

## RESEARCH ARTICLE

# Reproductive consequences of female size in haematophagous ectoparasites

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## ABSTRACT

To test relationships between maternal size, egg size and size of new offspring, we studied (a) the effect of maternal size on egg size and number, and maternal survival after oviposition and (b) the effect of egg size on the 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, the 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 a 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 a 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 the number of eggs (Lack, 1947; Smith and Fretwell, 1974; Parker and Begon, 1986; Reiss, 1989; Honěk, 1993). On the 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 probably 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, the 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. 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 the size of their eggs have been investigated (e.g. Poulin, 1995). One of the sharpest differences between the 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 they are likely to 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 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 for suppressed ectoparasite reproduction on resistant hosts could be an increase in mass-specific metabolic rate of individual parasites exploiting resistant as compared with immune-naïve hosts (Rechav and Fielden, 1995), which, in turn, might result from a higher metabolic cost of blood digestion when the source of this blood was a resistant rather than immune-naïve host (Khokhlova et al., 2008a, 2010a). Given the positive intraspecific allometry of metabolic rate in ectoparasitic arthropods (e.g. Rechav and Fielden, 1995), this effect may be more pronounced in larger than

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in smaller females. As a result, the positive relationship between the size of a female and the 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 that are most abundant and diverse on small and medium-sized burrowing mammals. These insects are holometabolous with pre-imaginal development occurring mainly off-host in a 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 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 the 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 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 of exposure 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 the quantity and quality of food resources and patterns of resource acquisition for both adult and pre-imaginal fleas (Krasnov et al., 1997). The ability to adjust offspring size and number dependent on the 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 in smaller but numerous offspring because smaller fleas are more difficult to dislodge by grooming, so a higher number of offspring will survive. Investment in larger (although fewer) offspring would be advantageous if, for example, mother fleas exploit hosts dwelling in sandy habitats because of locomotory considerations. Indeed, flea take-off from a mobile sand substrate probably 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* (Rothschild 1904) is both host and habitat generalist, found in many host species in a variety of habitats. *Synosternus cleopatrae* (Rothschild 1903) is a host generalist but a habitat specialist, exploiting multiple host species but only in sandy habitats. *Parapulex chephrenis* (Rothschild 1903) is both a host and a habitat specialist parasitic mainly on rodents of the 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*, weakest in *P. chephrenis* and in a moderate form in *S. cleopatrae*.

Allocation of large amounts of maternal resources to 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 investment in current versus future reproduction would be manifested by a negative relationship between egg number and/or size and the duration 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 versus 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 the 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 1 week old and had never fed. We used male immune-naïve rodents (6–8 months 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

**Table 1. Variation in female body size, egg number and egg volume among conspecific females and within a clutch in three flea species**

Species	Variable	Source of variation	Minimum	Maximum	CV
<i>X. ramesis</i>	Body size (mm)	Among females	1.27	1.72	0.06
	Egg number	Among females	1	5	0.40
	Egg volume (mm <sup>3</sup> )	Among females	0.015	0.022	0.08
<i>S. cleopatrae</i>	Body size (mm)	Within a clutch	0.015–0.020	0.015–0.022	0.003–0.12
		Among females	1.31	2.20	0.08
	Egg number	Among females	1	6	0.42
	Egg volume (mm <sup>3</sup> )	Among females	0.002	0.03	0.44
<i>P. chephrenis</i>	Body size (mm)	Within a clutch	0.002–0.028	0.019–0.03	0.001–0.68
		Among females	1.31	2.25	0.10
	Egg number	Among females	1	3	0.39
	Egg volume (mm <sup>3</sup> )	Among females	0.007	0.02	0.12
		Within a clutch	0.007–0.14	0.007–0.02	0.001–0.45

Data are shown for *Xenopsylla ramesis*, *Synosternus cleopatrae* and *Parapulex chephrenis*. CV, coefficient of variation.

belonged to species considered to be 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×23 cm×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 blood meal 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 2 or 3 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) to ensure that all of them were fully engorged; that is, they had blood in their midguts. Then, we transferred female fleas

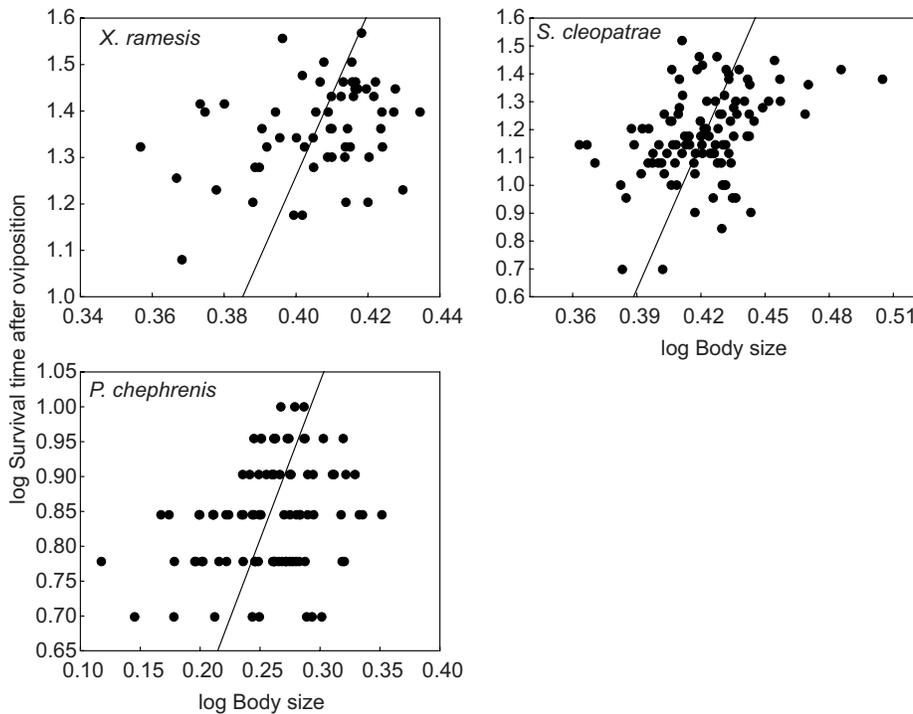
into individual Petri dishes (40 mm diameter) and placed them in an incubator (FOC225E, Velp Scientifica srl, Milan, Italy) at 25°C air temperature and 90% relative humidity (RH). Temperature and humidity were monitored using a Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, NJ, USA). After 24 h, we checked Petri dishes, recorded the 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 Co., 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 the vial 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 the Moticam 2000 camera (see above). Then, we measured its maximal body length from the frons to the most distal edge of the sensillum as described above.

To maximize the chances of pre-imaginal fleas surviving till adulthood, we selected the largest egg among all eggs produced by each female and discarded all remaining eggs. This procedure was unlikely to introduce bias into the data because the size range of the 'largest' egg was within the size range of all eggs (Table 1). Then, we added a 2 mm layer of clean sand and larval food medium (94%

**Table 2. Results of model II (major axis) regressions of the pairwise relationships between maternal body size, total egg volume, egg number and size and survival time of mothers after oviposition in three flea species**

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

MB, maternal body size; TEV, total egg volume; EN, egg number; ES, egg size; ST, survival time of mothers after oviposition. Asterisks denote relationships that remained significant after Holm–Bonferroni correction of the alpha-level.



**Fig. 1. Relationship between female body size and survival time after oviposition in three flea species.** Data are shown for *Xenopsylla ramesis*, *Synosternus cleopatrae* and *Parapulex chephrenis*. Fitted functions are model II (major axis) regression lines.

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 1 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, and photographed and measured it as described above (measurements did not cover claspers in male new imagos).

### 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  $V=1/6\pi \times W^2 \times L$ , where  $V$  is egg volume,  $W$  is maximal egg width and  $L$  is maximal egg length. Duration of development of each new imago was assessed as the number of days from the day of oviposition until the day of emergence.

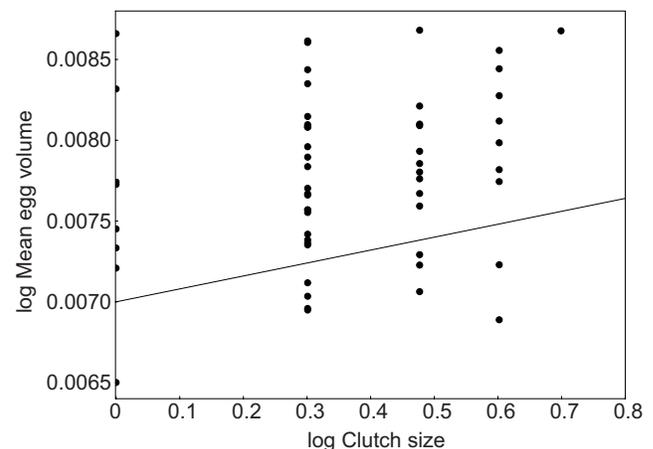
Continuous variables used in our analyses (e.g. egg size and body size) were random because they could not be controlled for. Therefore, we tested the relationships between variables using model II (major axis) regressions (Legendre and Legendre, 1998). We applied model II regressions using the package `lmodel2` (R package version 1.7-2; <http://CRAN.R-project.org/package=lmodel2>) implemented in R 3.2.3 (R Development Core Team 2015; <http://www.R-project.org/>). Significance of the slopes and correlation coefficients was estimated using a 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, required adjustment of the alpha-level due to an 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; García, 2004). Nevertheless, we performed sequential Holm–Bonferroni corrections of the significance level (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 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 mothers of each flea species, 57 *X. ramesis*, 48 *S. cleopatrae* and 69 *P. chephrenis* produced eggs (140, 132 and 85, respectively) in the 24 h after removal from the host. Female fleas of



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

**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**

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

F, female; M, male; ES, egg size; DD, duration of development; NIS, new imago size. Asterisks denote relationships that remained significant after Holm–Bonferroni correction of the alpha-level.

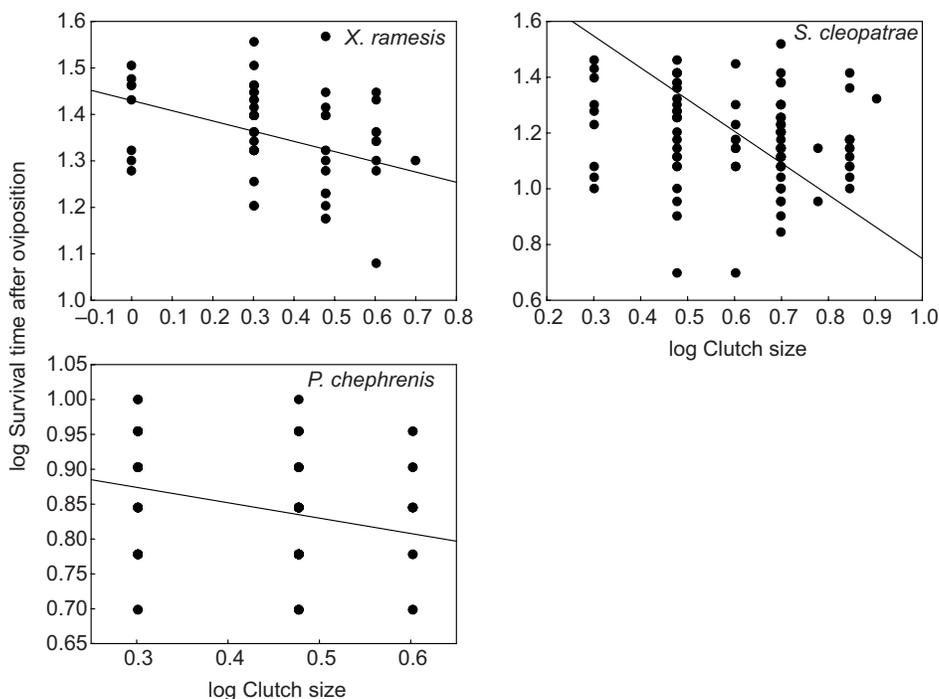
each species varied in their body size and the number of eggs they produced (Table 1). Furthermore, egg volume varied both between conspecific females as well as within a clutch (Table 1). Among females, variation in body size was not especially high; nevertheless, it was lowest in *X. ramesis* and slightly higher in the two other 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 the number or total volume of eggs produced during 1 day after an 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 the significance of this relationship disappeared after Holm–Bonferroni correction of the alpha-level (Table 2). Female body size was positively correlated with survival

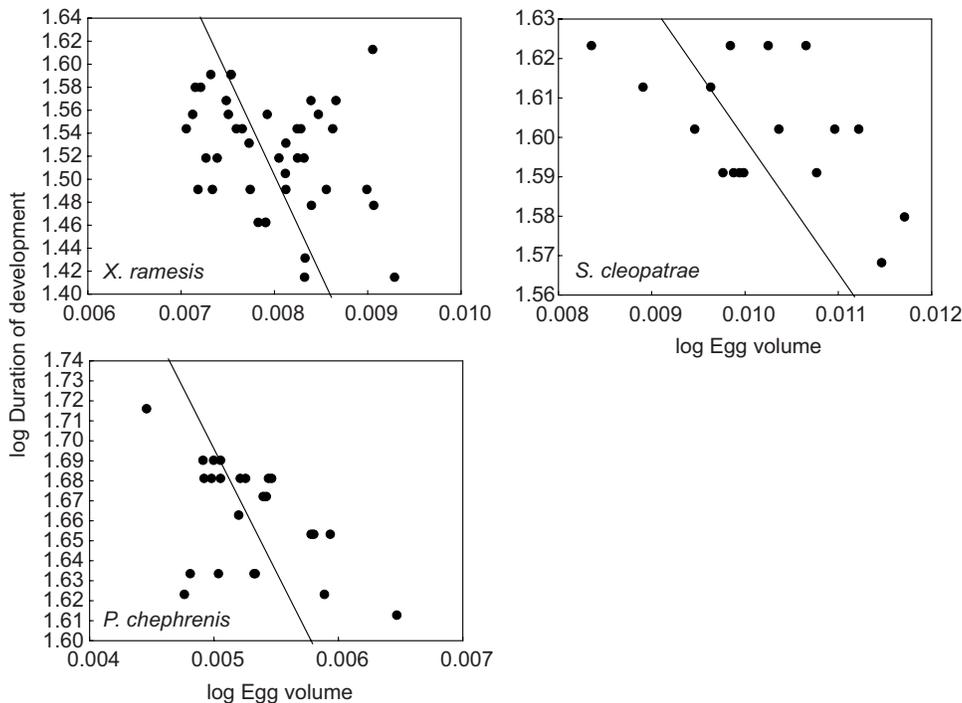
after oviposition. In all three flea species, larger females survived longer after oviposition than smaller females (Table 2, Fig. 1). No trade-off between egg number and their mean volume was found in any of the species (Table 2). In contrast, *X. ramesis* eggs from larger clutches were, on average, larger than eggs from smaller clutches (Table 2, Fig. 2), although this relationship appeared to be non-significant after correction of the 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 correction of the alpha-level in *X. ramesis* only (Table 2), whereas it was only marginally significant in *P. chephrenis* even prior to Holm–Bonferroni correction (Table 2).

Some eggs did not develop into new imagoes. There was no significant size difference 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;



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



**Fig. 4.** Relationship between egg volume and duration of preimaginal development in three flea species. Fitted functions are model II (major axis) regression lines.

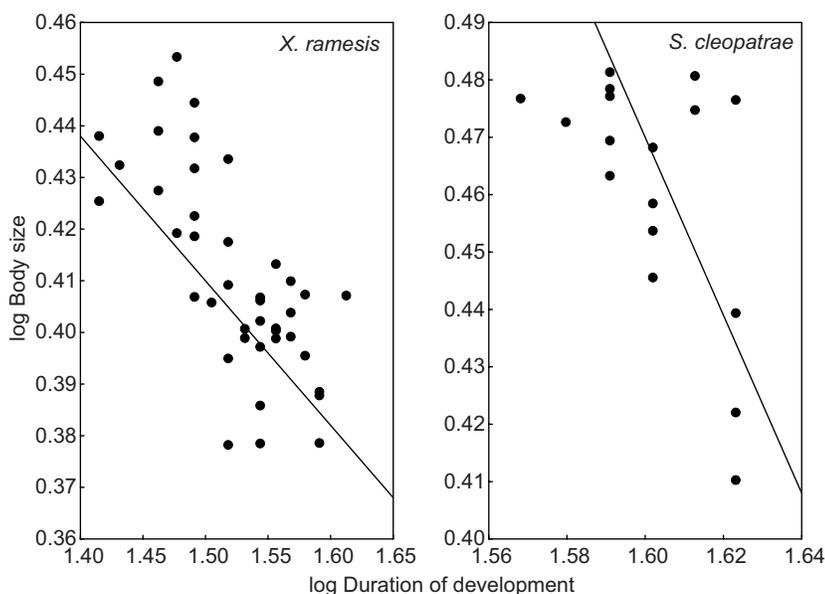
$t=6.41$ ,  $t=6.01$  and  $t=9.69$  for *X. ramesis*, *S. cleopatrae* and *P. chephrenis*, respectively;  $P<0.001$  for all). However, eggs which developed into females or males did not differ in size ( $t$ -tests;  $t=1.78$ ,  $t=0.005$  and  $t=0.33$  for *X. ramesis*, *S. cleopatrae* and *P. chephrenis*, 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 non-feeding imagoes; Hebert, 1983). As a result, the size of the



**Fig. 5.** Relationship between duration of development and female new imago body size in *X. ramesis* and *S. cleopatrae*. Fitted functions are model II (major axis) regression lines.

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 probably subjected more to host grooming than their smaller counterparts because they (a) are more conspicuous, (b) may inject a higher amount of allergen-containing saliva into 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 adjustment of the alpha-level. This lack of maternal effect on egg number/clutch volume could be explained in the same way as the lack of a 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 the one hand, their reproductive effort is not as limited by their body reserves as 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 number of eggs 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, factors. Firstly, the 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 et al.'s (2014) experiments than in this study [0.66 calculated from data of Khokhlova et al. (2014) versus 0.40 from the present study], resulting in the occurrence of a trade-off between the number of eggs and size of new imagoes in the former and the lack of a 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 per group in Khokhlova et al.'s (2014) design, presumably increasing among-larva competition for food (Khokhlova et al., 2010b), which, in turn, might result in a 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 a heterogeneous environment and not manifested in a stable environment) and (b) manifestation of the trade-off may depend on the stage of reproductive cycle being considered (e.g. trade-off between the number of eggs and their size versus trade-off between the number of eggs and size of the 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 future fitness. This also suggests that larger females invested mostly in 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 a peak when a flea is 6–20 days old and decreasing sharply in fleas older than 31 days (Korneeva and Sadovenko, 1990; see Krasnov, 2008, for additional references). This suggests that decreased investment in first clutches might enhance the reproductive output at peak reproduction. For example, female *Xenopsylla skrjabini* produced, on average, 5.5 eggs day<sup>-1</sup> until 5 days of age and 8.0 eggs day<sup>-1</sup> at the age of 6–20 days (approximately 30% increase in egg production; Korneeva and Sadovenko, 1990). Assuming a similar pattern in *X. ramesis*, though this has never been specifically studied, a female investing heavily in early reproduction (e.g. 3–4 eggs per clutch) will probably produce 10–28 fewer eggs during its lifetime than a female with a first clutch of 1–2 eggs (on average, survival was 12–16 days versus 22–30 days, respectively). Although this calculation may not be highly accurate, our results suggest that flea offspring resulting 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 the 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 larva, 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 in 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-biased 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 stages of pre-imaginal development later than egg (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-imagos 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 probably 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 lifetime 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.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

B.R.K., I.S.K. and D.K. conceived and designed the experiments, D.K. conducted the experiments, B.R.K. and E.M.W. analysed the data, all authors drafted the paper.

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