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## Sperm allocation in relation to male traits, female size, and copulation behaviour in a freshwater crayfish species

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**Abstract** Sperm competition is a well-recognised agent in the evolution of sperm and ejaculate structure, as well as variation in female quality. Models of the evolution of ejaculate expenditure predict that male body condition, female fecundity and the risk and intensity of sperm competition may be the ultimate factors shaping optimal ejaculate size. We investigated sperm allocation in *Austropotamobius italicus*, a freshwater crayfish exhibiting a coercive mating system and external fertilisation, in relation to male and female traits and copulation behaviour under laboratory conditions. We found that mating males were sensitive to female size and produced larger ejaculates when mating with larger females, which were more fecund in terms of number of eggs produced. We found no evidence for female egg production being sperm-limited, as the number of eggs was not dependent on male sperm expenditure. Copulation duration and number of ejaculations reliably predicted the amount of sperm transferred, and both these behavioural measures positively covaried with female body size. These results indicate that male freshwater crayfish can modulate their sperm expenditure in accordance with cues that indicate female fecundity. In addition, a novel finding that emerged from this study is the decrease in sperm expenditure with male body size, which may either suggest that large, old male crayfish are better able than small males to economise sperm at a given mating to perform multiple matings during a reproductive season, or that they experience senescence of their reproductive performance.

**Keywords** Arthropoda · Decapoda · Fecundity · Senescence · Sperm competition · Sperm economy · Sperm limitation

### Introduction

The traditional notion that male reproductive success is only limited by the number of mates he can encounter and fertilise, due to the huge amount of sperm cells produced (Bateman 1948), has been questioned in recent decades by recognising that sperm production inevitably entails some costs (Dewsbury 1982; Simmons 1988; Van Voorhies 1992; Gage and Cook 1994; Olsson et al. 1997; see also Wedell et al. 2002). Indeed, there is ample evidence that males have evolved strategies for allocating a finite number of sperm to each mating attempt to maximise their lifetime reproductive success (Dewsbury 1982; Parker 1990a,b; Shapiro et al. 1994; Parker et al. 1996). As a consequence, male ejaculate expenditure has been found to be dynamic in both space and time and sensitive to the risk and intensity of sperm competition (e.g. Gage and Baker 1991; Gage and Barnard 1996; Oppliger et al. 1998; Evans and Magurran 1999; Mougeot et al. 2001; Pilastro et al. 2002; Evans et al. 2003).

If females vary in reproductive quality, males may achieve a greater reproductive success by strategically allocating their sperm to females that provide the greatest fertilisation returns. Female egg production or fecundity may depend on condition or age (Trivers 1972; Arak 1988). Therefore, males in several species prefer larger or younger mates (e.g. in insects, see review in Bonduriansky 2001), and there is evidence that males also provide larger ejaculates to heavier females in insects, molluscs, and fish (e.g. Yusa 1994; Gage and Barnard 1996; Marconato and Shapiro 1996; Gage 1998; MacDiarmid and Butler 1999; but see Simmons and Kvarnemo 1997). Additionally, the energetic costs associated with sperm production may result in males being sperm-limited, which may in turn translate in female reproductive output being sperm-limited as well, if females can vary clutch size to match male

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ejaculate size or if fertilisation success (and, hence, fecundity) depends on the amount of delivered sperm (e.g. MacDiarmid and Butler 1999). This has been observed, for example, in arthropod species where females who mated with already-mated males show a reduced fecundity (Royer and McNeil 1993; Svensson et al. 1998; Rondeau and Sainte-Marie 2001; Torres-Vila and Jennions 2005).

Most data on sperm expenditure/allocation come from studies of insects. Some studies have also been conducted on marine crustaceans (e.g. Paul 1984; Paul and Paul 1990; Paul et al. 1991; Sainte-Marie 1993) but none on crayfish, despite several conditions that may promote strategic sperm allocation in this group. Crayfish offer an ideal opportunity to study reproductive investment due to their promiscuous mating system, with males and females obtaining multiple copulations during the same breeding season, and the occurrence of sexual coercion. We studied ejaculate allocation in the freshwater crayfish *Austropotamobius italicus* (Faxon 1914) (Decapoda: Astacidae), a species endemic to Italy (Grandjean et al. 2000), in relation to male and female traits, mating date and copulation behaviour. *A. italicus* is a long-lived (maximum life span 10–13 years), *k*-selected crayfish, which reproduces once a year in October–November (Matthews and Reynolds 1995). Mating consists in rapid exchanges of tactile and olfactory signals, after which males grasp females by means of their larger claws, turn them on their back and release spermatophores, which are attached to the thoracic sternites of females (Acquistapace et al. 2002). Fertilisation is therefore external to the female body, and spawning occurs within a few days or weeks from mating (up to 25 days in *A. italicus*, personal observation). Although females sometimes try to resist male advances, which could be a form of female preference, males eventually force them to copulate (personal observation and Reynolds 2002), thus causing females to actively search for multiple mates because they may be fertilised by non-preferred partners. The occurrence of sperm competition is suggested in *A. italicus* (and in other Astacidae) by previous studies reporting that males feed on the spermatophores deposited by other males before copulating (Villanelli and Gherardi 1998; Furrer 2004), although the ultimate consequences of this behaviour for the realised paternity of the first male are unknown. Additionally, the high variability in the time lag between copulation and egg release (see above) may leave ample chances for multiple matings by females.

Female egg production is positively correlated with body size in this species (Rhodes and Holdich 1982; this study); thus it should be advantageous for males to copulate with large females, provided that the sperm they release is sufficient to fertilise most eggs. A male preference for large females is widespread among arthropod taxa (Gwynne 1981; Rutowski 1982; Dick and Elwood 1989; Jormalainen et al. 1992; Sigurjonsdottir and Snorrason 1995; Uhl 1998; Harari et al. 1999), and males also generally allocate larger ejaculates to such females to sustain their greater egg production (e.g. Gage and Barnard 1996; Gage 1998; MacDiarmid and Butler 1999). However, in *A. italicus*, the restricted mating period (1 month) and the male-biased operational sex ratio (Brewis and Bowler 1985) may make it

difficult for males to find a mate in the wild mainly at the end of the breeding season. Therefore, *A. italicus* males seem to copulate indiscriminately with the first receptive female they encounter, regardless of body size (Woodlock and Reynolds 1988; Gherardi et al. 1997, 2006). Under this scenario, it may pay males to be cautious in sperm expenditure at the beginning of the breeding season, when mating opportunities are high, by partitioning their sperm budget to each ejaculate based on cues revealing female quality, risk/intensity of sperm competition and/or potential number of mating opportunities.

Our main aim was to analyse sperm allocation in relation to male morphological characteristics, female body size and copulatory behaviour by means of pairing experiments carried out with unmated females (i.e. females that did not carry spermatophores) in a non-competitive context. Specifically, we predicted that (1) sperm expenditure should be greater for larger males, assuming that spermatogenesis is costly and comparatively more costly for small than large individuals (Simmons 1988) because sperm reserves, ejaculate or spermatophore size positively covary with male size in several arthropod species, including crustaceans (e.g. Wedell 1993; Bissoondath and Wiklund 1996; MacDiarmid and Butler 1999; Gosselin et al. 2003; Jivoff 2003); and (2) males should allocate more sperm to large females, which release more eggs. Finally, we assessed whether copulation duration and number of ejaculations were reliable predictors of male ejaculate size, and examined the relationships between copulation behaviour and male and female traits.

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## Materials and methods

### Subjects and housing conditions

Sexually mature *A. italicus* (carapace length >30 mm) were collected from a stream in the northern Apennines (N. Italy) during September–October 2004, under licence from the local administration authorities. The area holds a healthy population of *A. italicus* (Nardi et al. 2004). Sexes were held separately under a natural light–dark cycle in opaque plastic jars (80×60×60 cm) filled with 150 l of recirculating, filtered water (20 individuals/jar). The crayfish were provided with a gravel substratum and shelter, and they were fed with dry crayfish food (Tetra WaferMix) twice a week. Water temperature ranged from 18°C in October to 12–14°C in November–December. Carapace length (CL) and right and left chelae length were measured for each individual using a digital calliper (accuracy 0.01 mm). As a measure of chelae length, we used the maximum chela length. Individuals with one or both chelae missing were not considered. Chelae asymmetry, which may be caused by loss and subsequent regeneration of one chela, was quantified as the absolute difference in length of the chelae and expressed as a percentage of the longer of the two chelae. Due to the large between-male variation in chelae asymmetry (range 0–63%), this measure was highly repeatable, as evaluated in a

random sample of ten males measured twice ( $F_{9,10}=238.03$ ,  $P<0.001$ ; repeatability=0.99 according to Sokal and Rohlf 1995). At the end of the experiments, all crayfish were returned to the stream of origin.

### Experimental setup

The analyses refer to 86 mating trials, where males were individually paired with receptive females, with the constraint that individual females were of similar size or smaller than males to maximise the probability of copulation (in fact, copulations are often forced in this species, and large females may prevent smaller males from copulating). This resulted in a positive correlation between male and female size ( $r=0.58$ ,  $P<0.001$ ). Mean male CL was 39.6 mm (range 31.5–52.3), whereas that of females was 37.5 mm (range 31.7–44.1), and the mean difference between male and female CL among copulating pairs was 2.1 mm (range -7.8–14.9). Receptive females were identified by means of the presence of whitish gelatinous patches along abdominal sternites, deriving from glair gland maturation. Mating trials were conducted in the evenings between 2 and 25 November 2004, during the mating season. Ten minutes before a trial started, pair members were placed separately in a 15-l plastic aquarium, provided with a gravel substratum and an opaque plastic divider forming two acclimation chambers. The divider was then removed and the animals were allowed to interact and were videotaped for 30 min. Pairs were observed under dim red light and their behaviour video recorded with a Sony DCR-TRV25E digital camera using the night shot function, mounted to allow for the simultaneous recording of four pairs of crayfish. If copulation and spermatophore deposition did not occur during the trial, a pair was placed individually in a rectangular plastic mesh cage within a 150-l plastic jar (see above) and checked daily until insemination occurred (for a maximum of 30 days), as judged from the observation of the white spermatophores attached to the thoracic sternites of females. After insemination, females were placed into 150-l spawning jars (see above) and checked for spawning every second day by removing them from the tank to examine their abdomen. Whole clutches were carefully removed from the female abdomen the day after spawning was completed, and eggs were counted to record clutch size.

### Measurement of sperm expenditure

We used an index of the area covered by spermatophores as a measure of sperm expenditure because this method was rapid, non-destructive and reliable, as documented in other decapod species (MacDiarmid and Butler 1999). As soon as possible after spermatophore deposition, we accurately drew the surface covered by spermatophores on the female ventral side on a standard realistic ventral outline printed on white paper, obtained by a digital picture of a dead

female. The CL of the standard female outline measured 57.9 mm on the paper sheet. The same observer reported the surface covered by sperm for all individuals. Female outlines were then scanned and the total surface covered by spermatophores (relative sperm area,  $\text{cm}^2$ ) was calculated by means of the Adobe Photoshop image processing software. Finally, this measure was transformed into a corrected sperm area index, to take into account the variation in female size, by means of the formula: corrected sperm area index=(relative sperm area×female CL)/57.9 mm. Therefore, as an index of sperm expenditure, the corrected sperm area index (sperm area hereafter for simplicity) was used in all analyses. Spermatophores, which consist of vermicular white filaments of variable length, were mostly deposited horizontally as a single layer on the female ventral side (on or around the female spermatophoric plate), with limited overlap (personal observation), so sperm area should represent a reliable index of sperm expenditure. Sperm expenditure could not be reliably assessed in eight females because eggs were extruded before sperm area could be recorded. Therefore, sperm area was not available for these individuals (see below).

### Behavioural observations

Video recordings were analysed to measure the latency to mounting, the interval between mounting and copulation, the latency to copulation, copulation duration and the number of ejaculations performed. In *A. italicus*, copulation is frontal, with the male holding firmly the female chelipeds and usually standing over the female with his gonopodia positioned over the female spermatophoric plate. During sexual encounters, a male approaches a receptive female touching her body with antennae and chelae. A female may then either show evasive behaviour or accept the male displays, probably depending on her reproductive status. In the first instance, the male may reach the female again, and, after repeated trials, he eventually grasps her chelae and obtains a forced copulation (although the occurrence of this behaviour may be overestimated in the confined space of an aquarium compared to natural conditions). In the second case, the male immediately grasps the female chelae and rapidly turns her on her back. When a female is turned, she may still show signs of resistance, but normally stands motionless until copulation is terminated. In addition, some time elapses between mounting and the start of spermatophore deposition, mainly because males are not in the correct copulating position or females resist. We considered that copulation started when a male reached the correct copulating position, with his copulating organs approximately coinciding with the female's spermatophoric plate. Ejaculations, consisting of rapid, sequential tail flips and simultaneous forward movements of the male's body, could be clearly observed and counted on video recordings. The same observer recorded all behavioural observations.

## Statistical analyses

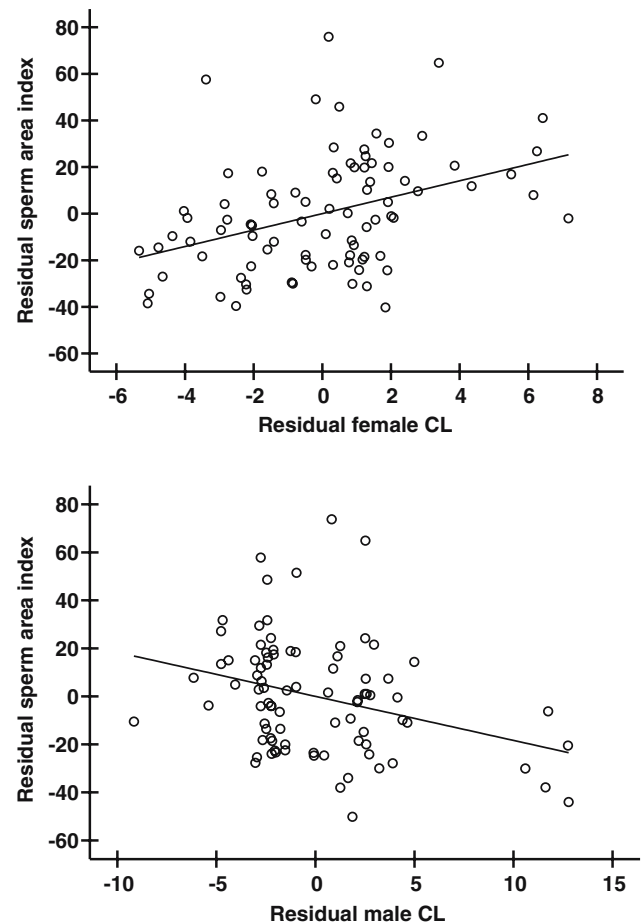
The relationships between sperm area, male traits (CL, representing a measure of body size, chelae length and chelae asymmetry) and female traits (CL) were analysed by multiple regression, where sperm area was the dependent variable and male traits, female CL and date of copulation (with day 1 = 1 November) were the independent predictors. Date of copulation was included in the analyses as a predictor variable because the probability of encountering unmated females may decrease as the season progresses. Chelae asymmetry (percentage) was square root–arcsine transformed to approach normality. The linear regression model was subjected to a step-down simplification procedure, where non-significant ( $P > 0.05$ ) terms were removed at each step starting from the least significant terms until a minimum adequate model, containing only significant ( $P < 0.05$ ) terms, was obtained (Crawley 1993). A similar regression analysis was conducted to study the effects of sperm area on female clutch size (see “Results” for details). The relationships between sperm area and selected measures of copulation behaviour (copulation duration and number of ejaculations) were analysed by simple correlation analyses. Finally, the relationships between behavioural measures (latency to mounting, interval between mounting and copulation, latency to copulation, duration of copulation and number of ejaculations), date of testing (with day 1 = 1 November) and male and female traits were addressed by means of step-down multiple linear regression analyses (see procedure above), where behavioural measures were entered as dependent variables and other measures as independent predictors. In all analyses, behavioural measures were  $\log_{10}$ -transformed to achieve normality. In multiple regression models, the high correlation between male CL and chelae length ( $r = 0.93$ ,  $P < 0.001$ ), due to the simultaneous growth of all body parts with age, could potentially result in collinearity and instability of regression coefficients. However, after the step-down procedure, these two variables were never included simultaneously in a simplified model. In any case, for all minimum adequate models including multiple variables, we noted the highest value of the variance inflation factor (VIF), a measure of the effect of collinearity, for the variables included. Although there is no general consensus on how large the VIF should be for a given multiple regression model to be regarded as unstable, it is generally accepted that VIF values below 5 or 10 indicate that regression coefficients are not so much affected by collinearity between independent predictors (McClave and Sincich 2003). In all step-down minimum adequate regression models including multiple predictors (see next Section), VIF values were always smaller than 1.53, and we are therefore confident that our results were not confounded by collinearity. Means and parameter estimates are reported together with their associated standard errors.

Sperm area was measured on 86 individual females, which were individually paired with 86 different males. Copulations observed during trials, for which we could obtain all measures of copulation behaviour, refer to 49

pairs. Of these, the sperm area could be measured on 41 females (see above).

## Results

The minimum adequate multiple regression model [ $F_{3,82} = 6.22$ ,  $P = 0.001$ ,  $R^2_{adj} = 0.16$ ; intercept =  $-31.4$  (32.69 SE)] revealed that sperm area increased with female CL [ $b = 3.54$  (0.92 SE),  $t = 3.82$ ,  $P < 0.001$ , Fig. 1], decreased with male CL [ $b = -1.84$  (0.62 SE),  $t = -2.96$ ,  $P = 0.004$ ; Fig. 1] and weakly increased with date of copulation [ $b = 1.19$  (0.58 SE),  $t = 2.05$ ,  $P = 0.043$ ]. The  $P$  value at removal for other terms (male chelae size and asymmetry) was always  $> 0.58$ . In addition, sperm area was not differentially affected by female size in relation to male size, i.e. females received similar amounts of sperm from both small and large males independently of their own size (male CL  $\times$  female CL,  $t = -0.92$ ,  $P = 0.36$ ). Results were qualitatively unchanged if



**Fig. 1** Relationships between residual sperm area index and residual female (*above*) and male (*below*) body size (expressed as carapace length, CL). Residual partial plots were obtained from the multiple regression model including male CL, female CL and date of copulation (see “Results”). Residuals for each variable are controlled for all the other terms in the model. The regression line is shown

sperm area was square root transformed to homogenize the dimensionality of variables (details not shown).

The number of eggs released by females did not depend on sperm area or male traits. In a step-down multiple regression model of female clutch size in relation to date of copulation, male body size, chelae length, chelae asymmetry, sperm area and female size, the latter variable was the only significant predictor of clutch size [ $b=5.61$  (1.24 SE),  $t=4.51$ ,  $P<0.001$ ,  $R^2_{adj} = 0.22$ , intercept (SE)=-114.68 (46.95),  $n=71$  females;  $P$  values at removal for other variables always  $>0.35$ ].

The mean duration of copulations was 279.5 s (26.2 SE, range 121–1,368 s), during which males performed a mean of 15.35 (0.84 SE, range 5–43) ejaculations. Both the duration of copulation and the number of ejaculations predicted sperm area ( $r=0.60$  and  $r=0.59$ , respectively,  $n=41$ ; both  $P<0.001$ ) (Fig. 2). The number of ejaculations and duration of copulation were also positively correlated ( $r=0.77$ ,  $n=49$ ,  $P<0.001$ ).

The mean latency between the beginning of a trial and mounting by males was 363.7 s (56.9 SE, range 12–

1,519 s). Male traits, female size or date of testing did not predict latency to mounting (all  $P$  values at removal  $>0.13$ , details not shown), whereas latency to copulation, which averaged 590.9 s (66.6 SE, range 51–2,094 s), was greater for increasing male chelae length [ $b=0.024$  (0.009 SE),  $t=2.55$ ,  $P=0.014$ ;  $R^2_{adj} = 0.10$ ; intercept (SE)=1.83 (0.31)]. In addition, the interval between mounting and copulation (mean 227.2 s, 41.1 SE, range 10–1,062 s) increased with male CL [ $b=0.061$  (0.019 SE),  $t=3.23$ ,  $P=0.002$ ;  $R^2_{adj} = 0.16$ ; intercept (SE)=-0.42 (0.75)]. Finally, both the duration of copulation and the number of ejaculations similarly increased with increasing female size and date of testing, but did not covary with male traits [duration of copulation: date,  $b=0.010$  (0.004 SE),  $t=2.20$ ,  $P=0.033$ ; female CL,  $b=0.019$  (0.008 SE),  $t=2.42$ ,  $P=0.020$ ; model  $F_{2,46}=4.79$ ,  $P=0.013$ ,  $R^2_{adj} = 0.13$ ; intercept (SE)=1.50 (0.32); number of ejaculations: date,  $b=0.008$  (0.003 SE),  $t=2.39$ ,  $P=0.021$ ; female CL,  $b=0.014$  (0.006 SE),  $t=2.29$ ,  $P=0.026$ ; model  $F_{2,46}=4.93$ ,  $P=0.011$ ,  $R^2_{adj} = 0.14$ ; intercept (SE)=0.47 (0.25)].

## Discussion

This study of a freshwater crayfish showed that males allocate more sperm to large females and at the end of the breeding season, and, intriguingly, that sperm expenditure decreased with male body size. In addition, sperm expenditure increased with copulation duration and the number of ejaculations a male performed, and both these variables covaried positively with female size and date of copulation. No effects of male chelae asymmetry on copulation behaviour or sperm production could be observed, indicating that these males were not handicapped by even severe chelae asymmetry (up to 63%), which theoretically could have impaired their ability to secure and position females for copulation (see also Galeotti et al. 2006).

The tailoring of sperm expenditure to female body size may be explained by the considerable variation of clutch size (28 to 299 eggs in this study), which is partly accounted for by variation in female CL. In fact, larger females produce larger clutches in this species and in other decapods (Annala and Bycroft 1987; Sainte-Marie 1993; Chubb 1994; MacDiarmid and Butler 1999). Therefore, the ejaculation of a huge amount of sperm when mating with a small female (laying fewer eggs) is likely to be an unnecessary energetic cost for a male (see also MacDiarmid and Butler 1999 for a similar reasoning in lobsters). Moreover, due to the restricted mating season, the costs associated with the loss of mating opportunities because of insufficient time to replenish sperm stores may outweigh the costs of sperm production. This may occur because the number of sperm decreases with successive ejaculates (Dewsbury 1982). Thus, under such conditions, it should pay males to ejaculate more sperm when paired to large females to maximise chances to fertilise most of their eggs, and expected female fecundity may be more important in determining ejaculate expenditure than any change in sperm competition risk or intensity associated with vari-

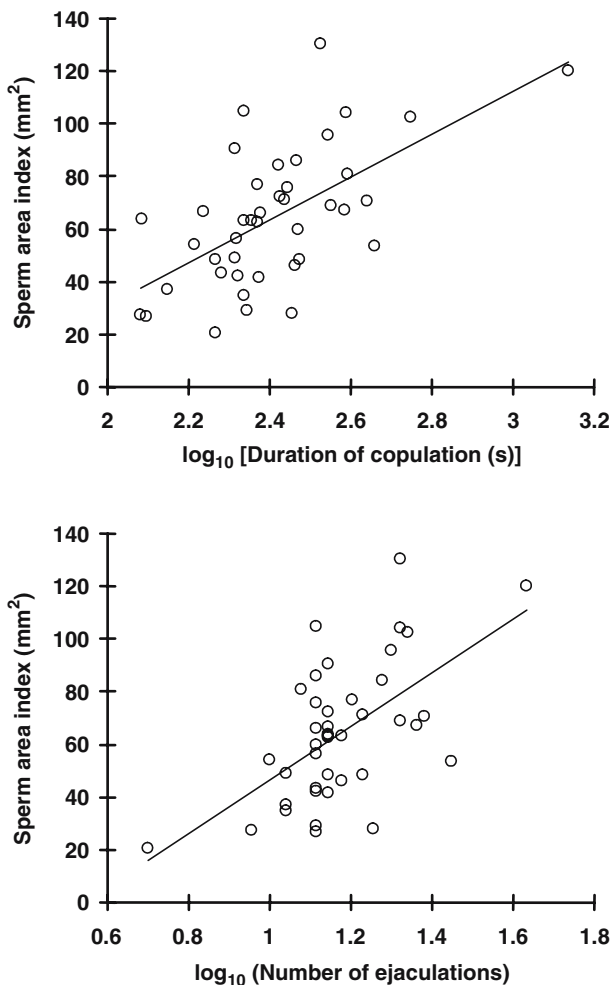


Fig. 2 Relationships between sperm area index, duration of copulation (above) and number of ejaculations (below). The regression line is shown

ation in female size (Wedell et al. 2002). By contrast, males may economise sperm when mating with small females, reserving it for future and potentially more rewarding matings.

An alternative explanation for the greater amount of sperm received by large females is that such females simply possess a larger thoracic area, which may allow males to deposit more sperm. However, this is highly unlikely because the surface covered by spermatophores was always much smaller than the female overall thoracic area (approximately 15%). In addition, males deposited their sperm mostly on or around the female spermatophoric plate, a small portion of the female thorax, which is possibly a specialised receptor for male spermatophores (e.g. George 2005).

The observed decrease of sperm expenditure with increasing male body size was unexpected. We may put forward three alternative explanations for such a striking result. Firstly, large males invest less per mating attempt to ensure that sperm is reserved for future mating opportunities. In fact, large males may forcefully copulate with a greater number of females than small ones, and a large size can thus potentially increase mating rates. Although rare in the population, large males dominate over small males in inter-male fighting (Villanelli and Gherardi 1998), possibly monopolising females in competitive contexts. Under such conditions, it may pay small males to maximally invest in the one or few matings they can obtain. Secondly, we may speculate that large males may cope with inter-male competition for fertilising females in a different way than smaller males; for instance, large males may release sperm of superior fertilisation capability (see e.g. Ceballos-Vázquez et al. 2003), which may allow them to economise the per-mating sperm expenditure. Finally, large, old males (CL > 43 mm, age > 6–7 years) could be senescent individuals, showing reduced sperm production (e.g. Cordero and Miller 1992; Radwan and Bogacz 2000; Radwan 2003). This would be in line with the increasing evidence that sperm production and quality decrease with age in various taxa (Kidd et al. 2001, Wolf et al. 2000), including arthropods (Schäfer and Uhl 2002). Large males are indeed sluggish and slower moving than small males, and in a few instances, they did not deposit any spermatophore, despite correctly turning females (personal observation). The additional finding that large males need more time than small ones to achieve a copulation, mainly because of an increasing time interval between mounting and actual spermatophore ejaculation, may also be consistent with the senescence hypothesis, suggesting that they may experience non-trivial difficulties to reach the correct copulating position. Furthermore, females also laid smaller eggs for such males, indicating that they did not favour them in their post-mating choice (Galeotti et al. 2006).

The topic clearly deserves more studies, and experiments on male sperm depletion after repeated matings may help to solve the question of senescence vs optimal sperm allocation among large males.

We did not find any evidence for sperm-limited egg production in females because sperm area did not explain a

significant amount of variation in clutch size, indicating that females did not adjust clutch size to the amount of sperm received from males. Female fecundity has been shown to be sperm-limited in other crustaceans, such as lobsters (MacDiarmid and Butler 1999). However, we could only assess whether the number of freshly released eggs was related to sperm area, whereas in lobsters the clutch size was measured long after fertilisation (MacDiarmid and Butler 1999). Because unfertilised eggs may be lost with time, it is indeed likely that the variation in the amount of sperm delivered by males would influence the number of eggs that are fertilised and reach maturity in our study species, too.

According to previous studies carried out in other arthropod species (including crustaceans, e.g. Sainte-Marie et al. 1997; reviewed in Simmons and Siva-Jothy 1998, Martin and Hosken 2002), copulation duration and number of ejaculations reliably predicted male ejaculate size, and males copulated longer and ejaculated more frequently when paired with larger females and late in the breeding season. Therefore, males may transfer larger ejaculates when the opportunities for mating decrease, as could happen at the end of the breeding season. In addition, longer copulations, besides providing more sperm, also result in monopolising receptive females for a longer time, which prevents copulation attempts from rival males, thus accruing a male's own chance of paternity. Furthermore, a greater amount of sperm delivered to large females may increase the probability of paternity for a given male by making it more difficult for other males to completely remove his spermatophores from the female underside.

In conclusion, our results confirmed the relevance of female traits in determining male mating behaviour and sperm allocation decisions, whereas the observed decrease in ejaculate size according to increasing male body size may either indicate size-dependent male mating strategies or that large males are subjected to senescence of the reproductive apparatus.

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