THE EFFECT OF MALE SIZE AND SPERMATOPHORE CHARACTERISTICS ON REPRODUCTION IN THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*

BY

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ABSTRACT

The average size of spiny lobsters (Decapoda; Palinuridae) has decreased dramatically worldwide as a result of the over-fishing of large individuals. Average male size is usually diminished more than that of females because of sexual dimorphism and this can impact reproductive success through sperm limitation. Using laboratory mating experiments and field comparisons of fished and unfished populations, we studied differences in spermatophore characteristics that may influence reproductive success in the Caribbean spiny lobster, *Panulirus argus*, in the Florida Keys, Florida (U.S.A.). We found that large males produce larger spermatophores with more sperm, resulting in operational sperm:egg ratios (range: 21-37:1) that were 40% lower in fished areas. Our experiments show that female mating receptivity is suspended upon receipt of a spermatophore and that this behavior is controlled by a combination of chemical and physical stimuli provided by the spermatophore. The distribution of sperm within spermatophores indicates that the fertilization of multiple clutches from one spermatophore is unlikely, as confirmed by laboratory observations. These results highlight the importance of spermatophore characteristics on fertilization success in spiny lobsters and suggest that reduced male size in the wild may limit reproductive success.

INTRODUCTION

Among the most universal effects of over-fishing is the depletion of large individuals within exploited populations, and spiny lobsters (Decapoda; Palinuridae) are no exception. Most fisheries for decapod crustaceans include a

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minimum size limit to protect non-reproductive individuals, but this focuses fishing effort on larger lobsters. Male lobsters grow larger than females, so when male size is reduced by over-fishing there are potential consequences on the reproductive success of the population. In several crab fisheries, for example, only large males attain the legal size limit and thus only they are extracted from the population (Sainte-Marie, 1993; Paul, 1984; Smith & Jamieson, 1991; Kendall & Wolcott, 1999; Sato et al., 2005, 2006, 2007; Sato & Goshima, 2006). This in turn leads to the increased participation of small males in reproduction (Smith & Jamieson, 1991; Sainte Marie, 1993; Kendall & Wolcott, 1999; Kendall et al., 2002). Similar situations exist for spiny lobster.

In Florida and New Zealand, male spiny lobsters (Caribbean spiny lobster *Panulirus argus* and Red Rock Lobster, *Jasus edwardsii*; respectively) are much larger than females in unfished populations (e.g., males are typically five times the mass of females), but in fished areas males and females are of equivalent size (MacDiarmid, 1989; Bertelsen & Matthews, 2001). When large males are unavailable, female fecundity can plummet (Sainte-Marie, 1993; Kendall et al., 2002; Sato et al., 2005, 2006, 2007; Sato & Goshima, 2006). In fact, when females of some spiny lobster species go unmated they release unfertilized eggs (e.g., *P. argus*), whereas other species (e.g., *J. edwardsii*) forgo egg deposition (MacDiarmid & Butler, 1999). The consequences of these actions range from the loss of a single clutch during one reproductive season in which multiple clutches will be produced (*P. argus*), to a severe reduction in future reproductive success resulting from damage to reproductive organs (*J. edwardsii*).

Lost mating opportunities are not the only reproductive problem faced by female lobsters in fished regions. Decapods may also experience sperm limitation if ejaculate size scales with male body size, mating history, expected female output, or future mating opportunities (reviewed by MacDiarmid & Sainte-Marine, 2006). Indeed, in the spiny lobsters *P. argus* and *J. edwardsii*, male size and spermatophore size are correlated, and spermatophore size explains nearly half of the variance in clutch size (i.e., number of eggs fertilized per clutch) (MacDiarmid & Butler, 1999). Appropriate sperm:egg ratios (S:E) are also crucial for the maintenance of high rates of fertilization, but S:E ratios vary greatly among organisms with external fertilization. In sea lamprey (*Petromyzon marinus*), for example, a S:E ratio of 50 000 provides maximal fertilization rates (Ciereszko et al., 2000), whereas a S:E ratio of 15 000:1 is optimal in African catfish (*Clarias gariepinus*) (Rurangwa et al., 1998). Studies of fertilization in the Crown-of-Thorns starfish, *Acanthaster*

planci reveal that fertilization rates can be high over a wide range of S:E ratios but drop precipitously at ratios less than 50. Estimates of S:E in decapod crustaceans range from 70:1 (*C. opilio*; Sainte-Marie & Lovrich, 1994), to "several":1 (*Libinia emarginata*; Hinsch, 1971), to approximately 2.5:1 (*Chionoecetes bairdi*; Paul, 1984). Inappropriately low S:E ratios can reduce the probability of fertilization success to such an extent that the females may delay egg deposition, as occurs in snow crabs when the S:E ratio drops below 7:1 (Sainte-Marie & Lovrich, 1994).

Sufficient access to suitable mates is clearly of prime importance for females and thus has strong selective consequences for female mate choice. In contrast, behaviors or mechanisms that ensure sole or majority paternity of offspring is important for males. Many brachyuran crabs and homarid (clawed) lobster males employ proximate mate guarding to reduce the likelihood of sperm competition and enhance their fertilization success (*C. sapidus*: Jivoff, 1997; *C. opilio*: Sainte-Marie et al., 1997; *H. americanus*: Atema & Voigt, 1995). An indirect means of post-copulatory mate guarding employed by many arthropods is the production of spermatophores that emit chemical signals or provide a physical cue that inhibits copulation (Roth, 1962; Sugawara, 1979). Whether similar mechanisms exist in Palinurid lobsters is unknown.

Given prior evidence that male size influences the reproductive success of spiny lobsters (MacDiarmid & Butler, 1999), we experimentally determined the impact of male size on sperm attributes in the Caribbean spiny lobster. We then examined the distribution of sperm cells within intact and used spermatophores to evaluate whether a single spermatophore can be used to fertilize multiple clutches, and tested whether the deposition of a spermatophore inhibits further mating through chemical or mechanical cues. Finally, we determined the operational S:E ratio of lobster populations in fished and unfished areas to assess the potential for fishing-induced sperm limitation.

MATERIALS AND METHODS

Male size and spermatophore characteristics

To determine the impact of male size on sperm and spermatophore attributes, we conducted mating experiments in the laboratory controlling for male and female size. Divers collected male and female lobsters from an unfished marine reserve (Dry Tortugas National Park, Florida, U.S.A.) and from various fished areas near the Florida Keys, Florida (U.S.A.) during February

and March of 1999-2001 prior to the reproductive season. The lobsters were transported in aerated live-wells to a laboratory at the Florida Fish and Wildlife Conservation Commission field laboratory in Marathon, FL where all experiments were conducted.

Laboratory mating experiments were designed to simulate the size structure of the reproductively active lobsters in both regions, and thus field mating size structure. Thus, small females (71-90 mm CL) were mated with small males (92-100 mm CL) and large females (95-140 mm CL) were mated with large males (120-155 mm CL). Males and females were kept in separate experimental tanks until approximately 1 week after capture when a single male and five females of the appropriate size class were placed in round experimental tanks (1.75 m diameter; 1500 liter) receiving aerated, filtered seawater from a flow-through system. Seawater temperatures (23-32°C) and photoperiod (12-13.5 hours of daylight) were at ambient conditions and lobsters were fed frozen squid and shrimp ad libitum. The lobsters mated in these tanks, and females were examined daily for the presence of a spermatophore — a paired, external sperm packet deposited by the male on the female's sterum. We used flat forceps to remove intact fresh spermatophores after they had hardened for 24 hours. The spermatophores were weighed to the nearest hundredth of a gram and stored at 5°C in labeled vials with $\sim$10 ml of sterile seawater (filtered to 0.2 μm) until the number of sperm cells contained within could be derived using the sperm counting procedure described below.

Spermatophores were sliced laterally into thin sections (approximately 0.5 mm) using a scalpel. The sections were placed in a known volume (10-15 ml) of sterile seawater and mechanically shaken for 3 minutes. Aliquots of 9.8 μl were removed from the shaken sample and placed into the wells of a hemacytometer. The number of sperm cells was then enumerated within each of four subsamples viewed in the hemacytometer. A preliminary evaluation of this sperm liberation technique, where a subset of eight spermatophores were subjected to sequential washings till less than 1% of cells remained in them, indicated that an average of 73% of the sperm within a spermatophore were removed using our technique. However, sperm counts were not adjusted in the formal study because all analyses were comparative. We then determined the weight (in g; measured with a top-loading balance) of the spermatophores, as well as the total sperm number and density of sperm per gram of spermatophore using only data collected from the first spermatophores deposited by each male (n = 28). The relationship between male carapace length and these three spermatophore variables (i.e., weight, sperm number, sperm concentra-

tion) was examined in three separate linear regression analyses; all regression assumptions were met, therefore no data transformations were necessary.

Sperm cell distribution

We examined the distribution of sperm cells within sections of spermatophores removed from females captured in the wild. In March of 1998, divers obtained spermatophore-bearing females from the Florida Keys and the Dry Tortugas National Park. The perimeters of the spermatophores on each female were traced onto acetate sheets and the intact spermatophores removed. The spermatophores were then bisected longitudinally and each resulting half was further divided into four to five sections laterally (fig. 3 inset).

The spermatophore sections were weighed and sperm density and total sperm number were then obtained from each individual section ($n = 5$) in a similar manner to that described above for whole spermatophores. We compared spermatophore weight, sperm density, and total number of sperm between left and right halves of the spermatophore using a 1-factor randomized block MANOVA. All MANOVA assumptions were met with the raw data, so no transformations were necessary.

Inhibition of female receptivity

We conducted a laboratory study to examine the influence of spermatophore presence on female receptivity to further mating. Spermatophore-bearing females were obtained by divers from the Florida Keys in May of 1999 and randomly distributed to each of four treatment groups: control, mechanical cue alone, chemical cue alone and a combination of chemical cue and mechanical cue.

For the control treatment, we removed the spermatophores from the females as described above and then reattached them in the same location on each female using a cyanoacrylate adhesive. The mechanical cue treatment was designed to provide a physical representation (stimulus) of a spermatophore without the chemical signals a spermatophore may provide. The mechanical cue was created by replacing spermatophores on females with artificial spermatophores made of silicone that were shaped, hardened in air, and cured overnight in seawater before being glued to the sternum of females. The chemical treatment provided the chemical stimulus of a spermatophore without the physical presence of a spermatophore on the female's sternum. To represent this, we removed the spermatophore from each female's sternum and

reattached it with adhesive on the side of the same female's carapace. Presumably, a chemical signal emitted by the spermatophore could be detected by the female via chemosensory aesthetascs on her pereiopods and antennules. To determine if the inhibition of female receptivity was controlled by a combination of chemical and mechanical signals, we devised a treatment that allowed a chemical signal to penetrate the sternum while preventing the detection of chemical stimuli to the female's pereiopods if she stroked the spermatophore. This treatment also provided a mechanical input simulating the presence of a spermatophore on the sternum. The combination treatment "experimental spermatophore" was created by first removing the spermatophore from the female (as described above), cutting away it's dorsal (outer) portion, and then completely covering it's outer surface with an artificial spermatophore made of silicone. Thus creating a bilayered spermatophore with an inner "natural" layer and an outer artifical layer. This was then reapplied back to the sternum of the original female.

Following the application of the spermatophore treatments to the experimental females, one female from each treatment was placed in an experimental mating tank (as previously described) with a single male (i.e., 4 females/male). The lobsters were checked daily to determine the presence of new spermatophores or damage to the manipulated spermatophore. If the lobsters had removed or severely damaged the spermatophore, this was noted and the treatment was reapplied accordingly. Animals were fed frozen squid and shrimp *ad libitum*. Differences in the frequency of mating by females, as determined by the presence of a newly-deposited spermatophore, in the four treatments were to be analyzed using a 2×4 contingency table analysis ($n = 24$; 6 per treatment).

Sperm:Egg ratios

We determined the operational S:E in fished and unfished wild populations of *P. argus* by enumerating the number of sperm in spermatophores found on females obtained by divers from the Florida Keys ($n = 62$) and the Dry Tortugas National Park ($n = 57$) during the spring of 2000. The spermatophores were removed from females and the sperm cells were counted as described above. Using an equation developed by Bertelsen and Matthews (2001) for lobsters from these regions, the expected egg output of each female was calculated based upon female carapace length. Based on the preliminary study of the sperm liberation technique outlined above, the S:E ratios were adjusted to compensate for the discrepancy between the number of cells enumerated

and the actual number present in the spermatophore. We used a two-sample t-test on log-transformed data to examine the differences in the mean S:E ratios between fished and unfished populations.

RESULTS

Male size and spermatophore characteristics

Male size had a significant effect on the weight of the spermatophore and number of sperm cells contained therein (fig. 1). Spermatophore weight increased with increasing male carapace length, and male size explained approximately 63% of the variation in spermatophore weight ($r^2 = 0.6330$; $F = 44.84$; $P < 0.0005$; $df = 1$) (fig. 2a). We also found a significant association ($r^2 = 0.2378$; $F = 8.110$; $P = 0.0085$; $df = 1$) between the number of sperm cells and male size with the total number of sperm cells increasing with increasing male size (fig. 2b). There was, however, no relationship between sperm cell density and male size (fig. 3c; $r^2 = 0.0299$; $F = 0.790$; $P = 0.382$; $df = 1$), perhaps a result of the greater rate of increase of spermatophore weight with male size than the rate associated with sperm number.

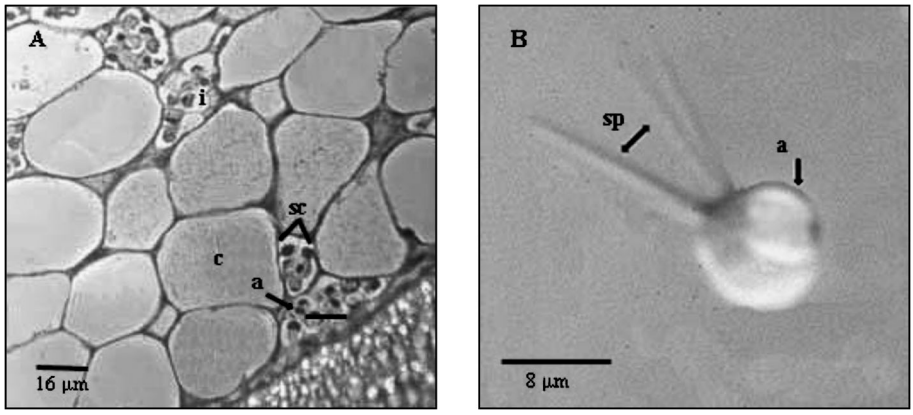


Fig. 1. Micrographs of spermatophore matrix with embedded sperm cells and an individual sperm cell. A, cross-section of primary spermatophore layer demonstrating association of sperm cell clusters with the acellular cavities in *P. argus*: a = acrosome, c = acellular cavity, i = interstices of cavities, sc = sperm cluster. 200×, hematoxylin and eosin staining; B, Nomarski-phase interference micrograph of *P. argus* spermatozoa: a = acrosome, sp = nuclear spikes. 1000×.

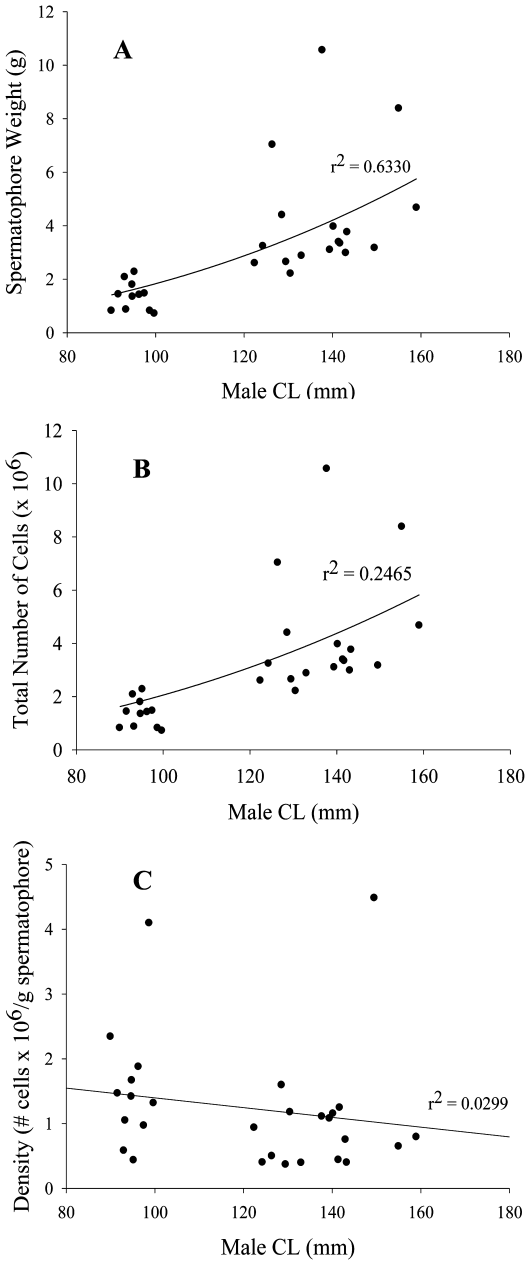


Fig. 2. The effect of male size on three spermatophore attributes. A, spermatophore weight; B, number of sperm cells; C, sperm cell density. Large males (>120 mm CL) were mated with large females (>95 mm CL) and small males (<100 mm CL) were mated with small females (<95 mm CL) (total n = 28) in these laboratory experiments and data on spermatophore attributes were measured for the first mating.

Sperm cell distribution

An analysis of sperm cell distribution with spermatophores revealed that the sperm cells are concentrated in the mid-posterior region of the spermatophore (fig. 3). There are noticeably fewer sperm cells in the anterior region, the region that often remains after spermatophore utilization at oviposition. Left and right spermatophore halves were not significantly different in weight, number of sperm cells, or sperm density despite the two halves of the spermatophore developing separately in the paired testes (table I; $F = 0.068$; $P = 0.974$; $df = 3$).

Inhibition of female receptivity

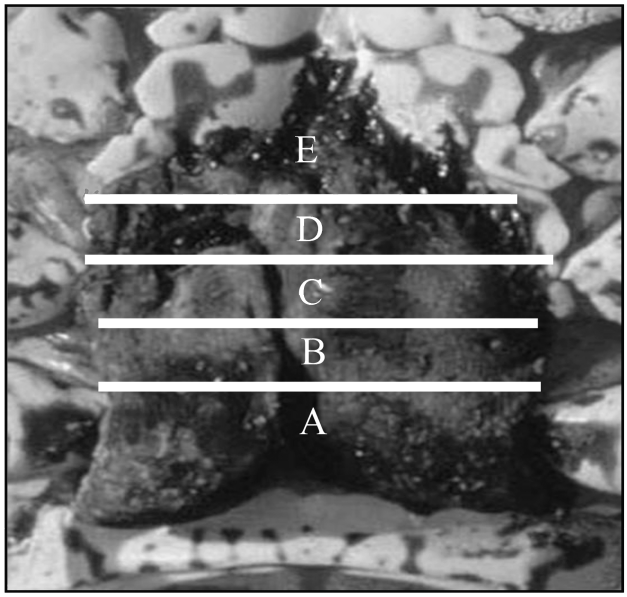
The only females to remate in the experiment where spermatophores were manipulated to test for post-copulatory mate inhibitory cues were those in the mechanical cue and chemical cue treatments (table II); half of the females in these treatments remated. The females assigned to the control treatment (intact spermatophore) and the chemical/mechanical cue combination treatment did not remate.

Sperm:Egg ratios

The mean S:E ratio in the fished population of lobsters in the Florida Keys was $21.5 \pm 10.6 : 1$ (mean $\pm$ 95% C.I.; $n = 62$). In the unfished lobster population in the Dry Tortugas Marine Reserve, the average S:E ratio was 40% higher ($37.4 \pm 18.3 : 1$; mean $\pm$ 95% C.I.; $n = 57$) (fig. 4), but this difference was not significant due to the large variability ($t = 0.075$, $df = 100$, $P = 0.941$). The lowest S:E ratios were similar in both populations, with several females in each population having an S:E ratio less than 5:1. However, only 3% of the females in the fished population had a S:E ratio greater than 50:1, in contrast to 23% of the females in the unfished population. The mean S:E ratio from females from fished populations that we collected with only partially eroded spermatophores was substantially lower than that seen in intact spermatophores from the same population of animals, averaging $3.8 \pm 3.2 : 1$ (mean $\pm$ 95% C.I., $n = 8$).

DISCUSSION

The impetus for this study was a concern that a reduction in the size of male spiny lobsters in populations subject to fishing could adversely affect



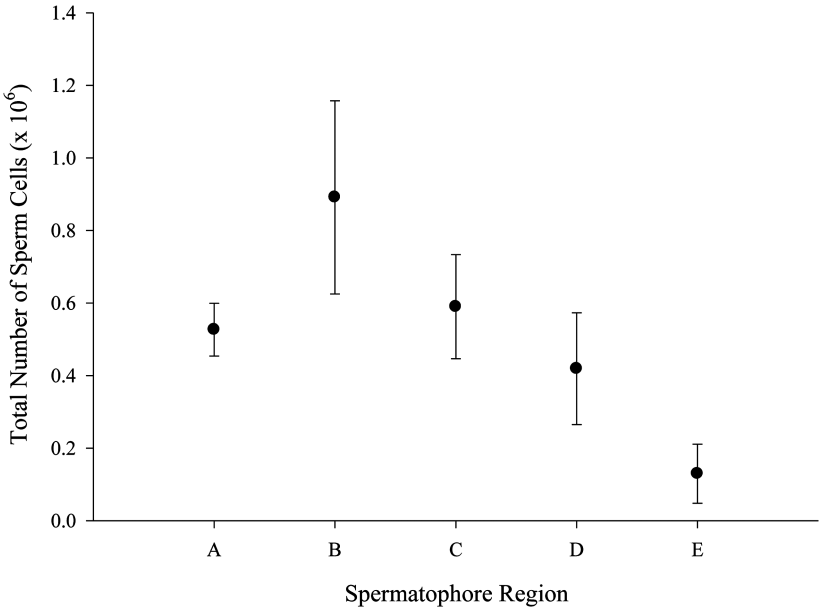


Fig. 3. Sperm cell abundance and distribution within the spermatophore of *P. argus*. Top: photo of a spermatophore attached to the ventral surface of the carapace of a female lobster showing the relative orientation of the regions sampled (A–E). Each region was roughly equal in area. Bottom: the number of sperm cells per region (mean ± 1 standard error of the mean).

TABLE I

Results of a MANOVA examining the difference between the left and right spermatophore halves for each of three spermatophore attributes: spermatophore weight, number of sperm, and sperm density. Pillai's Trace, Wilks' Lambda, Hotelling's Trace and Roy's Largest Root were calculated in the MANOVA. As all test statistics provided identical results, only those results associated with the Wilks' Lambda are displayed

	Hypothesis df	Error df	F Value	Wilks' Lambda	P
Spermatophore Half	3.000	4.000	0.068	0.951	0.974

TABLE II

Results of experiment examining the role of spermatophores in controlling female mating. Whether female *P. argus* remated or not in four spermatophore manipulation treatments is shown

Experimental Outcome	Spermatophore Manipulation Treatment			
	Control	Chemical Cue	Chemical+ Mechanical Cue	Mechanical Cue
Female Remated	0	3	0	3
Female Did Not Remate	6	3	6	3

fertilization success, hence the realized fecundity of exploited populations. Our results indicate that small males indeed deliver smaller quantities of sperm and smaller quantities of spermatophore matrix, that consequently may lead to lower S:E ratios in fished populations. Females are unable to counter a loss of large males by using a single spermatophore more than once, and we found no evidence that females mate more than once per clutch.

Our finding that male size significantly impacts spermatophore weight and the number of sperm cells transferred to female *P. argus* in Florida is consistent with findings by MacDiarmid & Butler (1999), who observed a positive relationship between spermatophore area and male size in both *P. argus* and the southern temperate rock lobster *J. edwardsii*. They also noted that spermatophore area varied more for large males, who apportioned spermatophores of different sizes to females of different sizes: large spermatophores to large females and vice versa. However, they did not measure spermatophore weight or enumerate sperm. Nor did Mauger (2001) when demonstrating that male size in *J. edwardsii* was positively correlated with vas defrens weight and with ejaculate recharge rate. The results for spiny lobsters contrast with findings in studies of the crabs *C. opilio*, *C. bairdi*, and *C. sapidus*, which show that

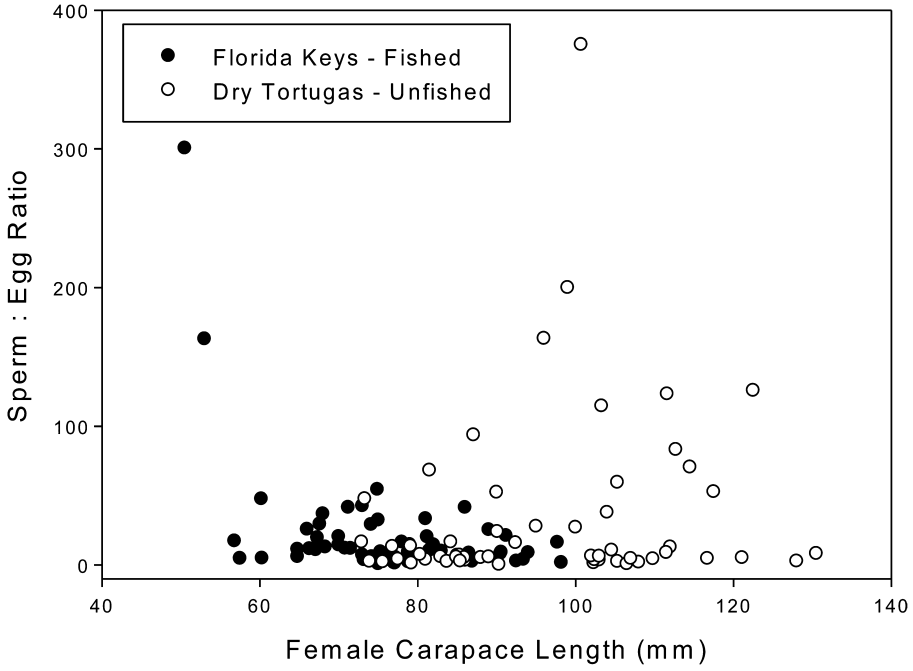


Fig. 4. Operational sperm:egg ratios plotted against female size (carapace length; mm) for *P. argus* collected from fished (closed circles; Florida Keys; n = 62) and unfished populations (open circles; Dry Tortugas; n = 57).

the number of sperm cells received by females is independent of either male and/or female size (Sainte-Marie & Lovrich, 1994; Kendall et al., 2002).

Although female *P. argus* mate many times in their lifetime, often more than once a season if they are large, our results suggest that only one spermatophore can be used to fertilize a single clutch of eggs. There are accounts of female *P. argus* possessing multiple spermatophores layered one atop another (e.g., Mota-Alves & Paiva, 1976), but our observations indicate that these instances simply represent the deposition of a second spermatophore over a previously used but incompletely removed one. When females fertilize their eggs, they use their legs to scratch open and often completely remove the posterior portion of the spermatophore to release the stored sperm, leaving an "eroded spermatophore" with the anterior-most region of the spermatophore intact. Eroded spermatophores are often found alone or beneath newly deposited spermatophores on females in the field, but it is unlikely that an eroded spermatophore could be used to successfully fertilize a second clutch. Sperm cells are evenly distributed among left and right halves of the spermatophore (similar to observations in both crabs and other lobsters; Sainte-Marie &

Lovrich, 1994; Mauger, 2001) and the majority (~80%) are located in the middle to posterior region of the spermatophore, leaving relatively few cells in the anterior region — the only portion that remains in eroded spermatophores.

Unlike the situation in brachyuran crabs and clawed lobsters where females mate multiple times and store sperm before fertilization (Sainte-Marie & Lovrich, 1994; Aiken & Waddy, 1980), our experiments show that the presence of an intact spermatophore inhibits further mating by females. That experiment also suggests that inhibition requires sternal contact from a chemical in the spermatophore.

The S:E ratios that we observed in *P. argus* (generally <40:1) are lower than those seen in most other species and >50% lower in heavily exploited populations subject to fishing compared to an unexploited population. In broadcast spawners, one would expect higher S:E ratios to combat the effect of dilution because fertilization declines with decreasing sperm concentration in the sea (e.g., Levitan et al., 1991; Benzie & Dixon, 1994; Tvedt et al., 2001). For example, a study on the fertilization kinetics in sea urchins revealed optimal fertilization rates at a S:E ratio of 72 000:1 (Levitan et al., 1991), whereas some starfish can tolerate greater dilutions of sperm, with S:E ratios as low as 50:1 (Benzie & Dixon, 1994). Studies of fertilization success in broadcast-spawning marine fish indicate that maximal fertilization occurs within a S:E ratio range of 9×10^5 to 5×10^4 (Tvedt et al., 2001; Ciereszko et al., 2000).

Because mating by *P. argus* involves a direct transfer of gametes, one assumes that the S:E may be lower than that of broadcast spawners. Yet, in humans, 200-300 million spermatozoa are deposited per ejaculate to fertilize a single egg, although only 300-500 of these survive to reach the site of fertilization (Sadler, 1990). In vivo studies of laboratory rodents also indicate that the S:E ratio at the site of fertilization is often only 1:1 (Gomendio et al., 1998). Gomendio and colleagues (1998) speculate that the high initial S:E ratios in these organisms are designed to combat low survival rates encountered in hostile reproductive tracts. During fertilization, decapod sperm encounter neither extreme dilution nor an environmentally hostile internal reproductive tract. Thus, it is not surprising that decapod S:E ratios are consistently low, with ratios varying from about 70:1 or lower in snow crabs (Sainte-Marie & Lovrich, 1994, 1999) to <5:1 in spider and tanner crabs (Hinsch, 1971; Paul, 1984). The high S:E ratio estimates obtained in a few in vitro fertilization studies of crabs and lobsters are probably an artifact, because the vast majority of the fertilized eggs observed in those studies

were polyspermic and not incubated long enough to obtain cleavage, thus yielded unreliable assessments of successful fertilization (Gomendio et al., 1998; Yanagimachi, 1994; Talbot et al., 1991).

CONCLUSIONS

There is growing evidence that over-fishing of decapod crustaceans can lead to reduced reproductive success, not only because of smaller female size but also due to sperm-limitation, which may ensue when small males come to predominate in the population (MacDiarmid & Saint-Marie, 2006). In not all situations, however, has intense exploitation resulted in sperm-limitation of fecundity, apparently because of differences among species in spermatophore characteristics and mating dynamics that in some instances may mitigate the effects of fishing on male size. Detailed investigations of the reproductive attributes and mating behavior of individuals in fished and un-fished areas are necessary if we are to discover why some decapods are more susceptible to fishery-induced sperm limitation of fecundity than others. What is clear, however, is that the role of male size in ensuring fertilization success, once viewed as demographically irrelevant, is indeed important to Palinurid population viability.

ACKNOWLEGEMENTS

We are appreciative of the laboratory and field assistance provided by: D. Behringer, S. Donahue, J. Goldstein, E. Ricelet and D. Robertson. John Hunt of the Florida Fish and Wildlife Conservation Commission graciously afforded us access to their laboratory in Marathon, FL for much of this work. This research was supported by a grant to M. Butler from The National Science Foundation (No. INT-9418306).

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First received 2 November 2009.
Final version accepted 20 December 2009.