Plausible causes for sperm-store variations in the coconut crab *Birgus latro* under large male-selective harvesting

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ABSTRACT: In populations subjected to size-selective harvesting of large males, remaining males are small and participate in more matings than in pristine populations. The reproductive rate of such harvested populations can be restricted due to sperm limitation, because the numbers of sperm passed to females decrease with decreasing male size and increasing male mating frequency. Japanese populations of the coconut crab *Birgus latro* are typical examples of populations that are subject to selective harvesting of large males. Reproductive output of the population may have declined via sperm limitation. However, there is no evidence that males exhaust sperm reserves through successive matings, and little is known about the factors causing variations in the number of sperm retained by females in the harvested population. Using field investigations and laboratory experiments, I examined (1) whether males exhaust sperm reserves and (2) what factors cause variations in the number of sperm retained by females. About half of the males had exhausted sperm reserves by the end of the mating season. These males were found to be incapable of further mating or of ejaculating larger numbers of sperm, which was inferred from laboratory experiments. The size of individuals of both sexes participating in mating decreased as the mating season progressed, and males did not adjust ejaculate size in response to female size. Thus, variations in the number of sperm retained by females may have mainly been due to a decline in the male ability to ejaculate large numbers of sperm, due both to increased male mating frequency and decreased male size.

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

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INTRODUCTION

Sperm limitation can arise in various animal taxa (Dewsbury 1982, Birkhead 1991, Pitnick & Markow 1994). The probability that females have an insufficient supply of sperm for fertilization is closely related to population demography, including the sex ratio and the mean male size (Sato & Goshima 2006). Male mating frequency and body size can influence the number of sperm passed to females. Male mating frequency increases if the population sex ratio is skewed toward females. Since successive matings deplete male sperm reserves and decrease ejaculate size (Birkhead 1991, Pitnick & Markow 1994, Jivoff 2003, Sato et al. 2005, Rubolini et al. 2007), the female fertilization rate may

be reduced in populations with female-biased sex ratios (e.g. Sato & Goshima 2006). In some species, larger males have larger sperm reserves (MacDiarmid 1989, Pitnick 1996, Kendall et al. 2001, Sato et al. 2005, Sainte-Marie et al. 2008) and pass larger ejaculates to successive mates than do smaller males (Jivoff 1997, Sato et al. 2006). Thus, overall, sperm supply is likely reduced in populations containing only small males.

In several biological resources, males are more likely to be harvested than females, and larger males are more likely to be caught than smaller males (Coltman et al. 2003, Rowe & Hutchings 2003, Fenberg & Roy 2008). Such harvesting affects population demography by skewing the sex ratio towards females and decreasing mean male body size (Paul & Adams 1984, Smith &

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Jamieson 1991, Ginsberg & Milner-Gulland 1994, Abbe & Stagg 1996, Sato et al. 2005). Females in populations subjected to such biased harvesting may receive less sperm, limiting their reproductive success and resulting in a decrease of the population reproductive rate. The reduction in female reproductive success due to sperm limitation has been documented in some crab populations from which large males are selectively harvested (Smith & Jamieson 1991, Hines et al. 2003, Carver et al. 2005, Sato et al. 2007, but see Hankin et al. 1997).

The coconut crab *Birgus latro* (Linnaeus, 1767) lives in coastal areas of the tropical Indo-Pacific region. Populations in most habitats have been severely depleted or have become virtually extinct (Brown & Fielder 1991). Overharvesting is considered one of the main causes for the present depletion of this resource (Fletcher 1993). On Hatoma Island, located southwest of Okinawa, Japan (24° 28′ N, 123° 49′ E), the large male-selective harvesting likely contributes to resource depletion (Sato & Yoseda 2010). This harvesting pattern skews sex ratios toward females and decreases mean male size in the harvested population (Sato & Yoseda 2010). Sato et al. (2010) described the size-dependent reproductive potentials of coconut crab males as follows: (1) smaller males provide females with smaller numbers of sperm than do larger males, which can result in lower fertilization rates due to insufficient sperm supply; and (2) although the number of ejaculated sperm decreases with increasing male mating frequency, regardless of male size, larger males always pass more sperm to successive mates than smaller ones. In the harvested population, more than half of the females were found to retain very low numbers of sperm, which would limit their fertilization rates (Sato et al. 2010). Therefore, the reproductive output of coconut crab populations subjected to large male-selective harvesting may have declined via sperm limitation (Sato et al. 2010). However, there is no evidence that males exhaust their sperm reserves through successive matings in the harvested population.

In the harvested population, the number of sperm retained by females decreases as the reproductive season progresses, and there are very large variations among females in the number of retained sperm (Sato et al. 2010). However, little is known about the factors causing large variations in the number of sperm retained by females in the harvested coconut crab population. Generally, males should partition their limited sperm reserves judiciously to maximize their reproductive success (e.g. Wedell et al. 2002). In some species in which female reproductive potential is dependent on body size, males provide larger ejaculates to larger, more fecund females, providing larger fertilization

returns (MacDiarmid & Butler 1999, Sato et al. 2006, Sato & Goshima 2007a). Coconut crab males should also adjust ejaculate size to female body size, because there is a positive relationship between female body size and the number of eggs extruded (Sato & Yoseda 2008), and the male sperm reserves available for mating are limited (Sato et al. 2010). The sperm allocation pattern can affect variation in the number of sperm retained by females. It is important to determine the factors that cause the large variation in the number of sperm retained by females in order to understand whether present large male-selective harvesting influences the reproductive rate of this resource.

To determine whether males exhaust their sperm reserves through successive matings in the Hatoma Island coconut crab population, which is presently subjected to large male-selective harvesting, I examined the temporal variation in male sperm reserves during the mating season. In addition, to determine the factors causing the large variation in the number of sperm retained by females, I examined (1) the temporal variations throughout the mating season in the body sizes of males and females participating in mating and (2) sperm allocation patterns by males of different body sizes as a function of female body size.

MATERIALS AND METHODS

Study species. Coconut crabs *Birgus latro* were captured by hand over as wide a coastal area as possible on Hatoma Island. Females in this population are regarded as attaining functional maturity when their thoracic length (TL), measured from the center of the anterior end to the center of the posterior end of the thorax, is 24.5 mm (Sato & Yoseda 2008), whereas all males with a TL > 25 mm are considered physiologically mature (Sato et al. 2008). The reproductive season for these crabs starts around early June and ends in late August, and most females finish extruding eggs by mid-July (Sato & Yoseda 2008). 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, which includes many spermatophores, is retained by the females until egg extrusion and external fertilization (Fletcher 1993). There is a positive correlation between female body size and number of eggs extruded (approximately 50 000 to 250 000 eggs), with females extruding only 1 clutch per reproductive season (Sato & Yoseda 2008). It is not common for females to extrude eggs without mating (author's pers. obs.). The incubation period is estimated to be from about 25 to 45 d, with the majority of eggs maturing 27 to 29 d

after extrusion (Schiller et al. 1991). Female pleonal expansion is strongly correlated with female reproductive activity, and the index of pleonal expansion (PE) can be used as a morphological criterion for selecting females that are able to mate (Sato & Yoseda 2009). All females with PE values >0.35 can mate, and some females with PE values around 0.30 can also mate (Sato & Yoseda 2009).

Collections and housing conditions. Males were captured in mid-May of 2009 and 2010, just before the start of the year's reproductive season, and females were captured in late May to mid-July in 2009 and 2010. The start of the reproductive season in each year was estimated by the date of first appearance of a female with a spermatophore mass. The reproductive season started on 24 and 25 May in 2009 and 2010, respectively. Captured crabs were sexed based on the presence of pleopods, which only females possess (Fletcher 1993). The TL of each crab was measured to the nearest 0.1 mm using Vernier calipers (Mitutoyo Corporation, CD-20PM). All laboratory experiments were conducted at the Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute (24° 34′ N, 123° 16′ E). In the laboratory, the crabs were kept individually in lidded plastic boxes (60 × 39 cm and 27 cm deep), through which fresh water flowed (up to 3 cm depth). Crabs were fed an artificial diet (dog food, CB Foods) every day. A plastic case (30.5 × 30.5 cm and 7.0 cm high) was placed inside each box to provide a space outside of the water for the crabs. In addition, each crab was provided with a plastic container (17 × 17 cm and 6 cm deep) filled with filtered seawater for drinking. The light:dark regime matched the local natural conditions, with natural light coming through a transparent roof.

Temporal variation in male sperm reserves. To investigate temporal changes in the number of sperm retained by males in harvested populations during the mating season, intact males with all limbs were captured just before (19 May) the start of the 2010 mating season, and during the early (28 May), middle (12 June), and late (26 June) parts of the mating season and brought to the laboratory. Ovigerous females constituted 10.0, 45.8, and 95.7 % of the female population, in which females were >25 mm TL during the early, middle, and late parts of the mating season, respectively (unpubl. data). Males were killed by puncturing a ganglion of the central nervous system with a needle through the shell underneath the point between the bases of the first pair of pereiopods. Each crab's vasa deferentia were removed, placed into a 50 ml tube, and the tube was filled to 40 ml with 20 % NaOH solution to extract the sperm (Sato et al. 2008). After >120 min, the contents of the tube were vortexed for 30 s, placed immediately in a Burker-Turk hemocytometer,

and allowed to settle for 3 min. Four aliquots of 0.1 µl were withdrawn from each tube, and sperm were counted under an optical microscope (Nikon, Eclipse E600) at 400× magnification. Sperm counts in the 4 aliquots were averaged, and the number of sperm in each tube was calculated as the average number of sperm per sample per 0.1 µl aliquot × the total volume of the tube (i.e. 40 ml). The relationship between male size and number of sperm reserves was determined by simple linear regression for each sampling date. There was no significant difference in the mean (±SD) TL of males captured before (43.0 ± 3.8 mm, n = 12) the mating season and those captured during the early (42.1 ± 3.1 mm, n = 10), middle (41.9 ± 2.4 mm, n = 10) and late (45.2 ± 4.6 mm, n = 10) parts of the mating season (1-way ANOVA, $F_{3, 38}$ = 1.78, p = 0.17; Levene's test, p = 0.16). The temporal variation in the number of male sperm reserves was analyzed using the Kruskal-Wallis test, because there was not always a significant correlation between male size and male sperm reserves on each sampling date (see 'Results: Temporal variation in male sperm reserves') and the data sets were not homogeneous for variance (Levene's test, p = 0.002). The Bonferroni multiple-comparisons test (Dunn 1961) was used to identify significant differences between the sampling dates.

Temporal variation in proportion of depleted males. To evaluate the proportion of males with depleted sperm reserves in the harvested population, the number of sperm retained by males incapable of further mating due to successive matings was estimated using 2 laboratory procedures. The crabs used in this experiment included 16 unmated males (TL: 41.5 ± 4.4 mm; range: 35.7 to 49.3 mm) and 84 unmated females (TL: 32.1 ± 0.5 mm; range: 31.0 to 33.0 mm) showing PE values >0.35. All crabs were intact.

Mating trials were conducted from late May to mid-July 2010. At about 20:00 h, a male was placed inside a 200 l (85 cm diameter × 55.5 cm high) circular tank in which artificial turf, about 15 mm high, had been laid down for traction. After allowing the male to acclimate for a few minutes, an unmated female was placed in the tank. The following morning at 08:00 h, each female's ventral surface was examined for an attached spermatophore mass to determine if mating had occurred. Regardless of mating success, the male and female were returned to their plastic lidded boxes after each mating trial. If no attached spermatophore mass was observed, the mating trial, using the same individuals, was performed the next night, for up to 3 trials. If the female had not mated by the third trial, the male was considered unable to mate, and these males were categorized as 'depleted males'. Each female was used in only 1 mating pair, whereas each male was used until he became depleted. Male mating intervals be-

tween different female partners were 7 d. In this experiment, the spermatophore masses of all mating pairs were correctly attached to the ventral surface of the female. Simple linear regression analysis was performed to determine the relationship between male body size and number of matings.

Subsequently, the vasa deferentia were removed from each depleted male, and sperm numbers were determined as in the above experiment. Simple linear regression analysis was performed to determine the relationship between body size and number of sperm in the vasa deferentia of depleted males. The number of sperm in the vasa deferentia of depleted males was compared with the number retained by males in the harvested population. If the number of sperm retained by a male in the harvested population was lower than the estimated regression line between body size and number of sperm in the vasa deferentia of depleted males (see 'Results: Temporal variation in the proportion of depleted males'), that male was defined as 'depleted'.

Sizes of individuals participating in reproduction. To investigate the temporal variation in the TL of females participating in reproduction, females in the harvested population retaining spermatophore masses on their ventral surfaces were captured from late May to late July in 2009 and 2010. To determine the temporal variation of TL of males participating in reproduction, the volume of spermatophores attached to female ventral surfaces was measured (Sato & Yoseda 2010). Using a pair of tweezers, spermatophores were collected randomly from several parts of the spermatophore mass attached to each female. The spermatophores were brought to the laboratory, and the volumes of 20 intact spermatophores from each female were measured, with the mean volume calculated as described in Sato et al. (2008). The TL of mated males can be estimated from the regression equation (r^2 = 0.83, Sato et al. 2008):

$$\text{Volume} = 0.017 \times \text{TL} - 0.031 \qquad (1)$$

where the volume is spermatophore volume in mm^3 and TL is measured in mm. Simple linear regression was used to determine the relationship between estimated TL of mated males and the time of capture of their mates, calculated as the number of days after the date on which the first female with a spermatophore mass was caught during each of the 2 mating seasons, 24 May 2009 and 25 May 2010. Simple linear regression was also used to determine the relationship between TL of females and the time of capture.

Effects of male and female size on number of ejaculated sperm. To examine the relationship between the sperm allocation pattern and female body size by males of different body sizes, 42 unmated males (TL: 39.8 ± 5.4 mm; range: 31.5 to 54.2 mm), 25 small females (TL:

30.5 ± 0.5 mm; range: 29.4 to 31.3 mm), and 17 large females (35.8 ± 0.8 mm; range: 35.0 to 37.0 mm) were used in the laboratory. All females were unmated, with no spermatophore mass attached, intact, and had PE values >0.35. Pairs were placed in mating tanks, as described in 'Materials and methods: Temporal variation in the proportion of depleted males', and the ventral surface of each female was examined for attached spermatophores the following morning. Crabs were assorted into 2 groups of male and female pairs, 25 pairs of males and small females and 17 pairs of males and large females, with each mating group consisting of males and females captured from late May to mid-July in 2009 or 2010. Females that had mated were killed by ganglion puncture, and the attached spermatophores were removed from the ventral surface by cutting out parts of the exoskeleton. These pieces of exoskeleton with attached spermatophore masses were placed into 50 ml tubes, and the number of ejaculated sperm was determined as described in 'Materials and methods: Temporal variation in male sperm reserves'. Replicates from different years were pooled for analysis because the number of ejaculated sperm did not differ significantly between years for each mating group (ANCOVA, male vs. small female mating group: year × male size, $F_{1,21}$ = 0.008, p = 0.93, year, $F_{1,22}$ = 0.91, p = 0.35; male vs. large female mating group: year × male size, $F_{1,13}$ = 0.066, p = 0.80, year, $F_{1,14}$ = 1.99, p = 0.18). Simple linear regression was used to determine the relationship between male size and the number of ejaculated sperm in each mating group. Using ANCOVA, male size was the covariate to determine the influence of female size on the relationship between male size and the number of ejaculated sperm.

RESULTS

Temporal variation in male sperm reserves

A significant relationship between retained male sperm reserves and male body size in *Birgus latro* was observed only before the reproductive season (linear regression, number of stored sperm = 3.88×10^7 male TL -135.6×10^7, r^2 = 0.86, n = 12, $F_{1,10}$ = 62.77, p < 0.001), but not during the early (n = 10, $F_{1,8}$ = 0.085, p = 0.78), middle (n = 10, $F_{1,8}$ = 0.053, p = 0.82), or late parts of the mating season (n = 10, $F_{1,8}$ = 0.87, p = 0.38) (Fig. 1). The number of sperm in male reserves differed significantly by sampling date (Kruskal-Wallis test, χ^2 = 21.2, df = 3, p < 0.001), with the number of sperm before the reproductive season being significantly higher than the numbers on other sampling dates (Bonferroni multiple-comparisons test [adjusted α = 0.0083], before vs. early: p = 0.001; before vs. middle: p = 0.001; before vs. late: p < 0.001).
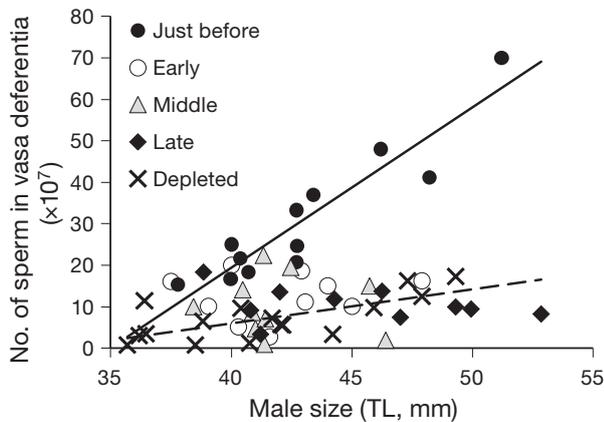
Fig. 1. *Birgus latro*. Relationship between male size (TL: thoracic length, mm) and number of sperm in the vasa deferentia retained either by males just before (19 May) the mating season or during the early (28 May), middle (12 June), or late (26 June) parts of the mating season in the harvested population and in depleted males. The solid line shows a significant positive relationship between male size and the number of sperm reserves just before the mating season. The broken line shows a significant positive relationship between male size and the number of sperm in the vasa deferentia of depleted males

Temporal variation in the proportion of depleted males

The number of matings before depletion and the number of residual sperm in depleted males increased with male body size (linear regression, number of matings = 0.17 male TL − 2.63, $r^2 = 0.32$, n = 16, $F_{1,14} = 6.45$, p = 0.024; Fig. 2; number of residual sperm = 0.83×10^7 male TL − 27.09×10^7, $r^2 = 0.49$, n = 16, $F_{1,14} = 13.26$, p = 0.003; Fig. 1). Although none of the 12 males captured before the mating season was depleted, 20, 40, and 50% of the males captured during each of the early, middle, and late parts of the mating season (n = 10

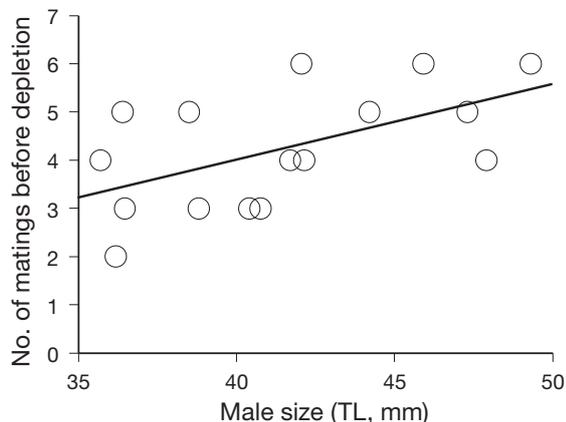each) were classified as depleted, indicating that half of the males in the harvested population had exhausted their sperm reserves by the end of the mating season and were incapable of mating.

Size of individuals participating in reproduction

In 2009 and 2010, the size of females participating in reproduction (i.e. females retaining spermatophore masses) decreased significantly as the mating season progressed (linear regression, 2009: estimated size of mated females = −0.15 no. days since start of mating season + 35.45, $r^2 = 0.31$, n = 22, $F_{1,20} = 9.10$, p = 0.007; 2010: estimated size of mated females = −0.16 no. days since start of mating season + 35.67, $r^2 = 0.43$, n = 20, $F_{1,18} = 13.815$, p = 0.002; Fig. 3), and spermatophores were collected from 21 and 20 females, respectively (mean volume of spermatophores ± 95% CI — 2009: 0.036 ± 0.008; 2010: 0.047 ± 0.006). The size of males participating in reproduction estimated from the volume of collected spermatophores also decreased significantly as the mating season progressed in both years (linear regression, 2009: estimated size of mated males = −0.43 no. days since start of mating season + 50.03, $r^2 = 0.30$, n = 21, $F_{1,19} = 8.26$, p = 0.010; 2010: estimated size of mated males = −0.37 no. days since start of mating season + 51.26, $r^2 = 0.35$, n = 20, $F_{1,18} = 9.49$, p = 0.006; Fig. 4).

Effects of male and female size on number of ejaculated sperm

Sperm allocation increased with male size for both small and large females (linear regression, male vs. small female mating group: number of ejaculated

Fig. 2. *Birgus latro*. Relationship between male size (TL: thoracic length, mm) and number of matings before sperm depletion
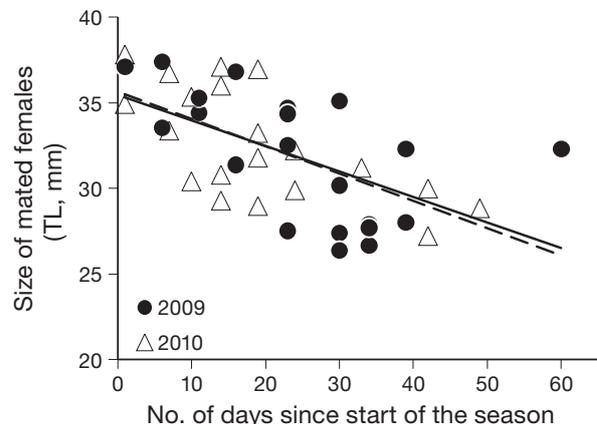

Fig. 3. *Birgus latro*. Relationship between the number of days since the start of the reproductive season (24 May 2009 or 25 May 2010) and the size of females participating in mating (TL: thoracic length, mm) in the harvested population
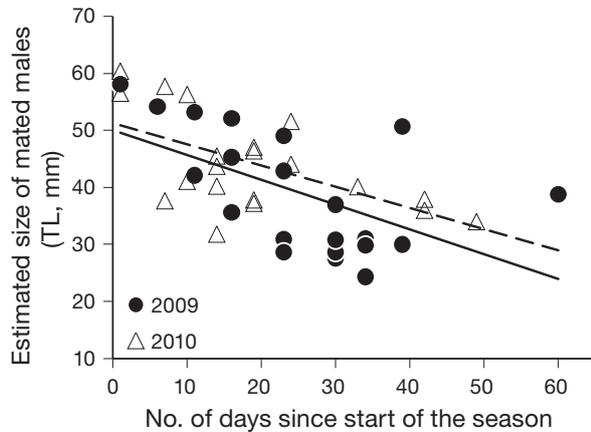
Fig. 4. *Birgus latro*. Relationship between the number of days since the start of the reproductive season (24 May 2009 or 25 May 2010) and the estimated size of males participating in mating (TL: thoracic length, mm) in the harvested population

Fig. 5. *Birgus latro*. Patterns of sperm allocation to large (mean [±SD] thoracic length [TL], 35.8 ± 0.8 mm; range: 35.0 to 37.0 mm) and small (TL: 30.5 ± 0.5 mm; range: 29.4 to 31.3 mm) females by males of different body sizes. The solid and broken lines show significant positive relationships between the number of ejaculated sperm and either large or small females, respectively

sperm $= 0.48 \times 10^7$ male TL $- 9.27 \times 10^7$, $r^2 = 0.46$, n $= 25$, $F_{1,23} = 19.29$, p < 0.001; male vs. large female mating group: number of ejaculated sperm $= 0.49 \times 10^7$ male TL $- 9.73 \times 10^7$, $r^2 = 0.59$, n $= 17$, $F_{1,15} = 21.52$, p < 0.001; Fig. 5). Female size, however, did not have a significant effect on the number of ejaculated sperm (ANCOVA, female size × male size, $F_{1,38} = 0.005$, p $= 0.94$; female size, $F_{1,39} = 0.001$, p $= 0.98$; Fig. 5).

DISCUSSION

Because coconut crab *Birgus latro* populations are selectively harvested for larger males (>40 mm TL), few large males with higher reproductive potential were present in the harvested population (Sato & Yoseda 2010) and those remaining would mate more often than would males of comparable sizes in nonharvested populations. Sperm reserves decreased sharply, and depleted males appeared soon after the start of the reproductive season in the harvested population. The proportion of depleted males increased steadily, up to 50%, toward the end of the mating season, strongly suggesting that some males had mated successively and depleted their sperm reserves to the point of sperm exhaustion; thus, they were no longer able to mate. Regardless of male body size, the number of ejaculated sperm decreases sharply as male mating frequency increases in coconut crab (Sato et al. 2010). This is especially true for males of TL <45 mm; these males ejaculate substantially smaller numbers of sperm during the second relative to the first mating, and ejaculate almost no sperm during the third mating (Sato et al. 2010). As in other anomuran crabs (Sato & Goshima 2006, Sato et al. 2006), sperm recovery rate is
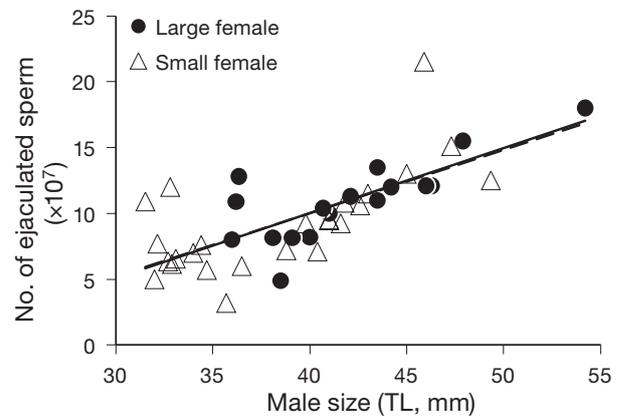
very slow in coconut crabs, and males cannot replenish any of their sperm reserves even after a 30 d recovery period (Sato et al. 2010). Results of the present study indicate that at least 50% of males pass substantially fewer sperm during and after their second mating. The number of sperm available to females may therefore be insufficient for complete fertilization in a harvested population.

More than half of the coconut crab females in the harvested population retained very low numbers of sperm ($<4 \times 10^7$), which would limit their fertilization rates (Sato et al. 2010), and the number of sperm retained by those females was almost equal to that retained by females that mated with smaller (<45 mm TL) males who had mated more than once (Sato et al. 2010). Moreover, 3 of 7 ovigerous females examined (42.8%) had incompletely fertilized clutches (fertilization rates: 73.3 to 84.0%) in this harvested population (author's unpubl. data). These results imply that reproductive success of females in the population subjected to large male-selective harvesting might decrease via sperm limitation. Although there are no data on fertilization rates or the number of sperm retained by females in unharvested coconut crab populations, this hypothesis appears to be supported by the observations of other anomuran crab species. Males of the spiny king crab *Paralithodes brevipes* show size-dependent reproductive potentials similar to those of coconut crab males (Sato et al. 2005, 2006). In Japan, spiny king crab and its populations are subjected to large male-only fishing, resulting in a skewed sex ratio toward females and a decrease in mean male size (Sato et al. 2005). In one such population, 42.2% of adult males depleted their sperm reserves by the end of the reproductive season,

and female reproductive success appeared to decrease via sperm limitation (Sato et al. 2007). The combination of (1) the decreased mean male size due to large male-selective harvesting and (2) the decrease in the number of ejaculated sperm due to successive male matings would be the primary cause of the temporal decrease in number of sperm retained by females throughout the reproductive season and of the large variation in the number of retained sperm among females in the harvested population reported in Sato et al. (2010).

Other factors can also cause the temporal decrease and the large variation in the number of retained sperm among females. Female multiple mating is common, as seen in other decapod crustaceans (Toonen 2004, Gosselin et al. 2005, Sainte-Marie 2007, Bailie et al. 2011), although little is known regarding female multiple mating in coconut crabs. Female multiple mating may be closely related to the variability in the number of sperm retained by females, as seen in snow crabs (Sainte-Marie et al. 2008). Further study focusing on the temporal patterns and frequency of occurrence of the multiple mating of coconut crab females is needed to determine the causes of (1) the temporal decrease and (2) the large variation in the number of retained sperm among females throughout the reproductive season and to verify the occurrence of sperm limitation in a harvested population.

In several animal species, including anomuran crabs, ejaculate size is scaled to female body size (Wedell et al. 2002, Sato et al. 2006, Sato & Goshima 2007b). Such a sperm allocation pattern in response to female body size could also be a factor causing the temporal decrease in the number of sperm retained by females, because smaller female coconut crabs commence mating and spawning later in the reproductive season. However, the number of sperm ejaculated by male coconut crabs was not adjusted in response to female body size. Therefore, the temporal decrease in the number of sperm retained by females did not result from the sperm allocation pattern by males. This hypothesis is supported by females of similar body size showing a large variation in the number of retained sperm (Sato et al. 2010).

Despite the positive relationship between female body size and the number of eggs extruded in coconut crabs, the number of sperm passed from males was not adjusted relative to female body size. The small (mean TL: 30.5 mm) and large females (mean TL: 35.8 mm) used in the present study spawned about 130 000 and about 200 000 eggs, respectively (Sato & Yoseda 2008). While the reason for this is unclear, it may be related to the reproductive habits of these crabs, namely that the spermatophore mass is attached on the ventral surface of the female and is retained externally until egg extru-

sion and external fertilization occurs (Fletcher 1993). Egg extrusion does not occur immediately after mating, though the time between mating and egg extrusion has been estimated to be <1 wk (Sato & Yoseda 2009). A portion of the spermatophore mass can be detached from the female ventral surface by physical contact before fertilization (Helfman 1977, author's pers. obs.), which may be a cause of incomplete fertilization. Therefore, males may pass larger numbers of sperm even to smaller females to assure a high fertilization rate. Alternatively, the lack of a relationship between number of sperm passed from males and female body size may be due to female multiple mating. Generally, the risk of sperm competition arising from female multiple mating induces an increase in ejaculate size per mating to achieve higher paternity relative to those of rival males (e.g. Jivoff 1997, Sato & Goshima 2007a). If most coconut crab females usually perform multiple mating, males may provide larger numbers of sperm regardless of female body size to assure a high fertilization rate.

The combination of (1) the decrease in the number of ejaculated sperm with decreasing male body size and (2) the decreased size of males participating in reproduction during the reproductive season may also be a factor causing the temporal decrease and large variation in the number of sperm retained by females. Why did the size of males participating in reproduction decrease with the progression of the mating season? Because larger coconut crab individuals are at an advantage over smaller individuals in physical contests for resources (Helfman 1979), larger males would be stronger competitors for mates. Moreover, coconut crab females would exhibit a mate preference for larger males (Sato & Yoseda 2010). Therefore, larger males would have more mating opportunities and mate successively early in the mating season, resulting in earlier depletion of their sperm reserves. Thus, subordinate (smaller) males may commence participating in reproduction, instead of larger males, as the mating season progresses, resulting in a temporal decrease in the size of males participating in reproduction. The temporal decrease in male size would more easily occur in populations subjected to large male-selective harvesting because larger males, which are able to mate with more females than smaller ones, are rarer in the harvested populations. The temporal decrease in the size of females participating in reproduction may also cause the decrease in male size throughout the mating season due to female mate preference (females mate only with males that are larger than or approximately equal to themselves; Sato & Yoseda 2010). Why larger females mate earlier than smaller females is not clear, although the temporal variation in female reproduction is seen in other anomuran crabs as

well (Yoshino et al. 2002, Sato & Goshima 2007b, Sato et al. 2007).

Coconut crab females in non-harvested populations may also be naturally subjected to sperm limitation, for instance due to spermatophore abrasion (Helfman 1977, author's pers. obs.). If this is so, it may be very difficult to determine the respective contributions of natural and anthropogenic sources to sperm limitation. But, considering the size-dependent reproductive potentials and limited sperm reserves of males, it is certain that sperm limitation frequently occurs in coconut crab, especially when larger males are selectively harvested from populations, and even when the sex ratio is not sharply biased towards females. These results signal a need for caution and reconsideration of the present harvesting practices. Investigations of the reproductive success of these crabs and comparisons of female reproductive success between areas or years with contrasting harvesting pressure may verify the hypothesis that selective harvesting of larger males has a negative impact on the reproductive rate of this species.

The protection of larger males with high reproductive potential would reduce the probability of sperm limitation and contribute to the sustainability of recruitment by increasing the probability that larger females encounter suitable mates of equal or larger body size. Larger coconut crab females produce offspring with superior qualities for recruitment (larger body size and longer survival periods under non-fed conditions) than do smaller ones (Sato & Suzuki 2010). In general, the protection of females from harvesting, thus allowing them to spawn, is effective in sustaining the reproductive rate of biological resources and allows them to recover from a depleted state. Furthermore, the protection of larger males with high reproductive potential would also reduce the probability of sperm limitation and help maintain sustainable recruitment by increasing the probability that larger females encounter suitable mates of equal or larger body size. Larger coconut crab females produce offspring with superior qualities for recruitment (larger body size and longer survival periods under non-fed conditions) than do smaller ones (Sato & Suzuki 2010). Therefore, male-only harvesting with slot size limits (i.e. allowing only a certain size range to be harvested) would be an effective management system for coconut crab resources.

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