

Variations in the reproductive strategies of three populations of *Phrynocephalus helioscopus* in China

Tao Liang¹, Lu Zhou^{1,2}, Wenfeng He¹, Lirong Xiao¹ and Lei Shi¹

¹ College of Animal Science, Xinjiang Agricultural University, Urumqi, Xinjiang, China

² Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China

ABSTRACT

Background. Egg size and clutch size are key life history traits. During the breeding period, it is possible for females to increase their reproductive output 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 are often influenced by animals' morphology and environment.

Methods. Here, we examined variation in female morphological and reproductive traits, tested for trade-offs between egg size and clutch size, and evaluated the relationship between egg size and female morphology in three populations of *Phrynocephalus helioscopus*.

Results. Female body size, egg size, and clutch size were larger in the Yi Ning (YN) and Fu Yun (FY) populations than in the Bei Tun (BT) population (the FY and YN populations laid more, and rounder eggs). Egg size was independent of female body size in two populations (BT and FY), even though both populations had an egg-size/clutch size trade-off. In the YN population, egg size and clutch size were independent, but egg size was correlated with female body size, consistent with the hypothesis of morphological constraint.

Conclusions. Our study found geographical variation in body size and reproductive strategies of *P. helioscopus*. Egg size was correlated with morphology in the larger-bodied females of the YN population, but not in the smaller-bodied females of the BT population, illustrating that constraints on female body size and egg size are not consistent between populations.

Subjects Ecology, Evolutionary Studies, Zoology

Keywords *Phrynocephalus helioscopus*, Optimal egg size, Morphological constraint hypothesis, Egg size-number trade-off, Life history, Reproduction

BACKGROUND

Reproductive traits are often variable in animals as a result of differences in the quality of resources and food availability in different habitats (Roff, 2002; Cruz-Elizalde & Ramirez-Bautista, 2016). Egg size and clutch size are key life history traits, and have received more attention than other reproductive traits (Amat, 2008; Qu et al., 2011; Lovich et al., 2012). When there is less food available, females may face the problem of limited available reproductive resources to invest in eggs. This results in a trade-off between (1) the energy allocated to each egg (egg size), and (2) the total number of eggs (clutch size). An increase

Submitted 13 April 2018
Accepted 6 September 2018
Published 24 October 2018

Corresponding author
Lei Shi, leis@xjau.edu.cn,
shileixj@126.com

Academic editor
James Roper

Additional Information and
Declarations can be found on
page 10

DOI 10.7717/peerj.5705

© Copyright
2018 Liang et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

in resources allocated to each egg will result in a decrease in clutch size (Roff, 1992; Kaplan & Phillips, 2006). This negative relationship between egg size and clutch size provides evidence for reproductive trade-offs (Rowe, 1992). Variation in female reproductive output is widespread, both interspecifically and intraspecifically. Especially for geographically widespread species, local genetic variation, short-term phenotypic plasticity, and the complex interactions between these two, contribute to variation in reproductive output (Brown & Shine, 2007).

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

Egg size is often correlated with female body size in reptiles (morphological constraint hypothesis), and both egg size and clutch size increase with an increase in female body size, contrary to OES theory (Dunham & Miles, 1985; Clark, Ewert & Nelson, 2001; Ryan & Lindeman, 2007; Mohamed et al., 2012). When resources are limited, reproductive output is directly correlated with the trade-offs between egg size and clutch size, and ultimately, with offspring survival (Congdon & Tinkle, 1982; Brown & Shine, 2009). The size of each egg normally determines the success of incubation and offspring survival (Angilletta et al., 2004; Räsänen, Laurila & Merilä, 2005). Females may increase energy allocation to eggs as a way to improve offspring quality.

P. helioscopus are small (mean snout-vent length (SVL): 47.5 mm) lizards that are 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, variation in female reproductive traits, and egg size-clutch size trade-offs, and the effects of female size on egg size have not been studied in geographically different populations in order to understand variation in investment in offspring production. In this study, we compared female morphological traits and the relationships among egg length (EL), egg width (EW), egg mass (EM), egg shape (ES), and clutch size (CS) in three populations of *P. helioscopus*. Specifically, we:

1. Tested whether reproductive female size differed between the three populations, and
2. Examined how that variation was associated with reproductive traits, especially fecundity, egg and clutch size, egg shape, and the trade-offs between egg size and clutch size.

MATERIALS AND METHODS

Ethical 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 study was conducted in compliance with current laws on animal welfare and research in China and the regulations set by the Xinjiang Agricultural University. After the research was completed, the lizards were released where they were captured.

Study site

The populations studied here are located in three ecologically distinct locations in the Xinjiang Uyghur Autonomous Region, China, and included locations near 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). The BT and the YN populations were located approximately 660 km apart, and the habitats were different at each location. The BT population was located in a typical gravel desert with little vegetation, while the YN population was located in a loam desert with abundant vegetation. The climate experienced by the YN population was hotter and wetter than that experienced by the BT population. The FY and BT populations were located approximately 160 km apart, and the FY and YN populations were located approximately 700 km apart. The vegetation and rainfall at the FY and YN locations were similar, while the temperature regimes were similar at the FY and BT locations (Figs. 1 and 2).

Animal and egg collection

In May 2014 and May 2017, we collected *P. helioscopus* individuals by hand from locations near BT (in 2014 Liang et al., 2015), FY (in 2017), and YN (in 2017). We collected lizards from 12:00 to 18:00, when they were most active (T Liang, pers. obs., 2015, 2017; S Lei, pers. obs., 2015), and we captured the lizards at random. Lizards were transported to Xinjiang Agricultural University, where female lizards were palpated to determine their reproductive state (Li et al., 2006). Fifty-three gravid females (BT: 13, FY: 24, YN: 16) were housed individually in plastic cages containing vermiculite in a room with ambient temperatures that varied from 20–28 °C 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 the 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*. Females dig before they lay eggs, and we observed females every 2 hours, which allowed us to collect eggs quickly, and prevented eggs from absorbing water in the moist vermiculite. All eggs used in this study were collected no more than 20 min after they were laid.

Morphology and reproductive traits

We measured female snout-vent length (SVL), tail base width (TBW), egg length (EL), and egg width (EW), using digital calipers. All measurements were accurate to within 0.1 mm. We also recorded the clutch size (CS). We weighed eggs (egg mass, EM) and clutches (clutch mass, CM) on an electronic balance to the nearest 0.01 g. The ratio of egg

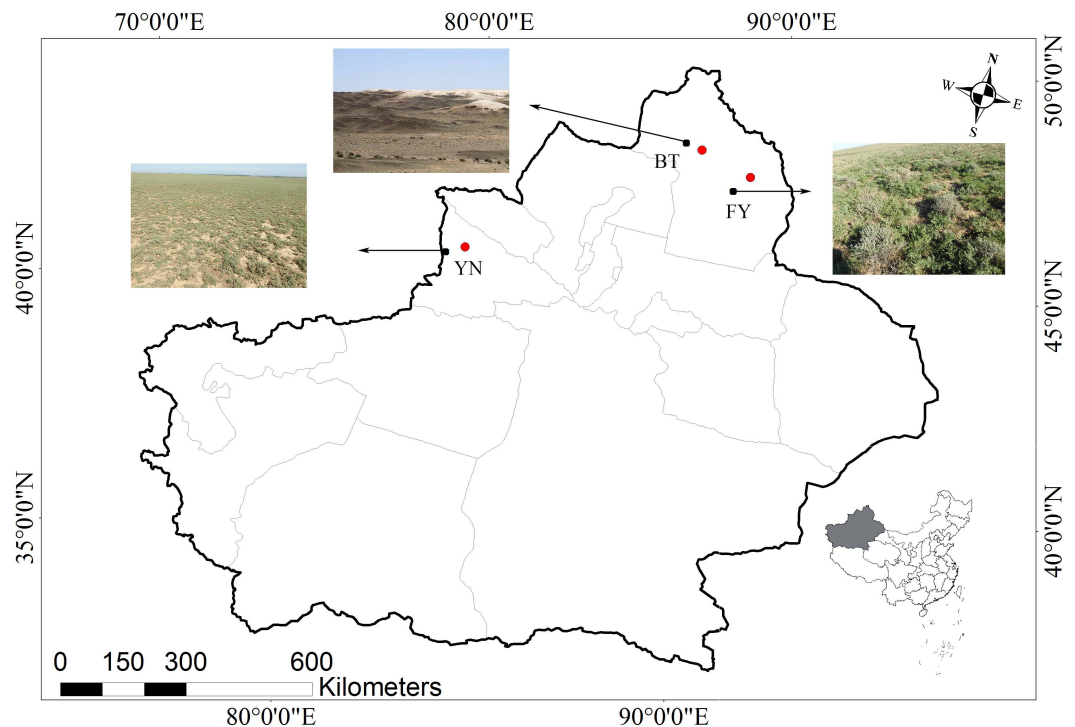


Figure 1 Map showing the three locations in the Xinjiang Uyghur Autonomous Region of western China where *P. helioscopus* were captured for this study. The nearest cities (BT, Bei Tun, FY, Fu Yun; YN, Yi Ning) are identified by the red dots, and collection locations are indicated by black dots with arrows. Photos indicate habitat types in each sampling location (Photo credit: Tao Liang).

Full-size [DOI: 10.7717/peerj.5705/fig-1](https://doi.org/10.7717/peerj.5705/fig-1)

length to egg width (EL/EW) indicates the general shape of the eggs (egg shape, ES), where 1 is a round egg, and larger values indicate increasingly elongate eggs (Ji & Wang, 2005; Kratochvíl & Frynta, 2006).

Statistical analyses

We used analysis of variance (ANOVA) to examine differences in SVL, EM, and ES, and we used analysis of covariance (ANCOVA) to examine differences in TBW, EL, EW, RCM, and CS between the three populations with *post hoc* Tukey's tests (multiple comparisons). We used Levene's tests to test for heterogeneity of variances. The residuals were tested by Kolmogorov–Smirnov tests for detecting normality. We log-transformed the variables to minimize the heterogeneity, where necessary (King, 2000). To test egg size-clutch size trade-offs, and analyze potential morphological constraints on optimal egg size, the relationships between EM and EL and CS, EM and EL, EL and CS and SVL, and EW and TBW were examined using reduced major axis (RMA) regressions rather than ordinary least squares (OLS) regressions, because RMA accounts for an error in the independent variable (Dunham & Miles, 1985). Historical climatic data (1990–2013) of the three study areas were taken from the Chinese National Climatic Data Center (<http://data.cma.cn>). Descriptive statistics are presented as mean adjusted (calculated using the *effect* function in the “effects” package, (Fox & Hong, 2009) \pm standard error (SE), except for SVL, EM, and

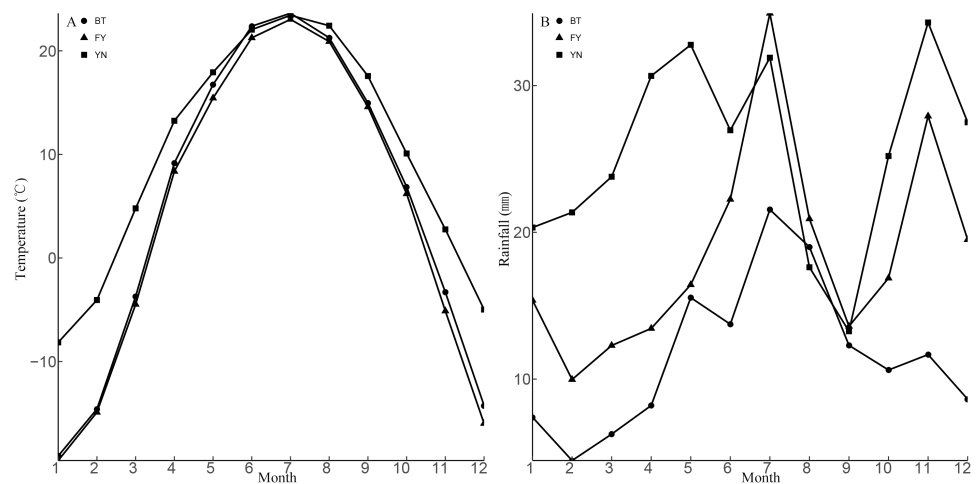


Figure 2 Means for monthly mean air temperature (A) and monthly mean rainfall (B) from 1990 to 2013 at three sampling locations (BT: Bei Tun, FY: Fu Yun, YN: Yi Ning) where *P. helioscopus* were collected. Note that months are numbered from 1 (January) to 12 (December).

Full-size [DOI: 10.7717/peerj.5705/fig-2](https://doi.org/10.7717/peerj.5705/fig-2)

ES, which are presented as the mean \pm SE. Differences were considered significant when $P < 0.05$.

All analyses were conducted using R v. 3.4.1 (*R Core Team, 2017*), using the packages “lmodel2” (*Legendre, 2011*), “ggplot” (*Wickham, 2015*), and “gplots” (*Warnes et al., 2011*).

RESULTS

Female morphological variation

SVL varied among populations, and was longest in the YN and FN populations, in which females had similar SVLs (YN: 51.23 mm; FY: 50.43 mm), and shortest in the BT population ($F_{2,52} = 20.75$, $r^2 = 0.45$, $P < 0.0001$, *Fig. 3A*). TBW varied among populations, and was smallest in the YN and FN populations, which had similar TBWs (YN: 7.20 mm; BT: 6.93 mm), and the largest in the FY population when controlling for SVL using ANCOVA ($F_{2,52} = 6.82$, $P = 0.002$, *Fig. 3B*).

Female reproductive traits

Females in the FY population laid heavier eggs than those in the BT and YN populations (*Table 1*). Eggs were similar in length in all three populations. Eggs were wider in the FY population, and narrower in the YN population. BT females laid smaller clutches than FY and YN females when controlling for SVL (*Table 1*).

Egg size-clutch size trade-offs

We found a positive relationship in all populations between EL and EM (*Fig. 4C*). In BT and FY populations, egg size decreased with clutch size, while in YN females, egg size was independent of clutch size (*Figs. 4A, 4B*).

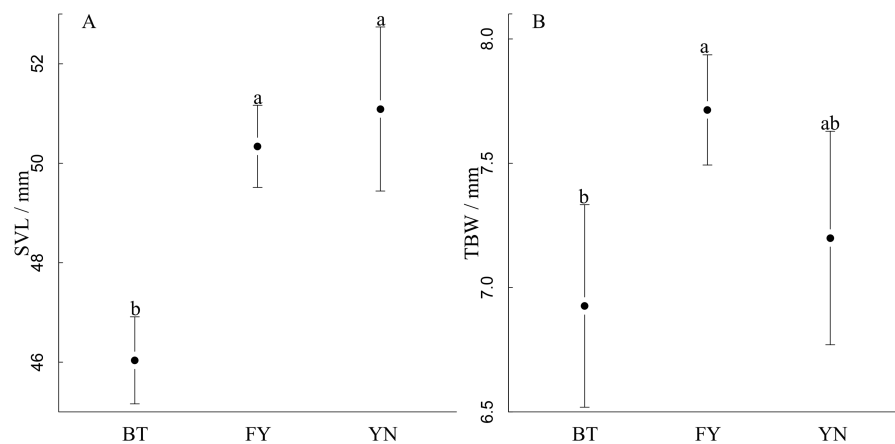


Figure 3 Comparisons between (A) snout-vent length and (B) tail width at base of gravid females in three populations (BT, Bei Tun; FY, Fu Yun; YN, Yi Ning) of *P. helioscopus*. Points indicate means with 95% confidence intervals. Different letters indicate significant differences at the $P < 0.05$ level.

Full-size [DOI: 10.7717/peerj.5705/fig-3](https://doi.org/10.7717/peerj.5705/fig-3)

Table 1 Descriptive statistics of female reproductive traits in three populations (BT: Bei Tun, FY: Fu Yun, YN: Yi Ning) of *P. helioscopus*.

	BT ($n = 35$)	FY ($n = 90$)	YN ($n = 63$)	<i>F</i> -level and <i>P</i> -value
EM (g) [†]	0.51 ± 0.02^b	0.61 ± 0.02^a	0.55 ± 0.01^b	$F_{2,187} = 11.67, r^2 = 0.11, P < 0.0001$
range	0.32 ~ 0.76	0.27 ~ 1.02	0.28 ~ 0.82	
EL (mm) [#]	15.7 ± 0.24^a	14.4 ± 0.17^a	14.9 ± 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) [#]	8.4 ± 0.08^b	8.5 ± 0.06^a	8.3 ± 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 [†]	1.8 ± 0.03^a	1.7 ± 0.02^b	1.8 ± 0.02^{ab}	$F_{2,187} = 6.71, r^2 = 0.06, P < 0.0001$
Range	1.44 ~ 2.27	1.43 ~ 2.18	1.47 ~ 2.11	
CS ^{#, *}	2.9 ± 0.13^b	3.7 ± 0.18^a	3.8 ± 0.14^a	$F_{2,187} = 10.93, P = 0.0001$
range	2 ~ 4	2 ~ 6	3 ~ 5	

Notes.

EM, egg mass; EL, egg length; EW, egg width; ES, egg shape; CS, clutch size.

Different letters indicate significance at the $P < 0.05$ level.

[†]ANOVA.

[#]One-way analyses of covariance (ANCOVAs) (for CS with snout-vent length (SVL) as the covariate, and for EL and EW with egg mass (EM) as the covariate).

*BT $n = 13$, FY $n = 24$, YN $n = 16$.

Relationships among egg size, clutch size, and female morphology

In the BT and YN populations, female body size was independent of EL, EW, and CS (Fig. 5). In the YN population, while CS was independent of female body size (Fig. 5C), EL was weakly correlated with SVL (Fig. 5A), and EW and TBW were correlated (Fig. 5B).

DISCUSSION

Females in our three populations varied in body size (SVL, TBW), and reproductive traits (EM, CS, RCM, and egg size), in ways that supported, with some exceptions, an egg size-clutch size trade-off in the three populations of *P. helioscopus*. Female body size, egg size, and clutch size were smaller in the BT population than in the FY and YN populations,

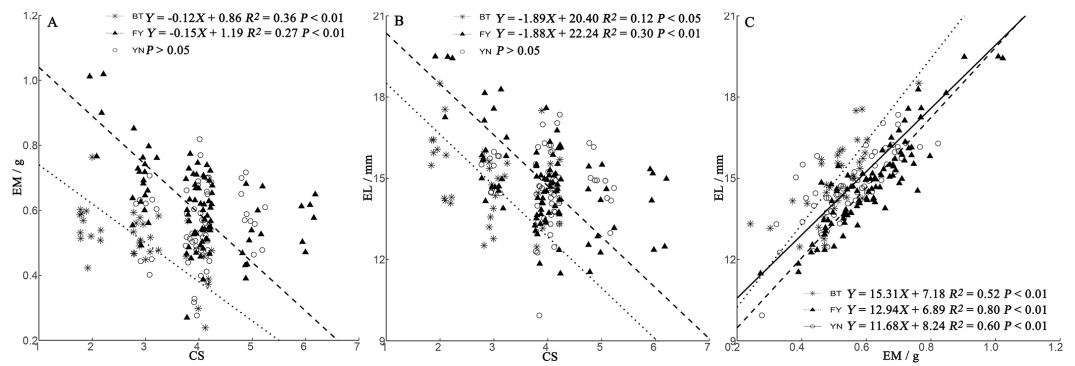


Figure 4 Regressions of egg mass (EM) (A) and egg length (EL) (B) and clutch size (CS) trade-offs, and relationship between EM and EL (C) in *P. helioscopus*. Fitted reduced major axis regression model and statistical significance ($P < 0.05$) are indicated in each case. BT, Bei Tun–Asterisk; FY, Fu Yun–Triangle; YN, Yi Ning–Circle. Points were jittered using the *geom_jitter* function.

Full-size [DOI: 10.7717/peerj.5705/fig-4](https://doi.org/10.7717/peerj.5705/fig-4)

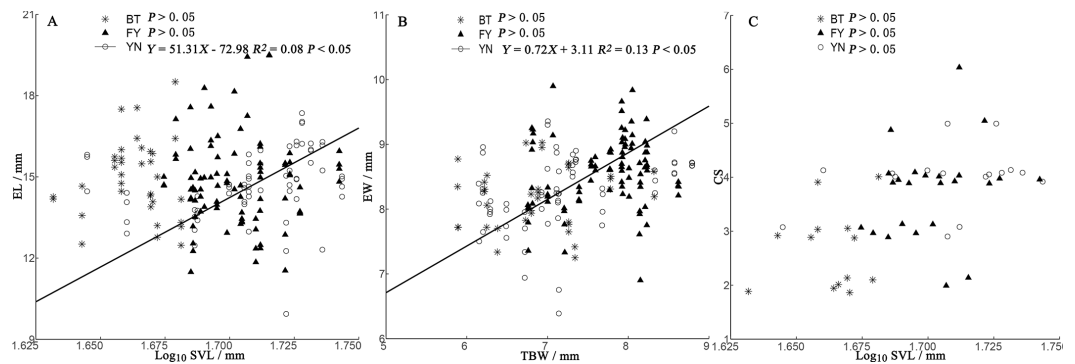


Figure 5 Regressions of egg length (EL) (A), egg width (EW) (B), and clutch size (CS) (C) and female morphological traits (SVL, snout-vent length; TBW, tail base width) from three populations of *P. helioscopus*. Fitted reduced major axis regression model and statistical significance ($P < 0.05$) are indicated in each case. BT, Bei Tun–Asterisk; FY, Fu Yun–Triangle; YN, Yi Ning–Circle. Points were jittered using the *geom_jitter* function.

Full-size [DOI: 10.7717/peerj.5705/fig-5](https://doi.org/10.7717/peerj.5705/fig-5)

and the FY and YN populations laid more, and rounder eggs. Egg size was not correlated with female body size in the BT and FY populations, but egg size-clutch size trade-offs occurred in both populations. Trade-offs between egg size and clutch size were not found in the YN population, but egg size was correlated with female body size in this population.

Morphological traits, such as body size and body shape, always vary among different populations of 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 females in the FY and YN populations were larger than females in the BT population. Longer activity seasons were assumed to be the cause of variation in the body size between the females of the YN and BT populations (*Liang & Shi, 2017*). Temperature, which is fundamentally important for lizards (*Grant &*

Dunham, 1990), was highest in YN, especially in March and November (*Fig. 2A*). In YN, *P. helioscopus* activity began in mid-March, and hibernation began in early November, which means that the activity period for lizards in YN was almost a month longer than in the other two sites (BT and FY). *P. helioscopus* in this population were larger, probably because of the longer growing season. It is possible that *P. helioscopus* age varied between the three study locations, and this may have influenced results. However, we have no reason to believe that the ages of the *P. helioscopus* captured from the three populations were very different. We could have tested for this possibility using mark and recapture methods, but unfortunately, this was outside the scope of this study.

The BT and the FY populations experienced similar temperatures, which raises the question as to what caused the differences in body size. One possibility is that food limitation might have resulted in reduced growth rates in the BT population. Rainfall is critical for habitat quality (e.g., vegetation cover and prey abundance (*Lorenzon, Clobert & Massot, 2001*)) and there was significant geographic variation in rainfall between BT and FY (*Fig. 2*). BT is sparsely vegetated, due to drier conditions in this location, whereas the vegetation is more abundant in the wetter FY and YN sites (*Figs. 1, 2B*). Humidity is the most important factor influencing the abundance and distribution of insects (*Savopoulou-Soultani et al., 2012; Cesne, Wilson & Soulier-Perkins, 2015*), and therefore drier conditions and sparse vegetation could be associated with less available food in the BT population.

Egg size varies among populations because of variation in the female body size, which is an important female trait that can affect offspring quality (*Steyermark & Spotila, 2001; Morrison & Hero, 2003; Olsen & Vøllested, 2003*). We found that egg size differed among the three populations, which suggests that larger females in the FY and YN populations were able to allocate more resources to egg production. In addition, egg size was also correlated with the incubation period, with smaller eggs having a relatively shorter incubation time. However, further studies are needed to determine whether the earlier hatching of the smaller eggs in the BT population could provide offspring with more time to forage before the hibernation period (*Thompson & Pianka, 2001*). EL was similar in the three populations (using EM as the covariate), but EW was not. However, egg shape is also related to clutch size, and larger clutches tend to have more rounded eggs (*Ji et al., 2002*). Eggs were narrower in the BT population, and both the FY and YN populations laid more, and rounder eggs (*Table 1*). Larger reptile females tend to lay more eggs (*Ryan & Lindeman, 2007; Amat, 2008*). Thus, the smaller CS of the BT population, associated with their smaller body size, may be due to limited resources (including food), as resource availability often varies among populations (*Liao, Lu & Jehle, 2014; Roitberg et al., 2015*).

The trade-off between egg size and clutch size is an important concept in life-history theory (*Kern et al., 2015*). Egg size (EM and EL) and clutch size were negatively correlated in the BT and FY populations, but not in the YN population. In the YN population, there was no egg size-clutch size trade-off, suggesting that patterns of variation in egg size and clutch size are not always consistent in different populations (*Liao, Lu & Jehle, 2014; Roitberg et al., 2015*).

Generally speaking, offspring phenotypes are influenced by female body size (e.g., SVL, *Krist & Remeš, 2004*). Body size and other factors affecting egg size will result in the

following five possible outcomes (Lovich *et al.*, 2012): (1) egg size is constrained by female size (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, and is not optimized, but rather is constrained by some other non-morphological factor (e.g., female age or clutch number Clark, Ewert & Nelson, 2001; Harms *et al.*, 2005; Paitz *et al.*, 2007), or (5) egg width is constrained and requires osteokinesis for oviposition (Hofmeyr, Henen & Loehr, 2005; Fehrenbach *et al.*, 2016).

Consistent with the predictions of the morphological constraint hypothesis, egg size increased as the size of the female increased (outcome 1) in the YN population. Although female body size in the BT population was smaller than that in the FY population, in both cases, egg size was not correlated with 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. Egg size (EL and EW) was not dependent on female body size in either the BT or FY population, but there were negative correlations between egg size and clutch size (Fig. 4), suggesting that egg size was constrained by CS (a non-morphological factor) in both populations (Brown & Shine, 2009; outcome 4). Unexpectedly, we found that egg size was correlated with body size in the larger-bodied females of the YN population. A positive relationship between egg size and female size indicates that there is no optimal egg size in the YN population (Escalona, Adams & Valenzuela, 2018). However, we found some support for the prediction that EW is constrained by TBW (Fig. 3), since eggs must fit the female tail base width, which they pass through on their smallest axis (i.e., EW). In some turtle species, EW but not EL increases with the size of the female (Rasmussen & Litzgus, 2010). There was a significant (but weak) positive correlation between EL and female SVL in the YN population, suggesting that EL is dependent upon on female SVL. 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 in the YN population. The specific mechanisms of the non-morphological factors require further study (Kern *et al.*, 2015).

CONCLUSIONS

We found geographical variation in the body size and reproductive strategies of *P. helioscopus*. Females in populations with longer growing seasons and abundant vegetation (the FY and YN populations) were larger. Lizards in the BT population were smaller, perhaps due to limitations on food availability, or limitations on activity seasons, and also had smaller clutches than the FY and YN populations. Females in the FY and YN populations produced rounder eggs, perhaps due to their larger body size. This study found that egg size was correlated with female body size in the larger-bodied females of the YN population—an anomaly for the morphological constraint hypothesis. Egg size was not correlated with female body size, and did not follow the optimal egg size hypothesis

in the BT and FY populations. Trade-offs between egg size and clutch size suggest that egg size was constrained by clutch size in both populations.

Potential genetic and age variation associated with females in these populations may have influenced our results. However, here we demonstrate that life histories, as measured by body size and clutch characteristics, can vary in surprising ways, sometimes supporting the possibility of trade-offs, and sometimes not.

ACKNOWLEDGEMENTS

We are grateful to James Roper, Prof. Lovich of the United States Geological Survey, the Southwest Biological Science Center, and anonymous reviewers for their excellent suggestions for improving this manuscript. We thank Luo D and Wang P for assistance during fieldwork, and we thank An J for help with the egg collection and lizard husbandry. Mr. T Martin provided professional advice regarding spelling and phrasing.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The research is funded by the National Natural Science Foundation of China (31660613). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
National Natural Science Foundation of China: 31660613.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Tao Liang conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Lu Zhou conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Wenfeng He performed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.
- Lirong Xiao performed the experiments, contributed reagents/materials/analysis tools, approved the final draft.
- Lei Shi conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

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 study was conducted in compliance with current laws on animal welfare and research in China and the regulations set by the Xinjiang Agricultural University. After the research was completed, the lizards were released where they were captured.

Data Availability

The following information was supplied regarding data availability:

The raw data are provided in the [Supplemental Tables](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.5705#supplemental-information>.

REFERENCES

- Amat F. 2008.** Exploring female reproductive tactics: trade-offs between clutch size, egg mass and newborn size in lacertid lizards. *Herpetological Journal* **18**(3):147–153.
- Angilletta MJ, Niewiarowski PH, Dunham AE, Leaché AD, Porter WP. 2004.** Bergmann's clines in ectotherms: illustrating a life-history perspective with Sceloporine lizards. *The American Naturalist* **164**(6):E168–E183 DOI [10.1086/42522](https://doi.org/10.1086/42522).
- Berven KA. 1982.** The genetic basis of altitudinal variation in the wood frog *Rana sylvatica* II. An experimental analysis of larval development. *Oecologia* **52**(3):360–369 DOI [10.1007/BF00367960](https://doi.org/10.1007/BF00367960).
- Brockelman WY. 1975.** Competition, fitness of offspring, and optimal clutch size. *American Naturalist* **109**(970):677–699 DOI [10.1086/283037](https://doi.org/10.1086/283037).
- Brown G, Shine R. 2007.** Rain, prey and predators: climatically driven shifts in frog abundance modify reproductive allometry in a tropical snake. *Oecologia* **154**(2):361–368 DOI [10.1007/s00442-007-0842-8](https://doi.org/10.1007/s00442-007-0842-8).
- Brown GP, Shine R. 2009.** Beyond size-number trade-offs: clutch size as a maternal effect. *Philosophical Transactions of the Royal Society B Biological Sciences* **364**(1520):1097–1106 DOI [10.1098/rstb.2008.0247](https://doi.org/10.1098/rstb.2008.0247).
- Cesne ML, Wilson SW, Soulier-Perkins A. 2015.** Elevational gradient of Hemiptera (Heteroptera, Auchenorrhyncha) on a tropical mountain in Papua New Guinea. *PeerJ* **3**(23):e978 DOI [10.7717/peerj.978](https://doi.org/10.7717/peerj.978).
- Clark PJ, Ewert MA, Nelson CE. 2001.** Physical apertures as constraints on egg size and shape in the common musk turtle, *Sternotherus odoratus*. *Functional Ecology* **15**(1):70–77 DOI [10.2307/826569](https://doi.org/10.2307/826569).

- Congdon JD, Gibbons JW. 1987.** Morphological constraints on egg size: a challenge to optimal egg size theory? *Proceedings of the National Academy of Sciences of the United States of America* **84**(12):4145–4147 DOI [10.1073/pnas.84.12.4145](https://doi.org/10.1073/pnas.84.12.4145).
- Congdon JD, Tinkle DW. 1982.** Energy expenditure in free-ranging sagebrush lizards (*Sceloporus graci*). *Canadian Journal of Zoology* **60**(6):1412–1416 DOI [10.1139/z82-190](https://doi.org/10.1139/z82-190).
- Cruz-Elizalde R, Ramirez-Bautista A. 2016.** Reproductive cycles and reproductive strategies among populations of the Rose-bellied lizard *Sceloporus variabilis* (Squamata: Phrynosomatidae) from central Mexico. *Ecology and Evolution* **6**(6):1753–1768 DOI [10.1002/ece3.1998](https://doi.org/10.1002/ece3.1998).
- Dunham AE, Miles DB. 1985.** Patterns of covariation in life history traits of squamate reptiles: the effects of size and phylogeny reconsidered. *The American Naturalist* **126**(2):231–257 DOI [10.1086/284411](https://doi.org/10.1086/284411).
- Escalona T, Adams DC, Valenzuela N. 2018.** A lengthy solution to the optimal propagule size problem in the large-bodied South American freshwater turtle, *Podocnemis unifilis*. *Evolutionary Ecology* **32**(1):29–41 DOI [10.1007/s10682-017-9922-3](https://doi.org/10.1007/s10682-017-9922-3).
- Fehrenbach AK, Louque I, McFadden SL, Huntzinger C, Lyons E, Shively SH, Selman W, Lindeman PV. 2016.** Habitat-related variation in body size and reproductive output and an examination of reproductive allometry in the Sabine map turtle (*Graptemys sabinensis*) across three river drainages. *Copeia* **104**(2):458–468 DOI [10.1643/CE-15-273](https://doi.org/10.1643/CE-15-273).
- Fox J, Hong J. 2009.** Effect displays in R for multinomial and proportional-odds logit models: extensions to the effects package. *Journal of Statistical Software* **32**(1):1–24.
- Grant BW, Dunham AE. 1990.** Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. *Ecology* **71**(5):1765–1776 DOI [10.2307/1937584](https://doi.org/10.2307/1937584).
- Harms HK, Paitz RT, Bowden RM, Janzen FJ. 2005.** Age and season impact resource allocation to eggs and nesting behavior in painted turtles. *Physiological and Biochemical Zoology* **78**(6):996–1004 DOI [10.1086/432920](https://doi.org/10.1086/432920).
- Hofmeyr MD, Henen BT, Loehr VJT. 2005.** Overcoming environmental and morphological constraints: egg size and pelvic kinesis in the smallest tortoises, *Homopus signatus*. *Canadian Journal of Zoology* **83**(10):1343–1352 DOI [10.1139/Z05-132](https://doi.org/10.1139/Z05-132).
- Horváthová T, Cooney CR, Fitze PS, Oksanen T, Jelić D, Ghira I, Uller, Jandzik D. 2013.** Length of activity season drives geographic variation in body size of a widely distributed lizard. *Ecology & Evolution* **3**(8):2424–2442 DOI [10.1002/ece3.613](https://doi.org/10.1002/ece3.613).
- Ji X, Huang HY, Hu XZ, Du WG. 2002.** Geographic variation in female reproductive characteristics and egg incubation in the Chinese skink, *Eumeces chinensis*. *Chinese Journal of Applied Ecology* **13**:680–684 [Abstract in English] DOI [10.1002/ece3.613](https://doi.org/10.1002/ece3.613).
- Ji X, Wang ZW. 2005.** Geographic variation in reproductive traits and trade-offs between size and number of eggs of the Chinese cobra (*Naja atra*). *Biological Journal of the Linnean Society* **85**(1):27–40 DOI [10.1002/ece3.613](https://doi.org/10.1002/ece3.613).

- Kaplan RH, Phillips PC. 2006.** Ecological and developmental context of natural selection: maternal effects and thermally induced plasticity in the frog *Bombina orientalis*. *Evolution* **60**(1):142–156 DOI [10.1111/j.0014-3820.2006.tb01089.x](https://doi.org/10.1111/j.0014-3820.2006.tb01089.x).
- Kern MM, Guzy JC, Lovich JE, Gibbons JW, Dorcas ME. 2015.** Relationships of maternal body size and morphology with egg and clutch size in the Diamondback terrapin, *Dalaclemys terrapin* (Testudines: Emydidae). *Biological Journal of the Linnean Society* **117**(2):295–304 DOI [10.1111/bj.12655](https://doi.org/10.1111/bj.12655).
- King RB. 2000.** Analyzing the relationship between clutch size and female body size in reptiles. *Journal of Herpetology* **34**(1):148–150 DOI [10.1111/j.0014-3820.2006.tb01089.x](https://doi.org/10.1111/j.0014-3820.2006.tb01089.x).
- Kratochvíl L, Frynta D. 2006.** Egg shape and size allometry in geckos (Squamata: Gekkota), lizards with contrasting eggshell structure: why lay spherical eggs? *Journal of Zoological Systematics and Evolutionary Research* **44**(3):217–222 DOI [10.1111/j.1439-0469.2005.00339.x](https://doi.org/10.1111/j.1439-0469.2005.00339.x).
- Krist M, Remeš V. 2004.** Maternal effects and offspring performance: in search of the best method. *Oikos* **106**(2):422–426 DOI [10.1111/j.0030-1299.2004.13373.x](https://doi.org/10.1111/j.0030-1299.2004.13373.x).
- Legendre P. 2011.** lmodel2: model II regression. R package version 1.7-1/r1794. Available at <http://R-Forge.R-project.org/projects/vegan/>.
- Li H, Ji X, Qu YF, Gao JF, Zhang L. 2006.** Sexual dimorphism and female reproduction in the multi-ocellated racerunner *Eremias multiocellata* (Lacertidae). *Acta Zoologica Sinica* **52**(2):250–255.
- Liang T, Lü SP, Wu KL, Shi L. 2015.** Sexual dimorphism and female reproduction of *Phrynocephalus helioscopus* (Agamidae). *Chinese Journal of Ecology* **34**(6):1602–1606 [Abstract in English] DOI [10.13292/j.1000-4890.2015.0144](https://doi.org/10.13292/j.1000-4890.2015.0144).
- Liang T, Shi L. 2017.** Sexual dimorphism and morphological variation of three populations of *Phrynocephalus helioscopus*: test of Bergmann's rule, Allen's rules and Rensch's rule. *Sichuan Journal of Zoology* **36**(3):249–257 [Abstract in English] DOI [10.11984/j.issn.1000-7083.20160314](https://doi.org/10.11984/j.issn.1000-7083.20160314).
- Liao WB, Lu X. 2011.** A comparison of reproductive output of the Omei Treefrog (*Rhacophorus omeimontis*) between high and low elevations. *Animal Biology* **61**(3):263–276 DOI [10.1163/157075511X584218](https://doi.org/10.1163/157075511X584218).
- Liao WB, Lu X, Jehle R. 2014.** Altitudinal variation in maternal investment and trade-offs between egg size and clutch size in the Andrew's toad. *Journal of Zoology* **293**(2):84–91 DOI [10.1111/jzo.12122](https://doi.org/10.1111/jzo.12122).
- Lorenzon P, Clobert J, Massot M. 2001.** The contribution of phenotypic plasticity to adaptation in *Lacerta vivipara*. *Evolution* **55**:392–404 DOI [10.1554/0014-3820\(2001\)055\[0392:TCOPPT\]2.0.CO;2](https://doi.org/10.1554/0014-3820(2001)055[0392:TCOPPT]2.0.CO;2).
- Lovich JE, Madrak SV, Drost CA, Monatesti JA, Casper D, Znrari M. 2012.** Optimal egg size in a suboptimal environment: reproductive ecology of female Sonora mud turtles (*Kinosternon sonoriense*) in central Arizona, USA. *Amphibia-Reptilia* **33**(2):161–170 DOI [10.1163/156853812X634035](https://doi.org/10.1163/156853812X634035).

- Mohamed N, Znari M, Lovich JE, Feddadi Y, Baamrane MAA. 2012.** Clutch and egg allometry of the turtle *Mauremys leprosa* (Chelonia: Geoemydidae) from a polluted periurban river in west-central Morocco. *Herpetological Journal* **22**(1):43–49.
- Morrison C, Hero JM. 2003.** Geographic variation in lifehistory characteristics of amphibians: a review. *Journal of Animal Ecology* **72**(2):270–279
DOI 10.1046/j.1365-2656.2003.00696.x.
- Olsen EM, Vøllested LA. 2003.** Microgeographical variation in brown trout reproductive traits: possible effects of biotic interactions. *Oikos* **100**(3):483–492
DOI 10.1034/j.1600-0706.2003.11900.x.
- Paitz RT, Harms HK, Bowden RM, Janzen FJ. 2007.** Experience pays: offspring survival increases with female age. *Biology Letters* **3**(3):44–46 DOI 10.1098/rsbl.2006.0573.
- Qu YF, Li H, Gao JF, Ji X. 2011.** Geographical variation in reproductive traits and trade-offs between size and number of eggs in the king ratsnake, *Elaphe carinata*. *Biological Journal of the Linnean Society* **104**(3):701–709 DOI 10.1111/j.1095-8312.2011.01749.x.
- Räsänen K, Laurila A, Merilä J. 2005.** Maternal investment in egg size: environment and population-specific effects on offspring performance. *Oecologia* **142**(4):546–553
DOI 10.1007/s00442-004-1762-5.
- Rasmussen M, Litzgus J. 2010.** Patterns of maternal investment in spotted turtles (*Clemmys guttata*): implications of trade-offs, scales of analyses, and incubation substrates. *Écoscience* **17**(1):47–58 DOI 10.2980/17-1-3298.
- R Core Team. 2017.** A language and environment for statistical computing. Vienna: the R Foundation for Statistical Computing. Available at <http://www.R-project.org/>.
- Roff DA. 1992.** *The evolution of life histories*. New York: Chapman & Hall.
- Roff DA. 2002.** *Life-history evolution*. Sunderland: Sinauer Associates.
- Roitberg ES, Eplanova GV, Kotenko TI, Amat F, Carretero MA, Kuranova VN, Bulakhova NA, Zinenko OI, Yakovlev VA. 2015.** Geographic variation of life-history traits in the sand lizard, *Lacerta agilis*: testing Darwin's fecundity-advantage hypothesis. *Journal of Evolution Biology* **28**(3):613–629 DOI 10.1111/jeb.12594.
- Rowe J. 1992.** Comparative life histories of the painted turtle (*Chrysemys picta*) from Western Nebraska. PhD dissertation. University of Nebraska-Lincoln, University of Nebraska-Lincoln.
- Ryan KM, Lindeman PV. 2007.** Reproductive allometry in the common map turtle *Graptemys geographica*. *American Midland Naturalist* **158**(1):49–59
DOI 10.1674/0003-0031(2007)158[49:RAITCM]2.0.CO;2.
- Savopoulou-Soultani M, Papadopoulos NT, Milonas P, Moyal P. 2012.** Abiotic factors and insect abundance. *Psyche* **2012**:1–2 DOI 10.1155/2012/167420.
- Smith C, Fretwell S. 1974.** Optimal balance between size and number of offspring. *American Naturalist* **108**(962):499–506 DOI 10.1086/282929.
- Steyermark AC, Spotila JR. 2001.** Body temperature and maternal identity affect snapping turtle (*Chelydra serpentina*) righting response. *Copeia* **2001**(4):1050–1057
DOI 10.1643/0045-8511.

- Thompson GG, Pianka ER. 2001.** Allometry of clutch and neonate sizes in Monitor lizards (Varanidae: *Varanus*). *Copeia* **2001**(2):443–458
DOI [10.1643/0045-8511\(2001\)001](https://doi.org/10.1643/0045-8511(2001)001).
- Wang YJ, Ji WH, Zhao W, Nannan Y, Liu NF. 2011.** Geographic variation in clutch and egg size for the lizard *Phrynocephalus przewalskii* (Squamata: Agamidae). *Asian Herpetological Research* **2**(2):97–102 DOI [10.3724/SP.J.1245.2011.00097](https://doi.org/10.3724/SP.J.1245.2011.00097).
- Wang Z, Ma L, Shao M, Ji X. 2013.** Differences in incubation length and hatchling morphology among five species of oviparous *Phrynocephalus* lizards (Agamidae) from China. *Asian Herpetological Research* **4**(4):225–232
DOI [10.3724/SP.J.1245.2013.00225](https://doi.org/10.3724/SP.J.1245.2013.00225).
- Warnes GR, Bolker B, Bonebakker L, Gentleman R, Liaw WHA, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, Venables B. 2011.** gplots: various R programming tools for plotting data. The comprehensive archive network. Available at <https://cran.r-project.org/web/packages/gplots/gplots.pdf>.
- Werner YL, Korolker N, Sion G, Göçmen B. 2016.** Bergmann's and Rensch's rules and the spur-thighed tortoise (*Testudo graeca*). *Biological Journal of the Linnean Society* **117**(4):796–811 DOI [10.1111/bij.12717](https://doi.org/10.1111/bij.12717).
- Wickham H. 2015.** ggplot2. *Wiley Interdisciplinary Reviews Computational Statistics* **3**(2):180–185 DOI [10.1002/wics.147](https://doi.org/10.1002/wics.147).
- Yom-Tov Y, Heggberget TM, Øystein W, Yom-Tov S. 2006.** Body size changes in the Norwegian otter: the possible effects of food availability and global warming. *Oecologia* **150**(1):155–160 DOI [10.1007/s00442-006-0499-8](https://doi.org/10.1007/s00442-006-0499-8).
- Zhong GH, Liu Q, Li C, Peng P, Guo P. 2017.** Sexual dimorphism and geographic variation in the asian lance-headed pitviper *Protobothrops mucrosquamatus* in the mainland china. *Asian Herpetological Research* **8**(2):118–122
DOI [10.16373/j.cnki.ahr.160011](https://doi.org/10.16373/j.cnki.ahr.160011).