



Sperm limitation: possible impacts of large male-selective harvesting on reproduction of the coconut crab *Birgus latro*

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ABSTRACT: For many biological resources, larger males are more likely to be harvested than smaller ones. In such harvested populations, the remaining males are relatively small and participate in more matings than in pristine populations. Because the number of sperm passed to females generally decreases with decreasing male body size and increasing male mating frequency, the reproductive success of females in populations subjected to such harvesting patterns can be restricted due to sperm limitation. The Japanese population of the coconut crab *Birgus latro* is typically subject to large male-selective harvesting, which may be one of the causes of the presently observed *B. latro* population decline. To examine the impact of harvesting on coconut crab populations, the effects of male size and mating frequency on male reproductive potential (number of ejaculated sperm and fertilization rate), sperm recovery rate, and the number of sperm received by females in a harvested population were investigated. Larger males provided females with larger numbers of sperm and achieved higher fertilization rates. Although the number of ejaculated sperm decreased with increasing mating frequency regardless of male size, larger males always passed more sperm to successive mates than smaller ones. The number of used sperm did not increase even after 30 d, suggesting a low sperm recovery rate. More than half of the females collected after the beginning of the reproductive season had very low numbers of sperm. These results suggest that the reproductive rate of the coconut crab has declined due to females suffering from sperm limitation.

KEY WORDS: *Birgus latro* · Ejaculated sperm · Fertilization rate · Large male-selective harvesting · Population structure · Sperm limitation · Sperm recovery rate · Slot size limits

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INTRODUCTION

Sperm limitation can arise in various animal taxa (Dewsbury 1982, Birkhead 1991, Pitnick & Markow 1994, Sato & Goshima 2007a) and is related to population demographic structure (Sato & Goshima 2006) as male mating frequency and male body size can influence the number of sperm passed to females. For example, male mating frequency increases if the sex ratio of a population is skewed toward females. Since males deplete their sperm reserves and decrease ejaculate size through successive matings (Birkhead 1991, Pitnick & Markow 1994, Sato et al. 2005), a population

with a female-biased sex ratio may have a reduced female fertilization success (e.g. Sato & Goshima 2006). Larger males have larger sperm reserves (Pitnick 1996, Kendall et al. 2001, Sato et al. 2005) and pass larger ejaculates to successive mates than do smaller ones in some species (Jivoff 1997, Sato et al. 2006), and thus sperm supply can be lower in populations with only small males. Therefore, the probability that females suffer from insufficient sperm supply is closely related to the structure of the population, namely the mean male size and the sex ratio.

Hunting and fishing are almost always non-random exploitations and often sex- and size-selective. For

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many biological resources, males are more likely to be harvested than females and larger males are more likely to be caught than smaller ones (Coltman et al. 2003, Rowe & Hutchings 2003, Fenberg & Roy 2008). Such harvesting, in addition to the obvious direct effect of reducing the abundance of the resource, also affects the demography of the population by skewing the sex ratio towards females and decreasing mean male body size (Smith & Jamieson 1991, Ginsberg & Milner-Gulland 1994, Sato et al. 2005). Females in populations subjected to such biased harvesting may receive reduced sperm supplies that limit their reproductive success, which can result in a decrease in the reproductive rate of the resource. Reduced female reproductive success via sperm limitation has been documented in some large male-only fished crab populations (e.g. *Paralithodes brevipes*, Sato et al. 2007; *Callinectes sapidus*, Carver et al. 2005; but see *Cancer magister*, Hankin et al. 1997) and population crashes in ungulate populations have been attributed to reduced fecundity due to selective harvesting of large males (Milner-Gulland et al. 2003, Milner et al. 2007). However, apart from in the above mentioned populations, the negative effects of large male-selective harvesting on the reproductive rate of resources have received far less attention than the direct effect of harvesting on abundance of many species.

The coconut crab *Birgus latro* (Linnaeus 1767) lives on oceanic islets and atolls as well as in coastal areas of the tropical Indo-Pacific region. Populations in most habitats have been severely depleted or become virtually extinct, but there have been few serious attempts based on scientific evidence to manage the resources (Brown & Fielder 1991). Overharvesting (Fletcher 1993) and habitat destruction (Eldredge 1996) are considered to be 2 of the main causes for the present depletion of the resource, as is the present harvesting pattern, namely large male-selective harvesting (Sato & Yoseda 2010). This harvesting pattern results in a sex ratio skewed towards females, and a mean male size decrease in harvested populations. These demographic changes may be detrimental to coconut crab populations because they cause a decline in reproductive output resulting from the reduced probability that females encounter suitable and preferred mates, which are males that equal or exceed the female's body size (Sato & Yoseda 2010). It is probable that skewed sex ratio and decreased mean male size due to present harvesting cause sperm limitation and reduce reproductive output, which may be one of the causes of the present depletion of the population.

In the present study, to determine the impact of large male-selective harvesting on a coconut crab population in Japan, we examined (1) the effect of male size on the number of ejaculated sperm and fertilization

rate, (2) the relationship between male mating frequency and number of ejaculated sperm, (3) the effect of male size on this relationship, (4) the sperm recovery rate, and (5) the number of sperm retained by females in a harvested population.

MATERIALS AND METHODS

Study species. Coconut crabs were captured on Hatoma Island, southwest of Okinawa, Japan (24° 28' N, 123° 49' E). The size of functional maturity in females is estimated at 24.5 mm thoracic length (TL) (Sato & Yoseda 2008), and all males larger than 25 mm TL are physiologically mature (Sato et al. 2008). On Hatoma Island, the reproductive season starts in early June and ends in late August (Sato & Yoseda 2008) and the maximum TL is 61.2 mm for males and 44.6 mm for females (Sato & Yoseda 2010). Females mate in the hard-shell condition (Helfman 1977). During mating, males deposit a spermatophore mass over the gonopores on the female ventral surface because females have no seminal receptacle. The attached spermatophore mass including many spermatophores is retained by the females until egg extrusion and external fertilization occurs (Fletcher 1993). The time lapse between mating and egg extrusion is estimated to be less than 1 wk (Sato & Yoseda 2009b). There is a positive correlation between female body size and number of eggs extruded (approx. 50 000 to 250 000 eggs) and females spawn only one clutch per reproductive season (Sato & Yoseda 2008). The incubation period is estimated to be about 25 to 45 d with the majority of eggs maturing 27 to 29 d after egg extrusion (Schiller et al. 1991).

Housing conditions. Males were captured in mid-May, before the start of the reproductive season and unmated females (i.e. without an attached spermatophore mass) were captured in late May to mid-August 2008 and 2009. Captured crabs were sexed based on the presence of pleopods, which only females possess (Fletcher 1993). The TL, from the center of anterior end to the center of posterior end of the thorax (Fletcher 1993), was measured to the nearest 0.1 mm using Vernier calipers (Mitutoyo, CD-20PM). All laboratory experiments were conducted at the Ishigaki Tropical Station, Seikai National Fisheries Research Institute (24° 34' N, 123° 16' E). In the laboratory, the crabs were kept individually in plastic lidded boxes (60 × 39 × 27 cm, length × width × depth) through which freshwater flowed up to 3 cm deep. Crabs were fed an artificial diet of dog food (CB Foods) daily. A plastic case (30.5 × 30.5 × 7.0 cm, length × width × height) was placed inside each box to provide a space outside of the water for the crab. Additionally, a plastic container

(17 × 17 × 6 cm, length × width × depth) filled with filtered seawater was also provided for drinking. The light:dark regime matched the natural local conditions with natural light coming through a transparent roof.

Mating trials and methods for sperm counts. Female pleonal expansion is strongly correlated with female reproductive activities, and the index of pleonal expansion (PE) can be employed as a morphological criterion for selecting females that are able to mate (Sato & Yoseda 2009b). Coconut crabs have 4 tergites, and the distance between the tergites increases when the pleon becomes enlarged. Thus the PE was calculated from the distances between the tergites. After measurement of female TL, the distances between the second and third tergites (*A*) and between the third and fourth tergites (*B*) were also measured to the nearest 0.1 mm using Vernier calipers. We then calculated the PE using the formula $PE = (A + B)/TL$. All females showing a PE value higher than 0.35 can mate and some females showing a PE value around 0.30 can also mate (Sato & Yoseda 2009b).

Mating trials were conducted from late May to mid-August, but most trials occurred before mid-July, which represents the end of the peak spawning period in nature (Sato & Yoseda 2008). Only individuals with a complete set of limbs were selected for mating. At about 20:00 h, a male was placed inside a 200 l (85 × 55.5 cm, diameter × height) circular tank in which artificial turf (~15 mm length) had been laid down for traction. After a few minutes for the male to acclimatize, an unmated female was placed in the tank. The pairs were observed for at least 3 h after transfer of the female. If the female mated during the observation period, she was removed from the tank. If no mating occurred during the observation, the female's abdomen was checked the next morning at 08:00 h for attached spermatophore mass to judge if mating had occurred.

If the female had mated, she was killed by ganglion puncture. A ganglion underneath the point between the bases of the first pair of pereopods was punctured with a needle through the shell. After the puncture, the attached spermatophores were removed from the ventral surface by cutting out parts of the exoskeleton. It was necessary to kill females to collect all attached spermatophores and this particular method was used in order to minimize suffering of the animals. All sperm was extracted from the spermatophores using a 20% NaOH solution (Sato et al. 2008). The pieces of exoskeleton were placed into 50 ml tubes containing 10 or 40 ml of 20% NaOH solution, depending on the volume of ejaculated sperm. After more than 120 min, the contents of the tube were mixed in a vortex-type mixer for 30 s without removing the piece of exoskeleton. The mixed solution was then placed immediately into a Burkert-Turk haemocytometer and the solution was

allowed to settle for 3 min. Sperm were then counted in aliquots of 0.1 µl of the solution under an optical microscope (Nikon, Eclipse E600) at 400× magnification. The total number of extracted sperm in the tube, i.e. number of ejaculated sperm, was calculated as the number of sperm counted/volume of NaOH containing the counted sperm (i.e. 0.1 µl) times the total volume (i.e. 10 or 40 ml). Four counts were made per sample and the average was taken as the number of ejaculated sperm.

When males were mated with several females successively, the male mating intervals ranged between 4 and 13 d. During mating intervals, males were returned to their respective plastic boxes and fed daily. In the present study, the spermatophores of all mating pairs were correctly attached to the ventral surface of the female. Mating trials were conducted in 2008 and 2009. Replicates from different years were pooled in analysis in each experiment because there was no significant difference in number of ejaculated sperm from males (2008: range = 36.0 to 47.2, mean ± SD = 42.1 ± 4.3 mm TL, n = 6; 2009: range = 40.0 to 54.2, mean = 46.8 ± 6.4 mm TL, n = 5) to females (2008: range = 34.8 to 37.2, mean = 36.0 ± 1.1 mm TL, n = 6; 2009: range = 34.5 to 36.9, mean = 35.8 ± 1.0 mm TL, n = 5) between years (ANCOVA, n = 11, $F_{1,8} = 2.13$, p = 0.29; T. Sato unpubl. data).

Effect of male size on number of ejaculated sperm and fertilization rate. In the first experiment, the following crabs were used: 6 small males (range = 32.0 to 34.0, mean = 33.0 ± 0.7 mm TL), 7 large males (range = 40.0 to 43.0; mean = 41.5 ± 1.1 mm TL), and 13 females (range = 30.1 to 32.7, mean = 30.9 ± 0.9 mm TL). Two groups of male and female pairs were formed: small male × female (n = 6) and large male × female (n = 7). Each mating was carried out either from 6 June to 20 June 2008 or from 30 May to 21 June 2009, and then ejaculated sperm were counted for each mating pair. Since female size may influence sperm allocation pattern as seen in other anomuran crabs (Sato et al. 2006, Sato & Goshima 2007c), differences in female size between treatments were removed in each experiment. There was no significant difference in female size between the groups in this experiment (mean, small male × female: 30.9 ± 1.0 mm TL; large male × female: 30.8 ± 0.5 mm TL; *t*-test, df = 12, *t* = 0.29, p = 0.38). The effects of male size on the number of ejaculated sperm were analyzed using a *t*-test, after testing for homogeneity of variance using Levene's test (p = 0.23).

In the second experiment, designed to examine the effect of male size on fertilization rate, the following crabs were used: 5 small males (range = 28.9 to 31.0, mean = 30.3 ± 0.8 mm TL), 6 large males (range = 40.7 to 46.0, mean = 43.5 ± 2.2 mm TL), and 11 females (range = 29.7 to 32.0, mean = 30.6 ± 0.7 mm TL). These

crabs were different crabs from the first experiment. Two groups of male and female pairs were formed: small male \times female ($n = 5$) and large male \times female ($n = 6$), and each pair was mated from 1 June to 14 July 2009. There was no significant difference in the size of females between the groups (mean, small male \times female: 30.6 ± 0.9 mm TL; large male \times female: 30.6 ± 0.6 mm TL; t -test, $df = 10$, $t = -0.03$, $p = 0.49$).

After checking the attachment of the spermatophore mass on the female ventral surface in the mating tank, each female was transferred into a separate tank ($145 \times 397 \times 90$ cm, length \times width \times depth), in which an artificial nest burrow was assembled ($\sim 150 \times \sim 10$ cm, length \times diameter) from several pieces of limestone (~ 15 to 20 cm in diameter) as an egg extrusion site for the mated female, because coconut crab females extrude eggs after mating in crevices or earth burrows near the seashore (Sato & Yoseda 2009a). Additionally, a plastic container ($17 \times 17 \times 6$ cm, length \times width \times depth) filled with freshwater, another plastic container ($87 \times 52 \times 21$ cm, length \times width \times depth) filled with seawater and artificial turf (~ 15 mm length) for traction were also placed in the tank. The females were observed every night when they left the burrow for about 5 to 10 d after being transferred, to see whether they had extruded and retained a clutch on their pleopods. If a transferred female had a clutch, more than 150 of the eggs were collected at random from 5 parts of the clutch using a pair of tweezers, and then cell division of a total of 150 eggs was observed under a stereomicroscope. The percentage of dividing eggs in the sample was taken as the fertilization rate for the clutch. The effect of male size on fertilization rate was analyzed using the Mann-Whitney U -test because data sets were not homoscedastic (Levene's test, $p = 0.002$).

Effect of male mating frequency on number of ejaculated sperm. In this experiment, the following crabs were used: 9 males (range = 39.1 to 42.6; mean = 40.9 ± 1.1 mm TL) and 27 females (range = 29.1 to 37.2; mean = 34.0 ± 2.0 mm TL). Each male mated successively with 3 females, and ejaculated sperm were counted after each mating. Each successive mating treatment was conducted either from 2 June to 12 July 2008 or from 30 May to 28 June 2009. There was no significant difference in the size of females among categories of male mating frequency (mean, first mating: 35.1 ± 0.9 mm TL; second mating: 34.7 ± 0.5 mm TL; third mating: 33.4 ± 2.5 mm TL; Kruskal-Wallis test, $df = 2$, $\chi^2 = 4.63$, $p = 0.10$; Levene's test, $p = 0.03$). The effect of male mating frequency on the number of ejaculated sperm was analyzed by repeated measures ANOVA and the Dunnett test. Huynh-Feldt (H-F) corrected probability was performed because the sphericity assumption was not met ($df = 2$, $W = 0.41$, $p = 0.04$; Huynh-Feldt epsilon = 0.69).

Effect of male size on ability to ejaculate successively. To investigate how male size affects the relationship between male mating frequency and number of ejaculated sperm, 42 males (range = 30.8 to 54.2; mean = 39.3 ± 6.7 mm TL) and 82 females (range = 27.9 to 37.5; mean = 32.9 ± 2.5 mm TL) were used. Three mating groups were formed: 14 males were each mated with 1 female, 16 males were each mated with 2 females successively, and 12 males were each mated with 3 females successively. Each mating group was formed either from 1 June to 13 July 2008 or from 27 May to 17 July 2009; sperm ejaculated at the last mating of each male was then counted. There was no significant difference in the size of females at last mating among the male groups (mean, first mating group: 34.0 ± 2.8 mm TL; second mating group, 32.1 ± 2.3 mm TL; third mating group, 32.3 ± 1.9 mm TL; 1-way ANOVA, $F_{2,39} = 2.77$, $p = 0.08$). The relationship between male size and the number of sperm ejaculated at each mating was determined by linear regression. An ANCOVA was conducted, assigning male size as the covariate to investigate the influence of male size on the relationship between male mating frequency and number of ejaculated sperm.

Sperm recovery rate. To examine sperm recovery rate, 26 males (range = 30.4 to 45.9; mean = 38.7 ± 4.6 mm TL) and 78 females were used (range = 27.9 to 37.2; mean = 32.7 ± 2.4 mm TL). Each male was mated successively with 3 females. Each successive mating treatment was conducted either from 1 June to 13 July 2008 or from 27 May to 12 August 2009. At 0 or 30 d after the third mating, the vasa deferentia, in which sperm available for mating are stored, were removed from each male and put into a 50 ml tube containing 20 or 40 ml of 20% NaOH solution, depending on the size of the removed vasa deferentia. The method of counting sperm in the vasa deferentia was the same as that described above for the spermatophores. There was no significant difference in the size of females mated in the successive mating between the 2 male groups with different recovery periods (mean, no recovery period group: 33.1 ± 2.4 mm TL; 30 d recovery period group, 32.4 ± 2.3 mm TL; 1-way ANOVA, $F_{1,76} = 1.514$, $p = 0.22$). In this experiment, replicates from different years were pooled in analysis in each group because there was no significant difference in number of sperm in vasa deferentia in each group between years (ANCOVA, no recovery period group: $n = 11$, $F_{1,8} = 0.14$, $p = 0.72$; 30 d recovery period group: $n = 15$, $F_{1,12} = 0.02$, $p = 0.90$). The relationship between male size and the number of retained sperm was determined by linear regression for each group. To examine whether the phase of the reproductive season in which males were allowed to recover sperm reserves influenced sperm recovery rate within the 30 d recovery

period, a multiple linear regression analysis of the number of sperm in the vasa deferentia after the 30 d recovery period with 2 influencing factors, male TL and the date on which males were dissected after the 30 d recovery period (number of days from date on which first male was dissected after the 30 d recovery period, 4 July in this experiment) for the 30 d recovery period group, was performed using the stepwise method. In this analysis, only variables significant at the 0.05 level are left in the model. In addition, an ANCOVA was conducted, assigning male size as the covariate to examine the influence of recovery time on the number of sperm stored in the vasa deferentia.

Number of sperm retained by females. To detect factors influencing number of sperm retained by females in the harvested population, non-ovigerous females retaining spermatophore mass were captured by hand on Hatoma Island between 24 May and 22 July 2009. The females were brought to the laboratory, and the number of sperm retained by each female was immediately counted using the same method used to count ejaculated sperm described above. Multiple linear regression analysis of the number of sperm retained by females with 2 possible influencing factors, the time when females were caught (number of days from date on which first crab was caught, 24 May in this experiment) and female TL, was performed using the stepwise method in which only variables significant at the 0.05 level are left in the model.

RESULTS

Effect of male size on number of ejaculated sperm and fertilization rate

The mean number of ejaculated sperm differed significantly between small and large males (t -test, $t = -8.1$, $df = 11$, $p < 0.001$), and large males passed more sperm (Fig. 1a). Fertilization rate also differed significantly with male size (Mann-Whitney U -test, $Z = -2.8$, $p = 0.005$), and females mated with large males showed a higher fertilization rate (Fig. 1b).

Effect of male mating frequency on number of ejaculated sperm

The number of ejaculated sperm decreased significantly with increasing male mating frequency (repeated measures ANOVA, $F_{1,38,11.05} = 73.35$, $p < 0.001$) (Fig. 2). The Dunnett test showed significant differences between the first and second ($p < 0.001$) and the first and third matings ($p < 0.001$).

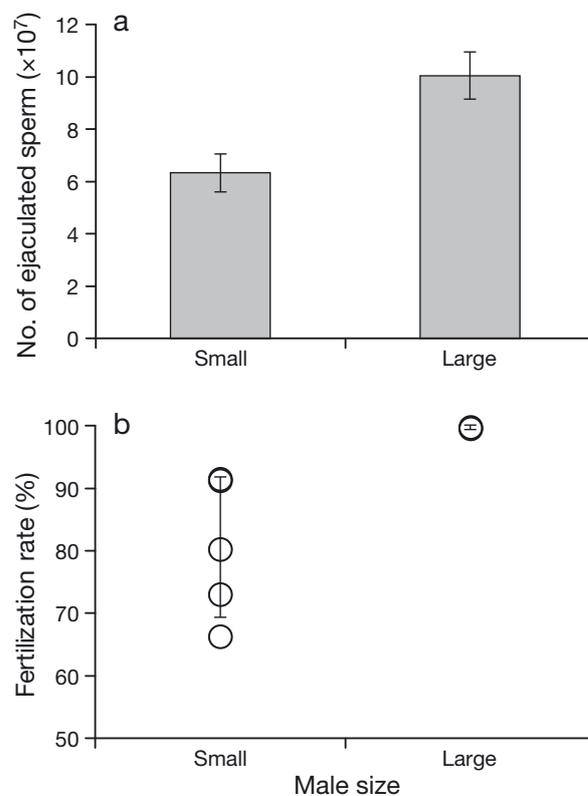


Fig. 1. *Birgus latro*. Effect of male size on (a) the number of ejaculated sperm (small males, mean TL = 33.0 ± 0.7 mm, n = 6; large males, mean TL = 41.5 ± 1.1 mm, n = 7) and (b) fertilization rate (small males, mean TL = 30.3 ± 0.8 mm, n = 5; large males, mean TL = 43.5 ± 2.2 mm, n = 6). Error bars are ±SD

Effect of male size on ability to ejaculate successively

Larger males passed more sperm to females, regardless of male mating frequency (linear regression, first mating: number of ejaculated sperm = 0.55 male TL - 11.71, $r^2 = 0.85$, n = 14, $F_{1,12} = 68.9$, $p < 0.001$; second mating: number of ejaculated sperm = 0.29 male TL - 8.08, $r^2 = 0.72$, n = 16, $F_{1,14} = 55.0$, $p < 0.001$; third mating: number of ejaculated sperm = 0.16 male TL - 4.70, $r^2 = 0.72$, n = 12, $F_{1,10} = 26.3$, $p < 0.001$) (Fig. 3). The difference between the number of sperm ejaculated on 2 consecutive matings was greater with larger males (ANCOVA, male size × male mating frequency: $F_{2,36} = 13.98$, $p < 0.001$; Fig. 3). Smaller males (<45 mm TL) passed only a small number of sperm to females in the second and the third matings.

Sperm recovery rate

The number of sperm retained in the vasa deferentia was greater in larger males not just after the third

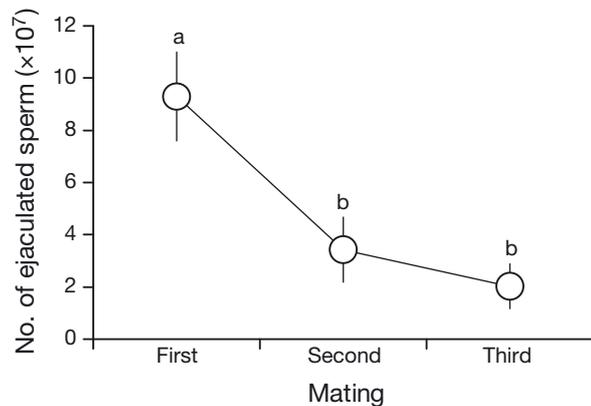


Fig. 2. *Birgus latro*. Relationship between male mating frequency and the number of ejaculated sperm (first mating, $n = 7$; second mating, $n = 7$; third mating, $n = 7$). Error bars are \pm SD. Different letters indicate a significant difference

mating but also after a 30 d recovery period (linear regression, after third mating: number of stored sperm = 0.65 male TL - 18.79 , $r^2 = 0.78$, $n = 11$, $F_{1,9} = 32.10$, $p < 0.001$; after 30 d recovery period: number of stored sperm = 0.67 male TL - 18.98 , $r^2 = 0.32$, $n = 15$, $F_{1,13} = 7.87$, $p = 0.014$) (Fig. 4). In multiple linear regression analysis, only male TL had a significant effect on the number of sperm in vasa deferentia after the 30 d recovery period, and the phase of the reproductive season when males were allowed to recover sperm reserves had no effect on sperm recovery rate within the 30 d recovery period (Table 1). Regardless of male size, there was no significant increase in the number of sperm in the vasa deferentia after the 30 d recovery period (ANCOVA, recovery period: $F_{1,22} = 0.34$, $p = 0.57$) (Fig. 4).

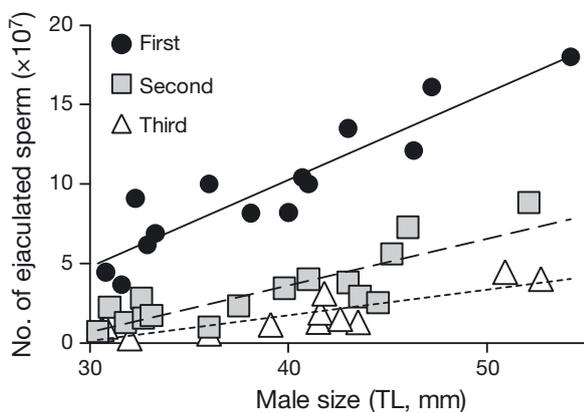


Fig. 3. *Birgus latro*. Relationship between male mating frequency by males of various body sizes (thoracic length, TL) and the number of ejaculated sperm (first mating, $n = 14$; second mating, $n = 16$; third mating, $n = 12$)

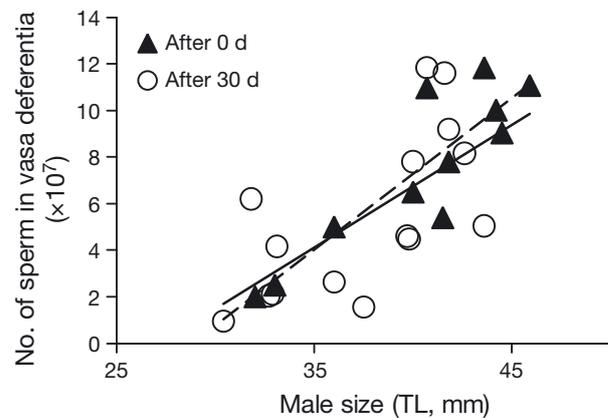


Fig. 4. *Birgus latro*. Recovery rate of number of sperm in the vasa deferentia retained by males of various body sizes (thoracic length, TL) after 0 d ($n = 11$) and after 30 d ($n = 15$)

Number of sperm retained by females

A total of 22 females carrying spermatophore mass were captured (range = 26.4 to 37.4 ; mean = 31.8 ± 3.6 mm TL). There was a very large variance in the number of retained sperm not only among females of different body sizes but also among females of similar body size (Fig. 5). Only the time when females were caught (i.e. number of days from 24 May) had a significant effect on the number of retained sperm in multiple linear regression analysis (Table 2). The number of retained sperm decreased significantly as the reproductive season progressed ($r^2 = 0.32$) (Fig. 6).

DISCUSSION

The results of the present study suggest that male size and male mating frequency are factors that greatly influence the probability of sperm limitation (shortage of sperm supply to females), in coconut crab populations. Large male coconut crabs provided more sperm to females than small males, as seen in other animal species (MacDiarmid & Butler 1999, Sato et al. 2006,

Table 1. Regression on effect of male thoracic length (TL) on the number of sperm in *Birgus latro* vasa deferentia after the 30 d recovery period. The phase of the reproductive season when males were allowed to recover sperm reserves was not a significant factor ($p > 0.05$) and was excluded

	Regression coefficient	SE	Standardised coefficient	t	p
Intercept	-18.978	8.998	0.000	-2.109	0.055
Male TL	0.667	0.238	0.614	2.806	0.015

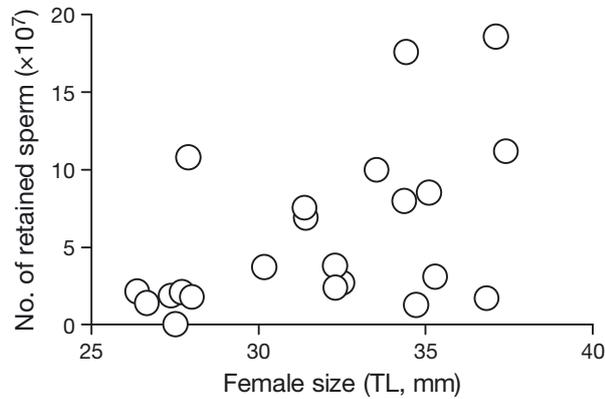


Fig. 5. *Birgus latro*. Relationship between female size (thoracic length, TL) and number of sperm retained by females in the harvested population ($n = 22$)

Table 2. Regression on effect of the time when *Birgus latro* females were caught (i.e. number of days from 24 May) on the number of sperm retained by females in the field. Female TL was not a significant factor ($p > 0.05$) and was excluded

	Regression coefficient	SE	Standardised coefficient	<i>t</i>	<i>p</i>
Intercept	11.085	1.963	0.000	5.647	<0.001
No. days	-0.218	0.071	-0.567	-3.077	0.006

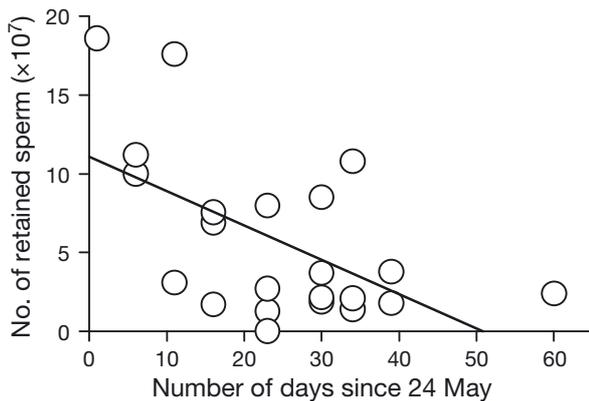


Fig. 6. *Birgus latro*. Relationship between the time when females were caught and number of sperm retained by females in the harvested population. Here, 24 May = 1 on the x-axis ($n = 22$)

Sato & Goshima 2007a, Lemaître et al. 2009, but see Hinojosa & Thiel 2003). The fertilization rate of females mated with small males was significantly lower than that of females mated with large males. The reduction of reproductive success of females mated with smaller males compared to those mated by large males was most likely due to insufficient sperm to fertilize all

eggs, i.e. sperm limitation, as has also been reported in other species (Bissoondath & Wiklund 1996, MacDiarmid & Butler 1999, Sato et al. 2006, Sato & Goshima 2007a, but see Kendall et al. 2002).

It has been widely observed among many species that male mating frequency strongly affects the number of ejaculated sperm (Dewsbury 1982, Birkhead 1991, Pitnick & Markow 1994, Sato et al. 2006, Hettyey et al. 2009, Smith et al. 2009). Male coconut crabs in the present study also showed a sharp decrease in number of ejaculated sperm with increasing mating frequency. Although decline in the absolute number of ejaculated sperm with increasing male mating frequency was greater in larger than in smaller coconut crab males, the former still passed larger ejaculates to successive mates than smaller males, regardless of whether they had mated twice or thrice. Compared to larger males, small males (<45 mm TL) showed more substantial decreases in the number of ejaculated sperm relative to the number ejaculated in the previous mating and they passed almost no sperm in the third mating. Thus larger coconut crab males have a greater capacity to successively mate with females and fertilize their clutches compared with smaller males, as seen in other crustacean species (Hinojosa & Thiel 2003).

Various taxa recover sperm reserves (e.g. Birkhead 1991, McWilliams 1992, Westneat et al. 1998). For example, male sperm reserve is completely replenished between 9 and 20 d after mating in the blue crab *Callinectes sapidus* (Kendall et al. 2001). After mating, male blue crabs may recover some sperm and seminal fluid before they mate again, because they typically guard females for 2 to 5 d before mating (Jivoff 1995). However, in the present study, the number of sperm in the vasa deferentia of coconut crabs did not increase even after a 30 d recovery period throughout the reproductive season, suggesting males are unable to produce available sperm packed in spermatophores for mating before the next mating. Sperm production is slow and may be costly for coconut crab males. Such a slow recovery rate of sperm was also observed in 2 anomuran crabs, *Paralithodes brevipes* (Sato et al. 2006) and *Hapalogaster dentata* (Sato & Goshima 2006). Coconut crab males recover their sperm reserves completely by the next reproductive season, in the following year. The slow sperm production leads to a sharp drop in number of ejaculated sperm with increasing male mating frequency. It is likely that coconut crab females suffer from sperm limitation when females mate with smaller males (<45 mm TL) who already mated within the reproductive season. Such limited sperm resources in coconut crabs could drive males to economize on their sperm reserves (Wedell et al. 2002) or females to prefer previously unmated males (Sato & Goshima 2007b).

For many biological resources, large males are more likely to be harvested than small males (Coltman et al. 2003, Rowe & Hutchings 2003, Fenberg & Roy 2008). Coconut crab populations are selectively harvested for large males (>40 mm TL), which skews the sex ratio towards females and leads to a decrease in mean male size (Sato & Yoseda 2010). Although selective harvesting of males can cause female-biased sex ratios, this does not necessarily lead to a reduction in the reproductive rate of a resource because most harvested species exhibit polygynous mating systems (Mysterud et al. 2002). In many cases, reproductive rates, therefore, are resilient to a skewed sex ratio (Milner et al. 2007) and may even increase (Solberg et al. 2000). However, this resilience is not applicable to species such as the coconut crab, in which male reproductive potentials (e.g. ability to mate with female and/or to fertilize female ova) are body size dependent.

In harvested coconut crab populations (Sato & Yoseda 2010), there are only a few larger males capable of providing high numbers of ejaculated sperm and complete fertilization of females. In addition, the small males that are left are likely to experience more matings than in non-harvested populations, and thus their ability to ejaculate large numbers of sperm is likely to be limited due to successive matings, as their sperm reserves do not recover within the reproductive season. As a result, females suffer from insufficient sperm supply to fertilize all their eggs and the reproductive rate of the harvested population is likely to decrease. This prediction is supported by the results of the present study: the number of sperm retained by females decreased rapidly in the field as the reproductive season progressed and there was very large variance in the number of retained sperm even among females of similar body size (Fig. 5). In addition, more than half of the females (13 of 22 females captured) received only a small number of sperm ($<4 \times 10^7$), and the number of sperm retained by those females was almost the same as that by females mated with smaller males (<45 mm TL) who had mated more than once (Figs. 5 & 6). Therefore, the decrease in the number of sperm retained by females as the reproductive season progresses can be attributed mainly to depletion of male sperm reserves due to male successive mating.

The selective removal of larger coconut crab males, which are preferred by females as mates, is also likely to decrease female reproductive success by reducing the probability of encountering potential mates (Sato & Yoseda 2010). In the field, some females in the harvested coconut crab population had clearly small or non-fertilized clutches (T. Sato unpubl. data), implying that they failed to receive sufficient sperm and/or to mate within their optimal mating period. In some species, clear declines in female reproductive success and

even some population crashes attributed to reduced fecundity due to large male-selective harvesting have been linked to, for example, sperm limitation and loss of mating opportunities (Hines et al. 2003, Milner-Gulland et al. 2003, Sato & Goshima 2006, Milner et al. 2007, Sato et al. 2007). The sex ratio of a population can fall below the threshold to sustain its reproductive rate through excessive large male-only harvesting (Sato & Goshima 2006, Fenberg & Roy 2008), which argues for caution with this practice. To sustain these resources, it is necessary to pay attention not only to direct overharvesting (absolute numbers and demography) but also to such negative impacts of large male-selective harvestings on reproduction, e.g. sperm limitation and loss of mating opportunities. Therefore, there is a need to investigate details of mating systems and reproductive ecology (Rowe & Hutchings 2003), including intra- and intersexual selection, size-dependent reproductive potential, and functional maturity size (size of males that participate in mating in the field), for each biological resource.

To avoid the anticipated negative impacts of large male-selective harvesting on the reproduction of coconut crab populations, it is necessary to establish management strategies that take into consideration the fact that male reproductive potential, in terms of successive mating and fertilization, is size-dependent. The results of the present study suggest that the protection of large males with high reproductive potential would contribute to sustaining the reproductive rate of populations. In addition, protection of females from harvesting so that they can spawn is in general an effective way to sustain the reproductive rate of biological resources and to allow them to recover from a depleted state. Therefore, male-only harvesting with slot size limits would be an effective management strategy for maintaining sustainable coconut crab populations: intermediate size males are harvested selectively, and all females, juvenile males, smaller adult males just after maturation, and large males with high reproductive potential are protected from harvesting. Furthermore, it is also essential to regulate the total number of harvested crabs within the determined size range, in order to allow intermediate-sized males to survive and become large males with high reproductive potential.

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