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Different sperm allocation strategies in two populations of the semiterrestrial crab *Neohelice granulata* (Brachyura, Grapsoidea, Varunidae)

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Abstract

Sperm reserves are costly to produce and so the development of different sperm allocation strategies may be advantageous. Knowledge of sperm allocation strategies is important from an evolutionary perspective because they contribute to characterize mating systems. However, population differences in sperm allocation strategies of the same species have rarely been assessed. Here, we studied the male sperm allocation strategies in two different populations of *Neohelice granulata* in Mar Chiquita Lagoon (MCL) and San Antonio Oeste (SAO), Argentina. We found that the quantity of ejaculate transferred by *N. granulata* males depended on different factors such as male size and female receptivity duration, while it was independent of female size, at both study areas. Regarding the operational sex ratio factor, the amount of ejaculate transferred depended on it in MCL but not in SAO. Thus, *N. granulata* males may have the capacity to regulate the quantity of ejaculate transferred based on individual factors such as their own size and female receptivity duration, and population factors such as operational sex ratio, which varies in the two populations studied.

Introduction

The diverse strategies of sperm allocation displayed by males have been frequently described in many marine taxa that spawn gametes into water (Levitan & Petersen 1995; Marconato & Shapiro 1996), in terrestrial insects (Pitnick & Markow 1994; Gage & Barnard 1996; Wedell & Cook 1999) and in birds (Pizzari *et al.* 2003). However, in crustaceans, there is limited evidence of the occurrence of sperm allocation strategies (Rondeau & Sainte-Marie 2001; Sato & Goshima 2007). The few studies that have been conducted have mainly focused on commercially fished decapod populations because only large males are selectively harvested (*e.g.* Smith & Jamieson 1991; Sainte-Marie & Hazel 1992; Paul & Paul 1997; Rondeau & Sainte-Marie 2001; Kendall *et al.* 2002; Ogburn *et al.* 2014).

The development of diverse male sperm allocation strategies has been favored because sperm, spermatophores and seminal fluid may all be limited in supply because of their high costs of production (Dewsbury 1982; Pitnick & Markow 1994). Depending on the mating system, male sperm allocation strategies can generate sexual conflicts that affect female fitness (Nakatsuru & Kramer 1982; Birkhead & Fletcher 1992), such as sperm limitation, which occurs when the number of sperm is insufficient to fertilize all eggs produced by a female (Pennington 1985; Pitnick 1993). Males may increase their reproductive success by regulating the quantity of sperm transferred to females based on the potential risks of sperm competition (when multiple mating occurs and sperm from numerous individuals occupy the female seminal receptacles, Parker 1970), which may vary as a function of sex ratio, potential for polyandry and/or female mating history (Parker *et al.* 1997).

It is assumed that large-sized males have higher reproductive success than smaller individuals because they may

have the capacity to transfer larger quantities of ejaculate to a single female or to many females, thus increasing the probability of fertilization of the female oocytes (Pitnick & Markow 1994; Gosselin et al. 2003). When the size of the ejaculate is correlated with male size, copulating with a small male may not be sufficient to fertilize all of a female's oocytes (McLain et al. 1990; Bissoondath & Wiklund 1996). However, in polygynous species, dominant males that mate at high frequencies sometimes become sperm depleted because of limited production and time to replenish reserves (Warner et al. 1995; Kendall et al. 2001, 2002; Preston 2001). The amount of ejaculate that males pass to females may decrease with increasing male mating frequency (Ryan 1967; Woodhead 1985; Svard & Wiklund 1986; Birkhead & Fletcher 1992; Pitnick & Markow 1994; Cook & Gage 1995).

In species in which females vary in reproductive quality, theoretical models predict that males should increase sperm investment with increasing female quality (Galvani & Johnstone 1998; Reinhold et al. 2002). There is evidence indicating that sperm competition and female quality modulate the optimal male sperm allocation strategies (Sato & Goshima 2007). For example, males have the capacity to regulate the size of their ejaculate based on female size (MacDiarmid & Butler 1999). It is predicted that when the risks of sperm competition and the fecundity of females are high, males may invest in transferring larger ejaculates (Wedell et al. 2002).

Males are also thought to modify the quantity of ejaculate transferred based on the operational sex ratio [OSR, defined as the relationship between the number of fertilizable (i.e. receptive) females and the number of sexually mature males in a determined space and time, Emlen & Oring 1977] and the risks of sperm competition (Gage & Barnard 1996; Wedell & Cook 1999). Generally, the OSR and the presence of potential rival males provide information about the probability of sperm competition at a particular mating event in polyandrous mating systems (Wedell et al. 2002). The capacity of males to regulate the quantity of ejaculate passed to females based on some social circumstances has been demonstrated in diverse animals such as birds (Birkhead & Fletcher 1992), crabs (Jivoff 1997a; Rondeau & Sainte-Marie 2001) and fishes (Shapiro et al. 1994; Marconato & Shapiro 1996; Warner 1997).

Knowledge of sperm allocation strategies is important from an evolutionary perspective because they presumably contribute to characterize mating systems (Wedell et al. 2002). Further, population differences in sperm allocation strategies of the same species have rarely been assessed (e.g. Hines et al. 2003; Gosselin et al. 2005). As males can exhibit variance in ejaculate allocation, there is the additional possibility that male responses to variance in sperm competition and female quality differ among populations, in the same way that variance in mating pattern varies in space and time within a species (Wedell et al. 2002).

The burrowing and semiterrestrial crab Neohelice granulata (Dana 1851) is considered a keystone species of South American salt marshes, mud flats and estuaries, ranging from Northern Patagonia, Argentina (42°25′ S, 64°36′ W), through Uruguay, to Rio de Janeiro, Brazil (22°57 ′S, 42°50 ′W) (Spivak 2010). The mating system of N. granulata is based on the defense of the resource 'burrows' constructed by the large males and used as a protected copulatory chamber (Sal Moyano et al. 2012a). Copulatory chambers are constructed only by large males, while small males construct burrows without chambers and their copulations occur more frequently on the surface by interception of receptive females (Sal Moyano et al. 2012a). Neohelice granulata females become receptive two to four times during the reproductive season, although for a limited period each time. Thus, the OSR is frequently male-biased and females may mate with several males (Sal Moyano et al. 2012b). The duration of the receptive period varies between two types of receptive females: (i) those with short receptivity [2–6 days in Mar Chiquita Lagoon (MCL) and 1–4 days in San Antonio Oeste (SAO)] and (ii) those with extended receptivity (7–16 days in MCL and 5–12 days in SAO) (Sal Moyano et al. 2012b). The seminal receptacles are expandable storage chambers and receptivity duration is dependent on seminal receptacle weight and the capacity to lay eggs: females with short receptivity have heavier seminal receptacles (0.059 ± 0.01 g in both MCL and SAO) and lay eggs, whereas extended receptivity females have lighter seminal receptacles (0.037 ± 0.01 g in MCL and 0.047 ± 0.01 g in SAO) and do not lay eggs (Sal Moyano et al. 2012b). The copulation duration is longer in MCL than in SAO and depends on factors such as the OSR (Sal Moyano et al. 2014a). While copulating, males transfer the ejaculate (spermatophores and seminal fluid) through the gonopods and no sperm loss occurs because the gonopods tip is deeply introduced inside the female vulvae. The existence of post-copulatory guarding behavior depends on factors such as female receptivity duration and OSR (Sal Moyano et al. 2014b). For example, if females have extended receptivity, males do not guard them until they become unreceptive; thus, females are able to mate with several males in close succession prior to egg-laying (see Sal Moyano et al. 2014b). No sperm plugs are made in this species.

In this context, the aim of the present study was to study the sperm allocation strategies in N. granulata considering different factors such as: male size, female size, female receptivity duration and OSR in crabs from two different populations, MCL and SAO. We hypothesize

that *N. granulata* individuals display different sperm allocation strategies in the two populations according to the different biological and population factors.

Material and Methods

Study site

We studied two *Neohelice granulata* populations located 800 km apart along the Argentinean coast and inhabiting contrasting habitats: one at Mar Chiquita Lagoon (MCL), which is a coastal lagoon located in Buenos Aires Province, Argentina (37°45′S, 57°19′W), and the other one at San Antonio Oeste (SAO), a bay located in the northwest of San Matías Gulf, Río Negro Province, Argentina (40°46′ S, 64°50′ W). Mar Chiquita is a body of brackish water (46 km^2) with low tidal amplitude (*c.* 1 m) permanently connected to the sea (Reta *et al.* 2001). By contrast, the other area, SAO, is a marsh connected to the sea but lacking freshwater input, except for the scarce rainfall (200 mm·$year^{-1}$), with a semidiurnal macrotidal (*c.* 9 m) regime.

Collection and maintenance of crabs

Neohelice granulata crabs were collected by hand from the field 1 month before the beginning of the reproductive season (August and October) of 2012/2013 and 2013/2014 in MCL and SAO, respectively, and transported to the laboratory. In the laboratory individuals were maintained in seawater aerated aquaria (0.3 × 0.35 × 0.25 m, containing a depth of 3 cm of seawater), and sexes were kept separate (four individuals per aquarium). Individuals were fed three times a week with pet-food pellets and water was changed weekly. Crabs were maintained under natural daylight conditions and temperatures that ranged between 22 and 28 °C (mean 24 °C). All individuals were measured (carapace width, CW) with a caliper accurate to 0.1 mm. Only morphologically and physiologically mature crabs of both sexes were used in the experiments (CW > 19 mm; López Greco & Rodríguez 1998). Based on the size of the mating partners observed copulating in the field, the size difference between males and females never exceeded 4 mm. Three categories of individuals of both sexes were used for the experiments: small (SM), medium (MM) and large (LM) males; and small (SF), medium (MF) and large (LF) females. SM were 22 < CW < 25 mm, MM 25 < CW < 28 mm and LM 28 < CW < 32 mm in MCL and SAO. SF were 20 < CW < 24 mm, MF 24 < CW < 27 mm and LF 27 < CW < 30 mm in MCL and SAO.

Immediately after capture, males of the three categories (n = 30, 10 males per each category at each study area) were killed by placing them in a freezer at –15 °C for approximately 30 min and their vas deferens (VD) dissected out. The right and left VD were weighed, and these weights were considered the 'standard' ones because individuals were captured before the beginning of the reproductive season; thus, their VD were full of spermatophores and not depleted (T. Luppi unpublished data). Additionally, those males were maintained in laboratory for a minimum of 1 month before beginning the experiments in order to reassure the absence of sperm depletion, because *N. granulata* males need about 10 days to recharge sperm reserves (T. Luppi unpublished data).

Experimental design

Experiments were carried out in plastic aquariums (1 × 0.4 × 0.15 m). Four experiments with two trials each were conducted to test the effect of the factors male size, female size, female receptivity duration and OSR as follows: (i) to test the male size factor we placed a LM + a receptive MF in an aquarium and a SM + a receptive MF in another aquarium; (ii) to test the female size factor we placed a receptive LF + a MM in an aquarium and a receptive SF + a MM in another aquarium; (iii) to test the female receptivity duration factor we placed a short receptivity MF + a MM in an aquarium and an extended receptivity MF + a MM in another aquarium; (iv) to test the OSR factor we placed a MM + a receptive MF in one aquarium and three MM + a receptive MF in another aquarium. Fifteen replicates were performed for experiments (i), (ii) and (iii), while 12 replicates were carried out for experiment (iv). A fifth experiment (v) was carried out to jointly test the effect of the factors male size and female receptivity duration and four trials were conducted: a LM + a short receptivity MF, a LM + an extended receptivity MF, a SM + a short receptivity MF, and a SM + an extended receptivity MF. Ten replicates were performed for experiment (v).

Females were checked daily and experiments began the same day that a receptive female was recognized. Receptive females were recognized by the presence of mobile vulvae opercula as it could be pushed inwards like a trapdoor when checked under a binocular microscope: the pleon was lifted and the two opercula were gently probed daily using fine forceps (Sal Moyano *et al.* 2012b). The different categories of individuals (depending on the treatment) were placed in the aquarium and observed for 2 h to detect copulation. Only experiments in which copulation occurred (when both the male and female pleon were observed open and the male gonopods were inserted into the female vulvae) were considered. Immediately after copulation ended and the pair separated, males were

killed, dissected and their right and left VD were weighed (these were the post-evacuated VD). These males were named 'experimental'. In experiments (iii) and (v), immediately after the pair separated, females were isolated in an aquarium until their opercula became immobile, and thus unreceptive, and were then characterized as having short or extended receptivity based on the number of days for which they were receptive (short: <6 days, extended: >6 days, for details see Sal Moyano *et al.* 2012b).

Statistical analyses

There was no difference in the weights of right and left VD in either standard (MCL: $t = 0.96$, $df = 19$, $P = 0.34$; SAO: $t = 0.54$, $df = 19$, $P = 0.59$; $n = 20$ for each category in both study areas) or experimental (MCL: $t = 1.12$, $df = 19$, $P = 0.28$; SAO: $t = 0.77$, $df = 19$, $P = 0.45$; $n = 20$ for each category in both study areas) males. We therefore calculated the mean VD weight for each male and used it for all analyses.

A two-way analysis of co-variance (ANCOVA) was performed to test for differences in the weight of the VD of standard males (dependent variable) in relation to the co-variate: carapace width, and the independent factors: male size and study area.

To test for the quantity of the ejaculate transferred (ET) we calculated the mean weight value for each category of standard male and these were considered as 'VD weight of standard males'. The difference between the mean VD weight value of standard males and the mean VD weight of each experimental male (post-evacuated VD) in the different experiments for each category was considered as the ET. We did not use the seminal receptacle weight to calculate the ET in order to avoid overestimating its weight because females can copulate with many males while being receptive, store sperm from previous copulas – even during the non-reproductive season after molting – and do not use all sperm stored after laying eggs.

For experiments (i–v), because the diagnostic residual plots indicated heteroscedasticity owing to the inherent heterogeneity of variance, a generalized least squares analysis (GLS) was conducted to analyse differences in the ET (dependent variable) in relation to the independent factors: study area and (i) male size, (ii) female size, (iii) female receptivity duration, (iv) OSR, (v) join male size and female receptivity duration. GLS allows the introduction of a range of variance–co-variate structures that model the variance structure. These models were compared with the equivalent model without the GLS extension through the examination of plots of residuals *versus* fitted values. The numerical output of the optimal model

was obtained using restricted maximum likelihood estimation (West *et al.* 2007). Analyses were performed with the 'nlme' package (linear and non-linear mixed effects models; Pinheiro *et al.* 2006) of the R software (R Development Core Team 2005).

A two-way ANCOVA was performed to test for differences in the weight of the post-evacuated VD (dependent variable) in relation to the co-variate: carapace width, and the independent factors: male size and study area. We considered a randomly selected subsample of males from the different experiments ($n = 10$ for each study area).

Results

The two-way ANCOVA showed differences in the VD weight of standard males between the factors male size and study area: LM had the heaviest VD, MM had an intermediate VD weight and SM had the lightest ones, in both study areas. However, LM and MM from MCL had heavier VD weights compared with those from SAO, while SM had similar VD weights in both study areas (Table 1, Fig. 1).

In experiment (i) the two-way GLS showed differences in the ET between the factors male size and study area: LM transferred larger ejaculates than SM in both study areas. However, LM from MCL transferred larger ejaculates than the ones from SAO, while SM transferred similar ejaculates in both study areas (Table 2A, Fig. 2A).

In experiment (ii) the two-way GLS did not show differences in the ET with respect to the factor female size: MM transferred similar ejaculates to LF and SF in both study areas. However, MM from MCL transferred larger ejaculates than MM from SAO (Table 2B, Fig. 2B).

In experiment (iii) the two-way GLS showed differences in the ET between the factors female receptivity duration and study area: MM transferred larger ejaculates to short receptivity MF in both study areas, but MM from MCL transferred larger ejaculates to short and

Table 1. Two-way analysis of co-variance showing the effects and interactions between the dependent variable, vas deferens weight, with respect to the co-variate, carapace width, and the independent factors: study area and male size class.

effect	SS	df	MS	F	P
carapace width	0.000003	1	0.000003	0.102	ns
(1) study area	0.000378	1	0.000378	13.262	***
(2) male size class	0.000488	2	0.000244	8.569	***
(1) × (2)	0.000434	2	0.000217	7.621	**
error	0.001510	53	0.000028		

Significant P values: ***P < 0.001; **P < 0.01.

MS = mean square; ns = non-significant; SS, sums of squares.
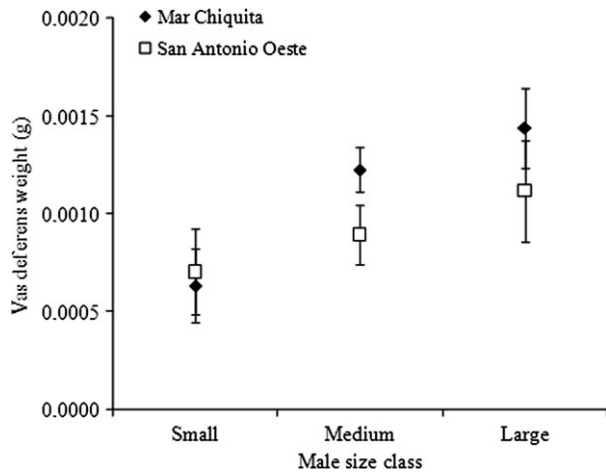
Fig. 1. Differences in the vas deferens weight (g) between the different categories of standard males (large, medium and small) in both study areas, Mar Chiquita and San Antonio. Error bars indicate the standard deviation.

Table 2. Two-way generalized least squares analysis showing the effects and interactions between the dependent variable, ejaculate transferred weight, with respect to the independent factors: study area and (A): male size, (B): female size, (C): female receptivity duration, (D): operational sex ratio (OSR).

	effect	df	SE	t	P
A	(1) study area	2	0.000580333	0.321	ns
	(2) male size	2	0.001873098	13.760	***
	(1) × (2)	1	0.002009150	5.758	***
B	(1) study area	2	0.000677263	21.277	***
	(2) female size	2	0.001028695	0.823	ns
	(1) × (2)	1	0.001360198	0.553	ns
C	(1) study area	2	0.000538056	22.758	***
	(2) female receptivity duration	2	0.001740879	5.422	***
	(1) × (2)	1	0.001908593	3.005	**
D	(1) study area	2	0.000919221	10.915	***
	(2) OSR	2	0.000206196	0.463	ns
	(1) × (2)	1	0.000221115	3.308	**

Significant P values: ***P < 0.001, **P < 0.01.
ns = non-significant.

extended receptivity MF than the ones from SAO (Table 2C, Fig. 2C).

In experiment (iv) the two-way GLS showed differences in the ET between the factors OSR and study area: MM from MCL transferred larger ejaculates to MF when there was a 1:1 OSR compared with a 1:3 OSR, while MM from SAO transferred similar ejaculates independently of the OSR, and MM from MCL transferred larger ejaculates than MM from SAO at both OSRs (Table 2D, Fig. 2D).

In experiment (v) the three-way GLS showed differences between the ET and the factors male size, female receptivity duration and study area: LM from both study areas transferred larger ejaculates to short receptivity MF than to extended receptivity ones, while SM from both study areas transferred similar ejaculates independently of the female receptivity duration (Table 3, Fig. 3).

The two-way ANCOVA showed no differences in the post-evacuated VD weight among the different categories of experimental males in MCL and SAO. However, differences were found between study areas: post-evacuated VD from SAO were heavier than the ones from MCL (Table 4, Fig. 4).

Discussion

As sperm reserves are costly to produce, males of many species have evolved the ability to use them judiciously by developing different sperm allocation strategies (Dewsbury 1982; Pitnick & Markow 1994; Wedell et al. 2002). Here, we found that the quantity of ejaculate transferred by N. granulata males depended on different factors such as male size and female receptivity duration, while it was independent of female size, at both study areas. Regarding the operational sex ratio factor, the amount of ejaculate transferred depended on it in MCL but not in SAO. Thus, N. granulata males may have the capacity to regulate the quantity of ejaculate transferred based on individual factors such as male size and the fullness of the female seminal receptacle (and thus, receptivity duration) and population factors such as OSR, which varies in the different populations studied.

The differences found in the VD weight of the different male categories for both study areas were similar to those described for other crab species: the ejaculate is correlated with male body size (e.g. Kendall et al. 2001; Sato et al. 2005). The results indicating that large and medium standard males from MCL have heavier VD than the ones from SAO, and also, that post-evacuated VD of experimental males from SAO are heavier than the ones from MCL, correspond with previous results obtained in studies conducted on N. granulata because females of the same size from SAO have been found to have lower fecundity compared with those from MCL (35–50% lower, Bas et al. 2007) and males from SAO to have shorter copulation durations than ones from MCL (Sal Moyano et al. 2014a). Thus, it appears that because females from SAO produce fewer oocytes, males reduce copulation duration and, consequently, transfer less sperm. The result indicating that small males had similar VD weights at both study areas indicates that they grow at a similar rate and achieve physiological maturity at a similar VD weight, although after this, males grow bigger
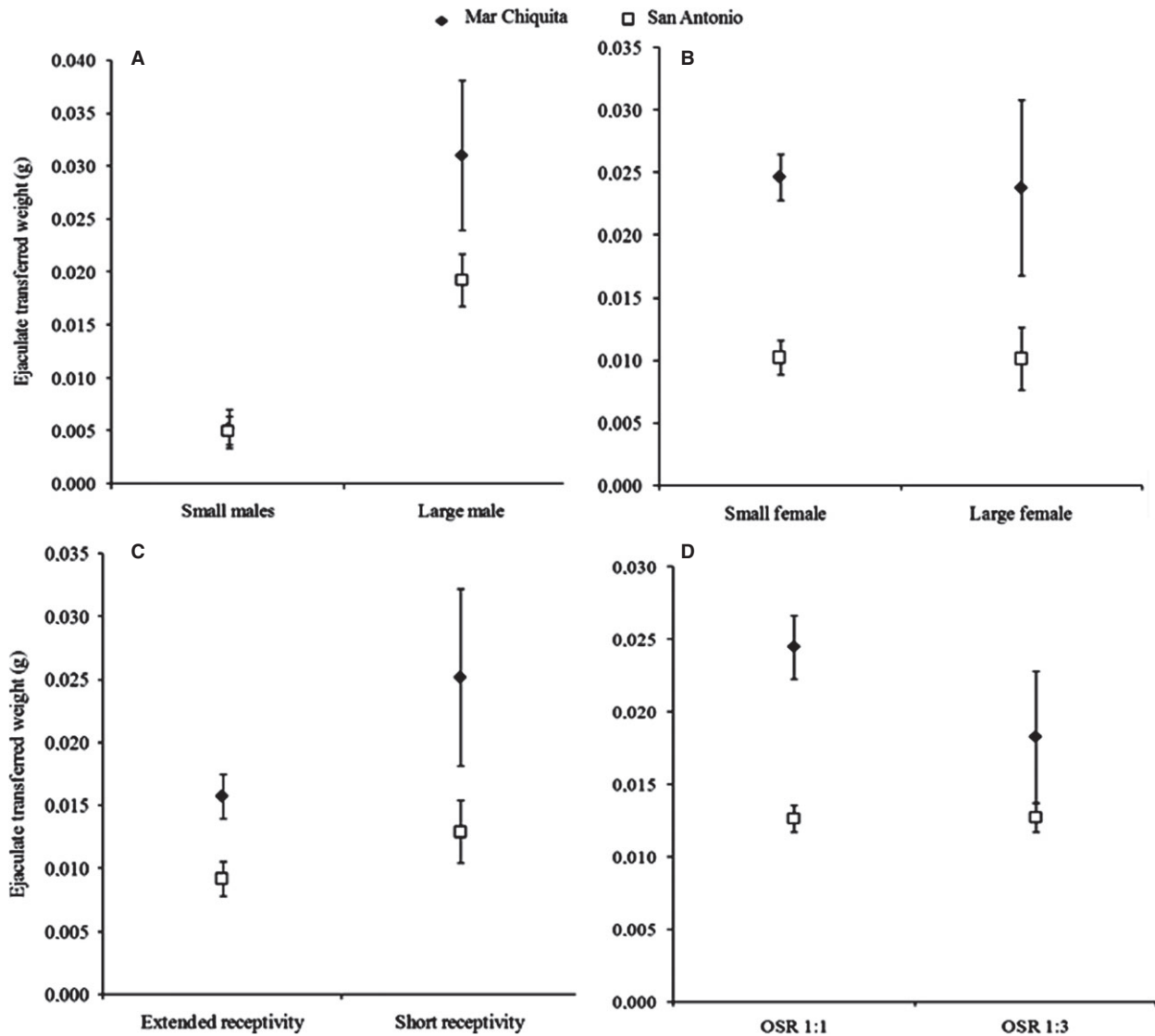
Fig. 2. Differences in the weight of the ejaculate transferred (g) in both study areas, Mar Chiquita and San Antonio, by (A): large and small males to medium females, (B): medium males to large and small females, (C): medium males to short receptivity medium females and extended receptivity medium females, (D): medium males when the operational sex ratio (OSR) was 1:1 and 1:3. Error bars indicate the standard deviation.

and become endowed with larger VD in MCL. Males from MCL reach larger sizes than the ones from SAO (and thus larger VD) probably as a result of differences in food supply because the amount of organic matter in the muddy substrata is higher in MCL (Bas *et al.* 2005). Diseases and/or food supply may affect male condition and compromise sperm supply (Zuk 1988; Proctor 1992; Olsson & Shine 1997).

The quantity of the ejaculate transferred depended on the male size: large males transferred larger ejaculates than small ones. This corresponds with previous studies conducted in different species of crustaceans (*e.g.* Jivoff 1997a; MacDiarmid & Butler 1999; Rondeau & Sainte-Marie 2001; Kendall *et al.* 2002; Gosselin *et al.* 2003;

Carver *et al.* 2005; Sato *et al.* 2006, 2010; Lemaitre *et al.* 2009). It must be pointed out that, although large and small *N. granulata* males have similar copulation durations in both study areas (Sal Moyano *et al.* 2014a), large males transferred larger ejaculates. By contrast, in other crab species large males may copulate for longer durations because they can resist disruptions and displacement during copulation (Abele *et al.* 1986). For *N. granulata*, it may be proposed that small males transfer less ejaculate once copulating because their smaller chela and gonopods may be less effective for manipulation and maintenance of the female in the copulation position than the large chela and gonopods of large males. Thus, large body size may give a male an advan-

Table 3. Three-way generalized least squares analysis showing the effects and interactions between the dependent variable, ejaculate transferred weight, with respect to the independent factors: study area, male size and female receptivity duration.

effect	df	SD	t	P
(1) study area	1	0.0004	9.6	***
(2) male size	1	0.0006	48.6	***
(3) female receptivity duration	1	0.0008	0.51	ns
(1) × (2)	2	0.0009	13.54	***
(1) × (3)	2	0.0011	2.21	*
(2) × (3)	2	0.0011	8.78	***
(1) × (2) × (3)	3	0.0017	3.07	**

Significant P values: ***P < 0.001, **P < 0.01, *P < 0.05.
ns = non-significant.

Table 4. Two-way analysis of co-variance showing the effects and interactions between the dependent variable, post-evacuated vas deferens weight, with respect to the co-variate, carapace width, and the independent factors: study area and male size class.

| effect	SS	df	MS	F	P
carapace width	0.000012	1	0.000012	2.941	ns
(1) study area	0.000040	1	0.000040	10.218	**
(2) male size class	0.000007	2	0.000004	0.917	ns
(1) × (2)	0.000012	2	0.000006	1.476	ns
error	0.000207	53	0.000004		

Significant P values: ***P < 0.001, **P < 0.01.
MS = mean square; ns = non-significant; SS, sums of squares.

tage in both forms of competition (male–male and sperm competition). In *Callinectes sapidus* ejaculate volume increased with the copulation duration although no size-related measures of copulation duration were assessed (Jivoff 1997a). Regarding the differences between study areas, we found that ejaculate transferred by MCL males was always larger than the ones from SAO males, independently of the studied factor. This could be related to the fact that males from MCL had initially heavier VD (possibly associated to food supply, see paragraph above) and/or because SAO males copulate for less time than MCL males (Sal Moyano *et al.* 2014a), and consequently transfer less sperm.

The amount of ejaculate transferred by *Neohelice granulata* males was independent of female size. By contrast, in some species of lobsters and crabs it has been demon-strated that males allocate more ejaculate to large females compared with small ones (Jivoff 1997a; Kendall & Wolcott 1999; Rondeau & Sainte-Marie 2001; Kendall *et al.* 2002; Gosselin *et al.* 2003; Sato *et al.* 2006), probably because female size is a good indicator of relative fecundity (MacDiarmid & Butler 1999; Wedell *et al.* 2002) or because large females are more likely to be promiscuous than small ones (Sainte-Marie *et al.* 2002). In *N. granulata*, our result may indicate that female seminal receptacle capacity is not related to their size. It is proposed that the seminal receptacle is expandable and sufficiently flexible to allow enlargement (Sal Moyano *et al.* 2012b). Indeed, the seminal receptacle of multiply mated females in *Chionoecetes opilio* can become huge (Sainte-Marie & Sainte-Marie 1998; Sainte-Marie *et al.* 2000). However, it should be pointed out that we never worked with a size difference bigger than 4 mm of CW between females and
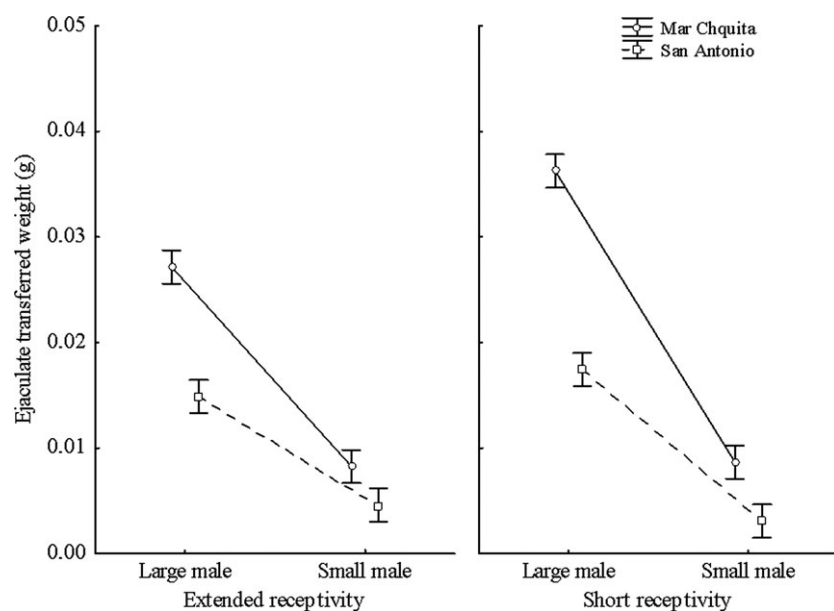
Fig. 3. Experiment (v). Differences in the weight of the ejaculate transferred (g) by large and small males to short and extended receptivity medium females in both study areas, Mar Chiquita and San Antonio. Error bars indicate the standard deviation.
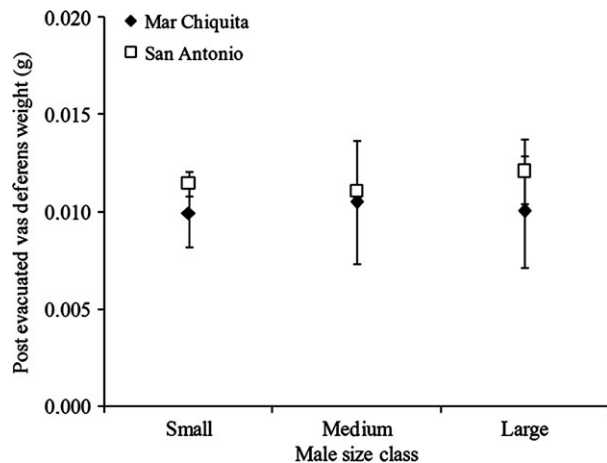
Fig. 4. Differences in the post-evacuated vas deferens weight (g) between the different categories of males class (large, medium and small) in both study areas, Mar Chiquita and San Antonio. Error bars indicate the standard deviation.

males because mating pairs observed in the field never exceeded this size difference.

Neohelice granulata males transferred larger ejaculates to short receptivity females than to extended receptivity ones. Short receptivity females may have more sperm from previous copulations inside their seminal receptacles, but their receptive period is shorter (Sal Moyano *et al.* 2012b). Besides, if males find this female type, they may prefer to copulate with them and guard them for less time (until oocyte fertilization) rather than copulating with extended receptivity females (Sal Moyano *et al.* 2014a,b). Theoretical models developed by Parker *et al.* (1996) suggest that first- and second-mating males should increase the size of their ejaculate to increase their fertilization success. Further, post-copulatory mate guarding during the short period between mating and egg-laying in the lobster *Jasus edwardsii* precludes multiple mating by females and thus eliminates the risk of sperm competition (MacDiarmid 1988). In *N. granulata*, females have a ventral-type seminal receptacle as defined by Diesel (1991) (López Greco *et al.* 1999; Sal Moyano *et al.* 2012b); thus, males may have more chances to fertilize oocytes if ovulation occurs shortly after copulation. It is proposed that males may have the capacity to detect the fullness of the female seminal receptacle. The existence of a mechanical signal used by males to recognize short receptivity females can also be suggested, given that while positioning the female in the copulation position, males could potentially manipulate the female using their gonopod and evaluate the quantity of sperm storage in the seminal receptacle before transferring the sperm (Sal Moyano *et al.* 2014b). For spiny lobsters it has been proposed that females may signal, perhaps via water-borne pheromones, the number

of ripe oocytes contained in the ovaries and that males respond by allocating smaller or larger amounts of ejaculate, maybe through the number of contractions of the muscular wall of the vas deferens (MacDiarmid & Butler 1999). Extended receptivity *N. granulata* females have seminal receptacles with few sperm and are not post-copulatory guarded by males because their receptive period is long; thus, they may be able to copulate with many males to fill their seminal receptacles, favoring sperm competition (Sal Moyano *et al.* 2014b). Consequently, *N. granulata* males may pass smaller amounts of ejaculate to extended receptivity females, which accords with models proposing that males reduce expenditure if the intensity of sperm competition is high, because the probability of fertilizing oocytes diminishes with the number of competing males that attempt to fertilize the same set of oocytes (Parker *et al.* 1996).

Males from MCL transferred larger ejaculates when the OSR was 1:1 compared with a 1:3 OSR. This result is consistent with those of a previous study in which males copulated for shorter times when the OSR was male-biased because of male–male interactions (Sal Moyano *et al.* 2014a). In the same way, copulating for less time may lead to the transfer of less ejaculate when the OSR is 1:3. By contrast, in SAO, males transferred similar amounts of ejaculate independently of the OSR. In this sense, although copulation duration is also shorter when the OSR is 1:3 compared with a 1:1 OSR (Sal Moyano *et al.* 2014b), post-copulatory guarding is not employed by SAO males because copulation occurs in burrows with open copulation chambers, where competing males can easily access and win females in male–male contests (Sal Moyano *et al.* 2012a). Thus, independently of the OSR, because male–male contests are frequent in SAO (Sal Moyano *et al.* 2012a), males should prefer to transfer large ejaculates once they gain a female, even when copulating for less time. This coincides with theory predictions because when different males copulate with a female, alternative mating strategies such as increased sperm allocation, sperm displacement or differences in sperm quality may be displayed (Parker 1990a,b; Simmons 2001). The ability of males to regulate the size of their ejaculate according to social circumstances has been demonstrated in blue crabs (Jivoff 1997b). By contrast, other studies conducted on crabs have recorded that the presence of potential rival males causes an increase in the number of ejaculated sperm per mating (Jivoff 1997b; Rondeau & Sainte-Marie 2001; Sato & Goshima 2007).

When we analysed male size and female receptivity duration jointly, we found that large males transferred larger ejaculates to short receptivity females compared with extended receptivity ones in both study areas, whereas small males transferred equal quantities of

ejaculate to both female types. As described above, large males are the dominant ones in both study areas, construct burrows with copulation chambers (Sal Moyano *et al.* 2012a) and may prefer to copulate with short receptivity females because they guard them for less time and because, if ovulation occurs shortly after copulation, they may secure paternity of the offspring. In both study areas small males copulate more frequently on the surface by intercepting receptive females because they do not construct burrows with copulation chambers as large males do (Sal Moyano *et al.* 2012a). Thus, because small male copulation opportunities arise from chance encounters of receptive females, once they find one, they copulate and transfer their ejaculate independently of the female's short or extended receptivity. In this way, small *Neohelice granulata* males may display an alternative mating strategy, defined as 'pure searching' by Christy (1987). This alternative mating strategy and plastic behavior developed by small males have been described in different species of crustaceans (*e.g.* Jormalainen *et al.* 2000; Thiel & Hinojosa 2003; Carver *et al.* 2005; Atema & Steinbach 2007; Sainte-Marie *et al.* 2007).

Theory suggests males should economize on sperm by allocating their supply among successive females depending on expected reproductive return and future mating opportunities (Dewsbury 1982; Parker 1990a,b; Pitnick & Markow 1994; Shapiro *et al.* 1994; Warner *et al.* 1995; Parker *et al.* 1996). However, sperm limitation occurs when males do not have time to recharge sperm reserves between matings (Pitnick 1993; Pitnick & Markow 1994; Warner *et al.* 1995; Jivoff 1997b; MacDiarmid & Butler 1999). Independently of the initial VD weight of small, medium and large *Neohelice granulata* males, the results obtained here show that all males depleted their sperm reserves until achieving a similar VD weight (although differences were found between study areas). Further, post-copulatory *N. granulata* males need about 10 days to recharge the VD (T. Luppi unpublished data). Pre-copulatory mate guarding may serve as a time to replenish sperm reserves, and thus its duration also influences a male's performance in sperm competition (Jivoff 1997a). However in *N. granulata* the pre-copulatory guarding duration is about 24 h (Sal Moyano *et al.* 2014a), which may not allow replenishment of sperm supplies. Thus, sperm limitation may occur in this species if a second female copulates with a recently mated male. In this sense, it has been demonstrated that *N. granulata* females choose unmated males (Sal Moyano *et al.* 2014c), thus avoiding sperm limitation. Sperm limitation is likely to be a strong selection factor for female choice, and females should be choosy in selecting mates to avoid the risk of sperm limitation (Sato & Goshima 2007). Similarly, the ejaculate of *Callinectes sapidus* represents on average 47%

of gonad volume and a severe depletion of sperm reserves occurs after just one mating, and males cannot equally inseminate two females in rapid succession (Jivoff 1997b; Kendall & Wolcott 1999).

Sperm economy is predicted by sperm competition theory when females can be polyandrous, have last-male sperm precedence and the probability that one male fertilizes a female's lifetime production of eggs is small (Pitnick & Markow 1994; Parker *et al.* 1997). Nevertheless, in species with sperm storage organs males do not always show an increase in the amount of ejaculate in response to the risk of sperm competition because the winner can be determined through several mechanisms, such as sperm stratification (Urbani *et al.* 1998; Sainte-Marie *et al.* 2000) or sperm removal (Waage 1979; Rubenstein 1989). Another important factor to consider is the viability of the sperm during storage in the seminal receptacle (Carver *et al.* 2005). In the case of *Neohelice granulata*, females can mate with several males while being receptive but there is no sperm stratification because females mix the sperm from different males inside the seminal receptacle and they can store viable sperm during consecutive reproductive seasons without re-mating, even after molting (López Greco *et al.* 1999; Sal Moyano *et al.* 2012b). Increasing the number or size of ejaculates may represent a swamping strategy in species where sperm mixing occurs and all sperm may potentially gain access to oocytes (Pitnick & Markow 1994). Moreover, sperm removal has not been described for *N. granulata*.

In conclusion, we propose that *N. granulata* females look for and choose large male burrows where they copulate safely and males guard them until egg-laying (Sal Moyano *et al.* 2012b), although they can store sperm from previous matings and sperm from different males is mixed inside the seminal receptacle 24–48 h after mating (Sal Moyano *et al.* 2012b). If copulation occurs shortly after ovulation (<24 h) males have a high probability of fertilizing the oocytes because of their ventral seminal receptacle and last-male precedence mechanisms (thus avoiding sperm competition). Large males invest energy in constructing burrows with copulation chambers, receptive females choose these burrows and males transfer large ejaculates to short receptivity females. By contrast, small males more commonly copulate by interception of females (Sal Moyano *et al.* 2012b) and transfer similar quantities of sperm to each female type, but in smaller quantities than large males (this study). We propose that extended receptivity females are prone to sperm limitation because (i) large males prefer short receptivity females (Sal Moyano *et al.* 2014b) and thus extended ones are more frequently copulated by small males who transfer less sperm (this study); (ii) if they copulate with a medium-sized male, they would receive less sperm

compared with a short receptivity female (this study). In SAO there is no post-copulatory guarding of either short or extended females and large males have more chance of finding short receptivity females; thus, for those with extended receptivity, sperm limitation should be increased.

Acknowledgements

References

Abele L., Campanella P.J., Salmon M. (1986) Natural history and social organization of the semiterrestrial grapsid crab *Pachygrapsus transversus* (Gibbes). *Journal of Experimental Marine Biology and Ecology*, **104**, 153–170.

Atema J., Steinbach M. (2007) Chemical communication and social behavior of the lobster, *Homarus americanus*, and other decapod Crustacea. In: Duffy J., Thiel M. (Eds), *Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms*. Oxford University Press, New York: 115–144.

Bas C., Luppi T., Spivak E. (2005) Population structure of the South American estuarine crab, *Chasmagnathus granulatus* (Brachyura: Varunidae) near the southern limit of its geographical distribution: comparison between northern populations. *Hydrobiologia*, **537**, 217–228.

Bas C.C., Spivak E.D., Anger K. (2007) Seasonal and interpopulational variability in fecundity, egg size, and elemental composition (CHN) of eggs and larvae in a grapsoid crab, *Chasmagnathus granulatus*. *Helgoland Marine Research*, **61**, 225–237.

Birkhead T.R., Fletcher F. (1992) Sperm to spare? Sperm allocation by male zebra finches. *Animal Behavior*, **43**, 1053–1055.

Bissoondath C.J., Wiklund C. (1996) Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). *Functional Ecology*, **10**, 457–464.

Carver A.M., Wolcott T.G., Wolcott D.L., Hines A.H. (2005) Unnatural selection: effects of a male-focused size-selective fishery on reproductive potential of a blue crab population. *Journal of Experimental Marine Biology and Ecology*, **319**, 29–41.

Christy J.H. (1987) Competitive mating, mate choice and mating associations of brachyuran crabs. *Bulletin of Marine Science*, **41**, 177–191.

Cook P.A., Gage M.J.G. (1995) Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). *Behavioral Ecology and Sociobiology*, **36**, 261–268.

Dana J.D. (1851) Conspectus Crustaceorum quae in Orbis Terrarum circumnavigatione, Carolo Wilkes e Classe Foedorate Duce, lexit et descripsit. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **5**, 267–272.

R Development Core Team (2005) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org, ISBN 3-900051-10-0.

Dewsbury D.A. (1982) Ejaculate cost and male choice. *American Naturalist*, **119**, 601–610.

Diesel R. (1991) Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda, Majidae). In: Bauer R., Martin J.W. (Eds), *Crustacean Sexual Biology*. Columbia University Press, New York: 145–163.

Emlen S., Oring L. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**, 215–223.

Gage A.R., Barnard C.J. (1996) Male crickets increase sperm number in relation to competition and female size. *Behavioral Ecology and Sociobiology*, **38**, 227–237.

Galvani A., Johnstone R. (1998) Sperm allocation in an uncertain world. *Behavioral Ecology and Sociobiology*, **44**, 161–168.

Gosselin T., Sainte-Marie B., Bernatchez L. (2003) Patterns of sexual cohabitation and female ejaculate storage in the American lobster (*Homarus americanus*). *Behavioral Ecology and Sociobiology*, **55**, 151–160.

Gosselin T., Sainte-Marie B., Bernatchez L. (2005) Geographic variation of multiple paternity in the American lobster, *Homarus americanus*. *Molecular Ecology*, **14**, 1517–1525.

Hines A.H., Jivoff P.R., Bushmann P.J., van Montfrans J., Reed S.A., Wolcott D.L., Wolcott T.G. (2003) Evidence for sperm limitation in the blue crab, *Callinectes sapidus*. *Bulletin of Marine Science*, **72**, 287–310.

Jormalainen V., Merilaita S., Hardling R. (2000) Dynamics of intersexual conflict over precoopulatory mate garding in two populations of the isopod *Idotea baltica*. *Animal Behaviour*, **60**, 85–93.

Jivoff P. (1997a) Sexual competition among male blue crab, *Callinectes sapidus*. *Biological Bulletin*, **193**, 368–380.

Jivoff P. (1997b) The relative roles of predation and sperm competition on the duration of the post-copulatory association between the sexes in the blue crab, *Callinectes sapidus*. *Behavioral Ecology Sociobiology*, **40**, 175–185.

Kendall M.S., Wolcott T.G. (1999) The influence of male mating history on male -male competition and female choice in mating associations in the blue crab, *Callinectes sapidus* (Rathbun). *Journal of Experimental Marine Biology and Ecology*, **239**, 23–32.

Kendall M.S., Wolcott D.L., Wolcott T.G., Hines A.H. (2001) Reproduction potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminal fluid. *Canadian Journal of Fisheries and Aquatic Science*, **58**, 1168–1177.

Kendall M.S., Wolcott D.L., Wolcott T.G., Hines A.H. (2002) Influence of male size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus*. *Marine Ecology Progress Series*, **230**, 235–240.

Lemaitre J., Rigaud T., Cornet S., Bollache L. (2009) Sperm depletion, male mating behavior and reproductive 'time-out' in *Gammarus pulex* (Crustacea, Amphipoda). *Animal Behavior*, **77**, 49–54.

Levitan D.R., Petersen C. (1995) Sperm limitation in the sea. *Trends in Ecology and Evolution*, **10**, 228–231.

López Greco L., Rodríguez E. (1998) Size at the onset of sexual maturity in *Chasmagnathus granulatus* Dana, 1851 (Grapsidae, Sesarminae): a critical overall view about the usual criteria for its determination. *Proceedings of the Fourth International Crustacean Congress*, 675–689.

López Greco L.S., López G.C., Rodríguez E.M. (1999) Morphology of spermathecae in the estuarine crab *Chasmagnathus granulata* Dana 1851 (Grapsidae, Sesarminae). *Journal of Zoology (London)*, **249**, 490–493.

MacDiarmid A.B. (1988) Experimental confirmation of external fertilisation in the southern temperate rock lobster *Jasus edwardsii* (Hrtton) (Decapoda, Palinuridae). *Journal of Experimental Marine Biology and Ecology*, **120**, 277–285.

MacDiarmid A.B., Butler M.J. (1999) Sperm economy and limitation in spiny lobsters. *Behavioral Ecology and Sociobiology*, **46**, 14–24.

Marconato A., Shapiro D.Y. (1996) Sperm allocation, sperm production and fertilization rates in the bucktooth parrotfish. *Animal Behavior*, **52**, 971–980.

McLain D.K., Lanier D.L., Marsh N.B. (1990) Effects of female size, mate size and number of copulations on fecundity, fertility and longevity of *Nezara viridula* (Hemiptera: Pentatomidae). *Annals of the Entomological Society of America*, **83**, 1130–1136.

Nakatsuru K., Kramer D.L. (1982) Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science*, **216**, 753–755.

Ogburn M.B., Roberts P.M., Richie K.D., Johnson E.G., Hines A.H. (2014) Temporal and spatial variation in sperm stores in mature female blue crabs *Callinectes sapidus* and potential effects on brood production in Chesapeake Bay. *Marine Ecology Progress Series*, **507**, 249–262.

Olsson M., Shine R. (1997) Advantages of multiple matings to females: a test of the infertility hypothesis using lizards. *Evolution*, **51**, 1684–1688.

Parker G.A. (1970) Sperm competition and its evolutionary consequences in insects. *Biological Reviews*, **45**, 525–567.

Parker G.A. (1990a) Sperm competition games: raffles and roles. *Proceedings of the Royal Society of London B: Biological Sciences*, **242**, 120–126.

Parker G.A. (1990b) Sperm competition games: sneaks and extrapair copulations. *Proceedings of the Royal Society of London B: Biological Sciences*, **242**, 127–133.

Parker G.A., Ball M.A., Stockley P., Gage M.J.G. (1996) Sperm competition games: individual assessment of sperm competition intensity by group spawners. *Proceedings of the Royal Society of London B: Biological Sciences*, **263**, 1291–1297.

Parker G.A., Ball M.A., Gage M.J.G. (1997) Sperm competition games: a prospective analysis of risk assessment. *Proceedings of the Royal Society of London B: Biological Sciences*, **264**, 1803–1812.

Paul A.J., Paul J.M. (1997) Breeding success of large male red king crab *Paralithodes camtsschaticus* with multiparous mates. *Journal of Shellfish Research*, **16**, 379–381.

Pennington J.T. (1985) The ecology of fertilization of echinoid eggs: the consequence of sperm dilution, adult aggregation, and synchronous spawning. *Biological Bulletin*, **169**, 417–430.

Pinheiro J., Bates D., DebRoy S., Sarkar D. (2006) nlme: an R package for fitting and comparing Gaussian linear and nonlinear mixed-effects models. http://www.stats.bris.ac. uk/R/.

Pitnick S. (1993) Operational sex ratios and sperm limitation in populations of *Drosophila pachea*. *Behavioral Ecology and Sociobiology*, **33**, 383–399.

Pitnick S., Markow T.A. (1994) Male gametic strategies: sperm size, testes size, and the allocation of ejaculate among successive mates by sperm-limited fly *Drosophila pachea* and its relatives. *American Naturalist*, **143**, 785–819.

Pizzari T., Cornwallis C.K., Løvlie H., Jakobsson S., Birkhead T.R. (2003) Sophisticated sperm allocation in male fowl. *Nature*, **426**, 70–74.

Preston B.T. (2001) Dominant rams lose out by sperm depletion. *Nature*, **409**, 681–682.

Proctor H.C. (1992) Effect of food deprivation on mate searching and spermatophore production in male water mites (Acari: Unionicolidae). *Functional Ecology*, **6**, 661–665.

Reinhold K., Kurtz L., Engqvist L. (2002) Cryptic male choice: sperm allocation strategies when female quality varies. *Journal of Evolutionary Biology*, **15**, 201–209.

Reta R., Martos P., Perillo G.M.E., Piccolo M.C., Ferrante A. (2001) Características hidrográficas del estuario de la Laguna de Mar Chiquita. In: Iribarne O. (Ed), *Reserva de Biosfera Mar Chiquita*. Editorial Martín, Mar del Plata: 31–52.

Rondeau A., Sainte-Marie B. (2001) Variable mate-guarding time and sperm allocation by male snow crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. *Biological Bulletin*, **201**, 204–217.

Rubenstein D.I. (1989) Sperm competition in the water strider, *Gerris remigis*. *Animal Behavior*, **38**, 631–636.

Ryan E.P. (1967) Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae) I. The male system. *Proceedings of the Symposium on Crustacea Marine Biological Association of India*, 506–521.

Sainte-Marie B., Hazel F. (1992) Molting and mating of snow crabs, *Chionoecetes opilio* (O. Fabricius), in shallow water of the north-western Gulf of Saint Lawrence. *Canadian Journal of Fisheries and Aquatic Science*, **49**, 1282–1293.

Sainte-Marie G., Sainte-Marie B. (1998) Morphology of the spermatheca, oviduct, intermediate chamber and vagina of the adult snow crab (*Chionoecetes opilio*). *Canadian Journal of Zoology*, **76**, 1589–1604.

Sainte-Marie G., Sainte-Marie B., Sevigny J.M. (2000) Ejaculate storage patterns and the site of fertilization in the female snow crab *Chionoecetes opilio* (Brachyura, Majidae). *Canadian Journal of Zoology*, **78**, 1902–1917.

Sainte-Marie B., Sevigny J.M., Carpentier M. (2002) Interannual variability of sperm reserves and fecundity of primiparous females of the snow crab (*Chionoecetes opilio*) in relation to sex ratio. *Canadian Journal of Fisheries and Aquatic Science*, **59**, 1932–1940.

Sainte-Marie B., Sevigny J.M., Gauthier Y. (1997) Laboratory behavior of adolescent and adult males of the snow crab *Chionoecetes opilio* mated noncompetitively and competitively with primiparous females (Brachyura: Majidae). *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 239–248.

Sal Moyano M.P., Gavio A., Luppi T. (2012a) Mating system of the burrowing crab *Neohelice granulata* (Brachyura: Varunidae) in two contrasting environments: effect of burrow architecture. *Marine Biology*, **159**, 1403–1416.

Sal Moyano M.P., Luppi T.A., Gavio M.A., Vallina M., McLay C. (2012b) Receptivity of female *Neohelice granulata* (Brachyura: Varunidae): different strategies to maximize their reproductive success in contrasting habitats. *Helgoland Marine Research*, **66**, 661–674.

Sal Moyano M.P., Gavio M.A., McLay C., Luppi T. (2014a) Habitat-related differences in the pre-copulatory guarding and copulation behavior of *Neohelice granulata* (Brachyura, Grapsoidea, Varunidae). *Journal of Sea Research*, **87**, 8–16.

Sal Moyano M.P., Gavio M.A., McLay C., Luppi T. (2014b) Inter-population variation in the post-copulatory guarding behavior of *Neohelice granulata* (Brachyura, Grapsoidea, Varunidae). *Marine Ecology*, doi: 10.1111/maec.12223.

Sal Moyano M.P., Silva P., Luppi T., Gavio M.A. (2014c) Female mate choice by chemical signals in a semi-terrestrial crab. *Journal of Sea Research*, **85**, 300–307.

Sato T., Goshima S. (2007) Effects of risk of sperm competition, female size, and male size on number of ejaculated sperm in the stone crab *Hapalogaster dentata*. *Journal of Crustacean Biology*, **27**, 570–575.

Sato T., Ashidate M., Wada S., Goshima S. (2005) Effects of male mating frequency and male size on ejaculate size and reproductive success of female spiny king crab, *Paralithodes brevipes*. *Marine Ecology Progress Series*, **296**, 251–262.

Sato T., Ashidate M., Jinbo T., Goshima S. (2006) Variation of sperm allocation with male size and recovery rate of sperm numbers in spiny king crab *Paralithodes brevipes*. *Marine Ecology Progress Series*, **312**, 189–199.

Sato T., Yoseda K., Okuzawa K., Suzuki N. (2010) Sperm limitation: possible impacts of large male-selective harvesting on reproduction of the coconut crab *Birgus latro*. *Aquatic Biology*, **10**, 23–32.

Shapiro D.Y., Marconato A., Yoshikawa T. (1994) Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. *Ecology*, **75**, 1334–1344.

Simmons L.W. (2001) *Sperm Competition and its Evolutionary Consequences in Insects*. Princeton University Press, Princeton: 448.

Smith B.D., Jamieson G.S. (1991) Possible consequences of intensive fishing for males on the mating opportunities of Dungeness crabs. *Transactions of the American Fisheries Society*, **120**, 650–653.

Spivak E. (2010) The crab *Neohelice* (=*Chasmagnathus*) *granulata*: an emergent animal model from emergent countries. *Helgoland Marine Research*, **64**, 149–154.

Svard L., Wiklund C. (1986) Different ejaculate delivery strategies in first *versus* subsequent matings in the swallowtail butterfly *Papilio machaon* L. *Behavioral Ecology and Sociobiology*, **18**, 325–330.

Thiel M., Hinojosa I. (2003) Mating behavior of female rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea) - indication for convenience polyandry and cryptic female choice. *Behavioral Ecology and Sociobiology*, **55**, 113–121.

Urbani N., Sainte-Marie B., Sevigny J.M., Zadworny D., Kuhnlein U. (1998) Sperm competition and paternity assurance during the first breeding period of female snow crab *Chionoecetes opilio* (Brachyura: Majidae). *Canadian Journal of Fisheries and Aquatic Science*, **55**, 1104–1113.

Waage J.K. (1979) Dual function of the damselfly penis: sperm removal and transfer. *Science*, **203**, 916–918.

Warner R.R. (1997) Sperm allocation in coral reef fishes. *BioScience*, **47**, 561–564.

Warner R.R., Shapiro D.Y., Marconato A., Petersen C.W. (1995) Sexual conflict-males with the highest mating success convey the lowest fertilization benefits to females. *Proceedings of the Royal Society of London B: Biological Sciences*, **262**, 135–139.

Wedell N., Cook P.A. (1999) Butterflies tailor their ejaculate in response to sperm competition risk and intensity. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 1033–1039.

Wedell N., Gage M.J.G., Parker G.A. (2002) Sperm competition, male prudence and sperm limited females. *Trends in Ecology and Evolution*, **17**, 313–320.

West B.T., Welch K.B., Gatecki A.T. (2007) *Linear Mixed Models: A Practical Guide Using Statistical Software*. Chapman & Hall/CRC, Boca Raton: 353.

Woodhead A.P. (1985) Sperm mixing in the cockroach *Diploptera punctata*. *Evolution*, **39**, 159–164.

Zuk M. (1988) Parasite load, body size and age of wild-caught male field crickets (Orthoptera: Gryllidae): effects on sexual selection. *Evolution*, **42**, 969–976.