Reptilia (Squamata, Lacertilia)* (Vol. 2, pp. 220–242). Beijing: Science Press.