

## REPRODUCTIVE CONSEQUENCES OF FEMALE SIZE IN HAEMATOPHAGOUS ECTOPARASITES

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Summary statement: Maternal size does not affect the number and size of eggs and new imagoes in fleas.

## ABSTRACT

To test relationships between maternal size, egg size and size of new offspring, we studied (a) effect of maternal size on egg size and number and maternal survival after oviposition in three species and (b) effect of egg size on duration of development and new imago size in three flea species (*Xenopsylla ramesis*, *Synosternus cleopatrae*, *Parapulex chephrenis*) with varying host and habitat specificity. In general, number and size of eggs as well as total egg volume appeared to be independent of maternal body size. There was no trade-off between egg number and size. However, female body size was related to post-oviposition survival with larger females surviving longer after oviposition than smaller females. In addition, females that produced more eggs died faster after oviposition in *X. ramesis* but not in the two other species. There were no significant size differences between eggs that developed into new imagoes and eggs that did not survive. Survivorship of male and female eggs did not differ; however, new adult females were significantly larger than new adult males. Female, but not male, new imagoes exhibited a significant positive relationship between egg size and duration of preimaginal development in all three species with larger eggs developing faster than smaller eggs. In *X. ramesis* and *S. cleopatrae*, faster developing eggs also developed into larger new imagoes. We conclude that these patterns were largely consistent among the three flea species suggesting that they result from the same mechanisms and are weakly affected by the ecological specialization of a given species.

**KEY WORDS:** Fleas, rodents, egg, body size, development

## INTRODUCTION

One of the most common manifestations of the maternal effect on the offspring phenotype is positive relationship between maternal body size and the size of a propagule (Berrigan, 1991; Roff, 1992). In oviparous species, larger females usually produce larger eggs (Berrigan, 1991; Hendry et al., 2001) because they have greater amount of resources to be allocated to offspring. These resources, however, can be allocated toward the number of propagules rather than toward their size, contributing to a well-known trade-off between size and number of the eggs (Lack, 1947; Smith and Fretwell, 1974; Parker and Begon, 1986; Reiss, 1989; Honěk, 1993). On one hand, reproductive investment into greater egg number increases the probability that many of the offspring will survive, increasing the potential number of descendants. On the other hand, investment into larger eggs will likely result in larger offspring which may outcompete smaller conspecifics and have higher reproductive success (Fox and Czesak, 2000; Krist, 2011; Koch and Meunier, 2014). Nevertheless, number and size of offspring are traded-off mainly because resources available for reproduction are limited (Stearns, 1992). As a result, females should make optimal allocation decisions that allow them to maximize their reproductive output (Stearns, 1992). Consequently, a female may lay many small or a few large eggs. However, the difference in resource acquisition as a function of female size may preclude the trade-off between egg size and egg number to occur. For example, larger females may acquire more resources than smaller females and thus face no limitations in resource allocation to both number and size of their eggs, so that larger females produce both more and larger eggs than their smaller counterparts (Berrigan, 1991; Roff, 1992; Hendry et al., 2001; Smiseth et al., 2014).

Relationships between maternal body size and egg size have been studied in various free-living taxa such as insects (e.g., Corkum et al., 1997; Fox et al., 1997), fish (e.g., Hendry et al., 2001) and amphibians (Räsänen et al., 2008). To the best of our knowledge, these relationships have never been studied in parasites, although other factors affecting size of their eggs have been investigated (e.g., Poulin, 1995). One of the sharpest differences between majority of free-living species and parasites is that the amount of resources acquired by a parasite and, consequently, invested in reproduction is a net result of two opposing processes: 1) extraction of resources from a host, which increases the amount of resources available for reproduction, and 2) defensive anti-parasitic response of a host, which decreases the amount of these resources. For example, mammalian and bird host auto-grooming is the major factor of mortality of ectoparasitic

arthropods such as fleas (Hawlena et al., 2007a). In addition to killing fleas, host grooming may disrupt flea feeding. Larger fleas are more conspicuous to a host than smaller ones, so that they likely suffer more from host grooming than smaller fleas (Poulin and Morand, 1997). Thus, negative effects of disrupted feeding on the number and/or size of the eggs may be more pronounced in larger than in smaller female fleas. Host immune defence also may inhibit both feeding and reproduction of ectoparasites (e.g., Fielden et al., 1992; Rechav and Fielden, 1997). One of the reasons of suppressed ectoparasite reproduction on resistant hosts could be an increase in mass-specific metabolic rate of individual parasites exploiting resistant as compared to immune-naïve hosts (Rechav and Fielden, 1995) which, in turn, might result from higher metabolic cost of blood digestion when the source of this blood was a resistant rather than immune-naïve host individual (Khokhlova et al., 2008a, 2010a). Given positive intraspecific allometry of metabolic rate in ectoparasitic arthropods (e.g., Rechav and Fielden, 1995), this effect may be sharper in larger than smaller females. As a result, the positive relationship between size of an ectoparasite female and size of its eggs, as found in many free-living arthropods (e.g., Berrigan, 1991), can be reversed or even absent in arthropod ectoparasites. Here, we tested this hypothesis, focusing on relationships between maternal size and egg size in three species of fleas (Insecta: Siphonaptera) parasitic on rodents. Fleas are obligatory haematophagous ectoparasites most abundant and diverse on small and medium-sized burrowing mammals. These insects are holometabolous with pre-imaginal development occurring mainly off-host in its burrow or nest. Larval fleas are generally not parasitic but feed on a variety of organic matter, including faeces of adult fleas, whereas adults alternate periods of stay on and off their host (see Krasnov, 2008 for a review).

We also tested whether fleas demonstrate a trade-off between the number and the size of their eggs. Our recent evidence supported a trade-off between the quantity and quality of the offspring in fleas when they exploit different host species (Khokhlova et al., 2014). However, this earlier study estimated the number of the offspring via the number of eggs but estimated offspring quality via size of the new imagoes and rate of their pre-imaginal development. In other words, we did not look at the direct response of a mother flea in terms of the offspring quality but rather on its delayed results. However, imaginal size and development can be influenced by a number of events occurring during development from egg to new imago such as hatching, larval feeding and moulting, cocoon construction, pupation and pupal development. In

this study we measured the size of each egg directly. In addition, Khokhlova et al. (2014) compared the number of eggs among fleas feeding on different host species and thus the quality and quantity of feeding resource differed among fleas. It remained unclear whether the trade-off between the quantity and quality of offspring occurs when resources are equal among females such as when they feed not only on the same host species but also on the same host individual at the same time. Furthermore, Parker and Begon (1986) argued that the trade-off between egg size and number may be adaptive and favoured by natural selection not always but rather when environmental conditions affect optimal offspring quality (e.g., size), so that condition-dependent adjustment of the size of the offspring is expected. This adjustment may be beneficial if both mothers and the offspring are exposed, at least potentially, to heterogeneous environments (Fox et al., 1995, 1997; Fox and Czesak, 2000) and not if the environment of mothers and offspring is stable. Regarding parasites, probability to be exposed to heterogeneous environments (i.e., different host species) could be relatively high for host opportunists, whereas it is undoubtedly low for host specialists. Environmental heterogeneity for fleas includes not only variation among host species but also variation among habitats occupied by these hosts. This is because host and habitat identities determine quantity and quality of food resources and patterns of resource acquisition for both adult and pre-imaginal fleas (Krasnov et al., 1997). Ability to adjust offspring size and number in dependence of host species can be beneficial for host-opportunistic fleas. For example, mother fleas feeding on a host with higher autogrooming abilities would benefit from investment into smaller but numerous offspring because smaller fleas are more difficult to dislodge by host's grooming, so higher number of offspring will survive. Investment into larger (although fewer) offspring would be advantageous if, for example, mother fleas exploit hosts dwelling in sandy habitats due to locomotory considerations. Indeed, flea take-off from a mobile sand substrate likely requires a higher energy investment than that from a hard substrate (Krasnov et al., 2003). Jumping ability in fleas (at least, interspecifically) correlates positively with resting metabolic rate which, in turn, correlates positively with larger body size (Krasnov et al., 2004a). Here, we studied relationships between maternal size, egg number and egg size in three flea species that differ in their host specificity and habitat specialization. *Xenopsylla ramesis* is both host and habitat generalist found in many host species in a variety of habitats. *Synosternus cleopatrae* is a host generalist but a habitat specialist exploiting multiple host species but only in sandy habitats. *Parapulex chephrenis* is both host and habitat specialist

parasitic mainly on rodents of genus *Acomys* and found mainly in rocky habitats (see detailed descriptions in Krasnov et al., 1999). We predicted that the trade-off between the number and size of the eggs will be strongest in *X. ramesis*, the weakest in *P. chephrenis* and in a moderate form in *S. cleopatrae*.

Allocation of large amounts of maternal resources into egg number and/or size is beneficial to the offspring because it can improve their survival and/or competitive and reproductive abilities. However, this investment may be costly to mothers because allocating large amounts of resources in a current reproductive bout may reduce their ability to invest in future offspring (Smith and Fretwell, 1974; Smiseth et al., 2014). We tested this trade-off using duration of maternal survival after oviposition, without access to food, as an estimate of further reproductive ability. The trade-off between investments into current versus future reproduction would be manifested by negative relationship between egg number and/or size and time that a female survives without food.

Larger eggs are thought to develop into larger and/or better performing offspring after controlling for confounding effects such as maternal age, microclimatic conditions and maternal care (e.g., Fox, 1994). However, the evidence for this relationship is contradictory (e.g., Wiklund and Karlsson, 1984; Fox, 1993 vs Fox, 1994; Fischer et al., 2003). Here, we also tested whether larger eggs indeed develop into larger offspring, all else [air temperature, humidity, lack of maternal care (see Krasnov, 2008)] being equal. We compared the size of an egg and the size of a new imago developed from this egg. A new imago does not hatch directly from an egg but rather there is a chain of developmental events between these two stages, so that the size of a new imago can be a result of interplay between the size of an egg and larval feeding performance. Consequently, we also measured time until each new imago emerged and examined relationships between egg size and duration of development as well as between duration of development and size of a new imago.

## MATERIALS AND METHODS

### Rodents and fleas

We used rodents and fleas from our laboratory colonies. Details on origin, rearing and maintenance of colonies have been described in earlier publications (e.g., Krasnov et al., 2001, 2003; Khokhlova et al., 2012). Fleas were reared and maintained on their natural rodent hosts, namely *X. ramesis* on *Meriones crassus* and *Gerbillus dasyurus*, *S. cleopatrae* on *Gerbillus andersoni* and *Gerbillus pyramidum*, and *P. chephrenis* on *Acomys cahirinus* and *Acomys russatus*. Every year, we added 100-150 field-collected fleas to each colony to maintain genetic heterogeneity of the colonies. For experimental trials, we randomly selected newly emerged fleas from each colony that were approximately one week old and had never fed. We used male immune-naïve rodents (6-8 mo old) randomly selected from colonies maintained in our lab. To avoid unnecessary sources of variation, experimental trials with fleas of the same species were carried out on the same host individual. Each host belonged to species considered as a characteristic host species for each flea species (see Krasnov et al., 1997, 1999); therefore, *X. ramesis* was tested on *M. crassus*, *S. cleopatrae* on *G. pyramidum* and *P. chephrenis* on *A. russatus*. This study was conducted under permits from Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments (IL-52-07-2012).

### Experimental design and procedures

Each rodent was placed in a separate plastic cage (33 cm by 23 cm by 13 cm) with a floor of 3-5 mm of clean sand covered by a wire mesh. Thirty male and 70 female fleas were released into the cage and allowed to feed for two (*X. ramesis* and *S. cleopatrae*) or three (*P. chephrenis*) days. Under these conditions, fleas take more than one bloodmeal and start to oviposit no sooner than the second day on a host (Khokhlova et al., 2012). We allowed *P. chephrenis* to stay on a host longer than the other two species because it requires more time for engorgement (Khokhlova et al., 2008b). Fleas were collected from both the rodent's body and cage substrate two or three days post-infestation as appropriate for each flea species. To collect fleas from the rodent's body, we brushed its fur over a white plastic pan with a toothbrush several times until no additional fleas were recovered. We then sieved sand from the floor of the rodent's cage to collect fleas from the substrate. After collection, we examined female fleas under light microscopy (40× magnification) and ensure that all of them were fully engorged, that is they had

blood in their midguts. Then, we transferred female fleas into individual Petri dishes (40 mm diameter) and placed them in an incubator (FOC225E, Velp Scientifica srl, Milano, Italy) at 25°C air temperature and 90% relative humidity (RH). Temperature and humidity were monitored using Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, NJ, USA). After 24 hours, we checked Petri dishes, recorded number of eggs produced by each female and measured the maximal length and width of each egg. Measurements were performed on-screen to the nearest 0.01 mm using a digital microscope camera Moticam 2000 with the Motic Images Plus 2.0ML program (Motic, Speed Fair Cp., Ltd., Causeway Bay, Hong Kong) under 40× magnification and calibrated using an object-micrometer. After oviposition, each female was transferred into an individual 500 µl Eppendorf vial filled with 3 mm of sand covering the tip and a perforated lid and placed into an incubator at 25°C and 90% RH (see above). Each vial was then monitored daily until the flea died. After death, the flea was mounted on a slide with the right lateral side facing upward and photographed using camera Moticam 2000 (see above). Then, we measured its maximal body length from frons to the most distal edge of sensillum as described above.

To maximize the chances of pre-imaginal fleas to survive till adulthood, we selected the largest egg among all eggs produced by each female and discarded all remaining eggs. This procedure unlikely introduced bias into the data because the size range of the “largest” egg was within size range of all eggs (Table 1). Then, we added a 2 mm layer of clean sand and larval food medium (94% dry bovine blood, 5% millet flour, and 1% ground excrements of the respective host) to a Petri dish with an egg, covered the dish with a perforated lid and transferred the dish to an incubator at 25°C air temperature and 90% RH. Starting from the 18th day after an egg was produced (approximately one week before metamorphosis; e.g., Khokhlova et al., 2014), we checked each vial daily until a new imago emerged or, in the case that a new imago did not emerge, for 30 consecutive days after the first new imago in each treatment emerged. Upon emergence, a new imago was killed by placing it into 70% ethanol. We then examined each imago under light microscopy to determine its sex, photographed and measured it as described above (measurements did not cover claspers in male new imagoes).



## Statistical analyses

Survival of mother fleas after oviposition was calculated as the number of days from the first oviposition event to death. Egg volume was used as an estimation of egg size and calculated after Berrigan (1991) as  $V = 1/6\pi * W^2 * L$ , where  $V$  is egg volume,  $W$  is maximal egg width and  $L$  is maximal egg length (Berrigan, 1991). Duration of development of each new imago was assessed as the number of days from the day of oviposition until the day of emergence.

Continuous variables used in our analyses (e.g., egg size and body size) were random because they could not be controlled for. Therefore, we tested the relationships between variables using model II (major axis) regressions (Legendre and Legendre, 1998). We applied model II regressions using package “lmodel2” (Legendre, 2014) implemented in R 3.2.3 (R Core Development Team, 2015). Significance of the slopes and correlation coefficients was estimated using permutation test with 9999 permutations.

In model II regression, only one response and one explanatory variable can be accepted. This necessitated running multiple models which, in turn, requested adjustments of alpha-level due to inflated Type I error rate (e.g., Bonferroni correction). However, this approach has been criticized by statisticians and ecologists because it often leads to the incorrect acceptance of the false null hypothesis (Perneger, 1999; Moran, 2003; Garcia, 2004). Nevertheless, we performed sequential Holm-Bonferroni corrections of the significance level using Holm-Bonferroni Sequential Correction: an Excel Calculator (Gaetano, 2013) and present our results with and without these corrections. Prior to the analyses, continuous variables were log- or log+1 transformed. Bivariate normality for each pair of variables was tested with Henze-Zirkler’s multivariate normality tests (Henze and Zirkler, 1990) using package “MVN” (Korkmaz et al., 2014) implemented in R. Each pair of the variables was found to be bivariate normally distributed (HZ statistics=0.22-0.71,  $p > 0.05$  for all).

## RESULTS

Among 70 mother females of each flea species, 57, 48 and 69 *X. ramesis*, *S. cleopatrae* and *P. chephrenis*, respectively, produced eggs (140, 132 and 85, respectively) during 24 hours after removal from the host. Female fleas of each species varied in their body size and the number of eggs they produced (Table 1). Furthermore, egg volume varied both between conspecific females as well as within a clutch (Table 1). Among females, variation in body size was not especially high; nevertheless, it was the lowest in *X. ramesis* and slightly higher in the two remaining species (Table 1). Among-female variation in egg number was similar in the three flea species, while variation in egg size was substantially higher in *S. cleopatrae* than in *X. ramesis* and *P. chephrenis* (Table 1).

Results of the model II (major axis) regressions of the pairwise relationships between maternal body size and egg number, total volume and size and survival time of mothers after oviposition are presented in Table 2. Maternal body size had no effect on number or total volume of eggs produced during one day after uninterrupted stay on a host in *X. ramesis* and *S. cleopatrae* (Table 2). Larger *P. chephrenis* tended to produce more eggs of larger total volume, although significance of these relationships disappeared after Holm-Bonferroni correction of alpha-level (Table 2). Female body size was positively correlated with survival after oviposition. In all three flea species, larger females survived after oviposition longer than smaller females (Table 2, Fig. 1). No trade-off between egg number and their mean volume was found in either flea (Table 2). On the contrary, in *X. ramesis* eggs from larger clutches were, on average, larger than eggs from smaller clutches (Table 2, Fig. 2), although this relationship appeared to be non-significant after corrections of alpha-level (Table 2). Females that produced more eggs tended to die more quickly after oviposition (Table 2, Fig. 3). This trend remained significant after corrections of alpha-level in *X. ramesis* only (Table 2), whereas it was only marginally significant in *P. chephrenis* even prior to Holm-Bonferroni corrections (Table 2).

Some eggs did not develop into new imagoes. There was no significant size differences between eggs that developed into new imagoes and eggs that either did not develop at all or died at larval or pupal stages (t-tests;  $t=0.98$ ,  $t=0.36$  and  $t=0.12$  for *X. ramesis*, *S. cleopatrae* and *P. chephrenis*, respectively;  $p>0.12$  for all).

New imagoes of all flea species demonstrated significant sexual size dimorphism with females being larger than males (t-tests;  $t=6.41$ ,  $t=6.01$  and  $t=9.69$  for *X. ramesis*, *S. cleopatrae*

and *P. chephrenis*, respectively;  $p < 0.001$  for all). However, eggs which developed into females or males did not differ in size (t-tests;  $t = 1.78$ ,  $t = 0.005$  and  $t = 0.33$  for *X. ramesis*, *S. cleopatrae* and *P. chephrenis*, respectively;  $p > 0.08$  for all). Furthermore, pre-imaginal development was shorter in female than in male imagoes of *X. ramesis* (t-tests;  $t = 6.08$ ,  $p < 0.001$ ), but the sexes did not differ in *S. cleopatrae* and *P. chephrenis* (t-tests;  $t = 1.30$  and  $t = 0.81$ , respectively;  $p > 0.20$  for both).

We observed significant relationships between egg size and duration of preimaginal development in female but not in male fleas of all three species (Table 3). Larger eggs developed faster than smaller eggs (Fig. 4). Body size of new imagoes correlated negatively with duration of development for females but not for males. In other words, faster developing eggs developed into larger new imagoes. However, this was true in *X. ramesis* and *S. cleopatrae* but not *P. chephrenis* (Fig. 5).

## DISCUSSION

We did not find any relationships between female flea size and mean egg volume. Positive effects of maternal size on egg size occur in some species of free-living arthropods but not others (see Fox and Czesak, 2000 for review). Such relationships also appear to be largely absent in blood-sucking dipterans (e.g., Bradshaw et al., 1993). One reason for these contrasting among-taxa patterns could be the provisioning of resources for egg maturation (Fox and Czesak, 2000). In some taxa, the main basis of resources for egg production is adult feeding (e.g., mosquitoes, lepidopterans with feeding imagoes), whereas in other taxa these resources mainly originate from larval feeding (e.g., lepidopterans with nonfeeding imagoes) (Hebert, 1983). As a result, the size of the eggs in the former depends more strongly on maternal diet rather than on maternal size (e.g., Steinwascher, 1984), whereas the opposite is true for the latter. Resources allocated for reproduction in fleas undoubtedly come from adult feeding. In fact, eggs do not mature until a female flea takes a blood meal (see Krasnov, 2008 for review). Moreover, multiple blood meals are needed for egg maturation in many flea species (Krasnov et al., 2004b; Krasnov, 2008). The lack of a positive relationship between maternal size and egg size in fleas could also be due to host defence efforts. For example, host grooming disrupts flea feeding, so that the engorgement rate of fleas exploiting grooming-restrained rodents was 4.5 times higher than that of fleas exploiting grooming-allowed rodents (Hawlena et al., 2007b). Larger ectoparasites are likely subjected more to host grooming than their smaller counterparts because they (a) are more conspicuous, (b) may inject higher amount of allergen-containing saliva in the bite wound, so that irritation caused by their bite is stronger (Lee et al., 1999) and (c) may be more easily removed by a host. As a result, larger fleas could lay smaller and/or fewer eggs than they are potentially able to produce if host defence efforts were neutralized. We also did not find any effect of maternal size on egg number or total volume except for a weak positive trend in *P. chephrenis* that appeared to be non-significant after the adjustment of alpha-level. This lack of maternal effect on the egg number/clutch volume could be explained by the same reason as the lack of the relationship between maternal size and egg size (i.e., host defence effort). In addition, larger female fleas could choose not to produce more eggs than smaller females at a given time because they preferred to invest a smaller proportion of their resources into current reproduction (Ellers and Jervis, 2003; Berger et al., 2012). On one hand, their reproductive effort is not as limited by their body reserves than that of smaller females. On the other hand, this would allow

them to overcome possible ecological limitations for reproduction (e.g., fluctuations in microclimate or host availability) (Berger et al., 2008, 2012).

Although a trade-off between the egg number and the size of new imagoes in *X. ramesis* was demonstrated in earlier experiments (Khokhlova et al., 2014), we did not find any trade-off between the number and size of eggs in the current study. On the contrary, in one of the three flea species (*X. ramesis*), larger clutches were composed of larger eggs. The contradiction between our current results and those of the earlier study (Khokhlova et al., 2014) could be caused by at least three main, though not mutually exclusive, reasons. Firstly, experimental designs of Khokhlova et al. (2014) and this study were substantially different. In particular, the occurrence of the trade-off between quantity and quality of offspring of *X. ramesis* in Khokhlova et al. (2014) was found when mother fleas exploited different host individuals belonging to different species, whereas in this study they fed not only on the same host species but also on the same host individual. As a result, variation in egg number among females was about 50% higher in Khokhlova's et al. (2014) experiments than in this study [0.66 (calculated from data of Khokhlova et al. (2014) versus 0.40, respectively] resulting in the occurrence of the trade-off between the number of eggs and size of new imago in the former and the lack of the trade-off between egg number and size in the latter. Secondly, the trade-off between egg number and size of new imagoes, but not egg size, suggests some delayed effects of postnatal development such as variation in larval growth (Fox and Czesak, 2000; Steiger, 2013). Finally, larvae in Khokhlova et al. (2014) were maintained in groups (from the same mother), whereas larvae were maintained individually in this study. Obviously, a higher number of eggs resulted in a higher number of larvae in a group in Khokhlova's et al. (2014) design, presumably increasing among-larva competition for food (Khokhlova et al., 2010b) which, in turn, might result in smaller size of each individual new imago. Comparison of the results of Khokhlova et al. (2014) and this study supports the idea that the trade-off between progeny size and number is not universal. Indeed, evidence for this trade-off in arthropods is conflicting and 11 out of 39 studies on this subject did not support the contention that progeny size is inversely related to number of offspring (Fox and Czesak, 2000). Furthermore, our results suggest that (a) the occurrence of this trade-off may be context-dependent even in the same species (e.g., manifested in heterogenous environment and not manifested in stable environment) and (b) manifestation of the trade-off may depend on the

stage of reproductive cycle being considered (e.g., trade-off between number of eggs and their size versus trade-off between number of eggs and size of new imago).

We found a strong effect of body size on the ability of an adult female to survive without feeding after oviposition with larger females surviving longer. Longer survival suggests that a larger female may have more opportunities for future reproduction and, thus, higher potential life-time fecundity than a smaller female (Berger et al., 2008). In other words, large female body size was not translated into immediate fitness benefits, but allowed a female to increase her fitness in future. This also suggests that larger females invested mostly into future, rather than current, reproduction (Berger et al., 2012). Indeed, investment into current reproduction decreased the likelihood of future reproduction as indicated by the negative relationship between number of eggs and duration of post-oviposition survival in all three flea species. Female fleas used in our study were newly emerged and did not reproduce prior to experimental treatments. In several flea species, including some *Xenopsylla*, fecundity has been found to vary with age (Cooke, 1990) reaching peak when a flea is 6-20 d old and decreasing sharply in fleas older than 31 d (Korneeva and Sadovenko, 1990; see Krasnov, 2008 for additional references). This suggests that decreased investment into first clutches might enhance the reproductive output at the peak reproduction. For example, female *Xenopsylla skrjabini* produced, on average, 5.5 eggs/d until 5 d of age and 8.0 eggs/d at the age of 6-20 d (about 30% increase in egg production; Korneeva and Sadovenko, 1990). Assuming similar pattern in *X. ramesis*, though this has never been specifically studied, a female investing heavily into early reproduction (e.g., 3-4 eggs/clutch) will likely produce 10-28 eggs less during its lifetime than a female with the first clutch of 1-2 eggs (on average, survival was 12-16 d vs 22-30 d, respectively). Although this calculation could be not highly accurate, our results suggest that flea offspring resulted from the first reproductive event of a mother have relatively low value relative to future reproductive prospects (Coleman and Gross, 1991).

The lack of sexual differences in egg size but not in new imago size suggests that larger eggs do not necessarily develop into large offspring. This is because the relationship between egg size and a new offspring size in holometabolous insects is mediated by changes during larval stage. In particular, the size of a new imago is determined by the size of a larva immediately prior to pupation which, in turn, depends on the size of the first instar larvae, its growth rate, critical weight and hormone-associated processes (Chapman, 1998; D'Amico et al., 2001; Davidowitz et

al., 2003). The fact that females and males developed from similarly sized eggs suggests that the first instar female and male larvae were, on average, similar in their body size too. It is unknown whether maternal fleas invest differently into female and male eggs in terms of egg composition. Assuming that this investment is similar, the main reason for sexual size dimorphism exhibited by imago fleas is thus differential larval growth. Although this has never been studied in fleas, other holometabolous insects with female-biased sexual size dimorphism exhibit this type of larval growth (Etilé and Despland, 2008). For example, female larvae possess significantly higher growth rates than male larvae in three mosquito species (Clark et al., 2004). However, differential growth rate of male and female larvae may vary due to environmental factors such as climate and seasonality of reproduction (e.g., Gotthard et al., 1994). Higher growth rate of female larvae may result in shorter development time (Vaughan and Coombs, 1979; Amin et al., 1993; *X. ramesis* in this study) which, in turn, is at least partially responsible for female-bias sex ratios in populations of many flea species (see Krasnov, 2008 for review). In addition, intersexual differences in growth rate may cause sexual size differences to appear at later than egg stages of pre-imaginal development (Castillo et al., 2015).

Among female fleas, larger eggs developed faster than smaller eggs in all studied species and, in two of the three species, faster-developing pre-imagines grew into larger adults. Seed beetles exhibit a similar pattern where offspring from larger eggs develop faster and attain larger adult body size than offspring from smaller eggs (Fox, 1994). The net result of these patterns, at least in *X. ramesis* and *S. cleopatrae*, was thus that larger new females were born from larger eggs. The mechanism behind this pattern could be associated with the positive correlation between the size of the first instar larvae and the size of a new adult (D'Amico et al., 2001). Larger first instar larvae hatch from larger eggs (e.g., Boivin and Gauvin, 2008) and they likely require less time to attain a critical weight prior to pupation than smaller larvae. In addition, larger larvae could also be more efficient foragers and assimilate resources faster than smaller larvae (Fox, 1994), or outcompete (Khokhlova et al., 2010b) and even cannibalize them (Lawrence and Foil, 2002).

Proximate reasons for the occurrence of these post-natal patterns in female but not in male fleas remain unclear and deserve further investigation. Ultimately, this might be associated with stronger selection pressure on larger body size in females than in males because larger body size in female fleas may result in higher life-time fecundity (Henry et al., 2009; Chown and Gaston,

2010; this study), whereas larger body size in males does not necessarily lead to fitness benefits such as higher abilities to overcome mate competition (Kelly et al., 2008).

In conclusion, the patterns found in this study were to a great extent consistent among the three flea species. This suggests that these patterns result from the same mechanisms and thus could be characteristic for the majority of flea species because of similarity in their life history (Krasnov, 2008) and unaffected by the level of ecological specialization of a given species.



## **Acknowledgements**

We thank Michal Segoli for helpful discussions. We thank three anonymous referees for helpful comments on the earlier version of the manuscript. BRK is incumbent of Benny Slome Chair for Wildlife Management and Conservation. This is publication no. XXX of the Mitrani Department of Desert Ecology.

## **Competing interests**

The authors declare no competing financial interests.

## **Funding**

This study was supported by the Israel Science Foundation (Grant no. 26/12 to BRK and ISK). DK received financial support from the Blaustein Center for Scientific Cooperation. EMW received financial support from the United States-Israel Educational Foundation (USIEF; Fulbright Post-Doctoral Fellowship) and the Swiss Institute for Dryland Environmental and Energy Research.

## **Author contributions**

BRK, ISK and DK conceived and designed the experiments, DK conducted the experiments, BRK and EMW analysed data, all authors drafted the paper.

## References

- Amin, O.M., Liu, J., Li, S.-J., Zhang, Y.-M. and Sun, L.-Z.** (1993). Development and longevity of *Nosopsyllus laeviceps kuzenkovi* (Siphonaptera) from Inner Mongolia under laboratory conditions. *J. Parasitol.* **79**, 193-197.
- Berger, D., Walters, R. and Gotthard, K.** (2008). What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. *Funct. Ecol.* **22**, 523-529.
- Berger, D., Olofsson, M., Friberg, M., Karlsson, B., Wiklund, C. and Gotthard, K.** (2012). Intraspecific variation in body size and the rate of reproduction in female insects – adaptive allometry or biophysical constraint? *J. Anim. Ecol.* **81**, 1244-1258.
- Berrigan, D.** (1991). The allometry of egg size and number in insects. *Oikos* **60**, 313-321.
- Boivin, G. and Gauvin, M.-J.** (2008). Egg size affects larval performance in a coleopteran parasitoid. *Ecol. Entomol.* **34**, 240-245.
- Bradshaw, W.E., Holzapfel, C.M. and O’Neill, T.** (1993). Egg size and reproductive allocation in the pitcherplant mosquito *Wyeomyia smithii* (Diptera, Culicidae). *J. Med. Entomol.* **30**, 384-390.
- Castillo, A., Serrano, A.K., Mikery, O.F. and Pérez, J.** (2015). Life history of the sand fly vector *Lutzomyia cruciata* in laboratory conditions. *Med. Vet. Entomol.* **29**, 393-402.
- Chapman, R.F.** (1998). *The Insects: Structure and Function*. Cambridge: Cambridge University Press.
- Chown, S.L. and Gaston, K.J.** (2010). Body size variation in insects: a macroecological perspective. *Biol. Rev.* **85**, 139-169.
- Clark, T.M., Flis, B.J. and Remold, S.K.** (2004). Differences in the effects of salinity on larval growth and developmental programs of a freshwater and a euryhaline mosquito species (Insecta: Diptera, Culicidae). *J. Exp. Biol.* **207**, 2289-2295.
- Coleman, R.M. and Gross, M.R.** (1991) Parental investment theory: the role of past investment. *Trends Evol. Ecol.* **6**, 404-406.
- Cooke, B.D.** (1990). Notes on the comparative reproductive biology and the laboratory breeding of the rabbit flea *Xenopsylla cunicularis* Smit (Siphonaptera, Pulicidae). *Austral. J. Zool.* **38**, 527-534.

- Corkum, L.D., Ciborowski, J.J.H. and Poulin, R.G.** (1997). Effects of emergence date and maternal size on egg development and sizes of eggs and first-instar nymphs of a semelparous aquatic insect. *Oecologia* **111**, 69-75.
- D'Amico, L.J., Davidowitz, G. and Nijhout, H.F.** (2001). The developmental and physiological basis of body size evolution in an insect. *Proc. Roy. Soc. Lond. B* **268**, 1589-1593.
- Davidowitz, G., D'Amico, L.J. and Nijhout, H.F.** (2003). Critical weight in the development of insect body size. *Evol. Devel.* **5**, 188-197.
- Ellers, J. and Jervis, M.** (2003). Body size and the timing of egg production in parasitoid wasps. *Oikos* **102**, 164-172.
- Etilé, E. and Despland, E.** (2008). Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. *Oikos* **117**, 135-143.
- Fielden, L.J., Rechay, Y. and Bryson, N.R.** (1992). Acquired immunity to larvae of *Amblyomma marmoreum* and *A. hebraeum* by tortoises, guinea-pigs and guinea-fowl. *Med. Vet. Entomol.* **6**, 251-254.
- Fischer, K., Brakefield, P.M. and Zwaan, B.J.** (2003). Plasticity in butterfly egg size: Why larger offspring at lower temperatures? *Ecology* **84**, 3138-3147.
- Fox, C.W.** (1993). The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia* **96**, 139-146.
- Fox, C.W.** (1994). The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. *Oikos* **71**, 321-325.
- Fox, C.W. and Czesak, M.E.** (2000). Evolutionary ecology of progeny size in arthropods. *Ann. Rev. Entomol.* **45**, 341-369.
- Fox, C.W., Waddell, K.J. and Mousseau, T.A.** (1995). Parental host plant affects offspring life histories in a seed beetle. *Ecology* **76**, 402-411.
- Fox, C.W., Thakar, M.S. and Mousseau, T.A.** (1997). Egg size plasticity in a seed beetle: an adaptive maternal effect. *Amer. Nat.* **149**, 149-163.
- Gaetano, J.** (2013). Holm-Bonferroni sequential correction: An EXCEL calculator (1.1) [Microsoft Excel workbook]. [https://www.researchgate.net/publication/236969037\\_Holm-Bonferroni\\_Sequential\\_Correction\\_An\\_EXCEL\\_Calculator](https://www.researchgate.net/publication/236969037_Holm-Bonferroni_Sequential_Correction_An_EXCEL_Calculator). doi:10.13140/RG.2.1.4466.9927

- García, L.V.** (2004). Escaping the Bonferroni iron claw in ecological studies. *Oikos* **105**, 657-663.
- Gotthard, K., Nylin, S. and Wiklund, C.** (1994). Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia* **99**, 281-289.
- Hawlena, H., Bashary, D., Abramsky, Z. and Krasnov, B.R.** (2007a). Benefits, costs and constraints of anti-parasitic grooming in adult and juvenile rodents. *Ethology* **113**, 394-402.
- Hawlena, H., Abramsky, Z., Krasnov, B.R. and Saltz, D.** (2007b). Host defence versus intraspecific competition in the regulation of intrapopulations of the flea *Xenopsylla conformis* on its rodent host *Meriones crassus*. *Int. J. Parasitol.* **37**, 919-925.
- Hebert, P.D.N.** (1983). Egg dispersal patterns and adult feeding behaviour in the Lepidoptera. *Can. Entomol.* **115**, 1477-1481.
- Hendry, A.P., Day, T. and Cooper, A.B.** (2001). Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *Amer. Nat.* **157**, 387-407.
- Henry, L.M., Ma, B.O. and Roitberg, B.D.** (2009). Size-mediated adaptive foraging: a host-selection strategy for insect parasitoids. *Oecologia* **161**, 433-445.
- Henze, N. and Zirkler, B.** (1990). A class of invariant consistent tests for multivariate normality. *Commun. Stat. Theor. Meth.* **19**, 3595-3618.
- Honěk, A.** (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**, 483-492.
- Kelly, C.D., Bussière, L.F. and Gwynne, D.T.** (2008). Sexual selection for male mobility in a giant insect with female-biased size dimorphism. *Am Nat.* **172**, 417-423.
- Khokhlova, I.S., Ghazaryan, L., Krasnov, B. R. and Degen, A.A.** (2008a). Does acquired resistance of rodent hosts affect metabolic rate of fleas? *J. Exp. Zool. A* **311**, 389-398.
- Khokhlova, I.S., Ghazaryan, L., Krasnov, B. R. and Degen, A.A.** (2008b). Effects of parasite specificity and previous infestation of hosts on the feeding and reproductive success of rodent-infesting fleas. *Funct. Ecol.* **22**, 530-536.
- Khokhlova, I.S., Ghazaryan, L., Degen, A.A. and Krasnov, B.R.** (2010a). Infestation experience of a rodent host and offspring viability of fleas: variation among host-parasite associations. *J. Exp. Zool. A* **313**, 680-689.

- Khokhlova, I.S., Hovhanyan, A., Degen, A.A. and Krasnov, B.R.** (2010b). The effect of larval density on pre-imaginal development in two species of desert fleas. *Parasitology* **137**, 1925-1935.
- Khokhlova, I.S., Fielden, L.J., Degen, A.A. and Krasnov, B.R.** (2012) Ectoparasite fitness in auxiliary hosts: Phylogenetic distance from a principal host matters. *J. Evol. Biol.* **25**, 2005-2013.
- Khokhlova, I.S., Pulosof, S., Fielden, L.J., Degen, A.A. and Krasnov, B.R.** (2014). A trade-off between quantity and quality of offspring in haematophagous ectoparasites: the effect of the level of specialization. *J. Anim. Ecol.* **83**, 397-405.
- Koch, L. and Meunier, J.** (2014). Mother and offspring fitness in an insect with maternal care: phenotypic trade-offs between egg number, egg mass and egg care. *BMC Evol. Biol.* **14**, 125.
- Korkmaz, S., Goksuluk, D. and Zararsiz, G.** (2014) MVN: An R package for assessing multivariate normality. *The R J.* **6**, 151-162.
- Korneeva, L.A. and Sadovenko, E.V.** (1990). Patterns of development of the fleas of the great gerbils in the laboratory. In *Advantages of Medical Entomology and Acarology in USSR* (ed. G. S. Medvedev), pp. 12-14. Leningrad, USSR: All-Union Entomological Society and Zoological Institute, Academy of Sciences of USSR (in Russian).
- Krasnov, B.R.** (2008). *Functional and Evolutionary Ecology of Fleas. A Model for Ecological Parasitology*. Cambridge: Cambridge University Press.
- Krasnov, B.R., Shenbrot, G.I., Medvedev, S.G., Vatschenok, V.S. and Khokhlova, I.S.** (1997). Host-habitat relation as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the Negev Desert. *Parasitology* **114**, 159-173.
- Krasnov, B.R., Hastriter, M., Medvedev, S.G., Shenbrot, G.I., Khokhlova, I.S. and Vatschenok, V.S.** (1999) Additional records of fleas (Siphonaptera) on wild rodents in the southern part of Israel. *Israel J. Zool.* **45**, 333-340.
- Krasnov, B.R., Khokhlova, I.S., Fielden, L.J. and Burdelova, N.V.** (2001). The effect of air temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: Pulicidae). *J. Med. Entomol.* **38**, 629-637.
- Krasnov, B.R., Burdelov, S.A., Khokhlova, I.S. and Burdelova, N.V.** (2003). Sexual size dimorphism, morphological traits and jump performance in seven species of desert fleas (Siphonaptera). *J. Zool. Lond.* **261**, 181-189.

- Krasnov, B.R., Khokhlova, I.S., Burdelov, S.A. and Fielden, L.J.** (2004a). Metabolic rate and jump performance in seven species of desert fleas. *J. Insect Physiol.* **50**, 149-156.
- Krasnov, B.R., Khokhlova, I.S., Burdelova, N.V., Mirzoyan, N.S. and Degen, A.A.** (2004b). Fitness consequences of density-dependent host selection in ectoparasites: testing reproductive patterns predicted by isodar theory in fleas parasitizing rodents. *J. Anim. Ecol.* **73**, 815-820.
- Krist, M.** (2011). Egg size and offspring quality: a meta-analysis in birds. *Biol. Rev. Camb. Phil. Soc.* **86**, 692-716.
- Lack, D.** (1947). The significance of clutch-size. *Ibis* **89**, 302-352.
- Lawrence, W. and Foil, L.D.** (2002). The effect of diet upon pupal development and cocoon formation by the cat flea (Siphonaptera: Pulicidae). *J. Vector Ecol.* **27**, 39-43.
- Lee, S.E., Johnstone, I.P., Lee, R P. and Opdebeeck, J.P.** (1999). Putative salivary allergens of the cat flea, *Ctenocephalides felis*. *Vet. Immunol. Immunopathol.* **69**, 229-237.
- Legendre, P.** (2014). lmodel2: Model II Regression. R package version 1.7-2. <http://CRAN.R-project.org/package=lmodel2>
- Legendre, P. and Legendre, L.** (1998) *Numerical Ecology*, 2<sup>nd</sup> edn. Amsterdam: Elsevier.
- Moran, M.D.** (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**, 403-405.
- Parker, G.A. and Begon, M.** (1986). Optimal egg size and clutch size - effects of environment and maternal phenotype. *Amer. Nat.* **128**, 573-592.
- Perneger, T.V.** (1999). Adjusting for multiple testing in studies is less important than other concerns. *British Med. J.* **318**, 1288.
- Poulin, R.** (1995). Clutch size and egg size in free-living and parasitic copepods: a comparative analysis. *Evolution* **49**, 325-336.
- Poulin, R. and Morand, S.** (1997). Parasite body size distributions: interpreting patterns of skewness. *Int. J. Parasitol.* **27**, 959-964.
- R Development Core Team.** (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Räsänen, K., Söderman, F., Laurila, A. and Merilä, J.** (2008) Geographic variation in maternal investment: acidity affects egg size and fecundity in *Rana arvalis*. *Ecology* **89**, 2553-2562.

- Rechav, Y and Fielden, L. J.** (1995). The effect of host resistance on the metabolic rate of engorged females of *Rhipicephalus evertsi evertsi*. *Med. Vet. Entomol.* **9**, 289-292.
- Rechav, Y and Fielden, L.J.** (1997). The effect of various host species on the feeding performance of immature stages of the tick *Hyalomma truncatum* (Acari: Ixodidae). *Exp. Appl. Acarol.* **21**, 551-559.
- Reiss, M.J.** (1989). *The Allometry of Growth and Reproduction*. Cambridge: Cambridge University Press.
- Roff, D.A.** (1992). *The Evolution of Life Histories*. NY: Chapman & Hall.
- Smiseth, P.T., Andrews, C.P., Matthey, S.N. and Mooney, R.** (2014). Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle. *J. Zool.* **293**, 80-83.
- Smith, C.C. and Fretwell, S.D.** (1974). Optimal balance between size and number of offspring. *Amer. Nat.* **108**, 499-506.
- Stearns, S.C.** (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.
- Steinwascher, K.** (1984). Egg size variation in *Aedes aegypti*: relationship to body size and other variables. *Am. Midl. Nat.* **112**, 76-84.
- Steiger, S.** (2013). Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proc. Roy. Soc. B.* **280**, 1225.
- Vaughan, J.A. and Coombs, M.E.** (1979). Laboratory breeding of the European rabbit flea, *Spilopsyllus cuniculi* (Dale). *J. Hyg.* **83**, 521-530.
- Wiklund, C. and Karlsson, B.** (1984). Egg size variation in satyrid butterflies: adaptive vs historical, “Bauplan”, and mechanistic explanations. *Oikos* **43**, 391-400.

## Tables

Table 1. Variation in female body size (mm), egg number and egg volume (mm<sup>3</sup>) among conspecific females and within a clutch in three flea species. *Xr* – *X. ramesis*, *Sc* – *S. cleopatrae*, *Pc* – *P. chephrenis*, CV – coefficient of variation.

Species	Variable	Source of variation	Minimum	Maximum	CV
<i>Xr</i>	Body size	Among females	1.27	1.72	0.06
	Egg number	Among females	1	5	0.40
	Egg volume	Among females	0.015	0.022	0.08
		Within a clutch	0.015-0.020	0.015-0.022	0.003-0.12
<i>Sc</i>	Body size	Among females	1.31	2.20	0.08
	Egg number	Among females	1	6	0.42
	Egg volume	Among females	0.002	0.03	0.44
		Within a clutch	0.002-0.028	0.019-0.03	0.001-0.68
<i>Pc</i>	Body size	Among females	1.31	2.25	0.10
	Egg number	Among females	1	3	0.39
	Egg volume	Among females	0.007	0.02	0.12
		Within a clutch	0.007-0.14	0.007-0.02	0.001-0.45



Table 2. Results of the model II (major axis) regressions of the pairwise relationships between maternal body size (MB), total egg volume (TEV), egg number (EN) and size (ES) and survival time of mothers after oviposition (ST) in three flea species. Asterisks denote relationships that remained significant after Holm-Bonferroni correction of alpha-level.

Species	Response variable	Explanatory variable	$r$	Intercept	Slope	$p$
<i>X. ramesis</i>	MB	EN	-0.13	33.07	-80.66	0.16
	MB	TEV	-0.12	0.05	-0.07	0.18
	MB	ST	0.33	-5.74	17.5	0.007*
	EN	ES	0.24	0.0007	0.0008	0.03
	EN	ST	-0.29	1.43	-0.22	0.01*
<i>S. cleopatrae</i>	MB	EN	-0.06	11.34	-103.84	0.26
	MB	TEV	0.02	0.021	0.017	0.45
	MB	ST	0.39	-6.23	17.58	0.001*
	EN	ES	0.13	0.009	0.009	0.08
	EN	ST	-0.15	1.89	-1.14	0.04
<i>P. chephrenis</i>	MB	EN	0.19	0.21	10.82	0.03
	MB	TEV	0.26	-0.003	0.01	0.02
	MB	ST	0.29	-0.32	4.52	0.002*
	EN	ES	0.12	0.005	0.0007	0.12
	EN	ST	-0.15	0.94	-0.22	0.07

Table 3. Results of the model II (major axis) regressions of the pairwise relationships between egg size (ES), duration of development (DD) and new imago size (NIS) in three flea species. F – female offspring, M – male offspring. Asterisks denote relationships that remained significant after Holm-Bonferroni correction of alpha-level.

Species	Response variable	Explanatory variable	Offspring sex	$r$	Intercept	Slope	$p$
<i>X. ramesis</i>	DD	ES	F	-0.44	2.89	-173.32	0.003*
			M	0.13	-1.08	336.70	0.27
	NIS	DD	F	-0.65	0.83	-0.28	0.001*
			M	0.07	0.35	0.03	0.37
<i>S. cleopatrae</i>	DD	ES	F	-0.54	1.94	-34.04	0.008*
			M	-0.42	2.38	75.79	0.09
	NIS	DD	F	-0.57	2.95	-1.55	0.01*
			M	-0.24	0.87	-0.28	0.22
<i>P. chephrenis</i>	DD	ES	F	-0.49	2.31	-122.83	0.01*
			M	-0.18	2.38	-133.54	0.37
	NIS	DD	F	0.07	0.37	0.07	0.38
			M	-0.23	1.00	-0.34	0.08

## Figures

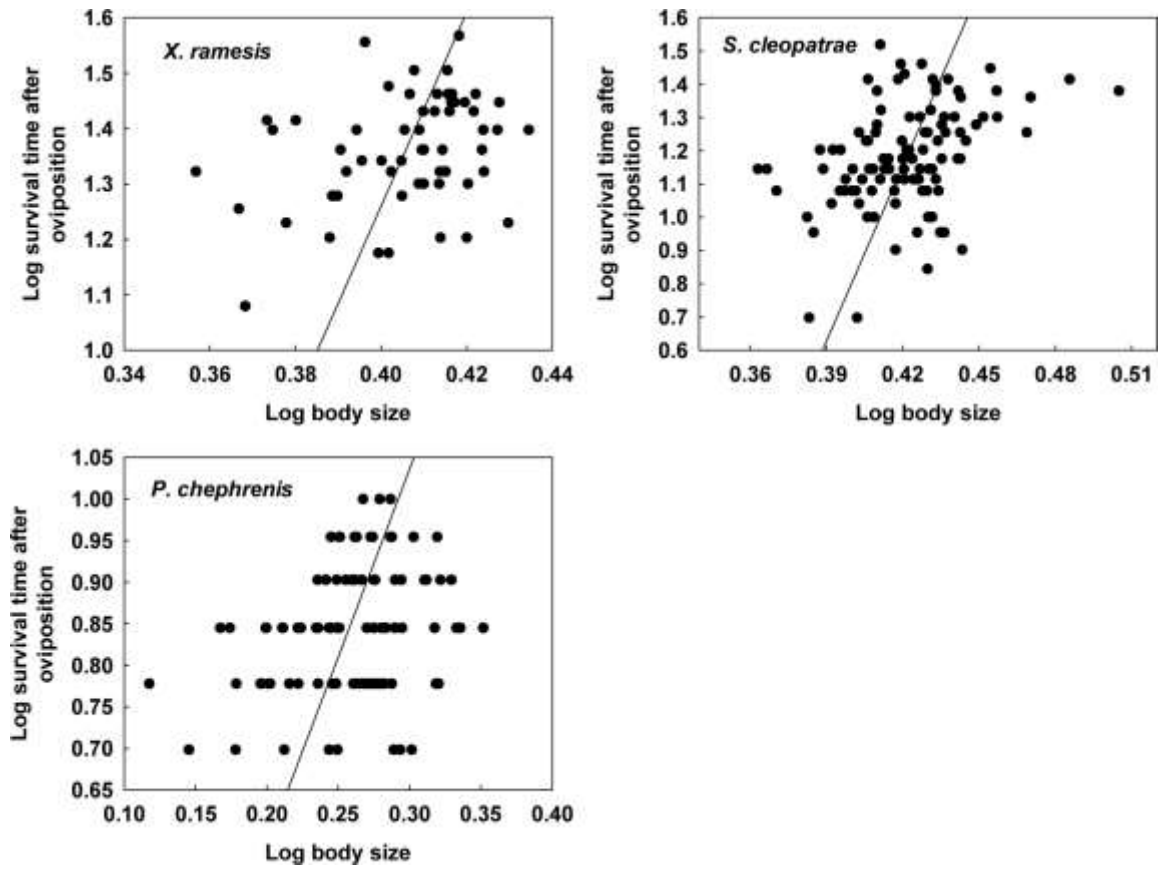


Fig 1. Relationships between female body size and time of survival after oviposition in three flea species. Fitted functions are model II (major axis) regression lines.

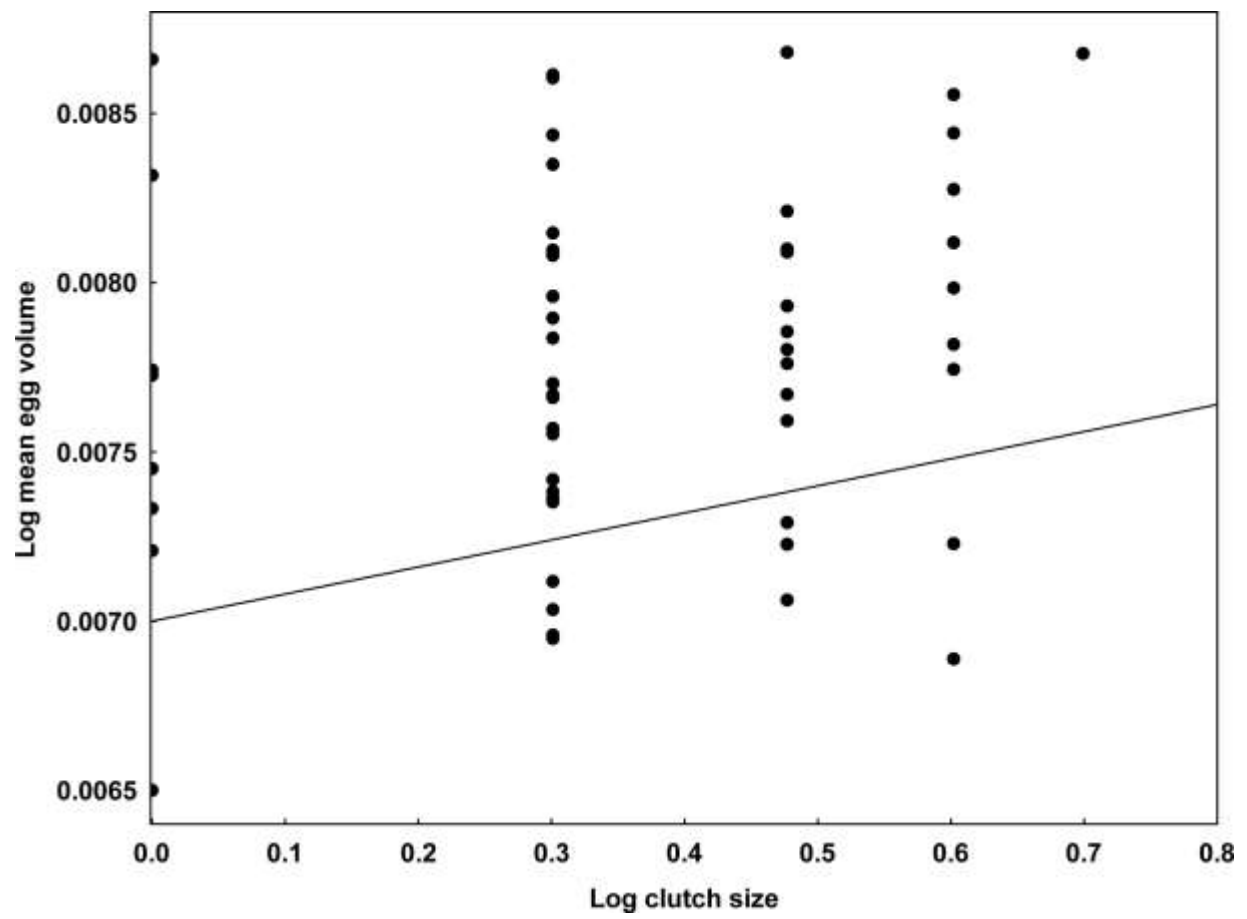


Fig 2. Relationships between clutch size and mean egg volume in *X. ramesis*. Fitted function is model II (major axis) regression line.

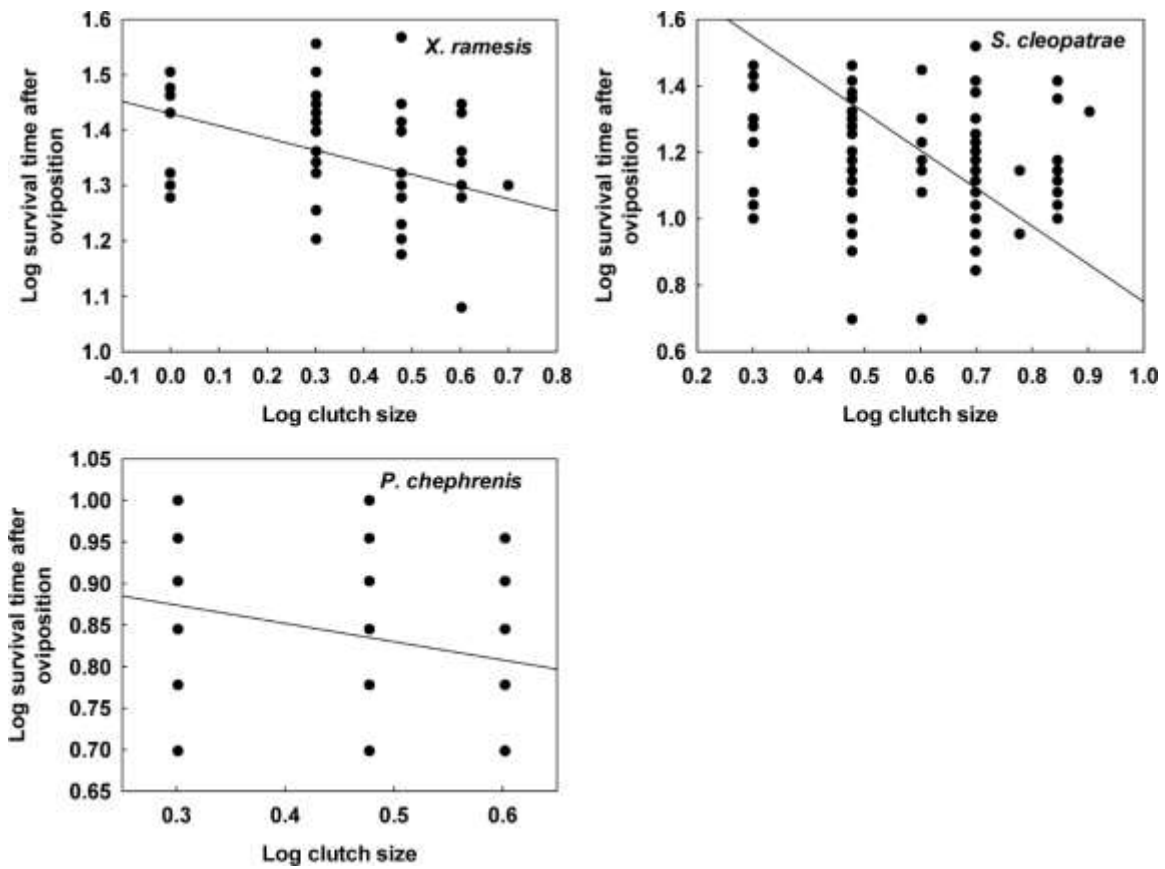


Fig 3. Relationships between clutch size and time of survival after oviposition in three flea species. Fitted functions are model II (major axis) regression lines.

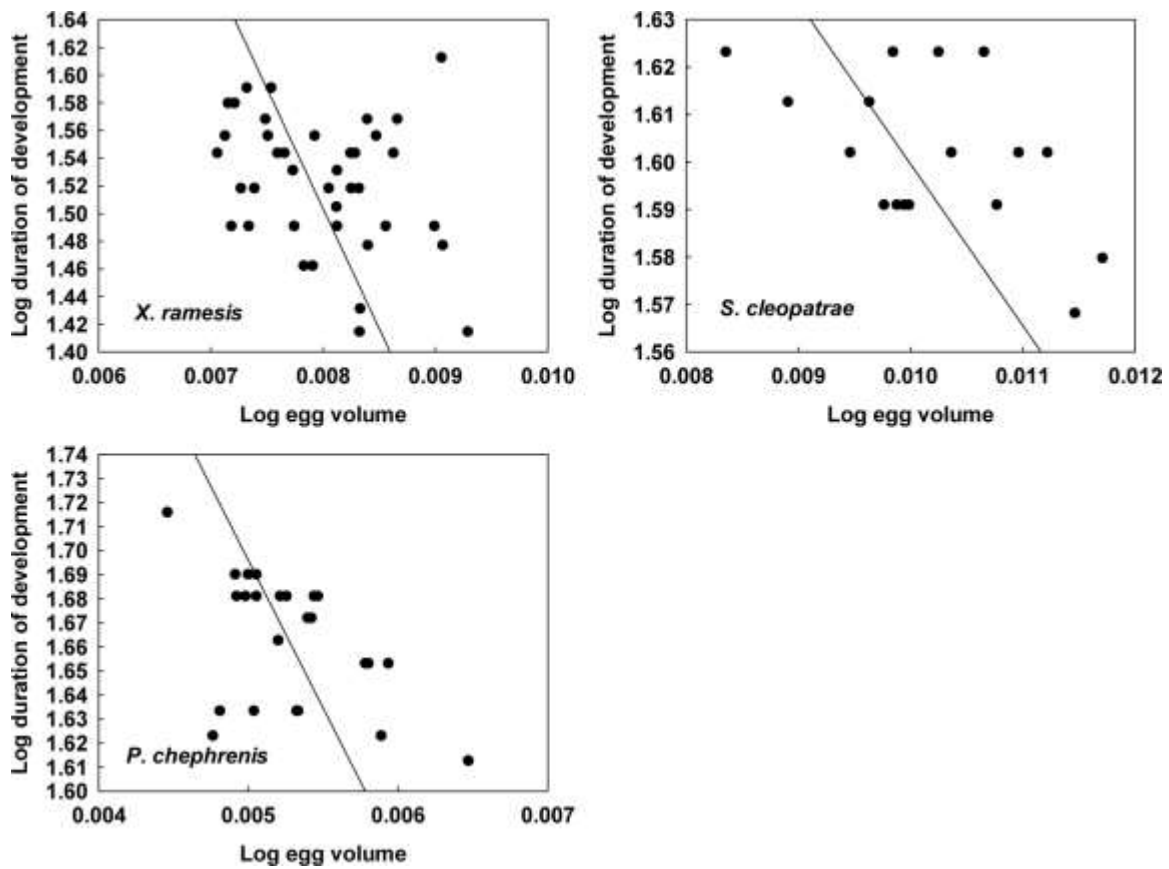


Fig. 4. Relationships between egg volume and its duration of development till a new imago hatches in female *X. ramesis* and *S. cleopatrae*. Fitted functions are model II (major axis) regression lines.

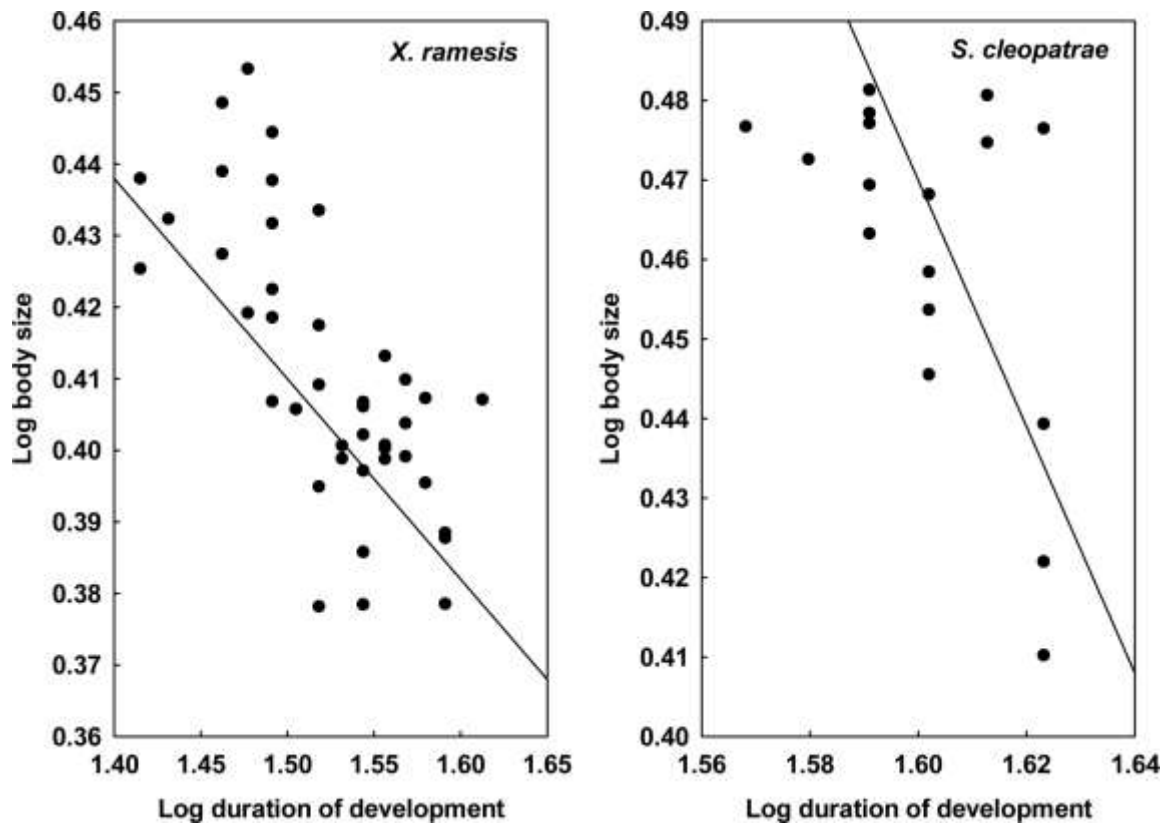


Fig. 5. Relationships between duration of development and body size of a new female imago in three flea species. Fitted functions are model II (major axis) regression lines.