Variation in Reproductive Strategies of Three Populations of *Phrynocephalus helioscopus* in China

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Abstract

Background. Egg size and clutch size are the key life history traits. It is possible during the breeding period to increase the fitness of the offspring either by increasing the number of eggs if the optimal egg size (OES) is maintained, or by increasing the allocation of energy to each egg. However, the strategies adopted ~~by animals~~ are ~~deeply~~ often influenced by ~~their~~ morphology and~~,~~ environment~~, or both~~.

Methods. In this paper, we studied variation ~~of~~in female morphological ~~traits,~~and reproductive traits, ~~the~~test fior an egg size-~~number~~clutch size trade-off~~,~~ and the relationship between egg size and female morphology in three populations of *Phrynocephalus helioscopus*.

Results. ~~In both the Yi Ning and Fu Yun populations, f~~Female body size, egg size, and clutch size were larger in the Yi Ning and Fu Yun populations than ~~that of~~ the Bei Tun population (~~, so the~~ reproductive output ~~of~~in the Bei Tun females was ~~the~~ smallest, and ~~both~~ the Fu Yun and Yi Ning populations ~~had~~laid more, ~~and~~ rounder eggs). Egg size was not constrained by female body size in the Beitun and Fuyun populations, but ~~there were~~ egg size-~~number~~clutch size trade-offs occured in both populations. Egg size-~~number~~clutch size trade-offs were not ~~present~~found in the Yining population, but egg size was correlated ~~to~~with female body size,

Future reproductive value? This is not necessarily true – that is, it is NOT increasing the fitness of the offspring, but rather increasing fitness of the female laying the eggs!
Unknown Author
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Here we examine
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consistent with the hypothesis of morphological constraint.

Conclusions. Our study found geographic variation in body size and reproductive strategies of the lizard *Phrynocephalus helioscopus*. Egg size was correlated with morphology in the larger-bodied females of the YN population but not in the small-bodied females of the BT population, ~~suggesting~~illustrating that constraints on female body size~~-specific constraints on~~ and egg size ~~in smaller-bodied females does~~do not always occur.

1. Background

Animals often exhibit variation in reproductive traits as a result of differences in the quality of resources and food availability of different habitats (Roff, 2002; Cruz-Elizalde & Ramırez-Bautista, 2016). Egg size and number are the key life history traits, and have received more attention than other reproductive traits (Qu *et al*., 2011; Amat 2008; Lovich *et al*., 2012). When food is less availab~~ility is poor~~le, females may face the problem of having ~~to allocate insufficient~~limited reproductive resources to invest in eggs, which results in a trade-off between 1) the energy allocated to each egg (egg size), and 2) the total number of eggs (clutch size, CS). An increase in resources allocat~~ed~~ion to each egg will ~~come at the cost of~~result in decreasing CS (Roff, 1992; Kaplan & Phillips, 2006). ~~In addition, a~~This negative relationship between egg size and clutch size provides evidence of reproductive trade-offs (Rowe 1992), and variations in reproductive output from different populations sometimes are correlated with varying relationships between egg size and ~~number~~clutch size (Thompson & Pianka, 2001).~~-~~

Variation in female reproductive output is ~~ar~~e widespread, ~~and exist not only~~ both interspecifically ~~but also~~and intraspecifically. Especially for ~~some ubiquitous~~widespread

A consequence of this trade-off.

Unknown Author
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You're being redundant here, basically repeating the idea of egg size – clutch size tradeoff without adding information.

Unknown Author
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species, local genetic variation~~ brought about by environmental factors~~, short-term phenotypic plasticity, and the complex interactions between these two~~, all~~ contribute to ~~those ~~variation in reproductive output (Brown & shine, 2007).

This paragraph only has two sentences, and could be joined to the following or the previous.

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Optimal egg size (OES) theory predicts that natural selection optimizes egg size within populations, ~~thus~~such that when ~~sufficient ~~resources are available (not limiting) for reproduction, clutch size or number of clutches may increase, ~~females may have larger CS (or more clutches)~~ rather than an increase in ~~larger ~~egg size (Smith & Fretwell, 1974; Brockelman, 1975). Natural selection predicts that females should optimize resources allocated to each egg, and CS should only increase ~~CS ~~after ensuring the production of high-quality offspring~~ fitness~~ (Lovich *et al*., 2012). In some reptiles, CS is positively correlated with ~~the maternal~~female morphological traits, ~~but~~while egg size is not, consistent with OES theory (Congdon & Gibbons, 1987). However, the relationship between egg size and number is determined by numerous factors, and the trade-offs between egg size and number are not always evident in natural populations (Berven, 1982; Liao & Lu, 2011; Wang *et al*., 2011).

In some reptiles~~, especially in some turtles,~~ egg size is corelated ~~to~~with female~~ maternal~~ body size (morphological constraint hypothesis), and both egg size and number increase with an increase in ~~maternal~~female body size, contrary to OES theory (Dunham & Miles, 1985; Clark, Ewert & Nelson, 2001; Mohamed *et al*., 2012; Ryan & Lindeman, 2007).

~~This type of c~~Constraints between female~~ maternal~~ morphological traits and egg size (e.g., egg width being constrained by the pelvic aperture width in some turtles and lizards) results in lack of fit with ~~some species not conforming to~~ predictions ~~of~~from OES theory. ~~Especially~~ ~~w~~When resources are limited, reproductive output is directly correlated with the trade-offs between egg size and ~~number~~clutch size, and ultimately with the ~~future~~offspring survival ~~of the population as well~~ (Brown & Shine, 2009; Congdon & Tinkle, 1982). The size of each egg normally determines the success of incubation and ~~the~~ offspring~~'s~~ survival (Angilletta *et al*., 2004; Räsänen, Laurila & Merilä, 2005). Females may allocate more energy to individual eggs, aiming for ~~higher fitness~~greater survival of their offspring.

Not necessarily, because eggs can still be smaller when resources are limiting.

Unknown Author
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Phrynocephalus helioscopus is a small (mean SVL 47.55mm) lizard that is widely distributed in Eurasia. Previous research on this species has focused on egg incubation (Wang *et al*., 2013) and female reproductive output (Liang *et al*., 2015). However, among the distinct populations of this widely distributed species, neither variation in the female reproductive traits and the egg size-number trade-off, nor the effects of ~~maternal~~female morphological traits on egg size have been studied. In this study, we compared ~~maternal~~female morphological traits and the relationships among their~~,~~ egg length (EL), egg width (EW), egg mass (EM), egg shape (ES) and clutch size (CS) ~~in~~among three populations. Specifically, ~~and examined the relationship of female morphological traits on egg size and CS with an aim toward~~we:

1. Test~~ed~~~~ing~~ whether reproductive female size differs among the three populations,
2. ~~When sizes vary between populations, to test~~Examined how that variation is associated with reproductive traits, especially in fecundity, egg and clutch size, egg shape, and the egg size-~~number~~clutch size trade-off~~s~~;

3. ~~To test whether variations exist in the~~examined the relationship~~s~~ of female traits to egg and clutch size ~~and egg number~~ in and among populations.

2 and 3 look essentially the same.
Unknown Author
06/21/2018 14:29

2 Materials and Methods

2.1 Study site

The populations studied here ~~occur at~~are in three ecologically distinct locations~~lities~~: Bei Tun city (BT: 87˚15" E, 47˚26' N), Fu Yun city (FY: 89˚05' E, 46˚36' N), and Yi Ning city (YN: 80˚47' E, 43˚40' N) of the Xinjiang Uyghur Autonomous Region, China. The distance between the BT and the YN populations is about 660 km and their habitats are different~~, and the geographic variation in their habitat is great,~~. The BT population ~~occupie~~is in a typical gravel desert with little vegetation, while the YN population ~~occupies~~is in ~~a~~ loam desert with abundant vegetation~~, especially shrubs~~. ~~Geographic variation in their climates also exist.~~ YN is hotter and wetter ~~has a higher mean air temperature and more precipitation compared to~~than BT. ~~The distance between the~~ FY and BT are separated by ~~populations is shorter (~~about 160 km~~)~~ ~~than that between~~and FY and YN by ~~(~~about 700 km~~)~~. ~~However, habitat and precipitation in~~ FY is similar to YN in vegetation and rainfall~~are similar to those in YN, while the mean air temperature of FY is similar to Bt~~while FY and BT have similar temperature regimes (Fig.1 and Fig.2).

2.2 Animal and egg collection

From May 2014 to May 2017, we collected ~~specimens of~~ *P. helioscopus* by hand from the outskirts of BT (in 2014, Liang *et al*., 2015), FY(in 2017), and YN (in 2017) and took them ~~. We transported the lizards~~ to ~~the~~ Xinjiang Agricultural University, where ~~the~~ female lizards were ~~individually~~ palpated to ~~assess~~determine their reproductive state (Li *et al*., 2006). Fifty-three gravid females (BT: 13, FY: 24, YN: 16) were housed individually in plastic cages~~. These cages were placed~~ in a room with~~here~~ ambient temperatures ~~were~~ never ~~higher than~~above 28℃ ~~and the room lights were programmed to create~~with a 12-hour light /12-hour dark cycle. A 250 W light bulb was suspended at one end of each cage, 20 cm above the cage floor and lizards could freely move to warmer and cooler places within the cage. Mealworms (larvae of *Tenebrio molitor*) and water enriched with vitamins and minerals were provided *ad libitum*. Female in cages ~~will continuously~~ dig before they lay eggs, which allowed us to ~~. This behaviour helped us~~ collect eggs quickly, ~~and prevented the~~ and prevented eggs from absorbing water in the moist substrate. The cages were checked every 2 hours for eggs. All eggs are used in this study were collected no more than 20 minutes after they had been laid.

2.3 Morphology and Reproductive Traits

We measured female snout-vent length (SVL), ~~body mass (BM, female post-oviposition mass, 0.01 g)~~, tail base width (TBW), egg length (EL), and egg width (EW) by using digital calipers (measured to the nearest 0.01 mm). We also ~~recorded~~noted clutch size (CS). We weighed females after oviposition, eggs ~~mass~~ (EM) and clutches ~~mass~~ (CM) ~~by~~ on an electronic balance ~~(measured~~ to the nearest 0.01g~~)~~. We calculated~~used~~ relative clutch mass (RCM, RCM = CM / BM) as a ~~proxy~~measure of ~~for~~ female fecundity (Shine, 1992). The ratio of egg length ~~/~~to egg width (EL / EW) ~~value represents~~indicates the general shape of the eggs (egg shape, ES), where 1 is a round egg, and larger values are increasingly elongate~~—a larger value means the egg has an elongated shape, and a smaller value indicates a rounder shape~~ (Ji & Wang, 2005; Kratochvíl & Frynta, 2006).

Minimum temperature?

And, how did you randomly collect animals? Is it possible that behavior of lizards influenced your probablity to get females of similar sizes/ages in all three locations?

Unknown Author
06/21/2018 14:36

What was the substrate?

Unknown Author
06/21/2018 14:37

You did not measure body mass with calipers…. You should put things measured together and things weighed together, but not mix the two.

Unknown Author
06/21/2018 14:40

Why? After all, you are going to compare all the principle variables. Calculating a ratio is unnecessary as a method.

Unknown Author
06/21/2018 14:43

2.3 Statistical analyses

Data were tested for normality using the Kolmogorov-Smirnov test, and for homogeneity of variance using Bartlett's test. The parametric tests will be applied when normality (and homogeneity of variance) assumptions are satisfied otherwise the equivalent non-parametric test will be used. For this reason, the Kruskal-Wallis test was used in conjunction with the *wmc* function (http://-www.statmenthods.net/RiA/wmc.txt), as multiple comparisons were necessary when examining variations in SVL, EM, and ES among the three populations. ANCOVA was used to examine variation in TBW, EL, EW, RCM, and CS among the three populations by *post hoc* Tukey's tests (multiple comparisons). To test egg size-number trade-off and analyze potential morphological constraint on optimal egg size, the relationships of EM and EL with CS, of EM with EL, of EL and CS with SVL, and of EW with TBW were examined using RMA (Reduced Major Axis regression) regression rather than OLS (Ordinary least squares) regression, because RMA accounts for an error in the independent variable (Dunham & Miles, 1985). ~~Data~~Variables (except CS) were $\log_{10}$-transformed to improve linearity and enable comparison with other studies (King, 2000), ~~except in CS, because it does not vary logarithmically~~. Historical climatic data (1990-2013) of the three study areas was taken from the Chinese National Climatic Data Center (http://data.cma.cn). Descriptive statistics were represented as follows: mean adjusted (calculate by the effect function of effect package) ± SE, except in SVL, EM, and ES, which are represented as the mean ± SE. Differences were considered significant when $P < 0.05$.

All analyses were conducted using ~~the software~~ R v.3.4.1 (R Core Team 2017),

"Data" do not need to be tested for normality. In regressions and ANOVA, the residuals need to be tested. It is likely that you did not need to use a nonparametric test, but rather you could have log10 transformed your measurements – which is the normal practice.

Unknown Author
06/21/2018 14:45

Is this spelled correctly? Is this a working link? I could not access it. Also, you should probably use Tukey for multiple comparisons.

Unknown Author
06/21/2018 14:47

You're not "examining" variation, but rather testing how each population differs.

Unknown Author
06/21/2018 14:52

Again, you're not examining variation.

Unknown Author
06/21/2018 14:53

employing the packages "lmodel2" (Legendre, 2011), "effects" (Fox & Hong 2009), "ggplot2" (Wickham 2015), "gplots" (Warnes *et al*., 2011).

Figure 1. Map, showing the three locations where lizards were captured for this study in the Xinjiang Uyghur Autonomous Region of western China. Closest Cities (BT, FY, and YN) are identified by the red dots, and the collecting locations are indicated by the black dots with arrows. Photos indicate habitat types in each sampling location (Photo credit: Tao Liang).

Figure 2 Means for monthly mean air temperature (A) and monthly mean rainfall (B) over the past 24 years (1990-2013) at the three localities, where females of *P. helioscopus* were collected. BT: pink; FY: green; YN: light blue.

3 Results

3.1 ~~Maternal~~female morphological variation

SVL varied between populations and was longest in the similar YN and FN populations (YN: 51.23 mm; FY: 50.43 mm), and shortest in the BT population

(χ^2 = 25.05, P < 0.0001). ANCOVA with SVL as a covariate revealed that TBW varied between populations and was smallest in the similar YN and FN populations (YN: 7.20 mm; BT: 6.93 mm), and largest in the FY population ($F_{2,52}$ = 6.82, P = 0.002) (Fig. 3).

Figure 3. Comparisons between A) snout-vent length and B) tail width at base, of gravid females in three populations of *Phrynocephalus helioscopus*. Points are means with 95% confidence intervals. Different lowercase letters means significant at the 0.05 level; different uppercase letters mean significant at the 0.01 level.

3.2 Female Reproductive Traits

~~EM differed significantly among the three populations, with~~Females in the FY ~~females~~population la~~idying~~id ~~significantly~~ heavier eggs than ~~both~~ those in the BT and YN populations. Eggs were similar in length in all ~~from the~~ three populations ~~did not differ from each other in EL~~. ~~EW varied between populations and was the widest~~Eggs were wider (rounder, EW) in ~~the~~ FN ~~population~~ and ~~the~~ narrower~~st~~ in ~~the~~ YN ~~population~~. ~~CS varied among the three populations, with~~ BT females la~~idying fewer eggs~~id smaller clutches than ~~the~~ FY and YN females ~~with the same~~when controlling for SVL. ~~There were significant differences in RCM among the three populations, with t~~The FY population had~~ving a~~ a larger RCM ratio than ~~the~~ BT ~~population~~. ~~There were significant differences in ES among the three populations, with t~~The BT ~~population having a longer~~had longer eggs, ~~ES than the~~ than FY ~~population~~ (Table1).

I don't understand why you inserted tables and figures into the text.
Unknown Author
06/21/2018 15:14

I don't think you need to worry about differences in 4% between the two levels, because they do NOT indicate importance. Rather, you should talk aboutr the r^2 values of each, because they DO indicate how much is being explained by location. I think you can just leave the alpha level at 0.05. Also, you should have lower case letters for figure 2A.
Unknown Author
06/21/2018 15:16

Unnecessary. You cannot say they are different if they are also not significantly different.
Unknown Author
06/21/2018 15:28

But, EW and ES are two ways to measure the same thing. So, if you are going to talk about ES, you need NOT talk about EW once you mention egg length. ES is just a combination of EL and EW! You can't talk about all three as if they were independent.
Unknown Author
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Table 1. Descriptive statistics of female reproductive traits in the~~of~~ three populations of *Phrynocephalus helioscopus*.

Variable	BT (n = 35)	FY (n = 90)	YN (n = 63)	*F*~~-level~~ and *P*~~-value~~
EM (g)†	$0.51±0.02^{B}$	$0.61±0.02^{A}$	$0.55±0.01^{B}$	$\chi^2 = 20.96$, $P < 0.0001$
range	0.32～0.76	0.27～1.02	0.28～0.82	
EL (mm)#	$15.66±0.24^{a}$	$14.39±0.17^{a}$	$14.91±0.16^{a}$	$F_{2,187} = 1.15$, $P = 0.318$
range	12.47～18.51	11.49～19.50	9.94～17.35	
EW (mm)#	$8.41±0.08^{B}$	$8.45±0.06^{A}$	$8.34±0.07^{B}$	$F_{2,187} = 19.42$, $P < 0.0001$
range	7.19～9.03	6.90～9.90	6.39～9.36	
ES	$1.83±0.03^{A}$	$1.73±0.02^{B}$	$1.78±0.02^{AB}$	$\chi^2 = 12.61$, $P = 0.002$
Range	1.44~2.27	1.43~2.18	1.47~2.11	
CS#*	$2.93±0.13^{B}$	$3.69±0.18^{A}$	$3.82±0.14^{A}$	$F_{2,187} = 10.93$, $P = 0.0001$
range	2～4	2～6	3～5	
RCM#*	$0.36±0.04^{B}$	$0.53±0.01^{A}$	$0.49±0.01^{AB}$	$F_{2,187} = 4.40$, $P = 0.018$
range	0.21～0.75	0.32～0.77	0.25～0.65	

Notes：Different lowercase letters means significant at the 0.05 level; different uppercase letters mean significant at the 0.01 level;
† Kruskal-Wallis test;
# One-way analyses of covariance (ANCOVAs) (for CS with SVL as the covariate, for EL and EW with egg mass as the covariate, and for RCM with BM as the covariate);
* BT n=13, FY n=24, YN n=16.

I don't understand why you embedded the tables. You also supplied them as attachments, so that was fine.
Unknown Author 06/21/2018 15:36

Because you have some of these comparisons in the figures, you do NOT need to repeat them in a table.
Unknown Author 06/21/2018 15:37

r^2 values would be useful.
Unknown Author 06/21/2018 14:57

Why chi-squared instead of F? After all, this should be a simple ANOVA.
Unknown Author 06/21/2018 14:56

Unnecessary. You can just use 0.05 and only one kind of letter.
Unknown Author 06/21/2018 15:38

3.3 Egg Size-~~Number~~clutch size Trade-offs

We found a positive relationship ~~I~~in all populations~~, there was a significant, positive relationship~~ between EL and EM. In ~~the~~ BT and FY females ~~population, there was a~~

Of course, because EW was relatively constant! So, the only way egg weight could vary is by differences in length.
Unknown Author 06/21/2018 15:39

~~significant negative relationship between~~ egg size decreased with ~~and number~~clutch size. ~~In the FY population, there was also a significant negative relationship between egg size and number~~, while in ~~. In the~~ YN females, egg size was independent of clutch size~~population, there was no significant relationship between egg size and number~~ (Table 2, Fig. 4). ~~The existence of this negative relationship between egg size and CS provides evidence of egg size-number trade-offs in both the BT and FY populations.~~

Discussion.

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Populations	Variables	Slope (95% CI)	Intercept (95%CI)	R^2	*P*~~-value~~
BT	EM-CS	-0.12 (-0.16~-0.09)	0.86 (0.78~0.98)	0.36	0.001
	EL-CS	1.89 (-2.61~-1.36)	20.40 (18.89~22.50)	0.12	0.039
	EL-EM	0.46 (0.36~0.59)	1.31 (1.28~1.35)	0.45	<0.001
FY	EM-CS	-0.15 (-0.17~-0.12)	1.19 (1.09~1.30)	0.27	<0.001
	EL-CS	-1.88 (-2.24~-1.57)	22.24 (21.04~23.68)	0.3	<0.001
	EL-EM	0.52 (0.47~0.57)	1.28 (1.27~1.29)	0.78	<0.001
YN	EM-CS	0.20 (0.16~0.26)	-0.26 (-0.49~-0.08)	0.004	0.602
	EL-CS	-2.36 (-3.04~-1.83)	24.18 (22.06~26.90)	0.008	0.471
	EL-EM	0.43 (0.37~0.51)	1.28 (1.26~1.30)	0.6	<0.001

Table 2. Relationships between EL and EM and egg size-number trade-offs.

If these were in the figures, you do not need to repeat them here. Also, the legend is in the wrong place. And, the table need not be embedded in the text.

Unknown Author
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Figure 4. Regressions of EL and EM and egg size-number trade-off of *Phrynocephalus helioscopus*. BT -Shaded triangles with dashed line, FY - Asterisk with solid line, YN - Unshaded triangle with dashed line.

If this is the legend, it is very incomplete. I know in R you can get the graphs to "jitter" the points (in the clutch size in A) so that it is easier to see the points. Also, the FY line in A is probably not significant or is in error. Either way, in regression, if a relationship is NOT statistically significant, you should not include a line for relationship. Also, in clutch size, your axis has freactions, and you can make the axis show the clutch size itself, and not the fractions between observed clutch sizes. That is, 2 instead of 1.5, 3 instead of 2.5 and so on. Also, in Figure 5.

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3.4 The Relationship Between Egg Size, Number and Female Morphology

In the BT and YN populations, ~~there were no significant relationships between maternal~~female morphological traits were independent of ~~and either~~ EL, EW~~, or~~ and CS. ~~In~~

the FY population as well, there were no significant relationships between maternal morphological traits and either EL, EW, or CS. In the YN population, while CS was independent of female measurements, there were no significant relationship between maternal traits and CS. A significant but week positive relationship existed between EL was weakly correlated with and SVL and ; EW and TBW were correlatedshowed a significant positive relationship as well (Fig. 5, Table 3).

Table 3 Relationship between egg and clutch size and female morphological traits of the three populations.

Populations	Variables	Slope (95% CI)	Intercept (95%CI)	rR^2	P-value
BT	EL-SVL	3.13 (2.21~4.43)	-4.03 (-6.20~-2.51)	0.0003	0.92
	EW-TBW	0.61 (0.43~0.86)	0.40 (0.20~0.55)	0.01	0.509
	CS-SVL	0.52 (0.28~0.96)	-21.19 (-41.73~-10.14)	0.006	0.787
FY	EL-SVL	2.84 (2.31~3.51)	-3.68 (-4.81~-2.76)	0.001	0.98
	EW-TBW	0.92 (0.75~1.14)	0.11 (-0.07~0.26)	0.03	0.123
	CS-SCL	0.46 (0.30~0.70)	-19.31 (-31.37~-11.40)	0.04	0.342
YN	EL-SVL	1.59 (1.24~2.03)	-1.55 (-2.30~-0.96)	0.04	0.039
	EW-TBW	0.66 (0.52~0.84)	0.35 (0.20~0.47)	0.12	0.004

You should indicate A, B and C when you talk about relationships. However, if the variables are independent, the figures should not have regression lines.

Unknown Author
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Looks redundant because of the figures.

Unknown Author
06/21/2018 15:53

When a relationship is NOT statistically significant, r^2 is meaningless and does not need to be reported.

Unknown Author
06/21/2018 15:54

	0.19			
CS-SVL	(0.11~0.31)	-5.53 (-11.97~-1.70)	~~0.1~~	0.221

Figure 5 Regressions of egg length (A), egg width (B), and clutch size (C) and ~~maternal~~female morphological traits from three populations of *Phrynocephalus helioscopus*. BT -Unshaded triangles with dashed line, FY - Asterisk with solid line, YN - Shaded triangle with dashed line.

Again, incomplete legend (you could include the regression lines) and if the regression lines are not statistically significant, you do not need the figure.

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4 DISCUSSION

~~Data on the reproductive ecology of *P. helioscopus* is relatively scant. Our interpopulation study showed~~We found variation in ~~maternal~~female morphological traits (SVL and TBW), reproductive traits (EM, CS, RCM, and egg size), in the relationship between reproductive characteristics and ~~maternal~~female morphological traits, and in egg size-number trade-offs among the populations of *P. helioscopus*.

The first paragraph of the discussion should emphasize your most important conclusions and should be a paragraph of more than two sentences. Then, you should follow with more context. Also, it is probably not necessary to divide your discussion into subparts.

Unknown Author
06/21/2018 15:57

4.1 Variation in female morphology the three populations

Morphological traits, such as body size and body shape always vary among different populations in animals (e.g. Snakes: Zhong *et al.*, 2017; Lizards: Horváthová *et al.*, 2013; Turtles: Werner *et al.*, 2016). Environmental factors that exert strong effects on animal life history traits include activity season length and food availability (Yom-Tov *et al.*, 2006; Horváthová *et al.*, 2013). Our study revealed that the FY and YN populations have significantly larger SVLs ($P < 0.01$). Longer activity seasons were assumed to be the cause of variation in the body size between the *P. helioscopus* of the YN and BT populations (lizards in the YN population have larger SVL, Liang & Shi, 2017). Temperature, fundamentally important for lizards ~~is a key factor in lizard activity~~ (Grant & Dunham 1990), was ~~Temperature~~higher in YN ~~is higher~~ than the other two sites ~~localities~~, especially in March and

November (Fig. 2-A). ~~According to observations i~~In YN city, *P. helioscopus* activity began ~~to be active~~ in mid-March and hibernation began ~~to hibernate~~ in early November, which means that the activity period for lizards here~~of this population have~~ is almost a ~~one-~~month longer ~~growing season~~ than ~~lizards~~ in the other two sites~~populations~~. It is conceivable that lizards in this population have a larger body size because of the longer growing season than the two populations.

The BT and the FY populations with ~~have~~ similar temperature~~s conditions, which~~ raises the question as to what causes their differenc~~ey variation exists~~ in ~~the~~ SVL ~~of the females of these two populations~~. One plausible explanation is that ~~poor~~ food limitation ~~availability (e.g. insect scarcity)~~ might have ~~resulted in the~~reduced growth rates in ~~smaller-bodied females of~~ the BT population. Rainfall is critical to habitat quality (e.g. vegetation cover and prey abundance, ~~see~~ Lorenzon, Clobert & Massot, 2001). ~~The g~~Geographic variation in rainfall in our study areas is great (Fig. 2) and~~. The~~ sparse vegetation in BT is ~~might be~~ due to ~~its~~ drier conditions versus the more abundant vegetation in ~~, while vegetation is abundant in the regions where the rainfall was abundant (~~the FY and YN sites ~~populations, (~~Fig. 1, Fig. 2-B). Humidity ~~stands out a~~is the most important factor ~~regarding the~~influencing abundance and distribution of insects (Savopoulou-soultani *et al.*, 2012; Cesne, Wilson & Soulier-Perkins, 2015) and so~~. Thus,~~ drier conditions and sparse vegetation should be associated with less food ~~dictate that food availability will be poor~~.

4.2 Variation in egg and clutch size, and fecundity among the three populations

Egg size varies among populations because of variation in ~~maternal~~female body size and is considered to be an important female ~~reproduction-related~~ trait ____that ~~which~~ can affect

But, this just means it should take the other population longer to reach the same size. If you collected similar AGED individuals, this makes sense, but if you collected adults of all ages, you do not expect this result UNLESS they also die at the same age.

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offspring size (Morrison & Hero, 2003; Olsen & Vollested, 2003; Steyermark & Spotila, 2001). Our data showed that egg size differed ~~significantly~~ among the three populations ~~(all cases $P < 0.05$, except for EL $P > 0.05$). The~~, which suggests that larger females of the FY and YN populations are able to devote more ~~energy to their eggs~~resources to egg production ~~(egg mass as a proxy for energy)~~. In addition, egg size is also correlated with the incubation period, with smaller eggs having a relatively short incubation time (Thompson & Pianka, 2001). Perhaps ~~It may be possible that in~~ the BT population smaller eggs hatch sooner providing offspring time to forage before entering hibernation~~, females obtain more growth time for their hatchlings in the field before hibernation by producing smaller eggs~~.

~~It is well known that~~Larger females ~~with a larger body size can~~tend to lay more eggs~~, which is commonly the case among~~ in reptiles (Amat, 2008; Ryan & Lindeman, 2007). Thus, the smaller CS of the BT population is associated with their smaller ~~smaller than that of the other two populations. This might be due to the BT population's small~~ body size~~, which cannot support the CS as much as the other two populations~~. CS ~~is also constrained by food resources~~can also be limited by food availability~~,~~ and ~~it~~ varies among populations ~~and species~~ (Liao, Lu & Jehle, 2014; Roitberg *et al*., 2015). Poor food availability then is likely another important factor for the smaller CS in the BT population. The BT population with the smallest RCM may also be influenced by habitat, specifically through variation in food availability (Shine, 2005; Pellerin *et al*., 2016).

4.3 Egg shape

You already said that smaller animals is probably due to limited food, now you're saying that smaller clutches due to food, but, smaller animals lay smaller clutches. You should simply say once and for all, that the BT population can have smaller females and clutches due to shorter growing seasons and limited food availability (all in one sentence). To present the two as separate arguments ignores their dependence.

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~~CS is also related to the~~Egg shape ~~of the eggs,~~is also related to clutch size and larger clutches tend to have more rounded eggs ~~females produce a larger CS when their eggs are rounded, and less when elongated~~ (Ji *et al*., 2002). EL did not significantly differ among the three populations (EM as the covariate), but ~~a variation did exist in~~ EW did. ~~The BT population's EW~~ Eggs were narrower in BT ~~is smaller than that of the other two populations, so the egg shape of the BT population females were more elongated than those laid by the YN and FY populations~~. On the other hand, ES is associated with female and clutch size~~related to the crowdedness of eggs in the female's uterus~~ due to available space in the uterus (Qu *et al*., 2011; Ji & Wang, 2005). Both the FY and YN populations lay more, ~~and~~ rounder eggs (Table 1). ~~Rounder eggs might indicate that the uteri of the females of these two populations were more tightly packed when they were gravid.~~

4.4 Variation in egg size-number trade-offs among the three populations

The trade-off between egg size and ~~number~~clutch size is ~~one of the central~~an important concept~~s~~ in life-history theory (Kern *et al*., 2015). ~~The egg size-number trade-offs among the three populations here are quite different. A significant negative relationship between e~~Egg size and clutch size were negatively correlated ~~number existed~~ in the BT and FY populations (EM and EL), but not in the YN populatio~~n, indicating that in the former populations the females with larger CS produce smaller eggs by reducing the length of the eggs~~. In the YN population there was no egg size-number trade-off ~~(*P* > 0.05)~~, ~~which is further evidence that~~and so intraspecific variation in the relationship between egg size and clutch size~~number~~ is widespread (Liao, Lu & Jehle, 2014; Roitberg *et al*., 2015).

4.5 Variation in the relationships between egg size, number, and ~~maternal~~female

morphology among the three populations

Generally speaking, offspring phenotypes are influenced by ~~maternal~~female body size (e.g., SVL, Krist & Remeš, 2004). ~~According to Lovich *et al.* (2012), m~~Morphological traits and other factors affecting egg size ~~will~~can result in the following five possible outcomes (Lovich *et al*. 2012): 1) egg size is constrained by female morphology (~~-~~not optimized), 2) egg size is unconstrained by female morphology (optimized), 3) egg size is unconstrained by female morphology and optimized only in the largest females ~~-~~(Fehrenbach *et al*., 2016), 4) egg size is not constrained by the pelvic aperture width, ~~but it~~and is not optimized, ~~as it is~~but rather is constrained by some other non-morphological factor (e.g., age or clutch number, Clark, Ewert & Nelson, 2001; Paitz *et al*., 2007; Harms *et al*., 2005), 5) egg width is constrained and requires osteo-kinesis for oviposition (Hofmeyr, Henen & Loehr, 2005; Fehrenbach et al., 2016).

Consistent with the prediction of the morphological constraint hypothesis, egg size increases as the size of the female increases (outcome 1) in the YN population. Although female body size in the BT population is smaller than in the FY population, in both cases, their egg size was unconstrained by ~~maternal~~female body size (outcome 2 or 4 above).

For some species with small body sizes, egg size is constrained by female morphology (Ryan & Lindeman, 2007). In small-bodied females, the body size-specific constraints on egg size coupled with selection towards an optimum egg size results in a positive correlation between body size and egg size. (Smith & Fretwell 1974; Congdon & Gibbons, 1987). Unexpectedly, our results revealed that constraint on egg size did exist in the large-bodied females of the YN population. A ~~P~~positive relationships between egg size and female

Bad word to use in this context. Unconstrained should mean that small females can lay large eggs or small eggs.
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I don't think you want to use the word constraint here.
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~~morphology~~size indicates that there is no optimal egg size, as in the YN population ~~is attained in the YN populations~~ (Escalona, Adams & Valenzuela, 2018). ~~Furthermore, our findings also~~But, wew found ~~provide~~ some support ~~to suggest that their~~for the prediction that EW was constrained by TBW (Fig. 3), since eggs must fit the female tail base width which they pass through on their smallest axis (e.g. EW). In some turtle species, EW but not EL increases with the size of the female (Rasmussen & Litzgus, 2010). There was a significant positive correlation between EL and female SVL in the YN population, suggesting that EL is dependent upon on female SVL. Egg size (EL and EW) was not dependent on female body size in either the BT or FY population, but there were significant negative correlations between egg size and number (Fig. 4), suggesting that the egg size was constrained by CS (non-morphological factor) in both populations (Brown & Shine, 2009, outcome 4). Overall, the relationship between egg size and SVL cannot be completely explained by female morphological constraints on egg size, especially for EL, because EL can be constrained by morphological factors, non-morphological factors (e.g. CS), or their interactions, which may indicate that a weak relationship ~~exists~~ between female morphology and EL exists in the YN population. The specific mechanisms of the non-morphological factors require further study (Kern *et al*., 2015).

Would you not have found it?
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CONCLUSIONS

In summary, ~~our study~~we found geographic variation in body size and reproductive strategies of the lizard *Phrynocephalus helioscopus*. Lizards in populations with longer growing seasons and abudant vegetation (the FY and YN populations) ~~exhibit~~are larger ~~body sizes~~ and have greater reproductive output. ~~The l~~Lizards of the BT population are smaller (perhaps due to food limitation or season limitation)~~, because they have the smallest body size and inadequate food availability, their~~ also have smaller clutches ~~CS was smaller~~ than ~~that of the lizards of~~ the FY and YN populations. ~~Due to their larger CS, the~~ FY and YN females~~populations~~ produce rounder eggs, perhaps due to larger body size. This study found that there were morphological constraints on egg size in the larger-bodied females of the YN population – an anomaly for the morphological constraint hypothesis. Egg size was not constrained by female body size and did not follow the optimal egg size hypothesis in the BT and FY populations. Egg size-number trade-off suggests that egg size was constrained by CS in both populations.

However, whether the existence of genetic variation is related to the differences in the life history traits of the three populations of this species has not been examined in this study and should be researched in the future.

Ethics approval

Specimens were collected following *Guidelines for Use of Live Amphibians and Reptiles in Field Research* (the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, 2004). This work was performed in compliance with the current laws on animal welfare and research in China. After the research was completed, the lizards were released where they were captured.

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