ORIGINAL ARTICLE

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Sperm economy and limitation in spiny lobsters

Abstract Sperm limitation, when female fertilisation success is constrained by the supply of sperm, is generally perceived to be an uncommon feature of reproduction in species which directly transfer gametes during copulation. Male size, previous copulations, and the balance of expected reproductive return and future mating opportunity may, however, limit the amount of sperm males transfer to females. We used laboratory experiments where mate size could be manipulated and its consequences on spermatophore size and clutch size determined, to show that in two genera of spiny lobsters (Crustacea: Palinuridae) male reproductive output limits the size of clutches brooded by females. In *Panulirus argus* from the Florida Keys, we show that while male size affects spermatophore area, males also vary the amount of ejaculate positively with female size. Furthermore, the area of the spermatophore has a greater influence than female size on subsequent clutch weight. In *Jasus edwardsii* from New Zealand, female size, male size and mate order all affect clutch weight. In both species, clutches fertilised by small males in the laboratory are significantly smaller than clutches fertilised by large males. These results suggest that to ensure they receive sufficient sperm, females should either mate several times prior to oviposition, mate as early as possible in the reproductive season, or choose large, preferably unmated males as partners and thus compete with other females for preferred males. Sperm-limited female fecundity has the potential to limit the egg production of fished populations where large males are typically rare.

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Introduction

Because sperm are small and numerous it is often assumed that variation in female reproductive success has little to do with male or sperm availability (Parker 1984; Levitan and Peterson 1995). However, since Dewsbury (1982) pointed out that sperm are delivered to females in ejaculates or spermatophores that may be costly or slow to produce, there is growing evidence that in certain circumstances sperm supply can limit fertilisation success and realised fecundity. Sperm limitation has, over the last decade, been found to be a common feature of reproduction in free-spawning marine taxa (Pennington 1985; Levitan and Peterson 1995), principally due to the dilution of gametes in the water column after spawning. In species in which males deposit an external or internal spermatophore during copulation with the female, sperm limitation may occur in a number of ways.

When ejaculate or spermatophore size is correlated with male body size (e.g. McLain et al. 1990; Bissoondath and Wiklund 1996), a single copulation by a small mature male may not provide enough sperm to fertilise all the eggs produced by a large female. In some polyandrous species, a single mating may not provide a female with enough sperm to fertilise all her eggs (Ridley 1988; Petersson 1991; Chen and Baur 1993). Repeated copulations of favoured males may deplete sperm supply or accessory glands resulting in inadequate numbers of sperm transferred to later-mating females (Dewsbury 1982; Nakatsura and Kramer 1982; Rutowski et al. 1987). Males with a high potential mating success (often the largest males) may submaximally allocate limited supplies of sperm across several females (Pitnick 1991; Pitnick and Markow 1994; Warner et al. 1995). There may be too few reproductively competent males to fertilise all the eggs females produce in some natural

populations (e.g. Kirkendall 1990; Pitnick 1993). Disease or food supply may affect male condition and compromise the supply of sperm (Zuk 1987, 1988; Proctor 1992; Olsson and Shine 1997). Sperm may die during storage in the spermatophore prior to use leading to low fertilisation rates (Paul 1984). In some species, although sperm may be stored internally, fertilisation of eggs occurs externally within a brood-chamber (Mann 1984; Subramoniam 1993) increasing the chance of sperm loss and depressed rates of fertilisation. The relative importance of these different possible mechanisms in limiting sperm supply and lowering fertilisation rates is not known.

Most data on sperm limitation among species in which there is direct exchange of gametes come from studies of terrestrial species, especially insects. Although many of the conditions promoting sperm limitation also occur in marine crustaceans, so far there is only limited evidence of this (McMullen and Yoshihara 1971; Powell et al. 1974; Paul 1984; Paul and Paul 1990; Paul et al. 1991; Saint-Marie 1993). This is surprising because selective fishing, as occurs in many species of crabs and lobsters for example, preferentially removes large males from the population (Miller 1976; Pollock 1986; Smith and Jamieson 1991; Saint-Marie and Hazel 1992). This practice may increase the likelihood of sperm limitation through lack of males during peak mating periods, sperm depletion of the few remaining large males (Paul and Paul 1997), submaximal allocation of sperm supplies by males amongst females, or inadequate sperm supply (from the females' perspective) from small males (Paul and Paul 1990).

In spiny lobsters (Crustacea: Palinuride), the number of eggs per clutch has been universally assumed to be a function of female size (Chubb 1994). For example, in *Jasus edwardsii*, egg production increases about seven times from 80,000 at 80 mm carapace length (CL) to 535,000 at 160 mm CL (Annala 1991). Much variation in size-specific female fecundity, however, often remains unexplained by the regression of clutch size on body size. The correlation (*r*) between these two variables in *J. edwardsii* can be as low as 0.592, for example (Annala and Bycroft 1987). Male size is not usually considered as a source of this variation, as it is generally assumed that males supply sperm in excess of the number of eggs to be fertilised. However, there is a very large change in sperm production with male size. In *J. edwardsii*, the concentration of sperm in the vas deferens is constant above a body size of 65 mm CL but the size of these organs increases 25-fold from 0.2 g at 100 mm CL to over 5 g at 180 mm CL (MacDiarmid 1989a), thus potentially determining the maximum size of spermatophore that can be deposited. Males may not deposit the entire contents of the vas deferens in a single mating. Theory and observations suggest that if the cost of sperm production is high, males should economise on sperm by allocating their supply among successive females dependent on expected reproductive return and future mating opportunity (Dewsbury 1982; Parker 1990a,b, 1993; Pitnick 1991; Pitnick and Markow 1994; Shapiro et al. 1994;

Warner et al. 1995; Ball and Parker 1996; Parker et al. 1996). If these are low and high, respectively, then males should deposit a small spermatophore, increasing the probability of sperm limitation.

The aim of this study was to test the hypothesis that sperm limitation in spiny lobsters is caused by submaximal allocation of sperm supplies by males amongst females, and inadequate sperm supply (from the females' perspective) from small males. We examined this question in two species, *J. edwardsii* from temperate Australia and New Zealand, and *Panulirus argus* from the tropical western Atlantic (Holthuis 1991), using controlled laboratory experiments where mate size could be manipulated and its consequences on mating behaviour, spermatophore size and ultimately on clutch size determined.

Methods

J. edwardsii and *P. argus* were chosen for study because they differ in a fundamental aspect of their reproductive biology. During copulation, male *P. argus* deposit a large (up to 11 g) external, tar-like spermatophore on the sternal region of the female (Lipcius et al. 1983; Martin et al. 1987). This is eventually (1–28 days after mating) scratched open by the female to expose the non-motile sperm (Talbot and Summers 1978) which fertilise her eggs externally within the brood-chamber formed by the reflection of the tail under her body (Kittaka and MacDiarmid 1994). The long-lived spermatophore provides an opportunity to measure male reproductive output. In the genus *Jasus* (and some species of *Panulirus*; e.g., *P. guttatus*), the spermatophore lacks the tough outer layer and disintegrates easily, so it is immediately used by the female to fertilise her eggs (Patterson 1969; Berry and Heydorn 1970; MacDiarmid 1988). The brief existence and fragility of the spermatophore makes it impossible to measure directly in *J. edwardsii*, and its size has to be inferred from the number of eggs it fertilises.

J. edwardsii

Mature males and pre-moult females were obtained from the Chatham Islands and the Wellington coastline in February 1995 and transferred to a flow-through sea water system on the shore of Wellington Harbour. Sexes were held separately until the start of the experiment. Lobsters were then measured (CL), individually marked with colour-coded antennae tags, and distributed among fifteen 1.8-m-diameter × 0.6-m-deep concrete tanks. Each tank contained a single small (90–105 mm CL), medium (120–130 mm CL) or large (180 + mm CL) mature male and five females, one from each of five size classes (75–89, 90–109, 110–129, 130–149 and 150 + mm CL) making five replicate tanks for each level of male size. The six lobsters in each tank had continuous access to live food (blue mussels, *Mytilus galloprovincialis*) and shelter from direct light. Tanks were monitored by direct observation once each day from late February to late July. We recorded if individuals had moulted, laid eggs or died since the last observation, or were courting or copulating. Moulted females were retagged. Three females and two males that died soon after the start of the experiment, but before the onset of moulting or reproduction, were replaced with others of an appropriate size held in reserve.

Whole egg clutches were removed from females approximately 76 days after egg laying to determine clutch weights. We used clutch weight rather than number of eggs per clutch as it is more quickly and accurately measured and the two variables are strongly correlated ($R^2_{adj} = 0.91$; $F_{1,56} = 580$, $P < 0.0001$; egg count = $39,576 + 2714 \times$ clutch weight (g):A.B. MacDiarmid, unpublished data).

Seven response variables were chosen for analysis on a per tank basis: mean Julian day (= day of year) of first courting (as defined by the frontal approach in Lipcius et al. 1983), mean number of days of courting, mean Julian day of mating, number of egg-bearing females, mean carapace length of mated females, mean clutch weight and total clutch weight. Response variables were broken into behavioural and physical groups and analysed in two separate MANOVAs. Roy's largest root (RLR) was selected as the test statistic because of its superior sensitivity (Hintze 1996).

To adequately describe the change in clutch weights across the size range of females mated by small, medium and large males, the experiment was repeated in 1996 with small males only to increase the pool of successful matings by males of this size. Experimental conditions and data collection were identical to those described for 1995, except unfertilised eggs loose on the floor of the tanks were collected and their settled volumes measured in a graduated measuring cylinder. For this pooled data set, we considered five factors that might affect clutch size: female CL, male CL, mate order (i.e. males' 1st, 2nd, and so on spermatophore deposited), Julian day of mating, and standardised day of mating (first mating by a male = day 0 for that male). We used best-subsets regression to identify which of the factors were important and determined the effects of only this subset on clutch weight using multiple linear regression.

P. argus

Intermoult females, without spermatophores or external eggs, and males were trapped in the Florida Keys in March 1996 and transported to a nearby flow-through sea water system in Marathon, Florida. They were measured (CL), antennal tagged and assigned among 12 1.5-m-diameter polyethylene tanks set up similar to those used for *J. edwardsii*. Each of the 12 tanks contained either one small (91–95 mm CL), medium (104–124 mm CL) or large (137–144 mm CL) male, making 4 replicate tanks for each level of male size. It was planned for each tank to also contain five females, one from each of five size classes (70–79, 80–89, 90–99, 100–109 and 110+ mm CL), but not all size classes were available in every tank due to a delay in the start of the experiment and its premature end due to a water system failure. This precluded analysis on a per-tank basis as for *J. edwardsii*.

Tanks were monitored each day as for *J. edwardsii*. The areas of fresh and used spermatophores were measured by tracing their outlines onto transparent overhead projector sheet using waterproof marker pens. Outlines were later scanned as digital images and their areas calculated using image analysis software (Jandel Scientific, San Rafael, Calif.). Whole egg clutches were removed and processed as described for *J. edwardsii* between 15 and 20 days after egg laying to determine clutch weights. Likewise, we used clutch weight rather than number of eggs per clutch as it is more quickly and accurately measured and the two variables are strongly correlated ($R^2_{adj} = 0.935$; $F_{1,41} = 601$, $P < 0.001$; egg count = $29,770 + 5009 \times$ clutch weight (g); A.B. MacDiarmid, unpublished data).

We considered four factors that might affect the size of the spermatophores deposited by the male in each tank: male CL, female CL, mate order (i.e. males' 1st, 2nd, 3rd, and so on spermatophore deposited) and standardised day of mating (first mating by a male = day 0). We used best-subsets regression to identify which of these factors were important and determined the relative effects of only this subset on spermatophore size using multiple linear regression.

Likewise, we considered five factors that might affect clutch size: female size, male size, total spermatophore size, scratched (used) spermatophore size and non-scratched (unused) spermatophore size. We used spermatophore area as the measure of spermatophore size because it was quick and non-destructive, and strongly correlated with spermatophore weight ($R^2_{adj} = 0.890$; $F_{1,58} = 477$, $P < 0.001$; weight (g) = $-2.036 +$ [spermatophore area(mm^2) $\times$ 0.0056]; A.B. MacDiarmid, unpublished data). We included scratched spermatophore area as a potential correlate of clutch size because sperm may have only been available from the portion scratched by the female during the fertilisation procedure. Similarly, we considered the unscratched area of the spermatophore because this may have been the reservoir from which sperm leaked out during egg laying once the "stopper" at the posterior end of the spermatophore had been scratched away. We used best-subsets regression to identify which of the factors were important and determined the effects of only this subset on clutch weight using multiple linear regression.

Results

J. edwardsii

The overall MANOVA model testing male size effects on three behavioural response variables was significant but of the three variables measured only one, the number of days courting (ln + 1 transformed), contributed significantly to this result (Table 1). Small males courted females for an average of 2 days while large males courted for 11 days on average before mating (Fig. 1). Male size did not significantly alter the mean Julian day of first courting and mean Julian day of mating, but the power of the tests was weak (0.15 at $\alpha = 0.05$). Male size also significantly affected three of the four physical response variables measured (Table 1). In tanks with small males, fewer females mated, these carried smaller clutches of eggs, and consequently overall egg production per tank was < 50% of those with large males (Fig. 1). The mean CL of females mated in each tank

Table 1 *Jasus edwardsii*: results of MANOVA on effects of three levels of mature male size on female behavioural and physical response variables (*RLR* Roy's largest root)

Variable	RLR value	df	F-ratio	P
Behavioural response variables				
Model	0.998	3,11	3.66	0.048
Mean Julian day of first courting	69.921	2,12	0.79	0.477
Mean (ln x + 1) number of days of courting	4.308	2,12	4.78	0.029
Mean Julian day of mating	95.641	2,12	0.77	0.486
Physical response variables				
Model	14.375	4,10	35.94	< 0.001
Number egg bearing	8.267	2,12	11.27	0.002
Mean CL of mated females	97.317	2,12	1.65	0.234
Mean (ln) clutch weight	0.389	2,12	5.01	0.026
Total egg weight	83,994	2,12	57.66	< 0.001

Fig. 1 Behavioural and physical responses of female *Jasus edwardsii* to three levels of male size. Data are mean response per tank ±95% confidence limits (*S* small male, *M* medium male, *L* large male, *CL* carapace length; $n = 5$)
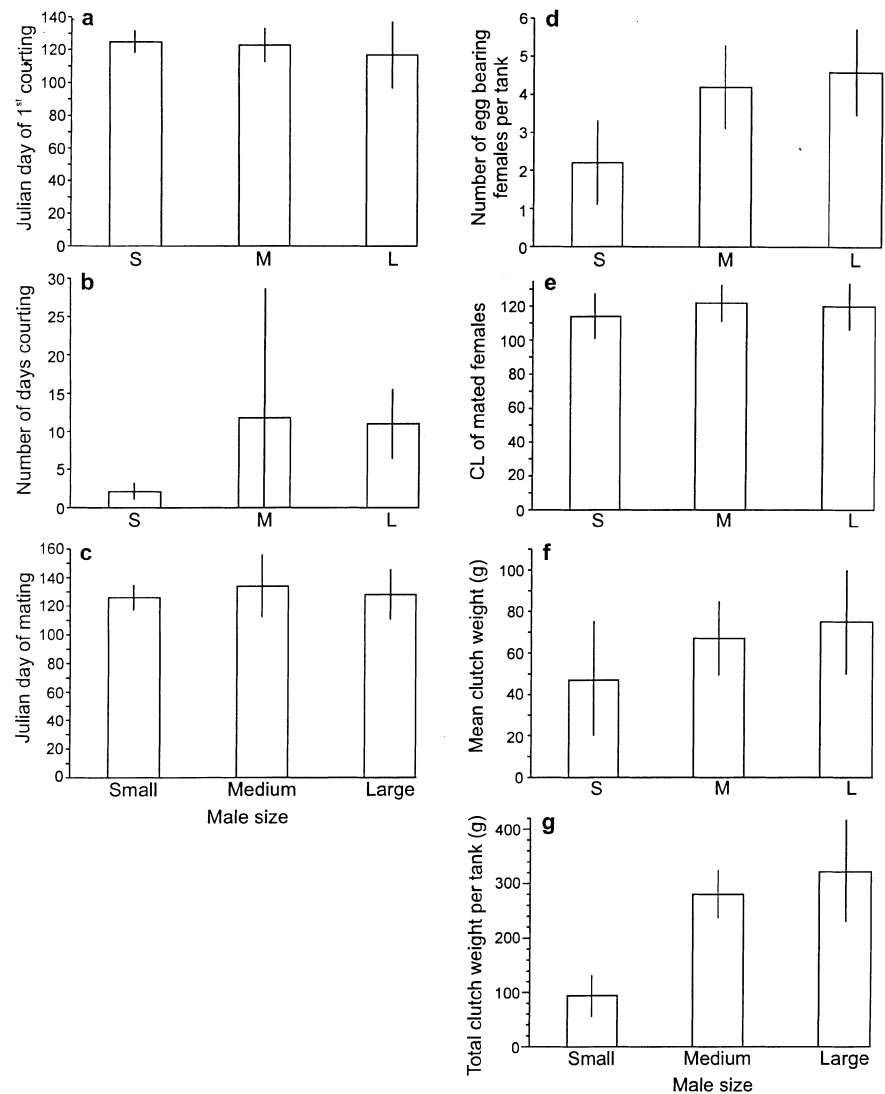
was unaffected by male size but the power of the test was low (0.28 at $\alpha = 0.05$).

Data from experiments in both 1995 and 1996 were pooled to describe the change in clutch weights across the size range of females mated by small, medium and large males. Three variables, female size, male size and mate order had significant effects on clutch weight (Table 2) and together explained about half of the observed variation ($R^2_{adj} = 0.424$). Female size accounted for 56% of the explained variation in clutch weight with the other two factors contributing equally to the remainder. Clutches fertilised by small males were highly variable in

weight and increased only slowly with female size (Fig. 2). Indeed, in this group of females, these two variables were only weakly correlated ($R^2_{adj} = 0.21$; $F_{1,56} = 16.4$, $P < 0.001$). In contrast, for females mated by large males, clutch weight increased more quickly with increasing female size and there was a strong correlation between these two variables ($R^2_{adj} = 0.76$; $F_{1,20} = 67.4$, $P < 0.001$). Females mated by medium-size males had intermediate results ($R^2_{adj} = 0.47$; $F_{1,19} = 18.5$, $P < 0.001$). This difference between large and small males is reflected in the slopes of clutch weight versus female CL which were significantly less for

Table 2 *J. edwardsii*: results of multiple regression on effects of male and female carapace length (*CL*) and mate order on clutch weight

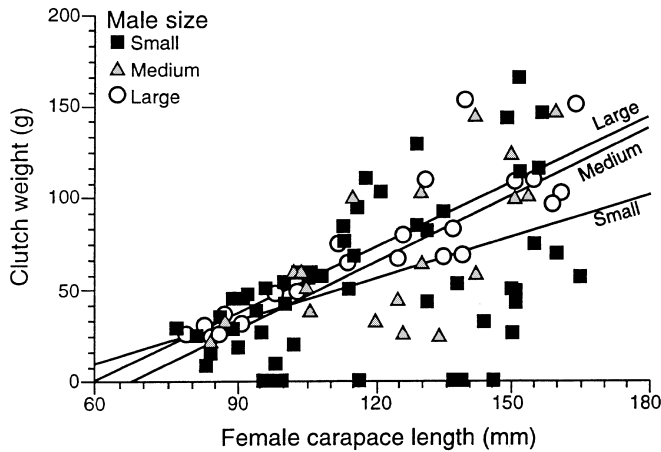| Variable	Regression coefficient	SE	Standardised coefficient	t-test value (Ho: $\beta = 0$)	P
Intercept	−50.2452	21.2144	0.000	−2.37	<0.05
Female CL	0.8313	0.1399	0.5139	5.94	<0.001
Male CL	0.2041	0.0820	0.1965	2.52	<0.05
Mate order	−6.0019	2.6035	−0.2005	−2.31	<0.05

Fig. 2 The relationship between clutch weight and female carapace length for female *J. edwardsii* mated by small, medium or large males. The regression lines for each male treatment are indicated. Regression equations for small, medium and large males respectively are: $y = 36.7 + 0.77x$; $y = 1.22x - 82.45$; $y = 1.19x - 70.77$

females mated by small rather than large males ($F_{2, 95} = 3.87$, $P < 0.05$).

We used multiple linear regression to determine the effect of the volume of loose eggs and female CL on the weight of egg clutches from females for which we were confident we had recovered most of the eggs lost after mating in the 1996 experiment. Together these two variables explained about a third of the variation in clutch weight of these 14 females ($R^2_{adj} = 0.36$; $F_{1,12} = 6.02$, $P < 0.05$), with only the volume of dropped eggs having a significant and negative effect ($t = -3.0522$, $P < 0.05$). The larger the volume of dropped eggs, the lighter the associated clutch of eggs [clutch weight (g) = 96.86 + (volume of dropped eggs (ml) × −0.53)].

P. argus

Spermatophore placement

With one exception, all spermatophores deposited by both large and small males were well formed, comprising distinct bean-shaped right and left parts and were correctly aligned forward of the trailing edge of the females' sternal plate, centred about the mid-line. The one exception occurred when a small male mated with a similar-sized female and about 5% of the total spermatophore area was misplaced on the base of the female's walking legs. In two cases, females completely removed the spermatophore within 2 days of copulation with a small male without depositing a clutch of eggs.

Spermatophore area

Of the four variables (male CL, female CL, mate order and standardised day of mating) considered that might influence spermatophore size, only male and female CL had significant effects (Table 3). Together these two variables explained about half the variation in spermatophore size ($R^2_{adj} = 0.509$) and accounted for 55% and 45% of the explained variation, respectively.

The size of spermatophores deposited by small males was only weakly related to female size ($R^2_{adj} = 0.10$; Fig. 3); indeed there was no significant relationship between these two variables ($F_{1,16} = 2.94$, NS), although the power of the test was weak (0.35 at a = 0.05). In contrast, for females mated by large- and medium-sized males, spermatophore size was more closely related to female size ($R^2_{adj} = 0.36$ and 0.39, respectively), although only for large males was this relationship significant ($F_{1,20} = 12.82$, $P < 0.01$). This difference between large and small males is reflected in the slopes of spermatophore area on female CL which were significantly less for females mated by small rather than large males ($F_{2,40} = 22.31$, $P < 0.0001$).

Males mated with more than one female, however. Thus, to determine the effect of within-male variation on the size of the spermatophore deposited, we analysed a balanced subset of the data using repeated-measures ANOVA. All males could not be used for this analysis because not all size classes of females were available to every male. There were three factors: male size class (fixed), male number (nested within male size class), and female size class (fixed). Like the previous multiple regression on the whole data set, the analysis showed significant effects of male size ($F_{1,4} = 8.72$, $P < 0.05$) and female size ($F_{1,4} = 33.8$, $P < 0.005$) on the area of the spermatophore. Individual males tended to deposit consistently smaller- or larger-sized spermatophores (Fig. 4).

Clutch weight

Of the five factors considered that might affect clutch weight (female CL, male CL, total spermatophore area, scratched spermatophore area and non-scratched spermatophore area), only total spermatophore area and female size had significant effects (Table 4). Together these two variables explained 75% of the variation in clutch weight ($R^2_{adj} = 0.763$) and accounted for 62% and 38% of the explained variation, respectively.

The increase in clutch weight with female size varied depending on the size of male that supplied the sperm

Table 3 *Panulirus argus:* results of multiple regression on effects of male and female carapace length (*CL*) on spermatophore area

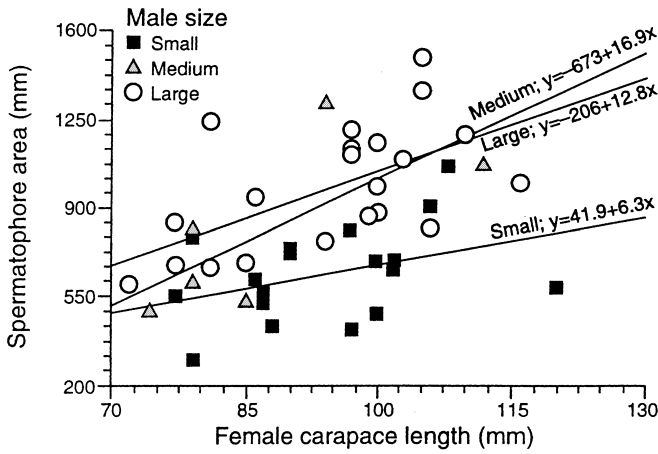| Variable	Regression coefficient	SE	Standardised coefficient	t-test value (Ho: $\beta = 0$)	P
Intercept	−7325	1225	0.000	−5.98	< 0.001
ln female CL	970	220	0.466	4.41	< 0.001
ln male CL	787	146	0.569	5.38	< 0.001

Fig. 3 The relationship between spermatophore size and female carapace length for female *Panulirus argus* mated by small, medium or large males. The regression lines and equations for each male treatment are indicated
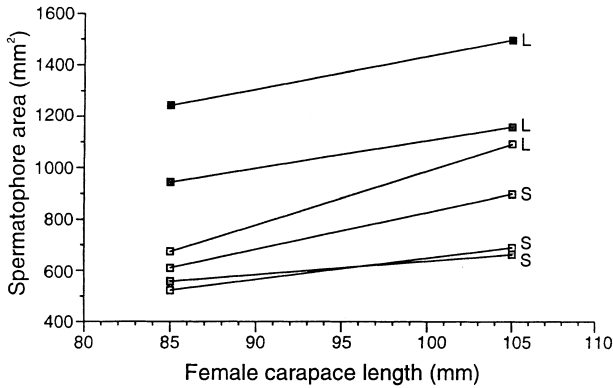

Fig. 4 Within-male variation of the size of spermatophores deposited on two sizes of female *P. argus* (*L* large male, *S* small male)

(Fig. 5). In females mated by small males, there was a relatively weak positive relationship between these two variables ($R^2_{adj} = 0.38$; $F_{1,12} = 9.01$, $P < 0.05$), indicating that fertilisation was constrained by the limited capacity of small males to increase the size of their
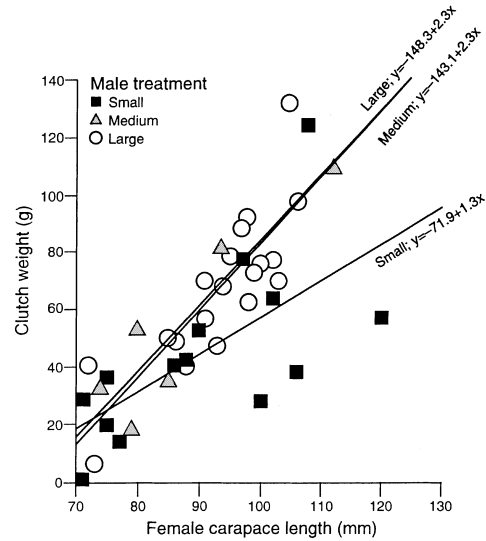

Fig. 5 The relationship between clutch weight and female carapace length for female *P. argus* mated by small, medium or large males. The regression lines and equations for each male treatment are indicated

spermatophore to match the size of their partner. For females mated by large and medium-sized males, clutch weight was more strongly related to female size ($R^2_{adj} = 0.68$ and 0.80; $F_{1,16} = 37.3$, $P < 0.001$ and $F_{1,16} = 21.5$, $P < 0.01$, respectively). This difference between large and small males was reflected in the slopes of clutch weight versus female CL which were significantly less for females mated by small rather than large males ($F_{2,32} = 5.93$, $P < 0.01$). Analysis of covariance (Table 5) showed that the size of the male producing the spermatophore had no significant impact on the positive relationship between spermatophore area and clutch weight (Fig. 6), although the power of the test was low.

The equations generated by the regression of: (1) spermatophore area on male and female carapace length, (2) clutch weight on spermatophore area and (3) egg number on clutch weight can be used to predict the impact of mate size on the egg production of females of average size (82 mm CL) in the fished Florida Keys population (R. Bertelsen and C. Cox, unpublished data)

Table 4 *P. argus*: results of multiple regression on effects of spermatophore area and female carapace length (*CL*) on clutch weight

Variable	Regression coefficient	SE	Standardised coefficient	*t*-value (Ho: $\beta = 0$)	P
Intercept	−75.6566	18.357	0.000	−4.12	0.0002
Female CL (mm)	0.9694	0.237	0.398	4.08	0.0002
Spermatophore area (mm²)	0.0605	0.010	0.582	5.98	< 0.0001

Table 5 *P. argus*: analysis of covariance on the effects of male size on clutch weights fertilised by spermatophores of varying size

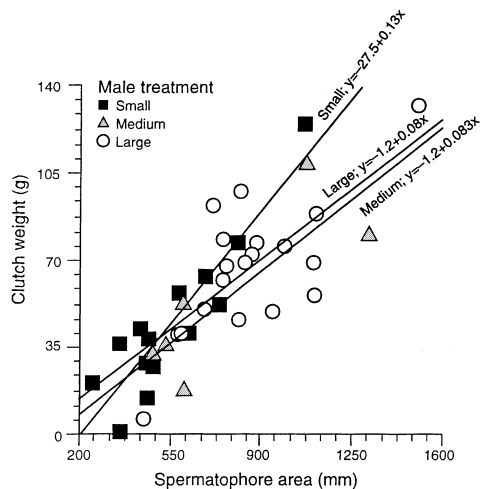| Source	df	MS	F-ratio	P	Power (at $\alpha_{0.05}$)
Spermatophore area (mm²)	1	19,778.2	62.5	< 0.0001	1.00
Male size	2	227.4	0.72	0.494	0.16
Residual	35	316.5			
Total	38				

Fig. 6 The relationship between clutch weight and spermatophore area for female *P. argus* mated by small, medium or large males. The regression lines and equations for each male treatment are indicated

(Table 6). Spermatophore area, clutch weight and number of eggs are all about 40% smaller when a female is mated by a 90-mm-CL male (mean size in the fished Florida Keys population) rather than a 145-mm-CL male (mean size in nearby unfished Dry Tortugus Marine Sanctuary, R. Bertelsen and C. Cox unpublished data).

Discussion

Spermatophore size

The pattern of increase in spermatophore area with the size of both the male and the female partner in *P. argus* strongly suggests that while small males have limited capacity to increase the size of their spermatophore, large males in particular adjust the size of their ejaculate dependent on the size of the female. Berry (1970) also noted that when female spiny lobsters, *P. homarus*, were mated by large males there was a constant relationship between female size and spermatophore size, but presented no data.

Alternative explanations for the relationship between female size and spermatophore area were not supported by our observations. There was no evidence that female size affected the correct placement of the spermatophore, or that large males when mating with small females misdirected their ejaculate and that it was partially lost.

In all but one case, the spermatophore was correctly positioned and well formed on the females sternal region with no ejaculate on the base of the walking legs, indicative of spillage. The one exception occurred when a small male mated with a similar-sized female and the amount of spillage amounted to less than 5% of the total spermatophore area. There was no physical evidence that females altered the size of the spermatophore to match her expected egg output, an alternative explanation of our results.

Sperm limitation

Our results strongly suggest that female clutch weight in two species of spiny lobsters is determined in large part by the size of the spermatophore deposited by the male. While recent work has documented the widespread occurrence of sperm limitation amongst free-spawning marine organisms (Levitan and Peterson 1995), it is generally held that sperm limitation is uncommon among species with internal fertilisation or direct exchange of gametes (Parker 1990a,b; Sneddon 1990; Birkhead and Hunter 1990; Birkhead and Møller 1992; Birkhead 1995, 1996; Harcourt 1997; Hosken 1997; Parker et al. 1997).

We sought alternative explanations for the relationship we observed between spermatophore size and clutch size in spiny lobsters. It is possible that females could signal, perhaps via water-borne pheromones, the number of ripe eggs contained in the ovaries and that males respond by laying down smaller or larger spermatophores. Thus, the size of the spermatophore which correlates so strongly with clutch size would be a male response to the female signal of egg number. Under this scenario, if we had measured the number of ovulated eggs in the ovaries of each female we would have obtained a similar high correlation with external clutch size. While it is possible that this explanation accounts for variation in spermatophore size by males of a certain size, it does not account for the variation in spermatophore area among males of different sizes when mating with the same-size females and the consequent effects of this on clutch weight. A more likely explanation is that female size, not ovarian egg number, is evaluated by the male either visually or chemically (perhaps via urine quantity) during the courtship phase, or tactually during the copulatory embrace when the male is usually upside down with the female clasped to his ventral surface, and that spermatophore size is a response to this. The number of contractions of the muscular wall of the vas deferens may dictate the size of

Table 6 *P. argus*: predicted effects of male size on the reproductive output of an 82-mm-CL female

Male size (mm CL)	Spermatophore area (mm²)	Clutch weight (g)	Number of eggs
Small (90)	493	33.6	188,192
Medium (120)	719	47.3	259,604
Large (145)	868	56.3	306,580

the ejaculate. The ability of males to regulate the size of their ejaculate according to social circumstances has been demonstrated in species as diverse as humans (Baker and Bellis 1989), rats (Bellis et al. 1990), flour beetles (Gage and Baker 1991), bush-crickets (Gage and Barnard 1996; Simmons and Kvarnemo 1997), birds (Birkhead and Fletcher 1992), blue crabs (Jivoff 1997) and fish (Shapiro et al. 1994; Marconato and Shapiro 1996; Stockely et al. 1997; Warner 1997).

It could be argued that our results are a consequence of confinement. The single male in each tank thus had an opportunity to evaluate the relative size, and thus the expected egg return from the females, and allocated his sperm accordingly. This, however, is similar to the wild situation where, because of inter-male aggression, large mature males are dispersed singly amongst the available dens during the mating season but cohabit simultaneously with up to 16 potential mates (MacDiarmid 1994), thus allowing ample time for males to evaluate the relative sizes of potential mates.

Another potential explanation of the association between smaller clutches and smaller males or smaller spermatophores is via differential investment strategies of females. Studies of birds show that females lay more eggs for preferred (often the largest) males (de Lope and Møller 1993; Petrie and Williams 1993). It is possible that female spiny lobsters extrude more or fewer eggs dependent on the size of the male supplying the sperm (as could be the case in *J. edwardsii*), or females extrude eggs until they detect that the supply of sperm is exhausted (as might occur in both species). In both cases, females would store, or resorb, any unused eggs. Evidence of partial egg retention or resorption would support female differential investment by either of these mechanisms. Alternatively, females may extrude the entire batch of ovulated eggs and these are fertilised as long as the supply of sperm from the spermatophore lasts. While unfertilised eggs may initially adhere to the setae on the female pleopods, they do not develop the attachment stalk (Silberbauer 1971) and soon dislodge during female ventilation and grooming of the clutch. Evidence of dislodged, unfertilised eggs would indicate the size of the brooded clutch is controlled not by differential investment strategies of females, but by the male supply of sperm.

The evidence overwhelmingly supports the latter mechanism. We observed quantities of newly extruded eggs on the bottom of tanks on several occasions during experiments on both species of spiny lobster. These eggs did not have an attachment "stalk" and were assumed to be unfertilised. The eggs were often difficult to detect among the debris on the tank bottom and in many instances were probably missed. In 1996, during experiments on *J. edwardsii* when these eggs were systematically collected, we found the larger the volume of loose eggs, the lighter the associated clutch of eggs. There was no evidence that females curtail egg extrusion once egg laying has begun. Unmated female *P. argus* extrude an entire batch of eggs (A.B MacDiarmid, unpublished data), indicating that resorption of unused eggs does not normally occur. Unmated *J. edwardsii* did not extrude any eggs. While histological examination of their ovaries indicated clear evidence of resorption, females which mated a small male and had a small egg clutch showed no evidence of resorption (A.B. MacDiarmid, unpublished data). To curtail egg extrusion once sperm supplies were exhausted would require females to detect the presence/absence of sperm either on the sternal plate or in the water mixing in the brood chamber during egg laying. No such detection method is known.

Sperm economy

Why do male spiny lobster ration their sperm? Female size, condition and size at onset of maturity dictate the maximum number of eggs which can be produced (Annala and Bycroft 1987; Annala 1991; DeMartini et al. 1993; Chubb 1994). A small (70 mm CL) female *P. argus* may lay as few as 1.5×10^5 eggs while a large female (140 mm CL) can produce as many as 2×10^6 (R. Bertelsen and C. Cox, unpublished data). In addition, smaller female *J. edwardsii* produce smaller eggs and larvae than larger females (T.H. Kendrick, unpublished data) although the consequences of this on larval quality are unknown. To eject a spermatophore capable of fertilising 2×10^6 eggs when mating with a small female with 13 times fewer and perhaps lower-quality eggs would be a considerable waste of sperm. Ejaculation of sperm carries associated costs not only of production but also of lost opportunity (Dewsbury 1982). For species with short breeding seasons, the energetic "cost" of sperm production may be secondary to costs associated with lost mating opportunities due to insufficient time to recharge sperm stores. The recharge rate of the vas deferens is unknown in spiny lobsters but in a wide range of other species the contents of successive ejaculates contain reduced numbers of sperm and the testes or vasa deferentia may take 2–7 days to refill (reviewed in Dewsbury 1982; LaMunyon and Eisner 1994; Bissoondath and Wickland 1996). The small but significant effect of mate order on clutch weight in *J. edwardsii* suggests that recharge rates are not rapid. The relatively short mating season in *J. edwardsii* (about 43 days, MacDiarmid 1989b) increases the possibility of sperm depletion of preferred males. The more extended mating period in *P. argus* (>150 days, Lipcius 1985) may make depletion of sperm supply less likely in this species.

Sperm competition

Another factor of potential importance in describing the evolution of sperm rationing in male *P. argus* is the occurrence of polygany and its ramifications for multiple paternity in an egg clutch. Up to 28 days may lapse between mating and egg laying in *P. argus* (Lipcius 1985) which provides opportunity for females to mate a

number of times and therefore increases the potential for sperm competition. In *P. argus* and *P. laevicauda* from Brazil, Mota-Alves and Paiva (1976) found the frequency of multiple mating, as evidenced by up to three overlaying viable spermatophores, to reach 43% and increase with female size. They suggested that larger females seek multiple copulations because more sperm are required to ensure fertilisation of their eggs.

First-mating males thus encounter a risk of later sperm competition and this increases with the size of the female he mates. Models developed by Parker et al. (1996) suggest in these circumstances that first- and second-mating males should increase the size of their ejaculate to increase their fertilisation success. This is consistent with our results where, with only one male per tank, all our males were first to mate with each female and increased the size of their ejaculate to match the size of the female or risk. Later-mating males encounter intense sperm competition from previous matings as well as the risk a female may mate again. Parker et al.'s (1996) models show that higher intensities of sperm competition favour reduced ejaculate expenditure by males. These models assume sperm mixing, however, and it is not clear what happens in the case of multiple spermatophores in spiny lobsters. Is the last, and thus outermost spermatophore the only one scratched open by the female, in which case first-mating males suffer from complete sperm competition, or do all layers contribute to fertilisations? Last-male precedence is well described in other groups (Birkhead and Hunter 1990; Birkhead and Møller 1992) and can lead to rationing of sperm amongst females (Parker 1990a; Simmons and Kvarnemo 1997). Describing the incidence of multiple spermatophores and their pattern of use by females in egg fertilisation is of obvious importance in understanding spiny lobster mating systems and could be addressed using the approach described by Cook et al. (1997).

Post-copulatory mate guarding during the short period between mating and egg laying in *J. edwardsii* (Mac-Diarmid 1988) precludes multiple mating by females and thus eliminates the risk of sperm competition. In these circumstances, males should deposit a spermatophore capable of fertilising most, but not necessarily all, the eggs the female is capable of producing (Ball and Parker 1996; Marconato and Shapiro 1996; Parker et al. 1996).

Female strategies

For female spiny lobsters, the consequences of sperm limitation and rationing by males are likely to be strong selection for female choice of mates, competition amongst females for the preferred males and multiple mating, especially by large females. Females should attempt to mate early in the mating period with the largest unmated male available and/or mate a number of times prior to oviposition to ensure adequate sperm supply (e.g. Mota-Alves and Paiva 1976). It is interesting to note that in both *P. argus* and *J. edwardsii*, large females mate earlier in the reproductive season than small females (Lipcius 1985; MacDiarmid 1989b), although the mechanism for this is unknown. Because there is little potential for multiple mating by female *J. edwardsii* (see above) they should be more choosy in selecting mates compared to female *P. argus* which have the opportunity to mate again. This is supported by field observations of courting activity and laboratory mate choice experiments which strongly suggest that female *J. edwardsii* prefer large males (A.B. MacDiarmid, unpublished data), but comparable data on *P. argus* are lacking. Competition among females by monopolising preferred males may account for the longer periods female *J. edwardsii* courted with large males than small males in our experiments.

Effects on wild populations

Fishing, especially when exploitation rates are high, not only decreases abundance and mean individual size, through the removal of larger, older individuals (e.g. Davis 1977; Cole et al. 1990; MacDiarmid and Breen 1993), but may also skew the population sex ratio when one sex is exploited more than the other (Chubb 1994; Jamieson et al. 1998). This is currently the case in New Zealand, where males make up 80% or more of the landed catch of *J. edwardsii* in some areas (Breen and Kendrick 1997), and in South Africa where male *J. lalandii* comprise almost 100% of the landed catch (Pollock 1986). In these situations, where large males are typically rare, sperm-limited female fecundity has the potential to limit egg production. However, other factors may work in the opposite direction. Enough large males may survive to mate many females, or in some species, females may have multiple small mates. In addition, there may be compensatory or density-dependent decreases in the size at onset of maturity (Polovina 1989; Chubb 1994) and increases in size-specific egg (De Martini et al. 1993) and sperm production. Field comparisons of female fecundity from areas dominated by small and large mature males are required to determine if sperm limitation, as found in this laboratory study, has any impact on wild fished populations of spiny lobsters.

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Communicated by L.W. Simmons