Energetically costly precopulatory mate guarding in the amphipod *Gammarus pulex*: causes and consequences

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Precopulatory mate guarding (PCMG) is thought to have evolved as a male mating strategy in species in which female receptivity is limited to a short time. It is common among crustaceans, and energetic costs associated with PCMG are thought to promote size-assortative pairing in such species, although direct evidence is lacking. Using both field surveys and laboratory experiments, we assessed the energetic costs of PCMG in *Gammarus pulex* and investigated their possible causes. Energetic costs were measured as differences in lipid and glycogen reserves. In field-collected samples, size-corrected lipid and glycogen reserves of paired males were both significantly higher than those of unpaired ones. In the laboratory, the energetic cost of PCMG was unrelated to its duration, but was strongly positively correlated with female size (relative to the size of the male). In addition, the increased energetic cost of guarding a larger female was independent of an induced starvation cost. Our results show for the first time an energetic cost of PCMG in male *G. pulex*, and indicate that such a cost is more likely to result from pair formation than from the cost of carrying the female, as has previously been assumed. We discuss our results in relation to sexual conflict over PCMG duration and the ability of males to overcome female resistance.

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When female receptivity is predictable, but limited to a brief time window during each reproductive bout, precopulatory mate guarding (PCMG) may evolve as a male competitive strategy (Parker 1974). It is particularly common in crustaceans because females can generally be fertilized only for a short period after the moult (Ridley 1983). Males benefit from PCMG by maximizing their chances of fertilizing females' eggs once they become receptive. However, the optimal time a male spends guarding each female will depend upon the costs associated with PCMG. Males that guard for longer will have less time available to find and fertilize other females (Parker 1974; Wickler & Seibt 1981; Grafen & Ridley 1983; Yamamura 1987), and the decreased mobility of precopula pairs (Adams & Greenwood 1983, 1987) may, in part, explain an increased predation risk (Strong 1973; Wickler & Seibt 1981; Ward 1986). Finally, PCMG may be energetically costly (Robinson & Doyle 1985; Elwood & Dick 1990; Sparkes et al. 1996; Jormalainen et al. 2001).

Determining the energetic cost of PCMG is important because the existence of body size-related differences in this cost is a fundamental, and generally untested,

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assumption of models that have previously examined optimal male time investment strategies (Grafen & Ridley 1983; Yamamura 1987; Jormalainen et al. 1994; Yamamura & Jormalainen 1996) and size-assortative pairing (Elwood & Dick 1990). What causes PCMG to be energetically costly is also unclear. Possible causal mechanisms include reduced feeding rates (Robinson & Doyle 1985; Sparkes et al. 1996), a cost of carrying a partner (Watson et al. 1998), or even a cost of overcoming a sexual conflict over mate-guarding duration, although this cost has been shown only in females (Watson et al. 1998; Jormalainen et al. 2001). Most studies have typically focused only on one causal mechanism at a time (Robinson & Doyle 1985; Sparkes et al. 1996), thereby ignoring the possibility that the energetic cost of PCMG may be multifactorial. Some of the potential causes of energetically costly PCMG may be sex specific. For example, Robinson & Doyle (1985) showed that in *Gammarus lawrencianus* the feeding rates of males were reduced while they were in precopula but female feeding rates were unaffected. Similarly, in the marine isopod *Idotea baltica*, struggles that preceded precopula resulted in an energetic cost for females but not for males (Jormalainen et al. 2001). Furthermore, the relative energetic cost of some of the processes associated with PCMG may vary with body size and PCMG duration in different ways. For example, in female water striders,

Aquarius remigis, the energetic cost of struggling with a male is positively related to the male's size (Watson et al. 1998). Thus, to verify the importance of energetic costs in male and female pairing decisions (Grafen & Ridley 1983; Yamamura 1987; Elwood & Dick 1990; Jormalainen et al. 1994; Yamamura & Jormalainen 1996) one must determine the relative influence of the causal mechanisms supposedly underlying this cost.

A second reason for studying the causal bases for energetically costly PCMG is that in crustaceans levels of stored organic reserves, such as lipid and glycogen, continuously cycle in response to an animal's proximity to moulting (Passano 1960; Chang & O'Conner 1983; Jormalainen et al. 2001). This may have two important implications for understanding male pairing decisions in *G. pulex*. First, if PCMG is energetically costly, there may be periods of the moult cycle during which all males are energetically constrained and, as such, are prevented from entering precopula (independent of other potential factors such as male body size, see Ward 1988; Elwood & Dick 1990). If so, we would predict that unpaired males should have lower size-corrected energy reserves than paired males. Second, the energetic costs of PCMG may result in a direct trade-off between current reproductive investment and growth, which is likely to be a major determinant of fitness in male *G. pulex*, since larger males are better competitors than smaller males (Ward 1988; Elwood & Dick 1990). Whether such a trade-off occurs is not clear. Robinson & Doyle (1985) showed that feeding rates of male *G. lawrencianus* were reduced while they remained in precopula, resulting in a 45% reduction in growth over time. However, Sparkes et al. (1996) showed that in the isopod *Lirceus fontinalis*, PCMG was associated with only a short-term (<36 h) reduction in glycogen reserves, and there was no reduction when pairs were given access to food. PCMG had no impact on lipid reserves in *L. fontinalis*, suggesting that the energetic cost of PCMG does not impact on growth in this species (Sparkes et al. 1996).

Few mating systems have been used more than that of the amphipod *G. pulex* for studying PCMG and size-assortative pairing (Grafen & Ridley 1983; Elwood et al. 1987; Ward 1988; Elwood & Dick 1990; Sutcliffe 1992, 1993; Ward & Porter 1993; Bollache et al. 2000). Grafen & Ridley's (1983) model predicted that larger males should have shorter precopula duration, because they can displace smaller males. In contrast to this prediction, experimental studies have shown that larger male *G. pulex* normally guard females for longer (Ward 1984; Elwood & Dick 1990). Elwood & Dick's (1990) 'guarding time' hypothesis proposes that this result may be explained if the costs of PCMG are less for larger males and increase at a lower rate than those of smaller males, thus enabling larger males to guard females earlier. However, no study has ever tested for an energetic cost of PCMG in this species. In this study, we measured the lipid and glycogen reserves of field-collected paired and unpaired male *G. pulex* to determine whether variation in energy reserves is correlated with male pairing ability. We then determined the relative influence of male and female body size, experimentally controlled PCMG duration and access to food on male lipid and glycogen reserves after one bout of PCMG. This laboratory experiment enabled us to separate and quantify the relative influence of the different mechanisms proposed to explain energetically costly PCMG, as well as to determine which energy reserves are used to pay this cost.

METHODS

Field Study

Using kick sampling and a hand net (Hynes 1954), we collected two separate samples of paired and unpaired male *G. pulex* on 19 and 20 April 2001 from the River Suzon, Burgundy, eastern France. Straight after collection all males were blotted dry and weighed (± 0.01 mg) on a Precisa 26ZSMA-FR balance. As in previous studies of *G. pulex* (Bollache et al. 2000), size was measured as the length of the fourth coxal plate, with a stereoscopic microscope Nikon SMZ-10A and a video analysis system VT0 232 from Linkam Scientific Instruments Ltd (Tadworth, Surrey, U.K.). We also measured the size (length of fourth coxal plate) of females with which males had paired.

Individuals from the first sample were killed by crushing the head with fine forceps, then stored in Eppendorf tubes in 0.2 ml of 2% sodium sulphate solution for 1 day before being analysed for glycogen content (see below). Individuals from the second sample were killed in the same way and stored in Eppendorf tubes containing 0.5 ml of a 1:1 chloroform/methanol solution for 3 days before being analysed for lipid content.

Lipid analysis

To measure lipid content we used a variation of the technique described by Van Handel (1985a). All samples were crushed in their Eppendorf tube with an Eppendorf pestle. The supernatants were then transferred to clean 16 × 100-mm culture tubes. The tubes were placed inside a fume cupboard in a water bath at 95 °C to enable any remaining solvent to evaporate. We then added 200 µl of concentrated (95%) sulphuric acid to each tube and left them for 10 min. The tubes were then removed from the water bath and left to cool before we added 5 ml of a vanillin–phosphoric acid reagent (Van Handel 1985b). All tubes were vortexed and left for 5 min to enable the colour to develop. We then pipetted a 0.3-ml subsample of the solution in each tube into a separate well of a 96-well microplate. The optical density in each well was read directly at 490 nm with a Dynex MRX plate reader with Revelation software. The lipid content in each sample was determined from a calibration curve constructed with 50-, 200-, 400-, 1000- and 2000-µg samples of commercial vegetable oil (linear regression: r^2=0.962, $F_{1,14}$=452.816, P<0.0001).

Glycogen analysis

Glycogen analysis was based on Van Handel (1985a). All samples were crushed in their Eppendorf tubes with an Eppendorf pestle. We then added 1 ml of methanol to

each Eppendorf tube, before vortexing them for 30 s and then centrifuging them at 2000 g for a further 2 min. The supernatant (containing sugars) from each tube was decanted into a separate 16 × 100-mm culture tube. The remaining *G. pulex* tissue and the glycogen (adsorbed on to the precipitated sodium sulphate) in the tubes were then washed into clean 16 × 100-mm culture tubes with 1 ml of Anthrone reagent (Van Handel 1985a). A further 4 ml of Anthrone reagent was then added to the tube before being placed into a water bath at 95 °C for 17 min. The tubes were removed from the water bath, vortexed for 20 s and left to cool. We then pipetted a 0.3-ml sub-sample of the solution in each tube into a well of a 96-well microplate. The optical density in each well was then read at 630 nm with Dynex MRX plate reader with Revelation software. The glycogen content of each sample was determined from a calibration curve, constructed with 25-, 50-, 100-, 150-, 200- and 400-μg concentrations of a standard glucose solution (Van Handel 1985a; linear regression: r^2=0.948, $F_{1,17}$=294.364, P<0.0001).

Because levels of male lipid and glycogen reserves are typically positively correlated with body size in *G. pulex* (Plaistow et al. 2001), we used an ANCOVA to compare the relative energetic status of field-collected paired and unpaired males independent of their body size. Because energy reserves should increase with body size in an allometric fashion, and ANCOVA requires relations to be linear (Sokal & Rohlf 1995), all measures of energy reserves and body size were log transformed before analysis. Deviations from normality were tested with Kolmogorov–Smirnov Liliefors test and homogeneity of variance was tested with Bartlett's test (Sokal & Rohlf 1995).

Laboratory Experiment

We collected a large sample of unpaired male and female *G. pulex* (about 150 individuals) from the field as described above. We used all the unpaired individuals we found to ensure that the natural ratio of unpaired males and females was maintained. They were immediately taken to the laboratory and placed in well-aerated aquaria (35 × 23 cm and 20.5 cm high) containing water from the collection site (at 12 °C) and excess food (elm leaves), under a 12:12 h light:dark cycle. During the first 3 days, all pairs that formed were removed to ensure that any pairs that had separated during the sampling and repaired at random were excluded from the experiment (see Elwood & Dick 1990). These animals were added to stock tanks that are kept in the laboratory. After this period, we checked the tank daily and sequentially assigned all newly formed pairs to one of six treatments: precopula duration of 2, 5, or 8 days either in the presence of ad libitum food (elm leaves) or with no food present. Individuals from each of the six treatment groups were evenly distributed within and between four aquaria (90 × 60 cm and 50 cm high) filled with constantly aerated stream water (12 °C) each containing 60 plastic tubes (diameter 3 cm, height 8 cm) that had the bottom cut away and replaced with a fine plastic mesh. After each male had completed its allotted precopula time, we

removed the pair and measured the fourth coxal plates of both the male and female as described for the field study. Females were added to laboratory stock. At this point females were added to laboratory stocks and were no longer used in the experiment. Males were killed by crushing the head with fine forceps and placed in an Eppendorf tube with either 0.2 ml of 2% sodium sulphate solution for 1 day for glycogen analysis, or 0.5 ml of a 1:1 chloroform/methanol for lipid analysis (see above). The experiment was repeated until we had obtained reasonable sample sizes for all of the three guarding treatments. Pairs that separated, moulted or died during the experiment were excluded from all further analyses and were added to laboratory stock tanks.

One problem with this experimental design is that the probability of pairs staying together (and therefore being included in the analysis) may not be the same for each of the treatments. For example, because larger males normally pair with larger females for longer (Elwood & Dick 1990), our experiment may have inadvertently selected for larger males in the longer guarding treatments. To test the relative influence of both the feeding treatment and the precopula duration treatment on the probability of pairs staying together, we carried out a logistic regression analysis using the 'Statview' 5.0 statistical software (SAS Institute Inc, http://www.statview.com). Feeding treatment and precopula duration treatment were entered into the model as separate fixed factors. However, for a subset of this experiment (77 pairs for lipid analysis and 82 pairs for glycogen analysis) we also measured male and female length (fourth coxal plate) whether or not the pair later split up. These data enabled us to carry out a second analysis in which male size, female size and female:male body size ratio were entered into a logistic regression model as continuous variables alongside 'feeding treatment' and 'precopula duration treatment'. After fitting the full model in each case, we used a backward elimination procedure to remove variables that had no significant influence on the model (Hardy & Field 1998).

As in the analysis of the field-collected data, all measures of size and energy reserves of males were log transformed before analysis to ensure linearity. However, we used only the length of the fourth coxal plate as a measure of size because weight might have changed during the experiment and varied between treatment groups. To analyse the data we used ANCOVA with 'feeding treatment' and 'precopula duration' entered into the model as fixed factors. Because males paired with females under simulated natural conditions (i.e. males competed for a natural size distribution of females at a natural sex ratio), pairs could have formed assortatively, that is, there would be a positive association between male and female body sizes. For this reason, we could not enter both male size and female size into the analysis as independent covariates. Instead, we controlled for the variation in male body size in all analyses by using residuals from the regression of female body size (mm) on male body size (mm) as the covariate, and the residuals from the regression of male energy reserves (log) and male body size (log) as the dependent variable.
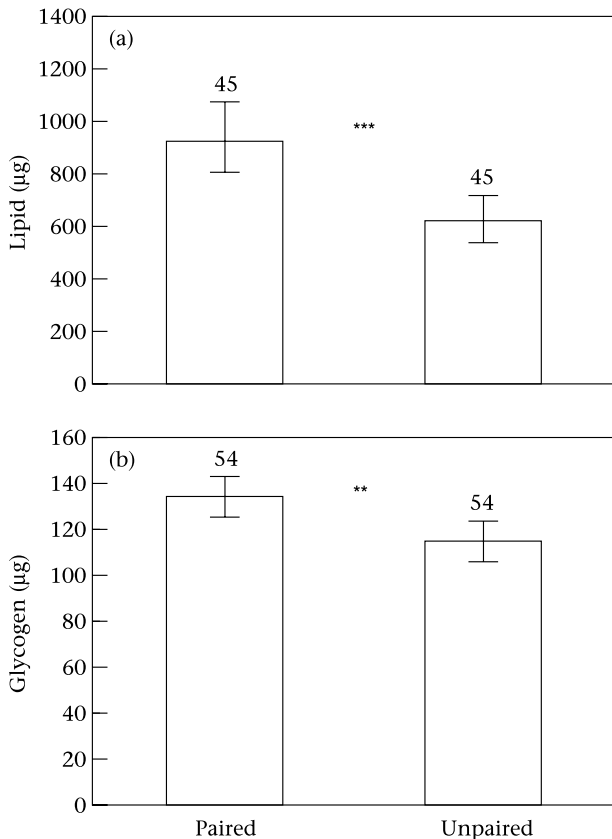
Figure 1. (a) Lipid and (b) glycogen reserves of males collected from the field either paired with a female or unpaired. All data were log transformed for analysis but here are expressed as antilogs of adjusted least square means after correction for male body size. Error bars represent antilogs of the (log) 95% confidence limits. Numbers above the bars refer to sample sizes. **$P<0.01$; ***$P<0.001$.

RESULTS

Field Study

Male lipid content (log) was not correlated with either male size (log; linear regression: $r^2=0.023$, $F_{1,89}=2.111$, NS) or male weight (log; linear regression: $r^2=0.04$, $F_{1,89}=3.667$, NS). The lipid content of paired males was significantly higher than that of unpaired males (unpaired t test: $t_{88}=4.212$, $P<0.0001$; Fig. 1a). For the paired males, male body size (mm) was positively correlated with female body size (mm; Pearson correlation: $r_{43}=0.508$, $P=0.0003$), that is, there was positive size-assortative pairing. Female body size (mm) had a marginally nonsignificant negative effect on male size-corrected lipid reserves (linear regression: $r^2=0.072$, $F_{1,44}=3.323$, $P=0.075$). This result was slightly stronger when female body size was also corrected for the size (mg) of the male with which she was paired, that is, when any biasing effect that size-assortative pairing may have had was removed (linear regression: $r^2=0.076$, $F_{1,44}=3.521$, $P=0.067$).

Glycogen content (log) was positively related to male body size (log: ANCOVA; $F_{1,105}=53.802$, $P<0.0001$) and male weight (log; ANCOVA: $F_{1,105}=52.195$, $P<0.0001$).

Male size (log) was a better predictor of male glycogen content (log) and was therefore used as the covariate for comparing the size-corrected glycogen content of paired and unpaired males. There was no difference in the slope of the relation between male body size and glycogen reserves between paired and unpaired males (ANCOVA: $F_{1,104}=0.121$, NS), but size-corrected glycogen content was significantly greater for paired than of unpaired males (ANCOVA: $F_{1,105}=9.839$, $P=0.002$; Fig. 1b). Female body size was positively correlated with the size of the male with which she had paired (Pearson correlation: $r_{52}=0.373$, $P=0.005$), resulting in positive size-assortative mating. However, there was no effect of female body size (mm) on male size-corrected glycogen content (residuals; $r^2=0.002$, $F_{1,53}=0.124$, NS). The effect of female body size remained nonsignificant after correction for the size of the male with which she had paired ($r^2=0.004$, $F_{1,53}=0.210$, NS).

Laboratory Experiment

Of the 627 pairs set up in this experiment, either the male or female died in 29 of them. From the remaining 598 pairs, 330 stayed together for their allocated amplexus duration and 268 split up. The full model explaining the probability of a pair remaining together was highly significant ($G_3=152.185$, $P<0.0001$). As expected, this probability was strongly influenced by their guarding duration treatment ($G_2=151.648$, $P<0.0001$). However, the feeding treatment had no influence on the probability of pairs staying together ($G_1=0.656$, NS).

The results were the same for the second analysis (175 pairs) in which male and female body sizes were included as covariates. The full model was highly significant ($G_6=69.321$, $P<0.0001$). Nevertheless, there was no influence of the ratio of female to male body size ($G_1=0.048$, NS), feeding treatment ($G_1=1.133$, NS), or female body size ($G_1=2.570$, NS), on the probability of a pair staying together. Thus we were left with a final model with just precopula duration treatment and male body size ($D_{102}=103.336$, NS). The probability of remaining paired increased with male body size ($G_1=12.001$, $P=0.0005$; Fig. 2) but declined significantly with increasing precopula duration ($G_2=57.861$, $P<0.0001$). For a male of average size ($\bar{X} \pm SE=2.6 \pm 0.02$ mm, $N=331$) the probability of remaining paired decreased from 96% in the 2-day treatment to 62% in the 5-day treatment, and 31% in the 8-day treatment (Fig. 2). These data show that for this population of *G. pulex*, at this time of year, PCMG duration rarely exceeded 8 days (at least in the laboratory), and confirm that the precopula duration chosen for the experiment was relevant. Differences in body size between the precopula treatment groups were tested with a planned linear contrast (8 days>5 days>2 days). There was no difference in body size in the males used for lipid analysis ($F_{1,172}=2.45$, NS; Fig. 3a), but there was a marginally significant increase in female size with longer precopula durations ($F_{1,170}=3.49$, $P=0.06$; Fig. 3a). In the glycogen replicate males in the 8-day group were larger
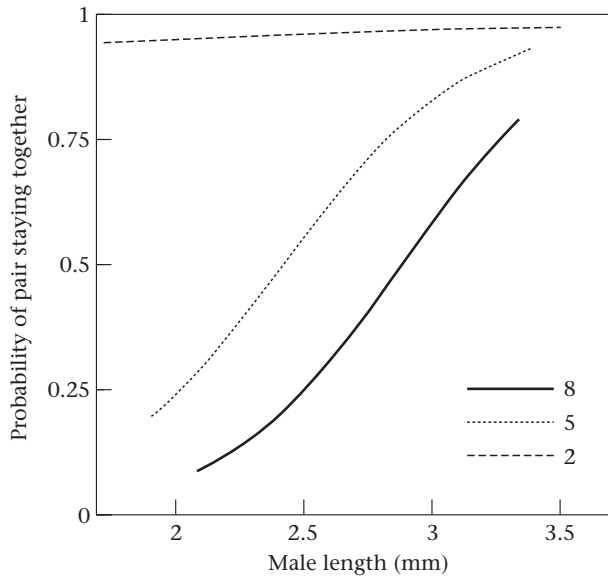
Figure 2. The effect of precopula treatment group (2, 5 or 8 days) and male body size (mm) on the estimated probability of a pair remaining together throughout the experiment. Estimated probabilities are plotted for the size range of males present within each precopula treatment group.
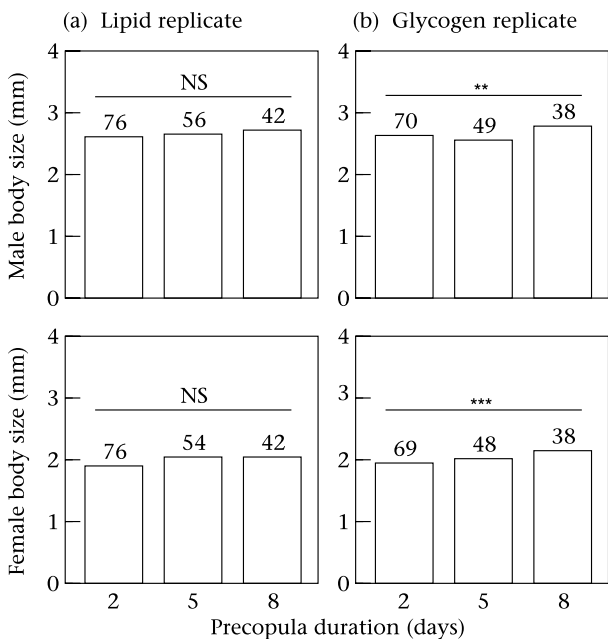

Figure 3. The body size (mm, $\bar{X}\pm SE$) of males and females that remained paired for either 2, 5, or 8 days in (a) the lipid replicate and (b) the glycogen replicate. Numbers above the bars refer to sample sizes. **$P<0.01$; ***$P<0.001$.
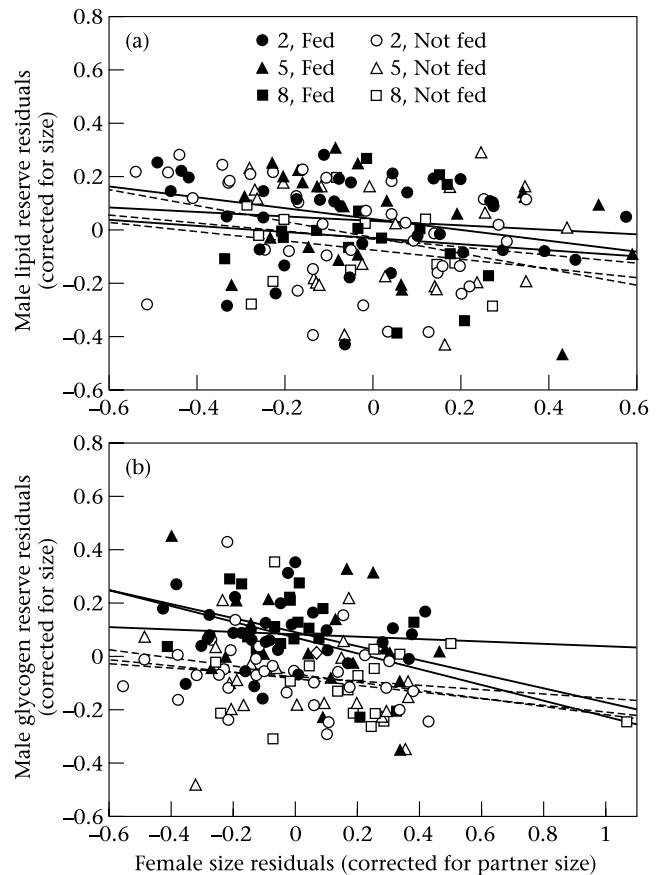

Figure 4. The relation between female body size and (a) male lipid reserves and (b) male glycogen reserves. To correct for size-assortative pairing, female body size is expressed as the standardized residuals from a regression of female size (mm). Measures of male energy reserves were also corrected for male body size and are expressed as residuals from a regression of (log) energy reserve over (log) male size (mm). Precopula duration was 2, 5 or 8 days and groups were either fed (———) or not fed (– – –).

increase with male body size (log; linear regression: $r^2=0.024$, $F_{1,152}=3.705$, $P=0.056$). We used residuals from this relation as a body size-corrected measure of male lipid reserves. Residuals from the relation between female body size (mm) and male body size (mm; linear regression: $r^2=0.04$, $F_{1,170}=7.063$, $P=0.009$) were used as a measure of female body size (relative to the size of the male with which she was paired). Relative female body size had a negative influence on corrected male lipid content (ANCOVA: corrected female body length: $F_{1,145}=8.152$, $P=0.0005$; Fig. 4a). There was no difference in slope between any of the treatment groups (ANCOVA: relative female body length × feed treatment: $F_{1,140}=0.221$, NS; relative female body length × guarding duration treatment: $F_{2,140}=0.021$, NS; relative female body length × feed treatment × guarding duration treatment: $F_{2,140}=0.460$, NS; Fig. 4a). Males in the fed treatment groups had slightly higher body size-corrected lipid contents than those in the nonfed treatment groups, although this difference was marginally nonsignificant (ANCOVA: $F_{1,145}=3.299$, $P=0.071$; Fig. 5a). However,

$(F_{1,155}=6.78$, $P=0.01)$, although males in the 2-day treatment were larger than those in the 5-day treatment (Fig. 3b). Female body size increased with precopula duration $(F_{1,153}=14.24$, $P=0.0002$; Fig. 3b).

Pairs from which males were used for lipid analysis showed positive size-assortative pairing $(r_{170}=0.2$, $P=0.009)$. Male lipid content (log) showed a weak positive
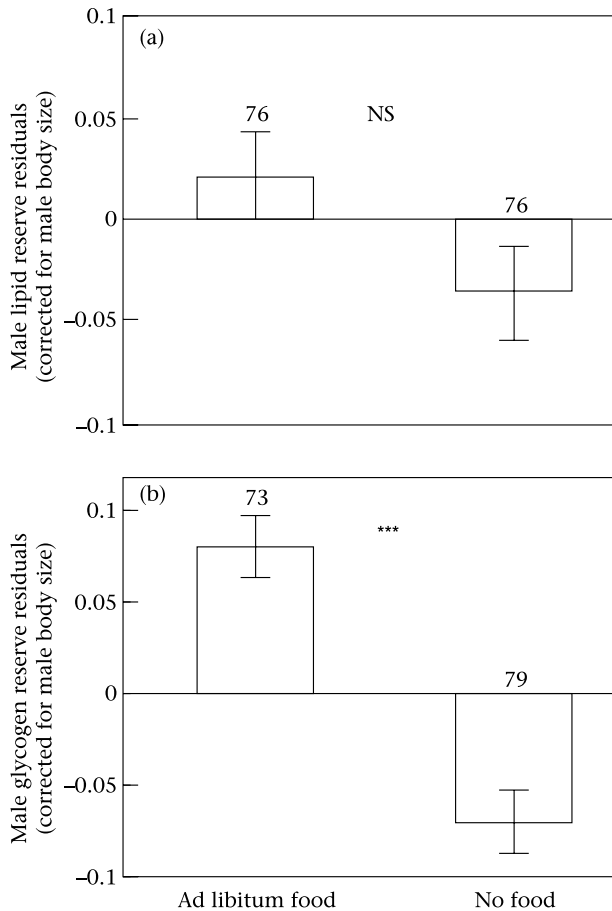
Figure 5. (a) Lipid and (b) glycogen reserves of males that were not fed or were fed ad libitum during the experiment to measure which factors influence the energetic cost of precopulatory mate guarding. Lipid and glycogen reserves are expressed as adjusted least square means following correction for the negative effect of partner size-corrected female body size on male energy reserves (Fig. 3). Numbers above the bars refer to sample sizes. ***$P<0.001$.
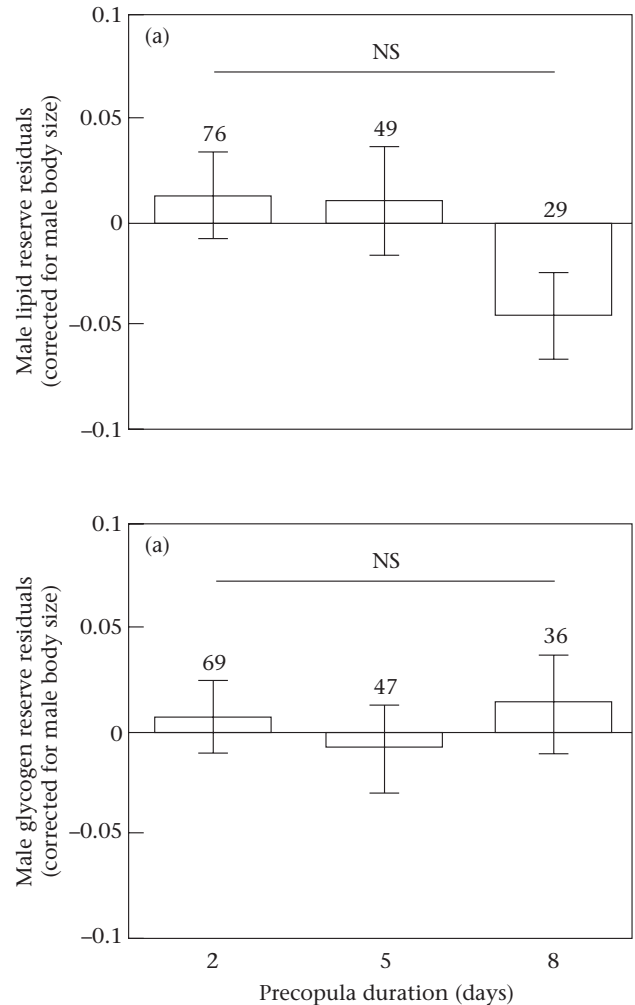

Figure 6. (a) Lipid and (b) glycogen reserves of males that were allowed to pair with a female for 2, 5 or 8 days. Lipid and glycogen reserves are expressed as adjusted least square means following correction for the negative effect of partner size-corrected female body size on male energy reserves (Fig. 3) Numbers above the bars refer to sample sizes.

guarding duration had no effect on male size-corrected lipid content (ANCOVA: $F_{2,145}=1.145$, NS; Fig. 6a) and the interaction between the two fixed treatments was not significant (ANCOVA: $F_{2,145}=0.051$, NS).

Pairs from which males were used for glycogen analysis also showed positive size-assortative pairing ($r_{153}=0.232$, $P=0.004$). Male glycogen content (log) increased with male body size (log; linear regression: $r^2=0.149$, $F_{1,152}=26.691$, $P<0.0001$; with outlier removed: $r^2=0.048$, $F_{1,151}=7.443$; $P=0.007$). The residuals taken from this relation were used as a body size-corrected measure of male glycogen reserves. Residuals from the relation between female body size (mm) and male body size (mm; linear regression: $r^2=0.054$, $F_{1,153}=8.739$, $P=0.0004$) were used as a measure of female body size relative to the size of the male with which she had paired. Relative female body size had a negative influence on corrected male glycogen content (ANCOVA: relative female body length: $F_{1,145}=7.948$, $P=0.0005$; Fig. 4b). There was no difference in slope between any of the treatment groups (ANCOVA: relative female body length × feed treatment: $F_{1,140}=$

0.643, NS; relative female body length × guarding duration treatment, $F_{2,140}=0.540$, NS; relative female body length × feed treatment × guarding duration treatment: $F_{2,140}=1.033$, NS; Fig. 4b). Males that had access to food had much higher body size-corrected glycogen contents than those that were not fed (ANCOVA: $F_{1,145}=39.428$, $P<0.0001$; Fig. 5b), but guarding duration had no effect on male size-corrected glycogen content (ANCOVA: $F_{2,145}=0.274$, NS; Fig. 6b). Furthermore, the interaction between the two fixed treatments was not significant (ANCOVA: $F_{2,145}=0.050$, NS).

DISCUSSION

Our results show that lipid and glycogen reserves were strongly correlated with a male's probability of being paired in the field. In contrast to the assumptions of previous models, the energetic cost of PCMG in *G. pulex*

was unrelated to the duration of male guarding but strongly positively correlated with the size of the female. This increased energetic cost of pairing with a larger female was independent of any cost associated with reduced feeding while in precopula, and resulted in a reduction of both glycogen and lipid reserves (Fig. 4).

The fact that field-collected paired males had higher size-corrected energy reserves than field-collected unpaired males supports our hypothesis that males that are in poor energetic condition may be less able to pay the energetic costs associated with PCMG. It is not known whether the observed variation in the energy reserves of *G. pulex* depends on the stage of the moult cycle, as observed in other crustaceans (Passano 1960; Chang & O'Conner 1983; Jormalainen et al. 2001), or whether other sources of variation are important. For example, the energy reserves of unpaired males might have been reduced if they had recently completed one or several bouts of PCMG. Since the extent of moult-induced variation in energy reserves and the average energetic cost of PCMG have not been measured in *G. pulex*, further study is required to isolate the causes of variable male energy reserves in this species.

Ultimately the influence of energetic costs on a male's pairing decision will depend on the magnitude of the cost and the factors that have most influence on it. The results of our laboratory experiment suggest that the major influence on the cost of PCMG is the size of the female relative to that of the male (Fig. 4), rather than the duration of male guarding (Fig. 6). The negative relation between male size-corrected energy reserves and female body size (relative to the size of the male) was observed in all six of the treatment groups, in both the lipid replicate and the glycogen replicate of the laboratory experiment (Fig. 4). Furthermore, the much smaller sample size of pairs from the field also suggested a nonsignificant trend ($P=0.06$) for males with relatively larger females to have lower lipid reserves, although there was no influence on male glycogen reserves. Finally, there was no significant interaction between guarding treatment and relative female size. Thus it is unlikely that this effect could have been caused by the inadvertent selection for larger individuals that was apparent in the 5- and 8-day treatment groups. The danger was that longer guarding duration would select for larger males that are able to guard females for longer. Although there was a tendency for male body size to increase with precopula duration, this effect was not as strong as the tendency for female size to increase (Fig. 3). If anything, the increased size of females in the longer guarding treatments should have increased the probability of guarding duration influencing male energy reserves. Because in *G. pulex* males normally guard larger females for longer (Elwood & Dick 1990), the probability of a larger female moulting before the end of the experiment might have been lower. This would explain why female size increased with precopula duration, but why the probability of not splitting up was also positively correlated with male body size in the 5- and 8-day treatment groups is not clear. It might be argued that only large males are able to guard females for longer. However, this seems unlikely given that only female size

and not the time spent in precopula had an influence on the energetic cost of precopula. Therefore, an alternative explanation is that only larger males are able to pair with larger females. The inadvertent selection for larger females in the 5- and 8-day treatments (as a result of proximity to moulting) may have led to selection for larger males as well.

Both the loading constraints hypothesis (Adams & Greenwood 1983, 1987) and the guarding time hypothesis (Elwood & Dick 1990) predict that in *G. pulex*, the cost of PCMG should increase with the size of the female that is guarded. However, neither of these hypotheses predicts that this cost should be independent of the duration of precopula, as we observed. One reason for the absence of an effect of guarding duration could be that our experiment was carried out in still water rather than flowing water, as would normally be found in a stream. However, because in the wild male/female size ratios are unaffected by current speed (Elwood et al. 1987; Elwood & Dick 1990), this explanation is unlikely. An alternative explanation is that the initial energetic cost of PCMG was greater than that later in the experiment. This result may have arisen because males initially paired with females in tanks that mimicked field conditions (density, sex ratio) but were later transferred to conditions where competition was absent (although chemical cues might still have been apparent). This would suggest that guarding a female is energetically costly, and that this cost increases with the size of the female being guarded. Alternatively, the energetic cost of PCMG may be the result of variation in the energetic cost of sexual conflict resolution over guarding duration (Jormalainen 1998; Watson et al. 1998; Sparkes et al. 2000) rather than a cost of carrying a female.

Female resistance is important for determining mating outcomes (Rowe et al. 1994; Clutton-Brock & Parker 1995; Jormalainen 1998; Watson et al. 1998; Jormalainen et al. 2000; Sparkes et al. 2000). Although we did not attempt to observe or measure female resistance to guarding in this experiment, it has been observed in *G. pulex* and was mainly associated with larger females (Thompson & Moule 1983; Ward 1984; Elwood et al. 1987). If this is the case, the reason why only larger male *G. pulex* are able to pair with large females may be that only larger males are able to keep hold of or subdue them. Such a large-male mating advantage is common in gerrids (see Arnqvist et al. 1996) and has also been seen in the seaweed fly, *Coelopa frigida* (Crean & Gilburn 1998). Why larger male *G. pulex* should have an advantage when overcoming female resistance is not yet clear. It could be that only larger males are able to pay the increased energetic cost of pairing with larger females. Alternatively, larger females may be less resistant to the advances of large males because they are unable to beat them, the cost of resisting them is greater, or because they would prefer to mate with larger males. These hypotheses might be distinguished by a study that closely examines male and female behaviour during pair formation, how this interaction is influenced by male/female size ratios, and how these differences influence the immediate energetic costs for males and females. The reason why the

energetic cost of PCMG does not increase with the duration of guarding may be that, once the initial conflict is resolved, females may become passive and the pair may rest on the substrate. Such behaviour is typical in *G. pulex* (L. Bollache, personal observation). Furthermore, Watson et al. (1998) showed that in the water strider, *Aquarius remigis*, females that carry males have a higher metabolic rate, that is, they pay an energetic cost. But this cost was influenced by the size of the male only during the initial, most energetically costly, stage of PCMG during which females tried to resist being guarded.

Elwood & Dick's (1990) guarding duration hypothesis suggests that larger male *G. pulex* guard larger females for longer because PCMG is costly and these costs accumulate with guarding duration at a faster rate for small males than for large males. Our finding that PCMG duration has no influence on the lipid and glycogen reserves of male *G. pulex* appears to reject this hypothesis. However, both theory (Jormalainen et al. 1994; Yamamura & Jormalainen 1996) and empirical studies (Jormalainen & Merilaita 1993, 1995; Sparkes et al. 2000) suggest that female resistance to being in precopula decreases as she approaches the moult. Furthermore, females may resist smaller males more (Jormalainen et al. 2000). If this is the case, then the energetic cost of PCMG will increase the further away a female is from moulting, and may still be higher for smaller males. Thus we suggest that the logic of the guarding time hypothesis may still be relevant, but size-related variation in the cost of PCMG may be the result of overcoming female resistance, rather than any direct cost of carrying females. If male *G. pulex* do pay an energetic cost of overcoming female resistance to PCMG, our results contrast with those of a study in the isopod *I. baltica* in which females paid an energetic cost of struggling with males, but males did not (Jormalainen et al. 2001). This difference may be caused by differences in the mating systems, since in *I. baltica*, there is apparently no male energetic cost of PCMG (Jormalainen et al. 2001), unlike in other mate-guarding crustaceans (Robinson & Doyle 1985; Sparkes et al. 1996).

In our study, males that had access to food during the experiment had higher glycogen reserves, and a tendency towards higher lipid reserves, than males that did not have access to food. These results clearly show that males are able to feed during precopula, but whether this is at a reduced rate compared with unpaired males, and whether this rate is reduced even further in the field, as shown by Sparkes et al. (1996), remains unclear. The results presented in Fig. 5 describe the 'worst case scenario' in which males are unable to feed at all. The lack of any significant interaction between feeding treatment and the effect of female body size on male energy reserves suggests that in *G. pulex* any reduced feeding cost is independent of the energetic cost of pairing with larger females. This result contrasts with Sparkes et al.'s (1996) study of the isopod *L. fontinalis*, which showed that when pairs had access to food during precopula, the cost of PCMG disappeared. The cost of PCMG appeared to be only a short-term energetic cost, as it had no influence on male lipid reserves (Sparkes et al. 1996). In contrast, our results show that in *G. pulex* both starvation and PCMG reduce

the glycogen and lipid reserves of males, although food deprivation had a larger impact on glycogen reserves, whereas PCMG may influence lipid reserves more, particularly in the field. Thus, it seems likely that in *G. pulex* the costs of PCMG are more than just short-term effects and may directly trade off with growth. This sort of reduction in energy reserves may explain why in Robinson & Doyle's (1985) study of *G. lawrencianus*, males that were permanently able to enter into PCMG had growth rates that were only 45% of those of males that were kept in the absence of females.

Acknowledgments

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