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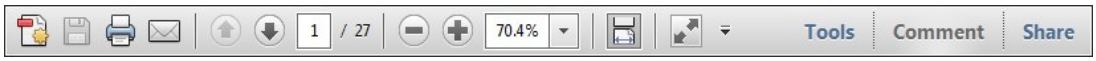

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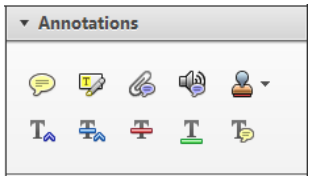

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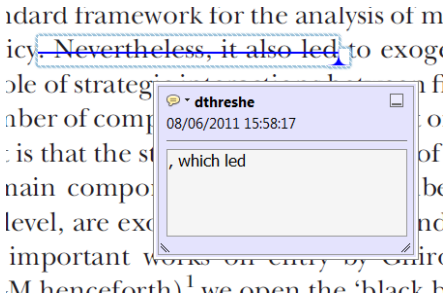

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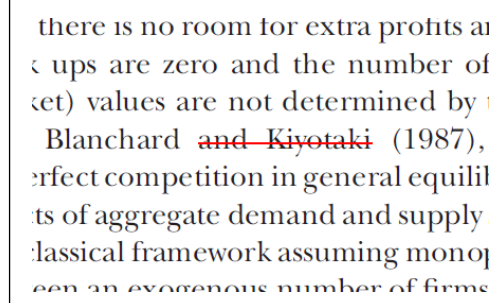

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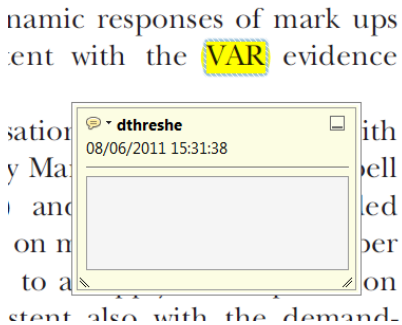

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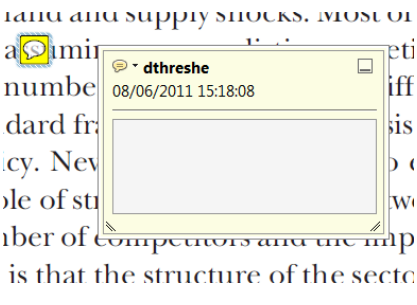

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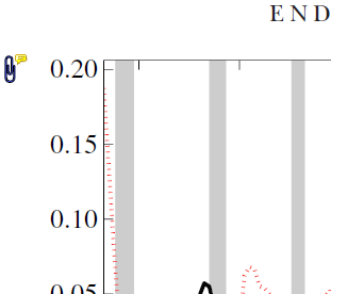

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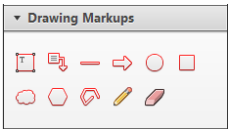

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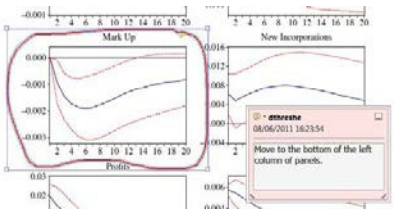

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ORIGINAL ARTICLE

Variation in the post-copulatory guarding behavior of *Neohelice granulata* (Brachyura, Grapsoidea, Varunidae) in two different habitats

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Abstract

Crab guarding behaviors are influenced by the biological and physical characteristics that each population experiences in a particular habitat. We studied post-copulatory guarding behavior and the factors affecting it, such as the presence of burrows, female receptivity duration, operational sex ratio (OSR) and male size, in two different populations of *Neohelice granulata*: Mar Chiquita coastal lagoon (MCL) and San Antonio Oeste (SAO). Laboratory experiments showed that post-copulatory guarding duration varied between the study areas. In MCL, it depended upon the presence of burrows and female receptivity duration, whereas it was independent of the OSR and male size. In SAO, it was dependent upon the OSR and female receptivity duration, whereas it was independent of the presence of burrows and male size. Thus, *N. granulata* males display different reproductive strategies related to their post-copulatory guarding behavior pattern in different habitats. These strategies are related to the fact that this species constructs and defends a burrow that provides shelter and a place to mate, something that other varunids do not have.

Introduction

Mate guarding is a reproductive behavior that is widely distributed in many taxa and has a important function in male reproductive success (Ridley 1983; Smith 1984). Specifically, effective post-copulatory mate guarding prevents rival males from mating with inseminated females, assuring paternity of the offspring and, consequently, increasing male reproductive success (Parker 1970). This is especially true in species in which females store sperm. In Brachyura, successful post-copulatory guarding behavior, based on the dorsal and ventral seminal receptacle types defined by Diesel (1991), favors the first or last male inseminating the female by conferring an advantage in fertilizing the oocytes in species in which females store ejaculates discretely (Diesel 1991). In Grapsoidea crabs, seminal receptacles described mostly correspond to the ventral type; thus, the development of a post-copulatory guarding behavior is expected to be a common strategy in order to ensure a male's paternity of the offspring. However, few studies have verified this prediction, perhaps because of the low number of studies performed in this superfamily (Brockerhoff & McLay 2005a).

Theoretical models predict the evolution of post-copulatory guarding patterns in populations with male-biased operational sex ratios and the existence of sperm competition (Parker 1974). The operational sex ratio (OSR) is defined as the number of fertilizable females relative to the number of sexually mature males in a defined space and time (Emlen & Oring 1977). Females only copulate when they are receptive. The timing and duration of female receptivity are important as these determine the OSR. When females become receptive asynchronously, the OSR is male-biased and there are more opportunities

for different males to find the same female; thus, sperm competition is favored, and consequently, the development of post-copulatory guarding is expected (Parker 1974). The post-copulatory guarding behavior would depend on the synchronization of the receptive period and/or an OSR (Yamamura 1986; Yamamura & Tsuji 1989). Thus, the OSR and the duration of female receptivity could affect post-copulatory guarding behavior.

Other factors such as resource availability and their value may also affect male guarding behavior (Dill 1978; Sih 1993). Burrows are a vital resource as they provide shelter from tidal inundation, predation and desiccation to semi-terrestrial crabs (Smith & Miller 1973; Koga *et al.* 2001), although they are also used during reproduction as a mating site (Backwell & Passmore 1996). If females require a resource for breeding (*e.g.* a burrow), ownership of this resource is essential for males to reproduce successfully (Kelly 2008). In species using burrows as mating sites, the presence of burrows may be an essential requisite to allow copulation, but also, for example, to favor the development of a successful post-copulatory guarding behavior because they are secure places in which to mate and males may easily confine females inside them.

Physical strength is the best predictor of fighting success in animal contests (Elias *et al.* 2010; Kasumovic *et al.* 2010). The size of the males and the potential relative ability of males to compete for a mate are other important factors that influence the success of a guarding reproductive behavior (Parker 1974; Grafen & Ridley 1983). Large males are commonly described as having an advantage in monopolizing and inseminating the majority of, and especially the larger, females (Abele *et al.* 1986; Donaldson & Adams 1989; Moriyasu & Comeau 1996; Jivoff 1997; Sainte-Marie *et al.* 1997; Kamio *et al.* 2003). Thus, the success of post-copulatory guarding behaviors may depend upon male size.

Several studies on *Neohelice granulata* (Dana 1851) have been performed in two different study areas: Mar Chiquita (MCL), which is a coastal lagoon located in Buenos Aires Province, Argentina (37°45′ S, 57°19′ W), and San Antonio Oeste (SAO), a bay located in the northwest of San Matías Gulf, Río Negro Province, Argentina (40°46′ S, 64°50′ W) (*e.g.* Ituarte *et al.* 2006; Bas *et al.* 2007, 2008; Sal Moyano *et al.* 2012a,b; Luppi *et al.* 2013). In both study areas the mating system of this species has been characterized as the resource-centered competition type (Christy 1987), considering burrows as the resource defended by males to attract and copulate with females (Sal Moyano *et al.* 2012a). Large males construct burrows with chambers where copulation occurs, whereas small males construct burrows without chambers and their copulations frequently occur on the surface (Sal Moyano *et al.* 2012a). However, at MCL small males can be found inside empty chambered burrows constructed by large males where they can copulate with females. Both study areas have different types of sediment (gravel content % grain >2 mm: MCL = 0, SAO = 13.3 ± 0.88; penetrability kp·cm^{-2}, MCL = 1.7 ± 0.48, SAO = 26.3 ± 1.1; organic matter content %: MCL = 4.1 ± 0.21, SAO = 0.9 ± 0.07). Thus, the burrow form differs: at MCL burrows are longer and have closed copulation chambers in the upper or middle region, whereas at SAO they are shorter and have open copulation chambers in their entrance (see Sal Moyano *et al.* 2012a). Field studies showed that females could be guarded more successfully in closed compared with open chambers (Sal Moyano *et al.* 2012a). Thus, the development of post-copulatory guarding would depend on the presence and form of burrows and/or on male size.

Neohelice granulata females become receptive asynchronously two to four times during the reproductive season, although each only for a limited period of time; thus the OSR is frequently male-biased (Sal Moyano *et al.* 2012b). Consequently, the OSR may be a factor affecting the post-copulatory guarding behavior. The duration of the receptive period varies, distinguishing two types of females: (i) with short receptivity (2–6 days in MCL, 1–4 days in SAO) and seminal receptacles well stocked with sperm and (ii) with extended receptivity (7–9 days in MCL, 5–7 days in SAO) and seminal receptacles empty or with few sperm (see Sal Moyano *et al.* 2012b). Thus, the duration of female receptivity (short *versus* extended) may be another factor influencing the optimal time of post-copulatory guarding.

The particular biological factors (female receptivity duration, OSR, male size) and the indirect effects of physical factors (*e.g.* presence of burrows with copulation chambers dependent upon the type of sediment) in a population will modulate the capacity of males to vary their guarding behavior pattern in order to maximize the number of oocytes fertilized, evaluating costs and benefits with respect to the time used to guard a mate or look for new mates. Burrow ownership means that it would be better to sit and wait for females to arrive rather than go in search of them. Previous work studied pre-copulatory guarding in *N. granulata* and demonstrated that copulation duration depend on diverse factors such as the OSR, female mating history and physical characteristics of the study area in different populations (Sal Moyano *et al.* 2014a). Thus, we hypothesize that post-copulatory guarding behavior of *N. granulata* varies according to the diverse biological and physical factors in different populations. In this context, the aim of the present work was to study post-copulatory guarding behavior and factors affecting it such as presence of burrows with copulation chambers, male size, a male-biased OSR and duration of

female receptivity in two different populations of *N. granulata*, one at MCL and another at SAO.

Material and Methods

General procedures

Experiments were carried out during the reproductive seasons of 2009/2010, 2010/2011 and 2011/2012 in MCL and SAO. Crabs were collected by hand from the field 1 month before the beginning of the reproductive seasons (August in MCL and October in SAO) and transported to the laboratory. Individuals were measured with a caliper accurate to 0.1 mm and only morphologically and physiologically mature crabs of both sexes were used in the experiments (>19 mm carapace width [CW]; López Greco & Rodríguez 1998). In the laboratory crabs were maintained in communal seawater aerated aquaria (0.3 × 0.35 × 0.25 m, containing 3 cm of seawater), and sexes were held separately. They were fed three times a week with pet-food pellets and water was changed weekly. Temperatures ranged between 22 and 28 °C (mean 24 °C) and natural daylight conditions prevailed.

Experiments were conducted in aerated plastic aquaria (0.4 × 0.5 × 0.2 m: width × length × height). Based on 23 previous experimental data (see Sal Moyano *et al.* 2012a), when burrows were required in the experiments, aquaria contained, on one side, a level of 20 (MCL) or 10 (SAO) cm of sediment obtained from each study area to allow the construction of burrows, and on the other side, a rock (approximately 10 × 20 × 6 cm). Given that only large males (LMs) construct burrows with copulation chambers, these males were placed in an aquarium and given between 1 and 3 days to construct a burrow. All constructed burrows included a chamber (closed entrance in MCL and open in SAO aquaria) in its upper region; thus if a successful copulation or a post-copulatory 24 guarding occurred, it could be visually monitored from the surface. The important difference between MCL and SAO burrows is that burrows with a closed chamber can be defended by MCL males by blocking the entrance (against male intruders or to prevent the female escaping), whereas males cannot defend SAO burrows with open chambers in their entrance (Sal Moyano *et al.* 2012a). A depth of 2 cm of sediment was used when burrows were not required in the experiments.

Females were monitored daily and used in the experiment on the first day that they were found to be receptive. Receptive females (RFs) were recognized by the detection of mobile vulvae opercula that could be pushed inwards like a trapdoor when checked under a binocular microscope (see Sal Moyano *et al.* 2012b). Based on the number of days that females had mobile opercula, they were characterized as having 'short' or 'extended' receptivity duration, following Sal Moyano *et al.* (2012b). In all experiments a male and a RF were added. After addition of individuals, successful mating was registered in all trials. Successful copulation was considered to have occurred when both the female and male pleons were opened and the male gonopods were inserted into the female vulvae. Experiments began immediately after copulation ended. All experiments were checked daily at the same time. Each time the experiment was checked, a post-copulatory guarding behavior was considered to occur successfully when the female was found still receptive (with mobile vulvae opercula) and in contact with the initial mating male (inside or outside of the burrow): the male held the female with his chelae or caged her within his legs. The experiment ended when upon checking the aquarium the female 25 was found to be unreceptive (with immobile vulvae opercula, ovigerous or not ovigerous). Unsuccessful post-copulatory guarding was considered to have occurred when upon checking the aquarium the female was found to still be receptive but not in contact with the initial mating male (in the burrow or on the surface), or being guarded by a competing male (in experiments where the OSR was male-biased), and the experiment ended. All experiments consisted of two treatments. Two-tailed Chi-square tests were performed to evaluate the overall difference in the frequency of successful post-copulatory guarding behavior between the two treatments.

Experiments with individuals from MCL

Earlier experiments conducted in the field at MCL dem- 26 onstrated that successful post-copulatory guarding depends upon the presence of burrows with closed copulation chambers (Sal Moyano *et al.* 2012a). Thus, the 'presence of burrows with copulation chambers' was considered as an independent factor (experiment 1), and also, as a factor interacting with the other ones: 'male-biased OSR' (experiment 2), 'duration of female receptivity' (experiment 3) and 'male size' (experiment 4).

Experiment 1: to test the independent factor 'presence of burrows with copulation chambers', the experiment consisted of two treatments: a LM (29 < CW<32 mm) in 27 a burrow plus a RF (25 < CW < 27.5 mm), and a LM without a burrow plus a RF. Fifteen replicates per treatment were performed.

Experiment 2: to evaluate the effect of the factor 'male-biased OSR' in the presence and absence of burrows, 28 individuals were arranged in four conditions: (i) a mating pair immediately after copulation ended inside a burrow plus three additional males of similar size compared with the male of the mating pair (n = 15); (ii) a mating pair immediately after copulation ended without a burrow

plus three additional males of similar size compared with the male of the mating pair (n = 12); (iii) a mating pair immediately after copulation ended inside a burrow (n = 15); and (iv) a mating pair immediately after copulation ended without a burrow (n = 12). Conditions i and ii were considered as treatment 1 (test of a male-biased 3:1 OSR in the presence or absence of burrows), whereas conditions (iii) and (iv) were considered as treatment 2 (test of a 1:1 OSR in the presence or absence of burrows). Additional males were added immediately after copulation ended and marked with a dot of colored nail polish on the carapace. Female CW ranged from 24 to 28 mm, whereas male ones ranged from 24 to 32 mm.

Experiment 3: to evaluate the effect of the factor 'female receptivity duration' in the presence or absence of burrows, individuals were arranged in two trials: (i) a male in a burrow plus a RF, and (ii) a male without a burrow plus a RF. When the trials ended, females were placed in single aquaria until they became unreceptive and were characterized as having short or extended receptivity by monitoring the mobility of their vulvae opercula. Based on these results, four conditions of receptivity duration were defined. Condition (i) was short receptivity females in the presence of burrows, whereas condition ii was short receptivity females in the absence of burrows. These two conditions were considered as treatment 1 (test of a short receptivity duration female in the presence or absence of burrows). Condition iii was extended receptivity females in the presence of burrows, whereas condition iv was extended receptivity females in the absence of a burrow. These two conditions were considered as treatment 2 (test of an extended receptivity duration female in the presence or absence of burrows). Ten replicates for each condition were obtained. Female CW ranged from 24 to 28 mm, whereas male ones ranged from 24 to 32 mm.

Experiment 4: given that small males (SMs) have previously been found inside burrows with copulation chambers constructed by LMs in MCL (Sal Moyano *et al.* 2012a), to evaluate the effect of the factor 'male size' in the presence or absence of burrows, a LM (29 < CW < 32 mm) or SM (24 < CW < 26 mm) were arranged in four conditions: (i) a LM in a burrow plus a RF (27 < CW < 29 mm), (ii) a LM without a burrow plus a RF (27 < CW < 29 mm), (iii) a SM in a burrow plus a RF (23 < CW < 25 mm), and (iv) a SM without a burrow plus a RF (23 < CW < 25 mm). Conditions i and ii were considered as treatment 1 (test of LM size in the presence or absence of burrows), whereas conditions iii and iv were considered as treatment 2 (test of SM size in the presence or absence of burrows). In the trials where a burrow and a SM were required, the LM was removed without modifying the form of the burrow after its construction, and a SM was added. Twelve replicates of each condition were obtained.

For the factors 'male-biased OSR', 'female receptivity duration' and 'male size', after the Chi-square tests that evaluated the overall difference between the two treatments were conducted, a two-tailed *Z*-test of comparison of two proportions (Zar 2010) was performed to analyse differences within each treatment, between the two conditions described.

Experiments with individuals from SAO

Earlier experiments conducted in the field at SAO demonstrated that post-copulatory guarding is not successful in the presence or absence of burrows with open copulation chambers (Sal Moyano *et al.* 2012a). Thus, the presence or absence of burrows with copulation chambers was considered only as an independent factor in these experiments. Four experiments were performed. Ten replicates per experiment were obtained.

Experiment 1: to evaluate the factor 'presence of burrows with copulation chambers', two treatments were performed, which consisted of placing a LM (27.5 < CW < 29 mm) in a burrow plus a RF (24 < CW < 26.5 mm) *versus* a LM without a burrow plus a RF.

Experiment 2: to evaluate the effect of the factor 'male-biased OSR', two treatments were conducted, which consisted of placing a mated pair immediately after they copulated *versus* a mated pair immediately after they copulated plus three additional males of similar size as the male that copulated with the female. Additional males, marked with a dot of colored nail polish on the carapace, were added immediately after copulation ended. Female CW ranged from 23.5 to 26 mm, whereas male ones ranged from 24 to 29 mm.

Experiment 3: to evaluate the factor 'female receptivity duration', two treatments were performed, which consisted of placing a male plus a short receptivity female (1–4 days) *versus* a male plus an extended receptivity female (5–7 days). When the trials ended, females were isolated in an aquarium until their opercula became immobile and they laid eggs. They were then characterized as having short or extended receptivity. Female CW ranged from 23.5 to 26 mm, whereas male ones ranged from 24 to 29 mm.

Experiment 4: to evaluate the factor 'male size', two treatments were conducted, which consisted of placing a LM (27.5 < CW < 29 mm) plus a RF (25 < CW < 27 mm) *versus* a SM (24 < CW < 25.5 mm) plus a RF (23 < CW < 25 mm).

Results

For all experiments with individuals from both MCL and SAO the post-copulatory guarding behavior developed by

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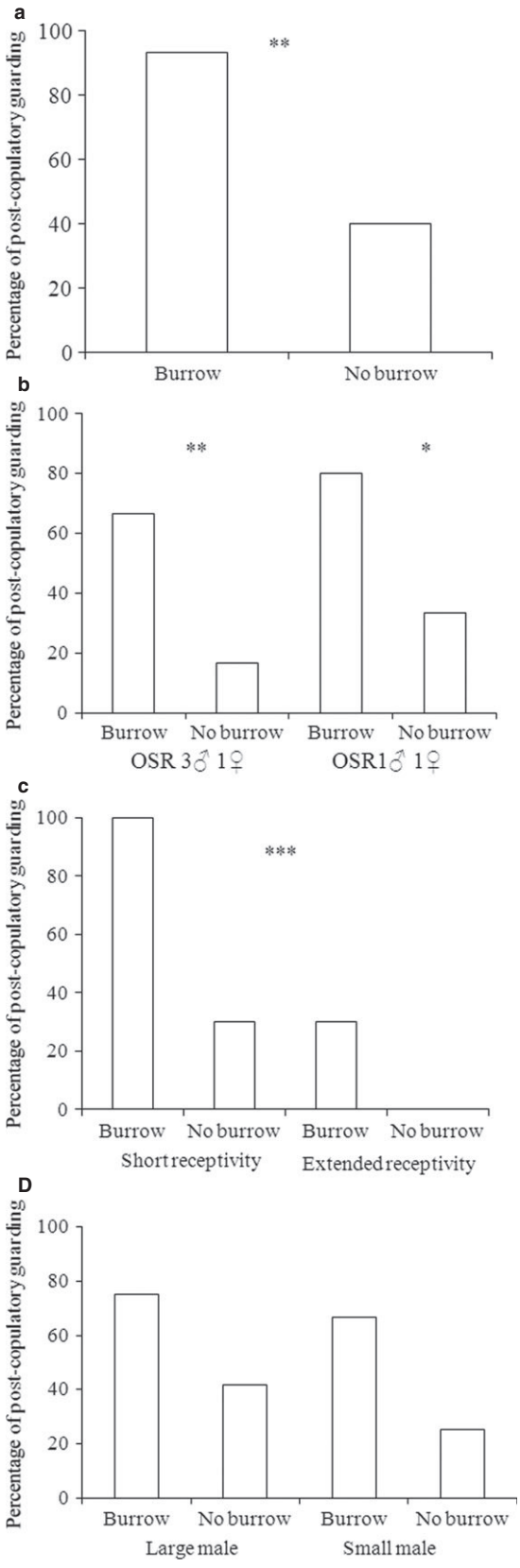

Fig. 1. Successful male post-copulatory guarding of *Neohelice granulata* females from Mar Chiquita Coastal lagoon dependent on the 74 factors: (a) presence *versus* absence of burrows: 'burrow' *versus* 'no burrow' (n = 15); (b) operational sex ratio (OSR) male-biased (3:1) *versus* OSR not male-biased (1:1) in the presence or absence of burrows: 'burrow' *versus* 'no burrow' (n = 12–15); (c) duration of female receptivity, short *versus* extended, in the presence or absence of burrows: 'burrow' *versus* 'no burrow' (n = 10); and (d) male size, large *versus* small, in the presence or absence of burrows: 'burrow' *versus* 'no burrow' (n = 15). (χ^2, *P < 0.05, **P < 0.01, ***P < 0.001).

males lasted until females laid eggs, and thus, became unreceptive (with immobile opercula).

Experiments with individuals from MCL

Experiment 1: males guarded females, showing a post-copulatory guarding behavior in the presence of burrows with copulation chambers (χ^2 = 9.6, P < 0.01, Fig. 1a). 39

Experiment 2: regarding the factor 'male-biased OSR', no differences were found between treatments (χ^2 = 0.007, P = 0.12, Fig. 1b). However, differences were found in the occurrence of post-copulatory guarding between the conditions when: the OSR was 3:1 male-biased (treatment 1) or it was 1:1 (treatment 2); males 40 guarded females in the presence of burrows (conditions i and iii), whereas they did not do so in the absence of burrows (conditions ii and iv) (Z = 2.6, P < 0.01; Z = 2.45, P < 0.05, respectively). Thus, the post-copulatory guarding behavior depended upon the presence of burrows but was independent of the OSR. 41

Experiment 3: differences between treatments were found regarding the 'female receptivity duration' factor (χ^2 = 22.5, P < 0.001, Fig. 1c). In treatment 1 (short receptivity females) differences were found in the occurrence of post-copulatory guarding behavior with respect to the presence of burrows: males guard females in the presence of burrows (condition i), whereas they did not do so in the absence of burrows (condition ii) (Z = 3.28, P < 0.01). However, in treatment 2 (extended receptivity females) no differences were found with respect to the presence or absence of burrows: males did not guard females in either case (Z = 1.12, P = 0.26). Thus, successful post-copulatory guarding was dependent upon the presence of burrows and upon the duration of female receptivity.

Experiment 4: no differences were found in successful post-copulatory guarding behavior between treatments regarding the factor 'male size' (χ^2 = 7.6, P > 0.05). However, differences in each treatment were found regarding both conditions: both the LM (treatment 1) and SM (treatment 2) guarded females in the presence of burrows (conditions i and iii), but they did not guard 42 females in the absence of burrows (conditions ii and iv)

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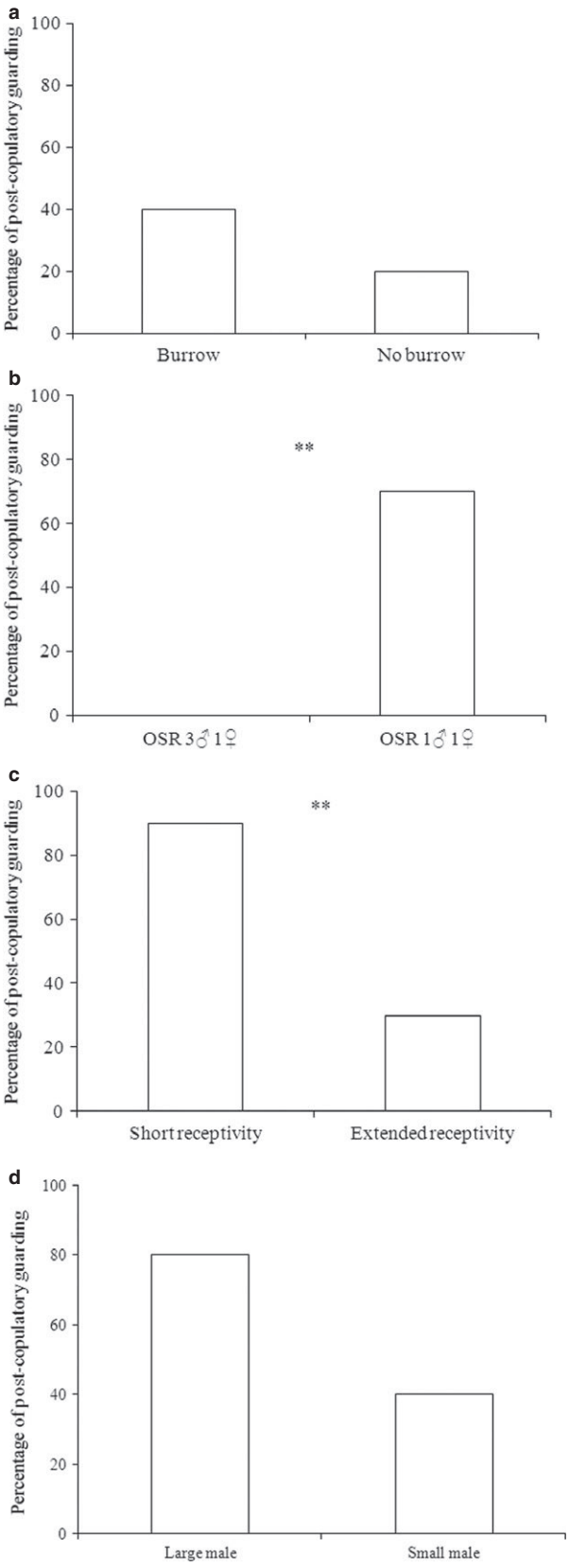

Fig. 2. Successful male post-copulatory guarding of *Neohelice granulata* females from San Antonio Oeste (SAO) dependent on the factors: (a) presence *versus* absence of burrows: 'burrow' *versus* 'no burrow'; (b) operational sex ratio (OSR) male-biased (3:1) *versus* OSR not male-biased (1:1); (c) female receptivity duration, short *versus* extended; and (d) male size, large *versus* small (χ^2, n = 10, **P < 0.01).

($Z = 1.65$, $P < 0.05$; $Z = 2.05$, $P < 0.05$, respectively). Thus, successful post-copulatory guarding depended upon the presence of burrows, although it was independent of male size.

Experiments with individuals from SAO

The occurrence of post-copulatory guarding was independent of the presence of burrows with copulation chambers (experiment 1, $\chi^2 = 0.95$, $P > 0.05$), whereas it was dependent upon the OSR (experiment 2, $\chi^2 = 10.7$, $P < 0.01$) and upon female receptivity duration (experiment 3, $\chi^2 = 7.5$, $P < 0.01$) (Fig. 2a,b): when the OSR was 1:1 and females had short receptivity duration, respectively, well-developed post-copulatory guarding was observed. No differences were found in post-copulatory guarding regarding the factor 'male size' (experiment 4, $\chi^2 = 1.33$, $P = 0.39$).

Discussion

The physical characteristics of a habitat and the social context have the capacity to modulate the strategies and sexual behavior of different populations in order to maximize reproductive success in each particular habitat (Chapman *et al.* 2003; Cordero & Eberhard 2005; Kokko 2005; Thiel & Duffy 2007). In *Neohelice granulata* previous studies showed that there are differences in some reproductive traits and in the reproductive strategies of the MCL and SAO populations (Ituarte *et al.* 2006; Bas *et al.* 2007, 2008; Sal Moyano *et al.* 2012a,b). In the present study, the success of post-copulatory guarding depended upon short female receptivity duration in both study areas although it also varied with the other factors studied. In MCL it depended on the presence of burrows, whereas it was independent of the OSR and male size. In SAO it was dependent upon the OSR, whereas it was independent of the presence of burrows and male size. Thus, the habitat characteristics, such as the form of burrows, and the biological factors, such as the OSR and the duration of female receptivity, of different populations modify male reproductive strategies and, specifically, the development and duration of post-copulatory guarding behavior in each particular habitat.

Neohelice granulata males guarded short receptivity duration females in the presence or absence of burrows

with copulation chambers at both study areas. These males would gain an advantage by copulating with short receptivity females given that the duration of guarding these females is shorter compared with guarding extended receptivity ones. In this way, males may increase their probability of looking for and mating with other females, increasing their reproductive success. Similarly, some ocypodid females lay more eggs at the beginning of the reproductive season compared with the peak of the season (Jormalainen 1998, 2007). Thus, ocypodid males that copulate with females at the beginning of the reproductive season would spend more time guarding, and thereby lose chances of mating with other females, thus creating a sexual conflict as copulating with the latter females would be more advantageous (Jormalainen 1998, 2007). Besides, in some ocypodid species post-copulatory guarding duration varies throughout the reproductive season with the number of ovigerous and non-ovigerous females (Christy 1982; Murai *et al.* 1987; Goshima & Murai 1988; Goshima *et al.* 1996). This pattern may also characterize *N. granulata* females because the number of extended *versus* short receptivity duration females fluctuates throughout the reproductive season: the former are more abundant at the beginning whereas the latter are at the end of the reproductive season (Sal Moyano *et al.* 2012b). Thus, males should develop longer post-copulatory guarding at the beginning compared with the end of the reproductive season.

Females of *N. granulata* have a ventral type seminal receptacle and store sperm from previous copulations (from a previous reproductive season or from a prior receptive period during the current reproductive season). The sperm is mixed in the seminal receptacle and so it is not possible to differentiate sperm packets corresponding to different males (López Greco *et al.* 1999; Sal Moyano *et al.* 2012b). This mixing occurs approximately 24 h after insemination, when spermatophores are broken (Sal Moyano *et al.* 2012b). However, it is expected that males guard short receptivity duration females because egg-laying occurs immediately after copulation (within 24 h), increasing the probability that the male's sperm fertilize the majority of the oocytes by avoiding too much sperm mixing. Moreover, extended receptivity females do not lay eggs because they may prefer to copulate with many males to (re)fill their seminal receptacles (Sal Moyano *et al.* 2012b), which reinforces our hypothesis. These results agree with the model proposed by Yamamura (1986), in which the duration of post-copulatory guarding and the costs for males varies also in relation to the female receptivity duration. In the case of SAO, the dependence of successful post-copulatory guarding on receptivity duration would be more intense given that the short receptive period is shorter compared with MCL (1–4 *versus* 2–6 days, respectively).

In *N. granulata* mate choice by females is based on chemical signals (Sal Moyano *et al.* 2014b). Thus, it is proposed that males should have the capacity to distinguish between short and extended receptivity duration females using some chemical signal. In the ocypodid *Uca lactea* males recognize chemically females that are close to laying eggs (Murai *et al.* 2002). Similarly, in *Halicarcinus cookii* males prefer to mate with females at an advanced stage of development of their eggs or with non-ovigerous females, for which post-copulatory guarding is the most extended (van den Brink & McLay 2010). Additionally, in *N. granulata* the existence of a mechanical signal used by males to recognize both female types can also be hypothesized given that while mounting the female in the copulation position, males could manipulate the female and evaluate the quantity of sperm stored in the seminal receptacle before transferring the sperm, for example using their gonopod.

Successful post-copulatory guarding behavior depended upon the presence of burrows with copulation chambers in MCL but not in SAO. In the ocypodid *Macrophthalmus hirtipes* males did not guard receptive females after copulating in the absence of burrows (Jennings *et al.* 2000). The results found here for *N. granulata* agree with a previous study conducted in the field because successful post-copulatory guarding depended on the form of the burrows: it occurred in closed copulation chambers of LMC burrows, whereas it did not occur in open copulation chambers of SAO burrows (Sal Moyano *et al.* 2012a). Moreover, this result may be related to the finding that post-copulatory guarding was independent of the OSR in MCL but dependent upon it in SAO. Thus, in MCL, independently of the OSR, the form of the burrow allows males to block its entrance, avoiding intrusion of rival males. This has also been recorded in some ocypodids in which males guarded females in their burrows until the extrusion of eggs (Goshima *et al.* 1996; Koga *et al.* 1999). By contrast, in SAO, open copulation chambers at the entrance of burrows do not allow males to guard females successfully, and thus, it is expected that post-copulatory guarding varies with additional factors such as the OSR. This result for the SAO population agrees with theoretical predictions that males should guard longer when the OSR favoring males increases (Grafen & Ridley 1983). For example, in *Chionoecetes opilio* in the presence of rival males, dominant males guard for longer after copulating and transfer larger ejaculates (Rondeau & Sainte-Marie 2001).

In crustaceans, different models have proposed that male size should modify the time of female guarding in response to diverse factors (Parker 1974; Grafen & Ridley 1983). When competition among males for access to females is high, time spent guarding should vary with the

relative competitive ability of males (Grafen & Ridley 1983). Large males should be the ones who guard females for a longer period of time, reflecting the importance of the size advantage to defending females and displacing small males (Sainte-Marie *et al.* 1997). For example, in *Austrohelice crassa* LMs mate with more females compared with SMs (Brockerhoff & McLay 2005b). Moreover, SMs should be unable to guard females for long periods owing to their small chela size, even when females do not show resistance to them (Minouchi & Goshima 1998; Kamio *et al.* 2003; Sal Moyano & Gavio 2012). In *N. granulata* individuals from MCL, post-copulatory guarding did not depend directly upon male size but upon the presence of burrows. Given that the burrows holding copulation chambers are only constructed by LMs, an indirect effect of male size on successful guarding is suggested. Besides, the result showing that SMs can be found in burrows with copulation chambers constructed by LMs in MCL (Sal Moyano *et al.* 2012a), where they copulate with females and successfully guard them, also indicates the independence of the male size factor and the dependence instead upon the presence and form of burrows. In SAO, although post-copulatory guarding was independent of male size and the presence of burrows, this could be because of the low number of replicates performed (n = 10, MCL: eight males post-copulatory guarding *versus* two males not guarding, SAO: four *versus* six, respectively). Given that at SAO post-copulatory guarding is not successful inside burrows because of its form, guarding outside them would depend on additional factors such as male size.

In some species post-copulatory guarding depends jointly upon OSR and male size: LMs guard females for a longer time in the presence of rival males (Kendall & Wolcott 1999). In this way, males would be capable of recognizing fluctuations in the OSR and in their relative competitive ability with respect to the size of rival males, and consequently, modify their guarding behaviors (Rondeau & Sainte-Marie 2001). In *H. cookii* the duration of the post-copulatory guarding was shorter when the OSR was female-biased, suggesting that in this species males display flexible reproductive behavior according to population fluctuations (van den Brink & McLay 2010). Similarly in *N. granulata*, it is proposed that male mating behavior is sufficiently plastic to allow the development of alternative reproductive strategies in different populations as was found here regarding the differences in post-copulatory guarding in both the MCL and the SAO populations.

Mating strategies among varunid crabs can be different even when they occupy the same habitat (Brockerhoff & McLay 2005a). For example in *Hemigrapsus sexdentatus* (3–4 receptive field cages) and *Hemigrapsus crenulatus* (2–4 days receptive) post-copulatory guarding occurs but in *Cyclograpsus lavauxi* (6 days receptivity) and *Austrohelice crassa* (12 days receptivity) it does not. This difference may be related to the duration of receptivity: 2–4 days in the two *Hemigrapsus* species, but 6–12 days in the latter two species. It may well repay a male to stay and guard if the female is only receptive for a short time, but if not then it may be more rewarding to go in search of other females. Here we compared the post-copulatory guarding behavior of the same species, *Neohelice granulata*, in two different habitats. The duration of receptivity in this species reflects the amount of sperm in the seminal receptacle and is dependent upon habitat (Sal Moyano *et al.* 2012b). Females with abbreviated receptivity (2–6 days in MCL, 1–4 days in SAO) laid eggs after mating but females with extended receptivity (7–9 days in MCL, 5–6 days in SAO) did not always lay eggs, a pattern that was the same in the estuarine lagoon and the marine embayment. Similar to New Zealand species, *N. granulata* males guard only short receptivity duration females in both populations. However, as we have shown here, an important difference between *N. granulata* and the four New Zealand species is that *N. granulata* constructs and defends burrows, which provide shelter and protection, as well as for males, a mating chamber. These cases illustrate the point that in varunid reproductive strategies there can be differences attributable to phylogeny (species with different evolutionary history do not do the same thing despite living under similar conditions) as well as differences within the same species living in different habitats. Such flexibility may be the key to adjusting to different habitats, given larval dispersal and colonization, as well as adjusting to long-term environmental change.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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30	**AUTHOR: Included has been changed to was directly after the condition number throughout this section. Please check and confirm if this is correct.**	
31	**AUTHOR: Given that small males (SMs) have previously been found inside burrows. This text has been revised for clarity. Please check and confirm if it is correct.**	
32	**AUTHOR: For the factors... Please split this long sentence into two (or more) separate sentences if possible for clarity.**	
33	**AUTHOR: Earlier has been added before experiments conducted here for clarity. Please check and confirm if this is correct in context.**	
34	**AUTHOR: Post-copulatory guarding is not successful in the presence or absence of burrows with open copulation chambers (Sal Moyano *et al.* 2012a). Thus, the presence or absence of burrows with copulation chambers was considered only. Please check this text. Is it appropriate to state the type of copulation chamber when it is absent? Please revise if appropriate. Should the type of copulation chamber also be added in the second sentence? Please revise text if appropriate.**	
35	**AUTHOR: Consisted of placing a mated pair immediately after they copulated *versus* a mated pair immediately after they copulated plus three additional males of similar size. Should the location where the animals were placed be added here for clarity? Alternatively can placing be revised for clarity? Please revise as appropriate.**	
36	**AUTHOR: Consisted of placing a male plus a short receptivity female (1–4 days) *versus* a male plus an extended receptivity female. As above, should the location where the animals were placed be added here for clarity? Alternatively can plus be revised to with for clarity? Please revise as appropriate.**	

37	AUTHOR: Females were isolated in an aquarium until their opercula became immobile and they laid eggs. They were then characterized. This text has been revised for clarity. Please check and confirm if it is correct.	
38	AUTHOR: Consisted of placing a LM (27.5 < CW < 29 mm) plus a RF (25 < CW < 27 mm) *versus* a SM (24 < CW < 25.5 mm) plus a RF (23 < CW < 25 mm). As above, should the location where the animals were placed be added here for clarity? Alternatively can plus be revised to with for clarity? Please revise as appropriate	
39	AUTHOR: Please note that in both Figures 1 and 2, Proportion has been changed to Percentage in all y-axis labels as the scale of the y-axes runs from 1 to 100 in all cases. Please check and confirm if this is correct.	
40	AUTHOR: Please note that the comma after (treatment 2) has been changed to a semicolon. Please check and confirm that this is your intended meaning or revise further.	
41	AUTHOR: Depended upon the presence of burrows but was independent. This text has been revised for clarity. Please check and confirm if it is correct.	
42	AUTHOR: Both the LM (treatment 1) and SM (treatment 2) guarded females in the presence of burrows (conditions i and iii), but they did not guard. This text has been revised for clarity. Please check and confirm if it is correct.	
43	AUTHOR: Quotation marks have been added around male size here for consistency with earlier text. Please check and confirm if this is correct.	
44	AUTHOR: The success of post-copulatory guarding depended upon short female receptivity duration in both study areas although it also varied with. This text has been revised for clarity of language. However, please check and confirm if the meaning is correct. Can the text be revised to success rates of post-copulatory guarding were significantly higher with females of short receptivity duration both study areas although they also varied with or an alternative for clarity? Please revise if appropriate.	
45	AUTHOR: Independent on changed to independent of here and in next sentence for clarity. Please check and confirm if this is correct.	
46	AUTHOR: Modify male reproductive strategies. Please check this text in context. Should modify be changed to influence or an alternative change be made for clarity? Please revise as appropriate.	
47	AUTHOR: The sperm is mixed... New sentence started here and the text of the new sentence revised for clarity. Please check and confirm if this is correct.	
48	AUTHOR: However, it is expected that males guard short receptivity duration females... The meaning of this sentence is unclear. Please check and revise as appropriate (please note that some changes have already been made for clarity - please check that these are correct or revise further).	
49	AUTHOR: Moreover, extended receptivity females do not lay eggs because they may prefer to copulate with. The meaning of this text is unclear in context. Please check and revise as appropriate.	

50	AUTHOR: Would be more intense given that the short receptive period is shorter compared with MCL. Can this be changed to should therefore be more intense than at MCL given that the receptive period is shorter or an alternative for clarity in the context of the previous sentence? Please check and revise as appropriate.	
51	AUTHOR: Days has been added after 2–6 here for clarity. Please check and confirm if this is correct.	
52	AUTHOR: Similarly, in *Halicarcinus cookii*... Please check this sentence and revise for clarity if appropriate. Please note that some changes have already been made. Please check that these are correct.	
53	AUTHOR: Can also be hypothesized. This text has been revised for clarity. Please check and confirm if it is correct.	
54	AUTHOR: Moreover, this result... This sentence has been revised for clarity. Please check and confirm if it is correct. As for earlier query, please also check the use of the phrase depended upon in context and revise if appropriate.	
55	AUTHOR: This has also been recorded... This sentence has been revised for clarity. Please check and confirm if it is correct.	
56	AUTHOR: This result for the SAO... This sentence has been revised for clarity. Please check and confirm if it is correct.	
57	AUTHOR: For example, in *Chionoecetes opilio* in the presence of rival males, dominant males guard for longer after copulating and transfer larger ejaculates. Please check this text. Should than when no other males are present or similar be added here? Please check and revise as appropriate.	
58	AUTHOR: That male size should modify the time of female guarding in response to diverse factors. The meaning of this text is unclear. Please check and revise as appropriate.	
59	AUTHOR: In *N. granulata* individuals from MCL, post-copulatory guarding did not depend directly upon male size but upon the presence. This text has been revised for clarity. Please check and confirm if it is correct.	
60	AUTHOR: The independence of the male size factor and the dependence instead upon. This text has been revised for clarity. Please check and confirm if it is correct.	
61	AUTHOR: Post-copulatory guarding is not successful inside burrows because of its form. The meaning of this text is unclear. Can the latter part be changed to because of their open form or an alternative change be made for clarity? Please check and revise as appropriate.	
62	AUTHOR: As was found here regarding the differences in post-copulatory guarding in both the MCL and the SAO populations. This text has been revised for clarity. Please check and confirm if it is correct.	
63	AUTHOR: Even when they occupy the same habitat. This text has been revised for clarity. Please check and confirm if it is correct.	
64	AUTHOR: 3–4 receptive field cages. The meaning of this text is unclear in context. Should this refer to the length of the receptive period as for the other text in parentheses in this sentence? Please revise as appropriate.	

65	AUTHOR: (2–4 days receptive). Can this be changed to (2–4-day receptive period) or an alternative for clarity? Please also check and revise (6 day receptivity) and (12 days receptivity) as appropriate for clarity and consistency.	
66	AUTHOR: 2–4 days in the two *Hemigrapsus* species, but 6–12 days in. Please note that the lengths of the receptive periods are given in the previous sentence. Can this or the previous sentence be revised to avoid repetition? Please check and revise if appropriate.	
67	AUTHOR: Receptivity in this species reflects the amount of sperm in the seminal receptacle and is dependent. This text has been revised for clarity. Please check and confirm if it is correct.	
68	AUTHOR: In the estuarine lagoon and the marine embayment. Can MCL and SAO be added as appropriate here for ease of reference? Please add if appropriate.	
69	AUTHOR: Similar to New Zealand species. It is unclear which species are being referenced here. Should of *Neohelice* or an alternative be added for clarity? Please revise if appropriate.	
70	AUTHOR: In Conflict of interest section, standard text has been used. Please check.	
71	AUTHOR: Jormalainen et al. (2000) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	
72	AUTHOR: Please check the expanded journal title 'Journal of Zoology (London)'.	
73	AUTHOR: Please provide the volume number, page range for reference Sal Moyano et al. (2014a).	
74	AUTHOR: Figure 1 is of poor quality. Please check required artwork specifications at http://authorservices.wiley.com/bauthor/illustration.asp	
75	AUTHOR: Figure 2 is of poor quality. Please check required artwork specifications at http://authorservices.wiley.com/bauthor/illustration.asp	