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Age-related reproduction of female Mongolian racerunners (*Eremias argus*; Lacertidae): Evidence of reproductive senescence

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Abstract

The reproductive maturation hypothesis, the terminal investment hypothesis, and the senescence hypothesis are the most extensively evaluated hypotheses proposed to explain age-related patterns of reproduction in iteroparous organisms. Here, we evaluated these hypotheses for the Mongolian racerunner (*Eremias argus*), a short-lived lacertid lizard, by comparing reproductive traits between females that completed reproductive cycles under the same laboratory conditions in two consecutive years (2008 and 2009). Reproductive females gained linear size (snout-vent length) not only as they got 1 year older but also during the breeding season. Larger females generally laid eggs earlier and invested more in reproduction than did smaller ones. Females switched from laying smaller eggs in the first clutch to larger eggs in the subsequent clutches but kept clutch size and postpartum body mass constant between successive clutches in a breeding season and between years. Females that laid more clutches or eggs in 2008 did not lay fewer clutches or eggs in 2009. Of the traits examined, only clutch frequency, annual fecundity, and annual reproductive output were susceptible to ageing. Specifically, the clutch frequency was reduced by 1.1 clutches, annual fecundity by 3.1 eggs and annual reproductive output by 1.0 g in 2009 compared with 2008. Our results suggest that the reproductive maturation hypothesis better explains patterns of reproduction in young or prime-aged females of *E. argus*, whereas the senescence hypothesis better explains reproductive patterns in old females. The terminal investment hypothesis does not apply to any trait examined because no trait value was maximized in old females.

KEYWORDS
annual fecundity, annual reproductive output, clutch frequency, clutch size, egg size, lizard, senescence hypothesis

1 | INTRODUCTION

One of the central goals of life-history studies is to assess changes in trait values associated with senescence (ageing) in animal populations (Stearns, 1992). Senescence, the decrease in functional capacities in old individuals, is nearly ubiquitous in natural populations of animals but varies in both timing and magnitude among species, across populations within a species, among individuals of the same population, and even among siblings of the same family (Berger, Lemaître, Allaine, Gaillard, & Cohas, 2015; Hamilton, 1966; Lemaître & Gaillard, 2017; Nussey, Froy, Lemaître, Gaillard, & Austad, 2013; Toïgo et al., 2007). Moreover, a growing number of studies provide evidence that sex is an important cause of variation in senescence (Austad, 2006; Berger et al., 2015; Clancy et al., 2001; Lemaître &

Gaillard, 2013; Nussey et al., 2013). Among traits that show senescence in nature, age-related declines in survival (actuarial senescence), and reproduction (reproductive senescence) have been most extensively examined largely because they are the important components of an organism's fitness. Actuarial senescence occurs in any free-ranging and age-structured population and focuses on whether or not an individual is alive or not at a given age; reproductive senescence involves a set of traits that covary in complex ways and, as such, its timing and magnitude are far more difficult to predict (Hamilton, 1966; Hayward et al., 2015; Jones et al., 2014; Lemaître & Gaillard, 2017; Nussey et al., 2013). For example, in species where offspring size covaries with offspring number the exact relationship between the two variables often cannot be detected without holding maternal size constant (Ji, Du, Qu, & Lin, 2009; Olsson & Shine, 1997; Wang, Xia, & Ji, 2011). Studies of age-related reproduction have shown diverse patterns. In insects, for example, egg size (a life-history trait highly associated with the fitness of both parents and offspring; Thorn & Morbey, 2018) declines with maternal age in *Apis mellifera* (Giron & Casas, 2003) and *Eupelmus vuilleti* (Al-Lawati & Bienefeld, 2009), but increases with maternal age in *Chorthippus hrunneus* (Butlin, Woodhatch, & Hewitt, 1987) and *Dysdercus fasciatus* (Kasule, 1991).

Several hypotheses have been proposed to explain age-related patterns of reproductive investment in iteroparous organisms, with the reproductive maturation (evolutionary restraint) hypothesis, the terminal investment (relative reproductive rate or residual reproductive value) hypothesis, and the senescence hypothesis having been most extensively evaluated. These hypotheses are not mutually exclusive but provide contrasting predictions regarding age-related patterns of reproductive investment. The reproductive maturation hypothesis predicts that reproductive investment increases with age in early adulthood because of improvements in physiology, morphology, and/or experience or because of high expectations of future reproduction (Clutton-Brock, 1988; Pärt, 1995; Rabon, 2013; Reid, Bignal, Bignal, McCracken, & Monaghan, 2003; Robertson & Rendell, 2001). The terminal investment hypothesis predicts that reproductive investment increases with age later in adulthood when the reproductive value of individuals decreases with age (Congdon et al., 2003; Descamps, Boutin, Berteaux, & Gaillard, 2007; Harms, Paitz, Bowden, & Janzen, 2005; Pianka, 1988; Pianka & Parker, 1975). The senescence hypothesis predicts that reproductive investment decreases in older versus younger females simply because the decline in functional capacities in old individuals leads to lower reproduction (Partridge, 2001; Williams, 1957).

Lizards are excellent models for experimental studies of age-related reproduction because many species are easy to breed in captivity and, unlike long-lived reptiles, such as turtles, tortoises, and crocodiles (Congdon et al., 2003; Congdon, Nagle, Kinney, & van Loben Sels, 2001; Harms et al., 2005; Miller, 2001; Warner, Miller, Bronikowski, & Janzen, 2016), have a short life span (3–15 years depending on the species, population, or sex) with evidence of age-related deterioration (Guarino, Crovetto, Mezzasalma, & Salvidio, 2015; Jesus, 2012; Olsson & Shine, 1996). However, few lizards have been used for studies on ageing, with the available data not permitting any generalization of ageing phenomena. To the best of our knowledge, the relationship between reproduction and ageing has been evaluated only in three species of lizards, the leopard gecko *Eublepharis macularius* (Rhen, Crews, Fivizzani, & Elf, 2006), the sand lizard *Lacerta agilis* (Olsson & Shine, 1996), and the common lizard *Lacerta* (*Zootoca*) *vivipara* (Massot et al., 2011; Richard, Lecomte, de Fraipont, & Clobert, 2005). In *E. macularius*, there is a trade-off between reproductive investment in the first reproductive season and the number of clutches produced in the second season (Rhen et al., 2006). In *L. agilis*, however, the observed positive relationship between reproductive success (as measured by the number of mates obtained for males, and the number of eggs produced for females) and age is merely an epiphenomenon, because it is a consequence of somatic growth (and thus increased body size) rather than a direct effect of age (Olsson & Shine, 1996). Ageing patterns have been found in multiple aspects in *Z. vivipara* . For example, increases in litter size (the number of offspring produced per reproductive episode) and offspring viability with age early in adulthood correspond to a pattern of reproductive maturation, the enhanced offspring quality (offspring size or corpulence) late in life corresponds to the terminal investment hypothesis, and a pattern of senescence is found in metabolic rate and immunity (Massot et al., 2011; Richard et al., 2005).

Here, we describe a study examining age-related reproduction by evaluating the aforementioned hypotheses using data collected from female Mongolian racerunners (*Eremias argus*; Lacertidae) that completed reproductive cycles under the identical laboratory conditions in two consecutive years (2008 and 2009). Previous studies of *E. argus* show that the unlimited food availability, the absence of predators, and the benign thermal environments provide females living under laboratory conditions the potential to allocate as a great fraction of available resources in reproduction as they can (Hao, Liu, & Ji, 2006; Sun et al., 2012; Sun et al., 2013; Wang, Xia, & Ji, 2009; Xia, 2010). We evaluate the hypotheses using multiple reproductive traits, including postpartum body mass, clutch size (the number of eggs produced per reproductive episode), egg size (clutch mean mass), clutch mass (the total mass of eggs produced per episode), clutch frequency (the number of clutches produced per year), annual fecundity (the number of eggs produced per year), annual mean egg mass, and annual reproductive output (the mass of eggs produced per year). Our goals were to find the reproductive traits that are susceptible to ageing, evidence for the likely hypotheses underlying age-related reproduction and the parsimonious explanation of age-related reproductive investment in *E. argus*.

2 | METHODS

2.1 | Study species

The Mongolian racerunner (*Eremias argus*) is a small (up to 70 mm snout-vent length [SVL]) oviparous lacertid lizard that is widely distributed in North-Northeast China (southwards to Jiangsu and westwards to Qinghai), Russia (region of Lake Baikal), Mongolia, and Korea (Zhao, 1999). The lizard has a relatively homogeneous genetic structure across its range in China (Qu, Zhao, Lu, & Ji, 2014; Zhao, Liu, Luo, & Ji, 2011) but shows substantial geographic variation in morphological, physiological, and life-history traits (Sun et al., 2012; Sun et al., 2013; Wang et al., 2011; Xia, 2010; Zhao, Wang, Liu, Zhao, & Ji, 2008). Energetic demands increase dramatically as body temperature increases, but food intake and food assimilation do not vary over a relatively wide range of body temperatures (Li, Wang, Mei, & Ji, 2009; Luo, Qu, & Ji, 2006). Eggs can be successfully incubated within the temperature range of 24–33°C, and daily exposure of eggs to temperatures outside this range for brief periods of time does not have adverse effects on hatching success and hatchling phenotype (Hao et al., 2006). Mongolian racerunners are easy to breed in captivity and have a short life span with evidence of age-related deterioration in survival and reproduction (Xia, 2010). In Handan, Hebei Province, North China where lizards in this study were collected, hibernation begins in late October and ends in mid-March, mating takes place soon after mid-March, egg-laying begins in late April and ends in early August, females become sexually mature at about 12-months old, females older than 3 years (often larger than 60 mm SVL) only account for 7.5% of the population, and prime-aged females (2 or 3-years old, with SVLs ranging from 55–63 mm) are more fecund than younger or older females (Wang et al., 2011; Xia, 2010).

2.2 | Animal collection and care

We collected 80 adults (40 females and 40 males) larger than 48 mm SVL in early April 2008 from a previously studied population in Handan (36°36'N, 114°28'E, ~70 m above sea level [Wang et al., 2011]). Lizards were brought to our laboratory in Hangzhou, where between 8 and 10 individuals, 4–5 of each sex, were housed in each 900 × 650 × 600 mm (length×width×height) communal cage with moist soil (50 mm depth) and pieces of clay tile served as shelters. Mealworm larvae (*Tenebrio molitor*) and house crickets (*Achetus domestica*) dusted with multivitamins and minerals and water were provided daily, so that excess food and water was always available in the cages. Thermoregulatory opportunities were provided during the daytime hours by a 100 W full-spectrum lamp, and overnight temperatures followed indoor temperatures varying from (22–28°C). We palpated gravid females every 3 days. Females with shelled oviductal eggs were housed individually in 200 × 150 × 200 mm egg-laying cages with moist soil (40 mm depth) and a 20 W heating lamp mounted in each cage to allow thermoregulation. Females never remained in egg-laying cages for longer than 5 days and often shorter than 3 days.

Eggs were collected and weighed within 3 hr after laying, thereby minimizing water exchange between the egg and the substrate (Hao et al., 2006). Egg-laying date, body mass, and SVL were recorded for each postpartum female. Females were then returned to the communal cages where they remained until they again carried shelled oviductal eggs, at that time they were once again moved to egg-laying cages.

We initiated lizard hibernation in late October 2008 by moving the communal cages to a 4 × 3 × 2.2 m artificial atmospheric phenomena simulator room inside which temperature, humidity, and light were automatically adjusted according to real-time weather reports released by Handan Meteorological Bureau. We ended lizard hibernation in mid-March 2009 by returning the communal cages to the room mentioned above. We allowed females to produce as many clutches as they could under the conditions described above for females collected in early April 2008.

2.3 | Data analysis

We excluded from analyses 14 females that laid abnormal eggs (four in 2008 and two in 2009) with condensed yolk at least once or died (two in 2008 and six in 2009) during the course of the experiment. Because of maternal effects, it is not valid to treat eggs of the same clutch as independent for the purposes of statistical analysis; thus, our statistical analyses were based upon mean values for egg sizes per clutch (hereafter egg mass). Clutch mass was calculated as the total mass of eggs in a clutch. Before parametric analyses, data were tested for normality using the Kolmogorov–Smirnov test, and for homogeneity of variances using Bartlett's test. We used linear regression analysis to see if an examined variable was related to female SVL. We used one-way analysis of variance (ANOVA) to see if female SVL, the timing of egg-laying, and annual mean egg mass differed between 2008 and 2009. We used one-way analysis of covariance (ANCOVA) to see if variables (clutch frequency, annual fecundity, and annual reproductive output) related to female SVL differed between the 2 years. For the same females that laid eggs normally in two consecutive years, we used repeated-measures ANOVA (female SVL and egg mass) or ANCOVA (postoviposition body mass, clutch size, and clutch mass) with female SVL as the covariate to see if the examined variables varied during the breeding season and between years. The homogeneity of slopes was checked before testing for differences in adjusted means. We used partial correlation analyses to examine correlations between the selected pairs of variables while holding other variables constant. All statistical analyses were performed with Statistica 8.0 (Tulsa, OK). Throughout this paper, values are presented as mean ± standard error, and the significance level is set at $p = 0.05$.

3 | RESULTS

Of the 40 adult females collected in 2008, 34 laid eggs normally throughout the breeding season. Of the 27 females that survived over the 2008/2009 winter, 20 laid eggs normally throughout the breeding season in 2009. Females laid up to five clutches in 2008, and up to four clutches in 2009 (Table 1). Females gained linear size (SVL) not only as they got 1 year older but also during the breeding season (Table 2). Larger females generally laid eggs earlier than did smaller ones as revealed by the negative relationship between the timing of egg laying and female SVL (linear regression analysis;

TABLE 1 Descriptive statistics, expressed as mean ± standard error and range, for reproductive traits of female *E. argus* that laid normal clutches throughout the breeding seasons in 2008 and 2009

	2008					2009			
	1st clutch	2nd clutch	3rd clutch	4th clutch	5th clutch	1st clutch	2nd clutch	3rd clutch	4th clutch
N	34	30	25	8	2	20	19	12	4
Snout-vent length (mm)	53.1 ± 0.5 48.8–58.9	54.9 ± 0.4 50.9–60.2	55.6 ± 0.4 52.1–61.2	57.5 ± 0.7 55.4–61.1	59.1 57.0, 61.2	57.1 ± 0.5 52.8–63.2	57.6 ± 0.5 53.0–63.0	57.9 ± 0.7 54.0–63.4	59.7 ± 1.5 56.5–63.7
Postoviposition body mass (g)	3.2 ± 0.1 2.0–4.1	3.4 ± 0.1 2.6–5.1	3.5 ± 0.1 2.8–4.7	3.9 ± 0.2 3.3–4.8	4.6 4.3, 4.8	3.9 ± 0.1 3.0–4.9	3.9 ± 0.1 2.9–5.3	4.0 ± 0.2 3.3–5.3	4.7 ± 0.2 4.3–5.1
Clutch mean egg mass (g)	0.35 ± 0.01 0.24–0.48	0.41 ± 0.01 0.29–0.52	0.40 ± 0.01 0.30–0.55	0.39 ± 0.03 0.29–0.60	0.36 0.26, 0.46	0.36 ± 0.02 0.23–0.53	0.43 ± 0.01 0.34–0.53	0.40 ± 0.02 0.28–0.52	0.42 ± 0.03 0.34–0.49
Clutch size	2.7 ± 0.1 2–5	2.9 ± 0.2 2–5	3.1 ± 0.2 2–5	2.9 ± 0.4 1–4	3.0 2, 4	3.5 ± 0.2 2–6	3.3 ± 0.2 1–4	3.3 ± 0.3 2–5	3.3 ± 0.6 2–5
Clutch mass (g)	0.94 ± 0.04 0.49 –1.84	1.15 ± 0.06 0.62–1.95	1.25 ± 0.08 0.61–2.20	1.06 ± 0.09 0.60–1.30	1.18 0.52, 1.83	1.25 ± 0.12 0.71–3.17	1.39 ± 0.10 0.41–2.08	1.32 ± 0.11 0.75–1.82	1.31 ± 0.15 0.98–1.69

$p < 0.001$ in both years). Females laid the first clutch an average of 10 days earlier in 2009 than in 2008, and the difference was significant (Table 3).

In both years postpartum body mass, clutch size, clutch mass, clutch frequency, annual fecundity, and annual reproductive output were positively related to female SVL (linear regression analysis; all $p < 0.003$), whereas egg size (both clutch mean egg mass and annual mean egg mass) was independent of female SVL (linear regression analysis; all $p > 0.549$). Females switched from laying smaller eggs in the first clutch to larger eggs in the subsequent clutches but kept clutch size and postpartum body mass unchanged between successive clutches in a breeding season and between the 2 years (Table 2). Neither clutch mean egg mass nor SVL-adjusted clutch mass differed between the 2 years (Table 2, Figure 1).

Annual mean egg mass did not differ between the 2 years, whereas clutch frequency, annual fecundity, and annual reproductive output were greater in 2008 than in 2009 after accounting for female SVL (Table 3, Figure 2). More specifically, the clutch frequency was reduced by 1.1 clutches, annual fecundity by 3.1 eggs and annual reproductive output by 1.0 g in 2009 compared with 2008. Controlling for SVL, we found that females that laid more clutches (partial correlation analysis; $r = -0.06$, $t = 0.26$, $df = 17$, $p = 0.797$) or eggs ($r = -0.08$, $t = 0.36$, $df = 17$, $p = 0.680$) in 2008 did not lay fewer clutches or eggs in 2009.

4 | DISCUSSION

As in other ectotherms with indeterminate growth, age, and SVL are highly correlated in lacertid lizards (Guarino et al., 2015; Jesus, 2012; Ljubisavllević, Polović, Kolarov, Džukić, & Kalezić, 2007; Olsson & Shine, 1996; Roitberg & Smirina, 2006) including *E. argus* (Kim, Song, Lee, & Park, 2010). Here, we did not assess adult age by skeletochronology but used body size (SVL) as a proxy of age. Females of *E. argus* from the Handan population mature at body sizes of 45–49 mm SVL, attained at age 10–12 months in their second activity season (Xia, 2010). Inconsistent with a study on *E. argus* from South Korea where females were estimated to live up to 11 years (Kim et al., 2010), most, if not all, of the females do not live up to 5 years in the Handan population (Xia, 2010). Body sizes in a sample of 93 adult females of *E. argus* randomly

TABLE 2 Results of repeated-measures ANOVAs (for female SVL and egg mass) or ANCOVAs (for postoviposition body mass, clutch size, and clutch mass) with female SVL as the covariate, clutch order as the within-subject factor and year as the between-subject factor

	Clutch order	Year	Interaction
Snout-vent length	$F_{1,47} = 80.78$, $p < 0.0001$	$F_{1,47} = 20.79$, $p < 0.0001$	$F_{1,47} = 10.03$, $p < 0.003$
	F < L	2008 < 2009	
Postoviposition body mass	$F_{1,47} = 0.72$, $p = 0.402$	$F_{1,46} = 1.28$, $p = 0.264$	$F_{1,47} = 4.25$, $p = 0.45$
Egg mass	$F_{1,47} = 27.45$, $p < 0.0001$	$F_{1,47} = 1.61$, $p = 0.211$	$F_{1,47} = 0.41$, $p = 0.525$
	F < S		
Clutch size	$F_{1,47} = 0.02$, $p = 0.889$	$F_{1,46} = 0.78$, $p = 0.381$	$F_{1,47} = 1.74$, $p = 0.193$
Clutch mass	$F_{1,47} = 6.98$, $p = 0.011$	$F_{1,46} = 1.57$, $p = 0.216$	$F_{1,47} = 0.77$, $p = 0.385$
	F < S		

Abbreviations: ANOVA: analysis of variance; ANCOVA: analysis of covariance; F: the first clutch; L: the last clutch; and S: subsequent clutches; SVL: snout-vent length.

TABLE 3 Descriptive statistics, expressed as mean ± standard error and range, for snout-vent length, timing of oviposition, clutch frequency, annual fecundity, annual mean egg mass, and annual reproductive output of female *E. argus* that completed egg-laying cycles in 2008 and 2009

	2008	2009	Results of one-way ANOVA or ANCOVA
N	34	20	
Snout-vent length (mm)	54.0 ± 0.5 49.5–60.7	57.2 ± 0.5 52.8–63.3	$F_{1,52} = 19.54$, $p < 0.0001$ 2008 < 2009
[a]Timing of oviposition (day number starting from 20 April)	30.2 ± 1.5 16–53	19.9 ± 1.8 9–40	$F_{1,52} = 6.41$, $p = 0.014$ 2008 > 2009
Clutch frequency	2.9 ± 0.2 1–5	2.8 ± 0.2 1–4	$F_{1,51} = 24.84$, $p < 0.0001$ 2008 > 2009
Annual fecundity (eggs)	8.4 ± 0.7 2–19	9.2 ± 0.9 3–18	$F_{1,51} = 17.49$, $p < 0.0002$ 2008 > 2009
Annual mean egg mass (g)	0.38 ± 0.01 0.32–0.44	0.40 ± 0.01 0.31–0.50	$F_{1,52} = 2.19$, $P = 0.145$
Annual reproductive output (g)	3.19 ± 0.25 0.66–6.99	3.69 ± 0.37 1.32–7.75	$F_{1,51} = 11.77$, $p < 0.002$ 2008 > 2009

Abbreviations: ANOVA: analysis of variance; ANCOVA: analysis of covariance.

[a]Timing of oviposition was negatively related to female SVL, with large females generally laying eggs earlier than did small females ($r = -0.54$, $F_{1,52} = 21.37$, $p < 0.0001$). We, therefore, used residuals from the regression of day number starting from 20 April against female SVL to test the difference in timing of oviposition between the two years.

collected from the Handan population ranged from 48–66 mm SVL (Xia, 2010). Accordingly, most, if not all, of the females collected in April 2008 were at young (1-year old) or prime-aged stages (mostly 2-year old), because their SVLs ranged from 49–59 mm (Table 1). Data from this study where females completed reproductive cycles in two consecutive years under the identical laboratory conditions showed that: (a) reproductive females gained linear size (SVL) but kept SVL-specific postpartum body mass unchanged not only along with the growth of age but also between successive clutches in a breeding season; (b) the mean mass, total mass (clutch mass), and number (clutch size) of eggs produced in single reproductive episodes and their patterns of seasonal shifts did not vary with advancing age of the mother; and (c) larger/older females started to lay eggs earlier but reproduced less frequently than did smaller/younger females after accounting for SVL. Based on these findings, we can draw the following conclusions regarding age-related reproduction in *E. argus*.

Firstly, *E. argus* does not display evidence of senescence in postpartum body condition, as revealed by the fact that SVL-specific postpartum body mass (investment in body mass during reproduction) did not differ between females reproducing in 2008 and 2009. The extent of age changes in postpartum body condition of reptiles is virtually undocumented. A constant postpartum body condition and increased SVL during the breeding season we observed in this study provide an inference that, as in other animal taxa that confront the conflicting demands of maintenance, body growth, and reproduction (Congdon, Dunham, & Tinkle, 1982; Lucas, 1996; McNab, 2002; Sibly et al., 2013; Zhang, Guo, Zhang, Lin, & Ji, 2018), somatic maintenance has a higher priority over reproduction in energy allocation in *E. argus*. Such a hierarchical pattern of energy allocation does not vary with age in *E. argus* (Table 2). The hierarchical allocation of energy to growth and reproduction has been described in three species of lizards, the Madagascar ground gecko *Paroedura picta* (Kubička & Kratochvíl, 2009), the northern grass lizard *Takydromus septentrionalis* (Luo, Ding, & Ji, 2010), and the white-striped grass lizard *Takydromus wolteri* (Ma et al., 2019). Reproductive females of these three species, as what we observed in *E. argus* (Table 2), gain linear size and keep postpartum body condition almost constant during the breeding season because they do not store energy but divert their current surplus energy to the production of eggs (Kubička & Kratochvíl, 2009; Luo et al., 2010; Ma et al., 2019). The age-independency of postpartum body condition highlights the importance of maternal body condition in initiating vitellogenesis of ovarian follicles and suggests that the threshold of body condition needed to initiate reproduction in females is fairly constant in *E. argus*. Evidence has been presented showing that female lizards with poor body condition tend to produce fewer and/or smaller offspring or even not to reproduce (Ballinger, 1983; Ji, Du, Lin, & Luo, 2007; Lu, Lin, Li, & Ji, 2014; Luo et al., 2010; Olsson & Shine, 1997). The age-related decline in the growth rate is the most widely held and firmly documented ageing phenomenon in reptiles (Patnaik, 1994). This appears to be true for *E. argus* because the significant clutch order × year interaction as a source of variation in female SVL provides an inference that young females grew during the breeding season at a faster rate than old females.

Secondly, egg size (both clutch mean mass and annual mean mass), clutch size, and clutch mass are also not susceptible to ageing in *E. argus*. Egg size did not vary with female SVL or age (Table 2), signifying that none of the aforementioned three hypotheses applies to the trait. Producing smaller eggs in the first clutch and larger eggs in the subsequent clutches could be a life-history strategy adopted by female *E. argus* to maximize their fitness. Dividing accessible resources into smaller or optimal amounts for individual eggs in the first clutch allows female lizards to increase the number of eggs produced per clutch or per year by channeling current surplus energy into the next clutch (Ji et al., 2007; Luo et al., 2010; Ma et al., 2019), and this is also true for *E. argus* (Wang et al., 2011). There has been evidence that female *E. argus* from the Handan population tends to divert an optimal, rather than a higher-than-usual, fraction of the available resources to individual eggs in any given clutch, thereby maximizing the number of eggs produced per year (Wang et al., 2011). Larger individuals are generally older than smaller conspecifics in taxa with indeterminate growth (Tinkle & Ballinger, 1972), and this is also true for *E. argus* (Xia, 2010). Here, we found that clutch size and clutch mass were positively related to female SVL but

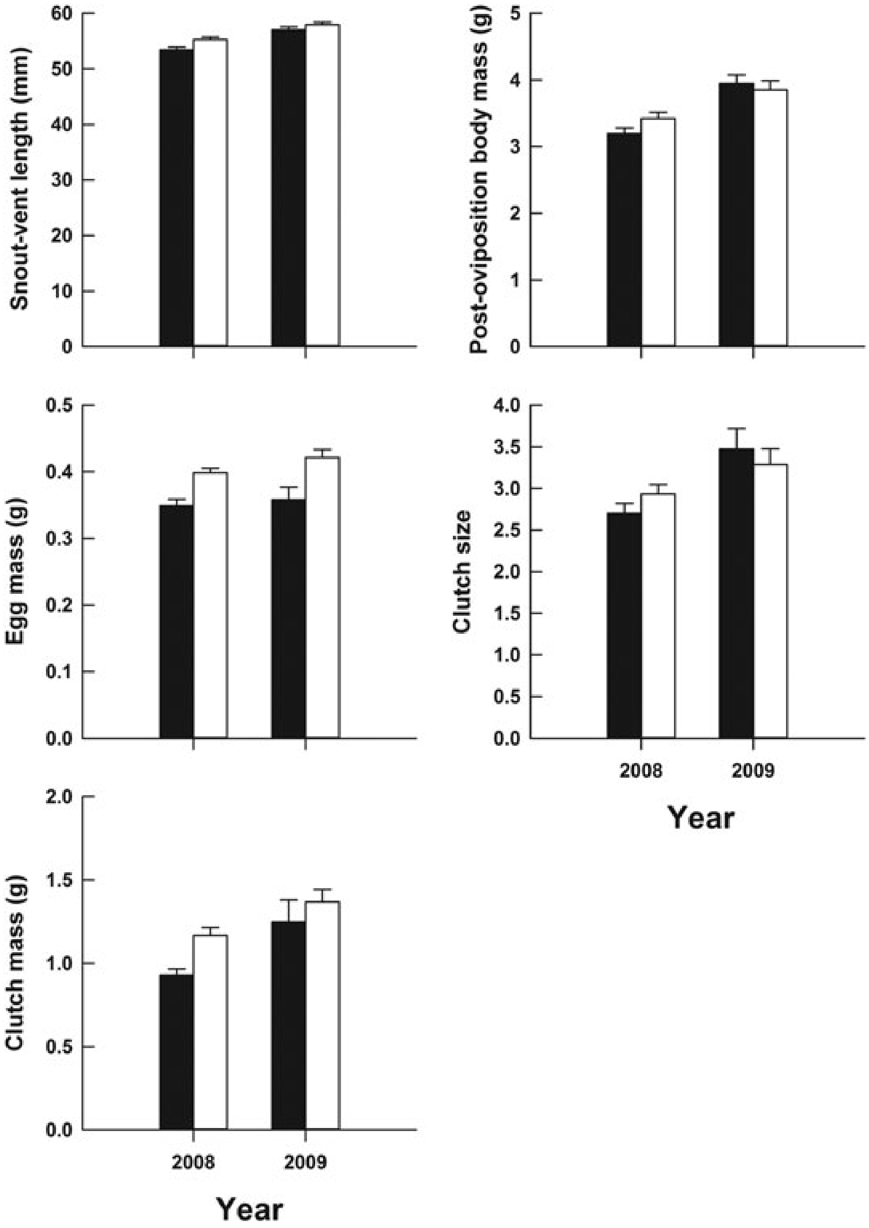

FIGURE 1 Mean values (+SE) for snout-vent length, postoviposition body mass, egg mass, clutch size, and clutch mass. Solid bars represent the first clutch and open bars represent the subsequent clutches. SE: standard error

both traits did not vary with advancing age of the mother after accounting for female SVL (Table 2). From these findings, we conclude that the reproductive maturation hypothesis applies not only to clutch size but also to clutch mass in *E. argus*. The explanation for why clutch size and clutch mass were greater in larger/older females at least partly lies in that, as in other lizards (Bleu, Massot, Haussy, & Meylan, 2012; Du, 2006; Finkel & Holbrook, 2000; Goodman, Hudson, Isaac, & Schwarzkopf, 2009; Olsson, Shine, Wapstra, Ujvari, & Madsen, 2002; Shine, 1992; Vitt & Congdon, 1978), the amount of abdominal space available to hold the clutch and/or the ability to acquire energy for reproduction is greater in larger/older females of *E. argus* (Luo et al., 2010; Sun et al., 2012; Sun et al., 2013).

Thirdly, clutch frequency, annual fecundity, and annual reproductive output are susceptible to ageing in *E. argus*. The reproductive maturation hypothesis generally applies to these three traits because in both years they were greater in larger/older females. However, despite the greater mean SVL, females in 2009 reproduced less frequently and thus invested less in reproduction during the breeding season than did females of the same SVL in 2008 (Table 2, Table 3). This suggests that the senescence hypothesis also applies to clutch frequency. As the size and number of eggs produced in any given clutch and annual mean egg mass did not vary with age (Table 2, Table 3), the reduced annual fecundity and annual reproductive output in older females primarily resulted from the reduced clutch frequency. Thus, the senescence in annual fecundity was dependent on the senescence in clutch frequency, and so was the senescence in annual reproductive output. Clutch frequency is much more variable than egg size and clutch size in multiple-clutched lizards. In *T. septentrionalis*, for example, manipulation of temperature or food availability in

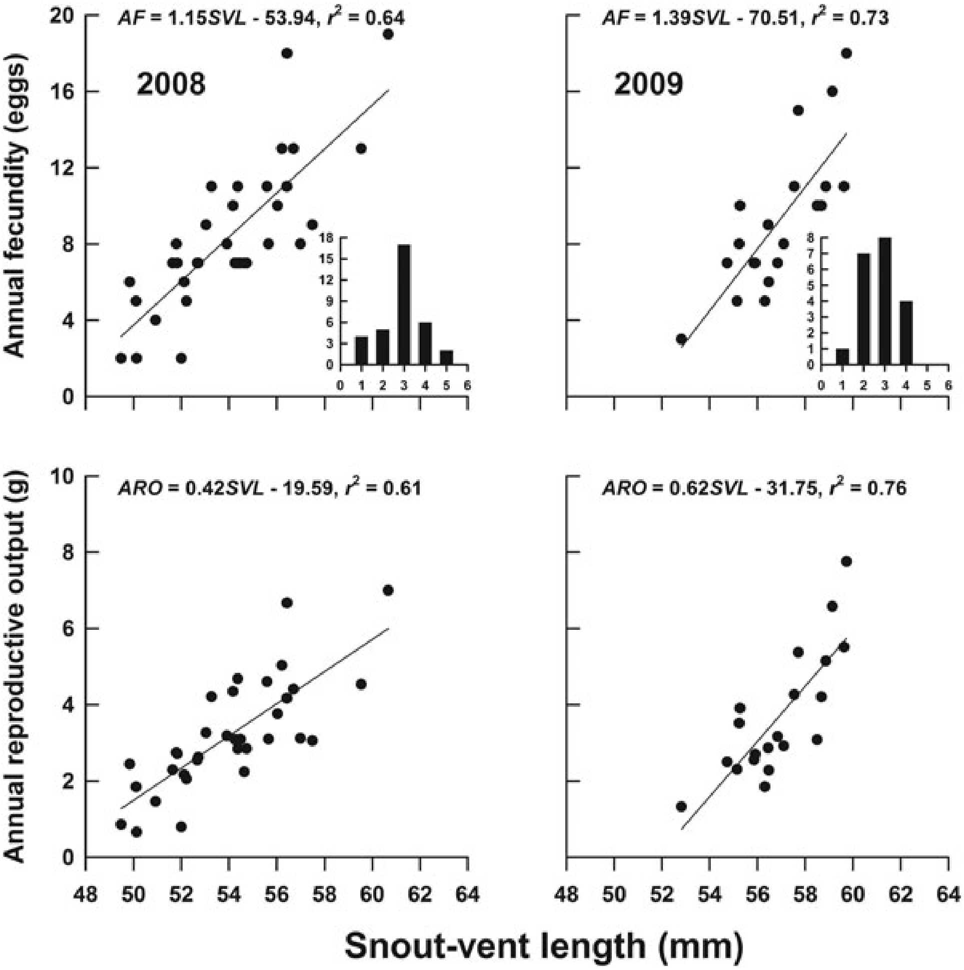

FIGURE 2 Regressions of annual fecundity (the total number of eggs produced per breeding season) and annual reproductive output (the total mass of eggs produced per breeding season) against female snout-vent length. Regression equations and coefficients are given in the figure. Two smaller plots show frequency distributions (vertical axis) of clutch size (horizontal axis)

reproducing females influences clutch frequency but not the size and number of eggs produced in single reproductive episodes (Du, 2006; Luo et al., 2010). One possible explanation for the reduced clutch frequency in older females could involve the increased maintenance costs in senescent individuals that have increased energy demands of cell and tissue repair (Finkel & Holbrook, 2000). Reptiles with high maintenance costs are less likely to invest more energy in reproduction (Zhang et al., 2018).

5 | CONCLUSIONS

Of the traits examined in this study, only clutch frequency, annual fecundity, and annual reproductive output were susceptible to ageing in *E. argus*. Young females were constrained or restrained in their reproductive investment, producing fewer eggs per reproductive episode and per year while growing during the breeding season at a faster rate than old females. Old females showed reproductive senescence, as indicated by the fact that they reproduced less frequently and invested less in reproduction per year than did young or prime-aged females after accounting for SVL. Our results provide evidence that the reproductive maturation hypothesis better explains patterns of reproduction in young or prime-aged female *E. argus*, and the senescence hypothesis better explains reproductive patterns in old females. The terminal investment hypothesis does not apply to any trait examined because no trait value was maximized in old females. Our study is the first to demonstrate that a reproductive trait susceptible to ageing may correspond to different hypotheses regarding reproductive senescence in short-lived reptiles. In *E. argus*, for example, increases in clutch frequency with age early in adulthood corresponds to a pattern of reproductive maturation and the relatively decreased clutch frequency late in life corresponds to the senescence hypothesis.

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